INSTITUUT VOOR BIOLOGISCH EN SCHEIKUNDIG ONDERZOEK
VAN LANDBOUWGEWASSEN
WAGENINGEN

MEDEDELINGEN
235-256

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with summaries

INHOUDSOPGAVE/*CONTENTS*

WOORD VOORAF

Veel van onze werkers bleken weer gegevens en gedachten uit hun lopende onderzoek beschikbaar te hebben voor verspreiding in ruimere kring. Deze zijn daarom gebundeld tot het nu gereedgekomen Jaarboek 1964. De behandelde onderwerpen liggen ver uiteen, maar we verwachten dat onze lezers zich met ons willen inspannen om het vermelde een plaats te geven in het geheel van kennis en ervaringen rondom de teelt der gewassen en het gebruik van de producten ervan.

Uit de auteurvermeldingen spreekt al een grote mate van samenwerking, doch daarin kon niet tot uiting komen dat de eindvorm mede te danken is aan de goede zorgen van Mej. A. H. VAN ROSSEM en de heren A. KLEINENDORST en G. BEEKHOF.

Drs. MAKKINK heeft ongetwijfeld weer een aantal buitenlanders aan zich verplicht, die slechts via het Esperanto tot de inhoud van dit jaarboek toegang hebben.

De Directeur van het Instituut voor
Biologisch en Scheikundig Onderzoek van Landbouwgewassen,

Prof. dr. ir. G. J. VERVELDE

Wageningen, oktober 1964.

NOTICE FOR FOREIGN SCIENTISTS

As in preceding years a mimeographed issue has been prepared with summaries and the explanation of figures and tables in Esperanto for countries where English is not generally understood. It will be sent on request.

AVIZO POR FREMDAJ SCIENCISTOJ

Kiel en antaŭaj jaroj mimeografita kajero estas preparita kun resumoj kaj la klarigo de la figuroj kaj tabeloj en Esperanto por landoj kie la angla ne ĝenerale estas komprenata. Ĝi estos alsendata post peto.

INSTITUUT VOOR BIOLOGISCH EN SCHEIKUNDIG ONDERZOEK VAN LANDBOUWGEWASSEN

31 JULI 1964

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Mededeling 235 van het I.B.S.

RESPONSES OF BEAN PLANTS TO ROOT TEMPERATURES

I. ROOT TEMPERATURES AND GROWTH IN THE VEGETATIVE STAGE

R. BROUWER

INTRODUCTION

Though the effect of root temperature on the growth of various crops has been investigated relatively often, it is as yet impossible to gain a clear impression of the causal relations which are of importance in this respect. RICHARDS *et al.* (11) stated after an elaborate review of the literature on this subject: 'Very limited data are available for only a few plants in certain stages of growth and under given environmental conditions'. As regards this little has changed since that time.

More detailed and elaborate investigations should be carried out on this subject. In the first place various activities of the same plant species need to be included in the investigations in order to get some idea of the various responses to different root temperatures. Until now data are extremely limited in this respect. It is impossible to estimate from data on the influence of root temperature on the ion uptake of a certain plant species anything as to the growth of another species. Obviously, there are considerable intervarietal differences, especially concerning the temperature responses (3, 9).

Secondly, it is important to examine the interaction of root temperature and other external factors. This also is a field in which little research has been carried out, although indications are to be expected that may add to the appreciation of the causal relations, as has been found for other growth factors as well (4). Evidently, the influence of the root temperature will decrease as other growth factors are more limiting. The greater part of the discrepancies in the results in literature probably is related to this fact.

Thirdly, the influence of root temperature should be investigated in the various growth stages. Literature generally assumes that the optimum changes to lower temperatures as the plant grows older (11, 12). In some cases it could be proved that this is not a question of sensitivity, but a change in the trend due to the duration of the application period (1, 8). Thus a root temperature of over 25°C decreases the potassium content in peas, because the potassium uptake initially lags behind as compared to growth. In the long run, this content grows so low that growth inhibition occurs. Peas also show growth depressions at the transition from the vegetative phase to the reproductive phase. This depression starts earlier as temperature is higher. However, so long as this change-over has not been completely realized transference to a lower temperature will induce the expected growth depression. The plant keeps the same optimum.

In so far as known the change of the optimum to lower values is always due to growth depressions caused in the long run by high temperatures.

In view of the extensive data of this investigation the results are published in a series of articles.

METHODS

The experiments were carried out with seedlings of *Phaseolus vulgaris* after germinating in moist sand at 20°C. When the midrib of the primary leaves had reached a length of about 25 mm the seedlings were put on a Hoagland nutrient solution, 750 ml per plant, refreshed three times a week. Air temperature in all experiments was 20°C, air humidity 65% and light intensity 40.000 ergs. $cm^{-2}.sec^{-1}$. at a length of 17 hours per 24 hours.

In part of the experiments the various root temperatures, *viz.* 5°, 10°, 15°, 20°, 25°, 30°, 35°, and when possible, 40°C were applied immediately after germination from the very beginning of the experiment. In another part of the experiments the various root temperatures were only applied after a preliminary period of 10 to 12 days at a root temperature of 20°C.

In both groups the nutrient solution was sometimes replaced by tap water in order to examine the influence of ion withholding on growth.

Growth was measured by frequent fresh-weight and dry-weight determinations and by daily measurements of the length of the midrib.

RESULTS

Growth of the young seedlings

The plants were put on nutrient solution at various root temperatures immediately after germination. Fig. 1 shows the fresh weights reached after 12 and 20 days respectively. Over a period of 12 days the optimal root temperature appeared to be 30°C. This holds for root growth as well as for shoot growth. During the next 8 days there was some change. At the end of this period there appeared to be only very little difference between the weights at the root temperatures 20°, 25° and 30°C during the whole 20 days' period. This means that the relative growth rate in the last part of the period was highest at 20°C (fig. 2). Fig. 2 also shows that the relative growth rate at root temperatures of 15° and 20°C was constant during the whole period. At the other root temperatures the growth rate at the beginning was higher than at the end of the period. This change was greatest at the temperature which was optimal during the first 12 days (30°C) so that the optimum temperature decreased gradually to

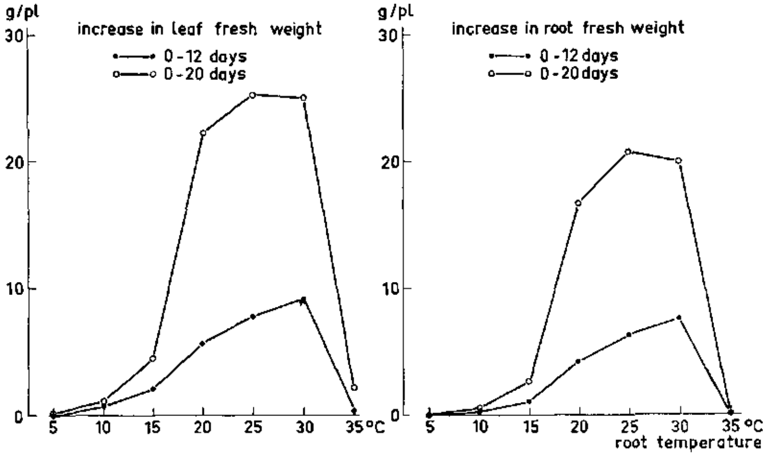
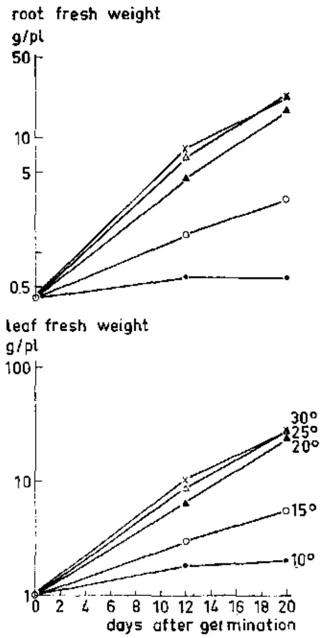


FIG. 1. Increase in fresh weights of bean plants grown on aerated Hoagland solution at different temperatures during 12 or 20 days after germination.

FIG. 2. Fresh weight of roots and leaves of bean plants grown at different root temperatures plotted against time.



25° and perhaps to still lower values when the experiment had been prolonged.

Fig. 3 reflects matters as they are when the length increase of the leaf is taken as a growth measure. Immediately from the beginning of treatment considerable differences occurred. At a root temperature of 5°C there was only some leaf growth in the first two days. In that period only cell division took place which was hardly influenced by root temperature. The succeeding cell elongation was completely inhibited at 5°C and more rapid as root temperatures were higher with an optimum at 30°C for the primary leaves, while the optimum for the first trifoliate leaf was 25° to 20°C. Growth at 35°C was about equal to that at a root temperature of 15°C as far as the primary leaves are concerned. However, this only holds if the final length is taken as a

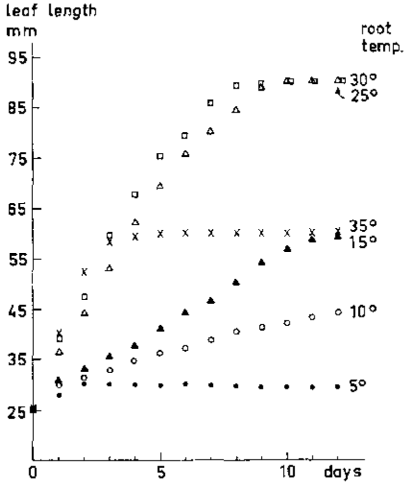


FIG. 3. Length of the midrib of the primary leaves of bean plants grown at different root temperatures plotted against time.

measure. The growth trend in time was most divergent and clearly showed that growth at this high root temperature gradually retarded. This effect is due to the fact that there hardly was any root growth at all and the roots gradually were loosing all uptake capacity as a result of the formation of impermeable layers (6).

Measuring the growth in length of the leaves is attractive as a growth measure, because this allows an examination of the growth responses to changing external conditions on the same plants. When plants have to be harvested the variability of the plant material will cause less clear cut results.

Growth responses to changes in root temperature

As has been mentioned above leaf growth is one of the most satisfactory properties to use in determining a rapid response of growth to changes in external conditions. In fig. 4 the length of the primary leaves has been plotted against time. After three days of growth at four different root temperatures the plants of each root temperature were distributed over all four root temperatures. Leaf growth during the first three days showed the normal response. After transferring the plants there were immediate changes in growth rate. Each symbol is the average of four plants. In transferring plants from

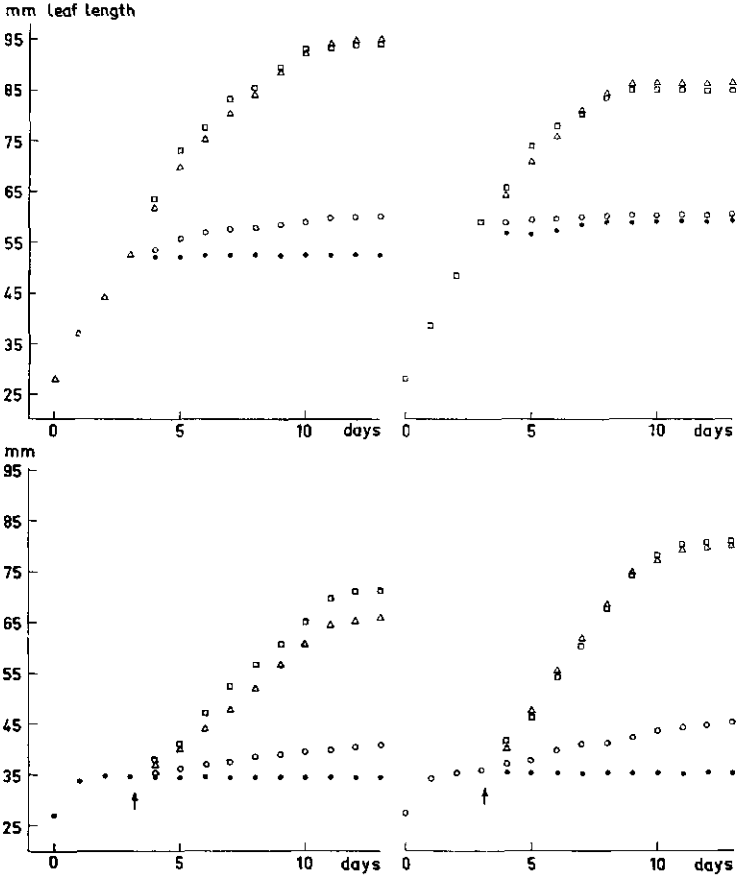


FIG. 4. Growth of the primary leaves before and after a change of root temperature (symbols as in fig. 3).

5° and 10°C to higher root temperatures growth was accelerated. At 10°C the growth rate normal for the new root temperature was immediately reached, at 5°C it seems to take somewhat longer. Apparently, the root system has changed to such an extent in the first three days of pre-treatment at 5°C that recovery of the normal activities demanded some time. A treatment of three days at 10°C was not long enough for this phenomenon to occur, but it did show after a prolonged treatment.

At transferring from higher root temperatures to those of 5° and 10°C immediate and complete growth depression took place. The plants showed immediate wilting at these temperatures, at 10°C recovery took place within the first 24 hours, while at 5°C after 5 days only the original leaf length was recovered. There was no question of growth being resumed at this temperature.

Fig. 5 represents the fresh-weight increase after changing the root temperature. In this experiment a great number of plants received a pre-treatment during 10 days at root temperatures of 10° or 20°C. On the tenth day both these groups were distributed over a range of root temperatures as indicated in the figure. The fresh-weight increase during the subsequent period of 10 days was determined. After a pre-treatment at 20°C the growth rate in the second period was almost the same at root temperatures of 20°, 25° and 30°C. The growth rates at all other root temperatures were lower, this being more apparent in root growth than in shoot growth. Plants transferred to the different root temperatures after a pre-treatment at a root temperature of 10°C showed a completely different response. The plants clearly showed differentiation over the whole temperature range with a root temperature of 35°C as an optimum.

The above has proved that growth at 20°C was logarithmic during the whole growth period of 20 days. At higher root temperatures the relative growth rate decreased with age. A comparison of fig. 5a with fig. 5b shows that this phenomenon in all probability was not directly related to the age of the plants. Apparently, the size of

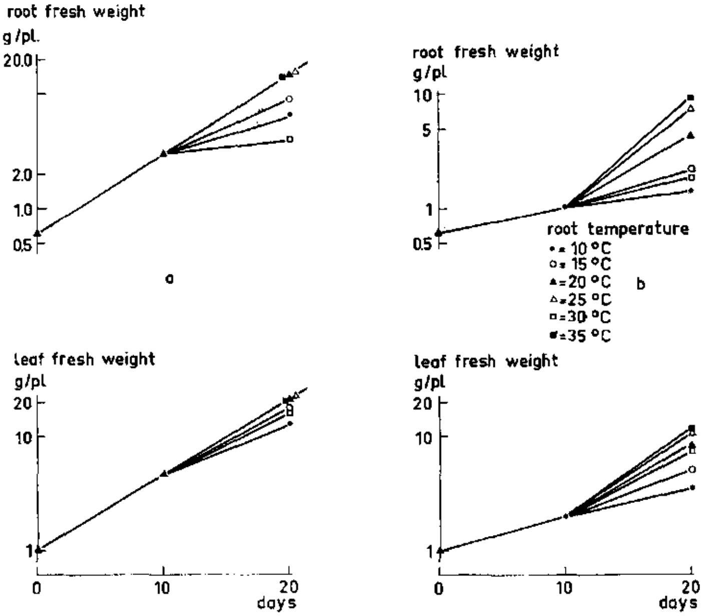


FIG. 5. Fresh weights before and after changing root temperatures plotted against time.
a. start at 10°C b. start at 20°C

the plant was more important. The decrease in relative growth rate may be due to mutual shading of the leaves. A shift in the optimum is less mysterious in this way.

Dry-matter content in the leaf

In the preceding the weight increase has been determined by fresh weight, because it is supposed to be the best growth indicator. In climate rooms where growth conditions are constant the use of fresh weights is quite well possible. Plotted against root temperatures the effect on dry weight does not seem to differ much from the effect on fresh weight (fig. 6). At closer inspection, however, some interesting differences emerge. Fig. 6b shows that these differences may be quite considerable. A low dry matter percentage was usually associated with rapid increase in fresh weight, a high one with a slow increase. This phenomenon is frequently found under all kind of conditions (3, 7 and 10). This is due to the fact that under less favourable conditions increase in fresh weight is much depressed or terminates completely, while photosynthesis continues. Accordingly growth measured as increase in fresh weight is more sensitive to changes in environmental conditions than assimilation. The leaf surface present continues photosynthesis, though at a retarded rate, even if there is no further increase in fresh weight (4). The relationship between dry matter content and fresh weight (fig. 7), may be considered as the relation between dry-matter content and growth, as all plants have been harvested at the same age. The plants grown at a root temperature of 35°C were an exception; considering their growth they had a surprisingly low dry-matter content. In view of this due care must be taken in the interpretation of the relation between the dry-matter content and growth, since the time factor is of importance. When growth is suddenly terminated the dry-matter content will gradually rise. Fig. 8 reflects how this dry-matter content increased with time when the plants were transferred to a root temperature of 10° or 35°C. The figure also shows the trend in the growth rate of the leaves. It is obvious that not growth and dry-matter content, but growth and increase in dry-matter content were each others opposites. After growth inhibition the dry-matter content continued to rise until a sort of maximum value was reached which is about 18 to 19% in beans (not shown in the

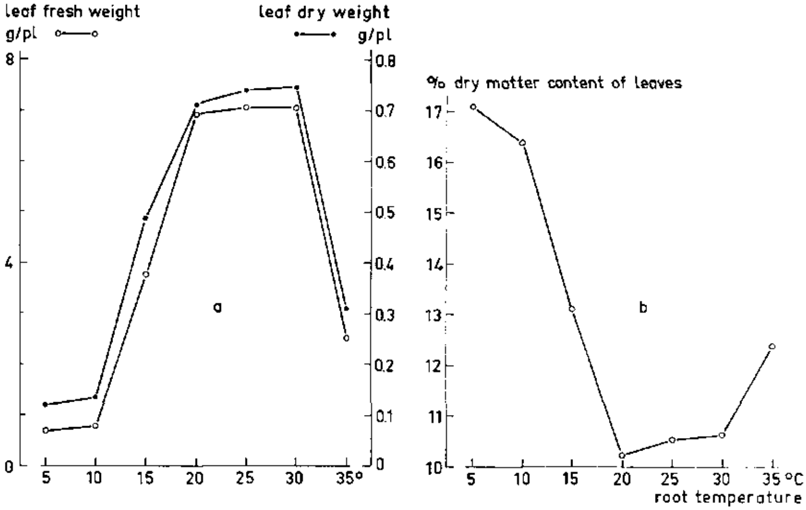


FIG. 6a. Fresh and dry weight of bean plants grown at different root temperatures.
FIG. 6b. Dry-matter content of the leaves.

FIG. 7. The relationship between dry-matter content and leaf-fresh weight.

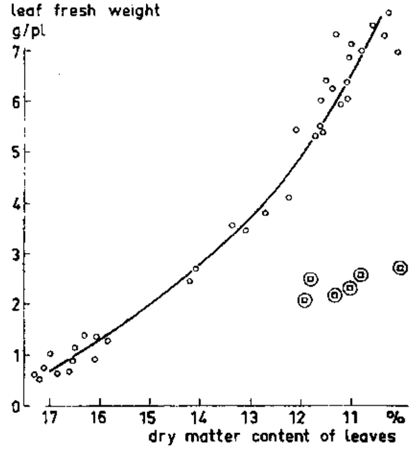


figure). It may be that photosynthesis terminates at that value. After some time however, the dry-matter content starts to increase again, but this time due to water loss caused by early maturation of the leaves at unfavourable root temperatures. The trend under different conditions has been treated more elaborately elsewhere (4).

Transferring plants from one root temperature to another effects a change in the growth rate and also in the dry-matter content. Under constant external conditions, extreme temperatures excluded, this leads to a kind of balance which is characteristic for the constellation of external conditions concerned. Obviously, the preceding history is of some importance as shown in fig. 9. This figure represents the dry-matter content of the leaves of plants which received a pre-treatment of 10 days at root temperatures of 10°C or 20°C. After this pre-treatment both groups of plants were distributed over the various root temperatures. Arrows in the figure indicate the dry-matter content directly after the pre-treatment. Evidently, the percentages changed during the experiment, that is they were higher as growth conditions were more unfavourable, while they decreased as conditions were more favourable. The data is from the experiment the fresh weights of which have been represented in fig. 5. After 10 days at various root temperatures there still proved to be differences due to the pre-treat-

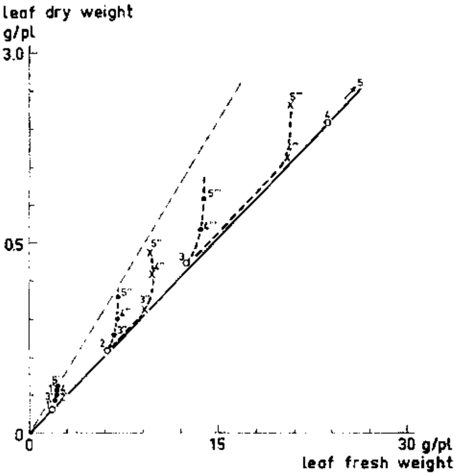


FIG. 8. Leaf-dry weight plotted against leaf-fresh weight; 1 to 5 subsequent harvest times; circles: root temperature 20°C throughout; black dots: over to a root temperature of 10°C at harvest times to which they are connected by a dotted line; crosses: over to a root temperature of 35°C at harvest times to which they are connected by a dotted line.

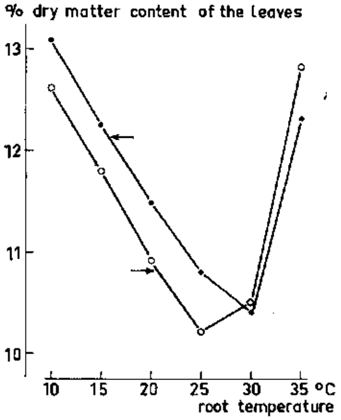


FIG. 9. Dry-matter content of the leaves of bean plants grown for 10 days at a root temperature of 10°C (black dots) or of 20°C (circles) and during the subsequent 10 days at different root temperatures. Arrows indicate dry-matter content at the time of transference.

ment. These differences, however, were small as compared to the changes taking place as a result of the treatment. The leaf material, present at the moment of transference, which had a high dry-matter content at 10°C is included in the average of all leaves. When the new leaves are taken separately these differences disappear. Evidently there is a fixed dry-matter content belonging to certain growth conditions. Any deviations are due to material present at the moment of changing, which was fully grown or which at least had terminated its participation in the growth process. In this respect there is much correspondence between the dry-matter content and the shoot/root-ratio.

Relation between leaf weight and root weight

Like the relation between fresh weight and dry weight at various root temperatures the relation between leaf growth and root growth was influenced in a similar way (fig. 1). Closer inspection, however, proves that there are considerable differences in the relation also in this case (fig. 10). It is striking that the ratio was much higher at 10° and at 35°C than at the other temperatures, with an intermediate value at a root temperature of 15°C. At both harvest times the ratio was the same for the root temperature 15° up to and including 30°C. At 10° and 35°C there was a clear difference between the two harvest times, indicating that the balance had as yet not been reached at these extreme temperatures. Comparison of the fig. 1 and 10 shows that the shoot/root-ratio was lowest at the root temperature with the most rapid leaf growth. This

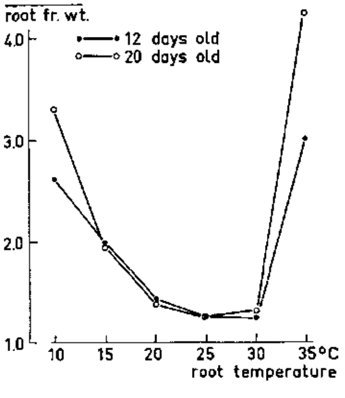
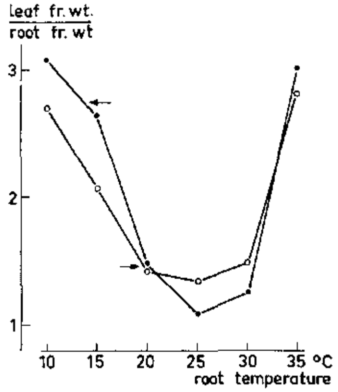


FIG. 10. Leaf/root-ratio of bean plants grown at different root temperatures.

FIG. 11. Leaf/root-ratio of bean plants grown for 10 days at a root temperature of 10°C (dots) or 20°C (circles) and during the subsequent 10 days at different root temperatures. Arrows indicate ratios at the time of transference.



means that the 'root value' was smallest when root growth as well as leaf growth was most rapid. The conception 'root value' suggests some sort of valuation number. In this case it means that the root under favourable growth conditions shows the slightest activity, expressed in leaf formation. Yet removal of a part of the root system under these conditions will immediately result in depressed leaf growth (4). Consequently, there is no surplus root formation, as sometimes suggested under similar conditions in literature. The phenomenon as such, however, is not explained in this way.

Fig. 11 shows how this relation is formed after transferring from one root temperature to another. The data are from the experiment of which fig. 9 represents the dry weights and fig. 5 the fresh weights. After transferring the plants to another root temperature the relative growth rate of the root and shoot changed in such a way that the relation associated with the new root temperature was reached as soon as possible. The values at the beginning of the experiment are indicated by the arrows. Comparison of the fig. 9 and 11 also shows the extent of the correspondence between the dry-weight percentage in the leaf and the leaf-root-ratio (4, 8, 10). At the same time it can be observed that the change from one relation into another takes place in the same way.

Growth on nutrient solution and tap water

Beans carry a considerable amount of nutrients necessary for growth in the cotyledons. This also holds for the minerals. To find the share of the ion and water supply in the effect of root temperature on shoot growth young seedlings have been placed at various root temperatures on nutrient solution and on tap water. The fresh-weight increase (fig. 12) did not show any difference between Hoagland solution and tap water during the first three days. Afterwards differences occur in favour of the nutrient solution. During the time of the experiment (12 days) growth on the nutrient solution was about logarithmic. The only exception was at a root temperature of 35°C. Leaf growth at this temperature was rapid initially, but an obvious depression occurred after six days. Thus it is evident from this experiment that growth was not limited by ion uptake in the first three days. Despite this a great difference in growth was found at the different temperatures which may be considered as due to the influence of water balance on growth. Without excluding any other causal relations at this moment, it is assumed for the time being that the influence of root temperature on the growth of beans for a great part was due to an influence on the water balance in the plant.

At a root temperature of 10°C there was no difference between the tap-water series and the Hoagland-solution series during the whole period of twelve days. Water

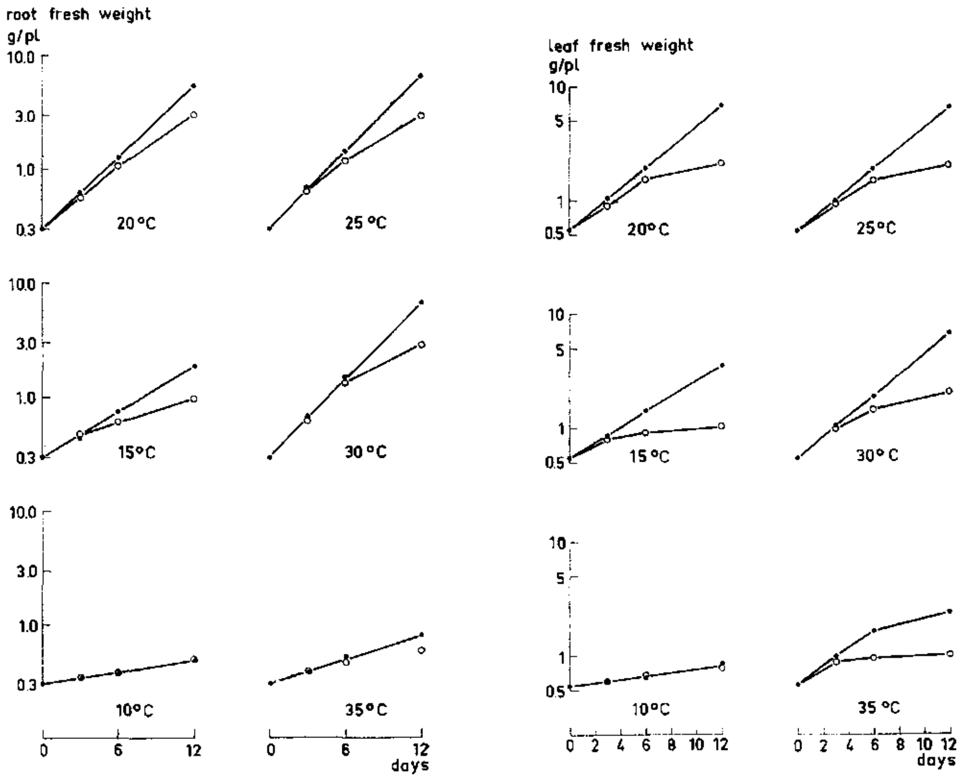


FIG. 12. Fresh-weight increase of roots and shoots of bean seedlings grown on Hoagland solution (dots) or on tap water (circles) at various root temperatures.

supply is supposed to be the limiting factor. A plausible inference was that the moment at which the difference between the two series showed for the first time should be earlier as growth was more rapid, for the ion supply in the cotyledons in all cases was the same and therefore should be sooner exhausted as growth was more rapid. However, the contrary was the case. It seems that a difference showed sooner at 15 °C than at 25° and 30 °C. This may be related to the fact that the transport from the cotyledons becomes the limiting factor. These things will be discussed in a separate article on radio-active phosphate applied to plants. This experiment proved that the transport of phosphate applied to the leaves was influenced to a great extent by root temperatures.

The influence of root temperatures on the growth of older plants during longer periods evidently was as large as that described here now for seedlings. A considerable number of plants is necessary to estimate this difference on basis of weight in short time experiments, however. Therefore, only leaf measurements were carried out in these experiments. Plants aged 15 days, which had formed a second and third trifoliate leaf were grown up to this time on nutrient solution at a root temperature of 20 °C. The length increase of the leaves present was measured daily. At an age of 15 days they were transferred to the various root temperatures on tap water or nutrient solution. The average growth rate was 20 mm per 24 hours on the day before transference and is given in fig. 13 (crosses) together with the growth rate on the first two days after transference. Obviously, there was an immediate response at trans-

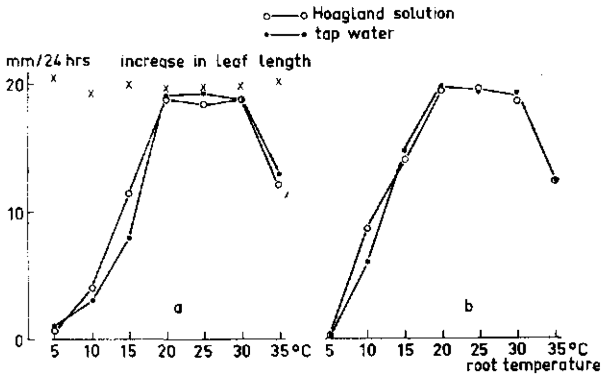


FIG. 13. Rate of increase in leaf length (2nd and 3rd trifoliate leaf) of bean plants grown on Hoagland solution at a root temperature of 20°C before (crosses) and after transference to various root temperatures (circles and dots).
a. first day after transference
b. second day after transference
circles: before and after transference Hoagland solution
dots: before transference Hoagland solution, after transference tap water.

ference from one root temperature to another (fig. 4). This already suggests that a growth factor was involved that may change very rapidly. As such the water balance is more likely than the ion balance. This was completely confirmed by the slight difference in response between these two groups of plants. The partial recovery of growth at 10°C and 15°C during the second day also indicates this. At transferring from 20° to 10° and 15°C water uptake is immediately hampered and as a result suction tension in the leaves increases. Due to this increased suction tension growth decreases (5). A secondary increase in the osmotic value of the cell sap induces a partial recovery. This recovery is supposed to continue until ion uptake becomes the limiting factor. Whether this stage is ever reached depends on the other conditions and will be treated in a next article.

DISCUSSION

On account of the experiments described above the following outline of the influence of root temperature on growth may be given.

The differences in root weights induced by root temperature prove to have consequences for shoot growth. It appears that weight increase of shoot and root roughly shows the same response to root temperature. Closer inspection furthermore shows that the quantitative response of the shoot is less than that of the root. This seems natural at first, for root growth is directly associated with the unfavourable environment, while shoot growth is only indirectly involved. On the other hand, it may be assumed that the shoot grows under all conditions in a measure as the supply of essential nutrients, provided by the root, allows (4). Seen in this way the relation as observed here is less rational. Obviously, the root is less developed at unfavourable root temperatures, while it may be expected also that it is less active per gram weight. This happens to be indeed the case (see subsequent articles in this series), and the more so, as the root forms impermeable layers under unfavourable conditions (6), which intensify the effect of temperature on its activity. The relation found in this case becomes more surprising as all these facts are taken into account. As has been found earlier for peas, these experiments have proved that root activity expressed in shoot growth per gram of root weight is largest at unfavourable temperatures (8). This is due to the fact that there is no straight-line relationship between root growth and shoot growth, when it is considered on a basis of weight. Root as well as shoot show anatomical and morphological differences at various root temperatures (6). This brings about great differences in the field of the various physiological activities as well. Thus the leaves grown at a root temperature of 10°C have a much more xeromorphic morphology than those grown at a root temperature of 20°C (6). The above-described relation is less surprising if the equilibrium between root and shoot is considered as a functional balance. More data concerning the water balance and ion balance will draw special attention to this aspect.

There are a few more aspects in the above-mentioned experiments that are interesting with regard to growth analyses.

Under certain conditions the dry-matter content of a plant is a very good indicator of growth conditions, *i.e.* both magnitudes are negatively correlated. However obviously, the time factor is most important in the quantitative relationship between these magnitudes. If the growth depression has occurred only recently it will not be immediately expressed in the dry-matter percentage. In prolonged treatments it will grow more apparent until it reaches an equilibrium value. This value is easier to handle in growth depressions occuring at a constant level, as e.g. with a root temperature of 15°C. This is probably why application of this percentage in practice, where more factors play a part in the realization, still meets with difficulties. However, the principle is of so much importance that closer investigation into this subject is justified.

The same holds for the shoot/root-ratio.

SUMMARY

Experiments are described in which the influence of a series of root temperatures (5°–40°C) at a constant air temperature (20°C) on root growth, shoot growth, shoot/root ratio and dry matter content was investigated. Quite considerable differences in each of these values were found.

It is assumed that the reduction in shoot growth reported was due to an unfavourable water balance induced by the treatments.

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Mededeling 236 van het I.B.S.

RESPONSES OF BEAN PLANTS TO ROOT TEMPERATURES

II. ANATOMICAL ASPECTS

R. BROUWER and ATJE HOOGLAND

INTRODUCTION

In earlier papers it has been assumed that at each set of conditions a functional equilibrium exists between shoots and roots. This might imply that at favourable root temperatures the shoot/root-ratio would be higher than at sub-optimal and supra-optimal root temperatures. The reverse appeared to be true (2, 3). It may be assumed that in evaluating the functional equilibrium the weight ratio is not always a reliable magnitude. Since in the functional equilibrium root functions play an important role several features may be of interest. Behind physiological features *e.g.* carbohydrate supply, also anatomical and morphological properties of the various plant parts may be of importance. This paper will deal with the latter aspect of the influence of root temperature on the growth of plants.

METHODS

The plants were grown at different root temperatures (2). The anatomical and morphological data were collected at different harvest times and compared with yield data.

RESULTS AND DISCUSSION

Roots

In fig. 1 a photograph is shown of root systems of plants grown at different root temperatures for 8 days after germination. As a comparison the root system of a seedling at the start of the experiment is shown. It is clear that hardly any growth takes place at root temperatures of 5°, 10° and 35°C, whereas at 40°C a decay of the root system and the base of the stem can be seen. This may be due to dying and subsequent contamination with micro-organisms. The optimal temperature was between 25° and 30°C. In addition to growth in length of main-, branch-, and adventitious roots there was also a clear influence on branching.

Fig. 2 shows different anatomical details of a root grown at 20°C at different distances from the apex. The diameter of the whole root as well as the diameter of the stele was about the same from the apex up to a distance of 20 cm. Roots grown at this temperature showed only suberization of the casparian strips up to 8 or 9 cm from the tip. At greater distances suberization of the other parts of the walls of the endodermal cells increased rather quickly. At a distance of 15 or 20 cm from the tip, practically the whole wall of these cells was suberized. Only the endodermal cells opposite the xylem rays remained unsuberized (passage cells).

Suberized endodermal cells are here considered to be cells the walls of which show distinct uninterrupted colouring with Sudan III.

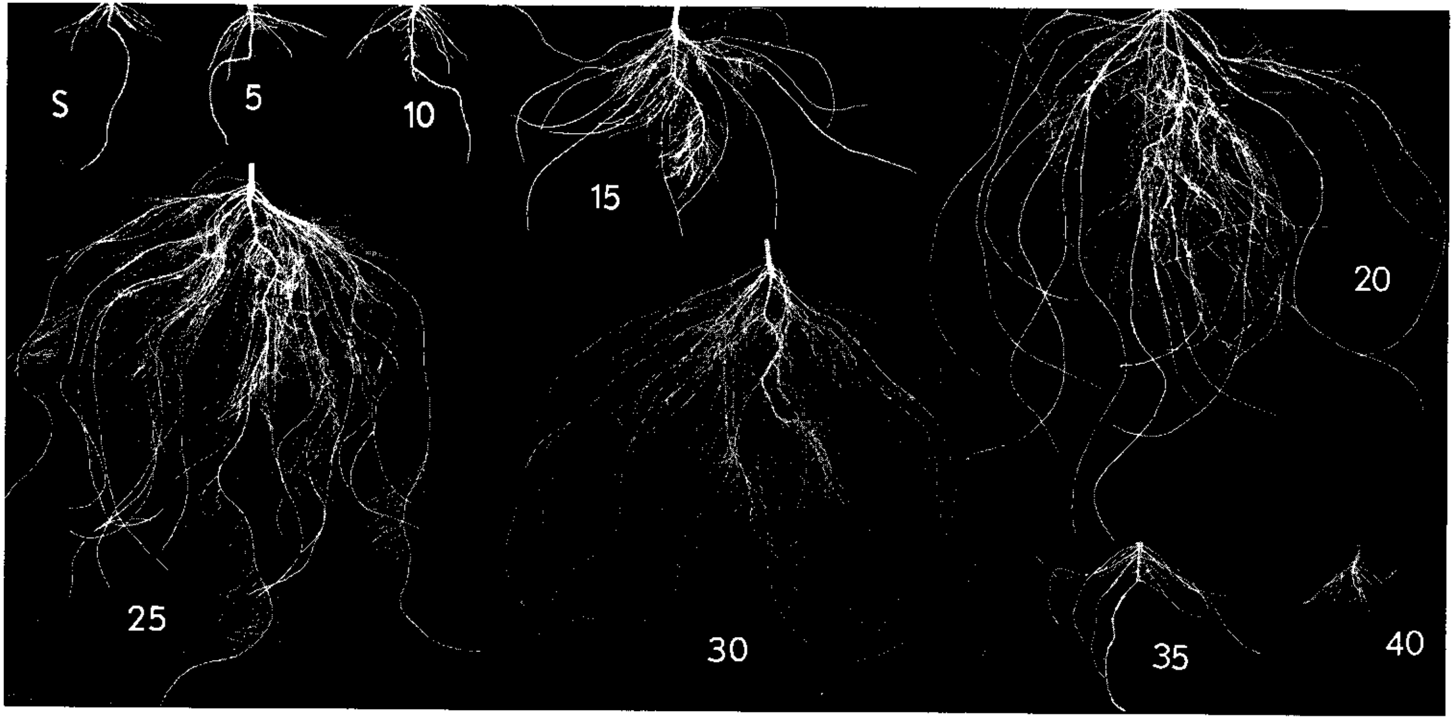


FIG. 1. Photograph of bean roots grown for 8 days on nutrient solutions of various temperatures after germination at 20°C. Upper left a seedling root (S) directly after germination.

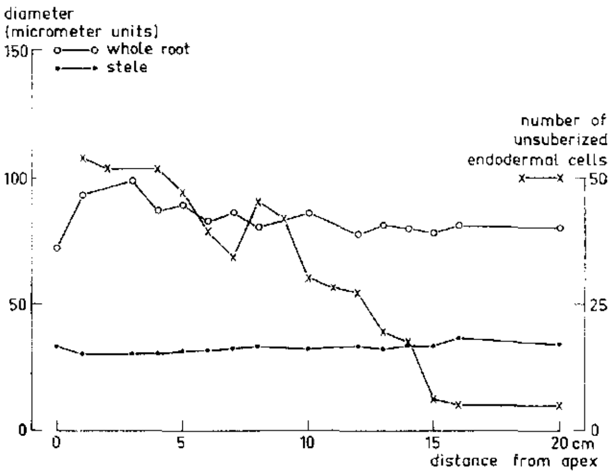


FIG. 2. Diameter of whole root and central cylinder of bean plants grown at 20°C at different distances from the root apex. In addition the suberization of the endodermal cells is indicated.

At this root temperature the first branch roots appeared at a distance of 5–6 cm from the apex. This was about the same distance at which the suberization of the endodermal cells rapidly increased. It is shown in fig. 3 that this distance was about the same between 15° and 35°C, but smaller at root temperatures of 5° and 10°C. These small differences in the distance between apex and the first macroscopically visible branch roots may be due to an after-effect of germination at 20°C.

There was a distinct difference in differentiation at the various root temperatures as shown in fig. 4. Compared with a root temperature of 20°C the suberization of the endodermal cells penetrated more closely to the apex at the lower as well as at the higher root temperatures. This was especially clear at 35°C. It appeared that there was complete suberization at this temperature at the distance of 5 cm from the apex. At 10°C this distance amounted to about 10 cm and at 15°C to 15 cm. This situation applies to plants that were pre-grown at 20°C during 7 days after germination. At root temperatures of 10° and 35°C there was hardly any growth in length and the suberization reached eventually the root tip. This was realized much more rapidly at 35°C than at 10°C. In addition to the suberization at 35°C the induction of meristems

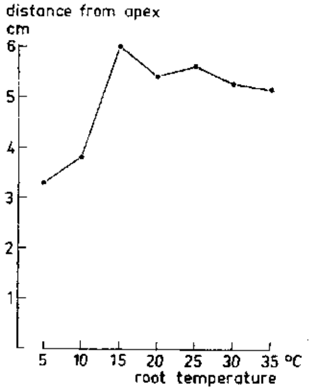


FIG. 3. Distance from root apex with first macroscopically visible branch roots.

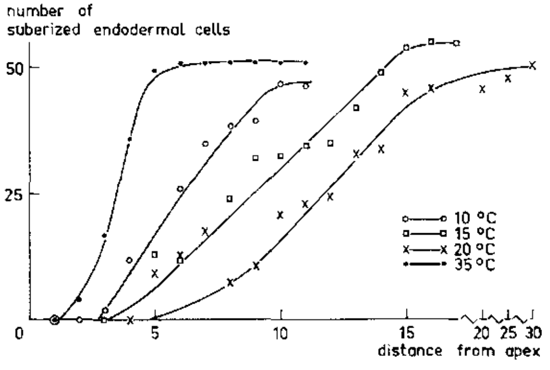


FIG. 4. Suberization of endodermal cells at different distances from the root apex of bean roots grown for 10 days at the root temperatures indicated after germination at 20°C.

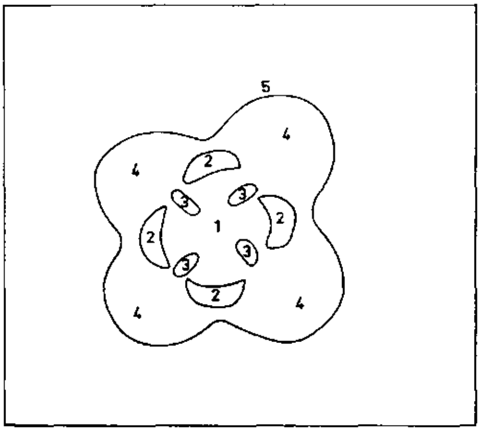


FIG. 5. A topographical drawing of a root tip 10 days after the seedlings were placed on a nutrient solution of 35°C.

1. pith
2. phloem
3. xylem
4. developing meristems of branch roots
5. endodermis

of branch roots in the pericambium proceeded steadily as well. It was a remarkable phenomenon that while at this temperature at which the meristems of the main roots lost all activity (cell division and cell elongation) in the centre of the same root cell divisions continued. In the absence of cell elongation the main roots contained a great number of meristems lying close together. These meristems made the roots ultimately somewhat angular (fig. 5). This figure shows that four meristems of branch roots were visible at the same height.

After transferring the plants from a root temperature of 20°C to a root temperature of 35°C leaf growth proceeded initially at the same rate or even at a somewhat higher rate. After a couple of days however a reduction of leaf growth appeared, ultimately

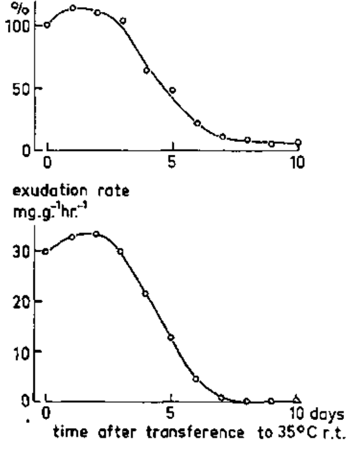
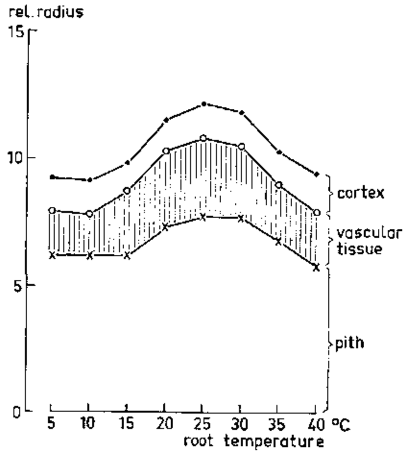


FIG. 6. A comparison between suberization of the endodermal cells, exudation rate and leaf growth of bean plants after transfer from a root temperature of 20°C to 35°C.

FIG. 7. Dimensions of various tissues within the radius of the whole stem.



leading to a complete stop. Fig. 6 shows a comparison between rate of leaf growth, rate of exudation and suberization of the endodermis after transference from 20 °C to 35 °C. There is a striking similarity between the three curves. No other anatomical or morphological features were found that showed such a close relation to leaf growth and root activity as suberization. It seems justified to conclude that the endodermis plays an important role as a transport barrier in the root, this in contrast to the assumption of various investigators (5) in recent papers.

Stem

At optimal root temperatures the cortex as well as the pith and the vascular tissue showed the largest diameter (fig. 7). The differences were small as far as the cortex was concerned and greatest for the vascular zone. Microscopic determinations revealed that the difference in diameter was predominantly determined by a difference in number of cells. Comparing the area of the cross section of the stem with the stem-fresh weight (fig. 8 **A** and B) reveals that these magnitudes do not parallel each other. This is due to the fact that both stem diameter and stem length react upon the root temperature.

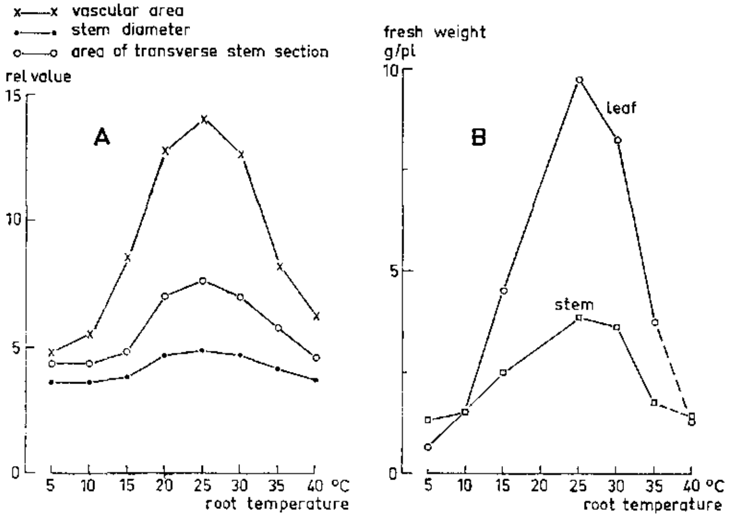


FIG. 8. A. Stem diameter, area of transverse stem section, and cross-sectional area of vascular tissue directly above the cotyledons at different root temperatures.
B. Fresh weight of stems and leaves at different root temperatures.

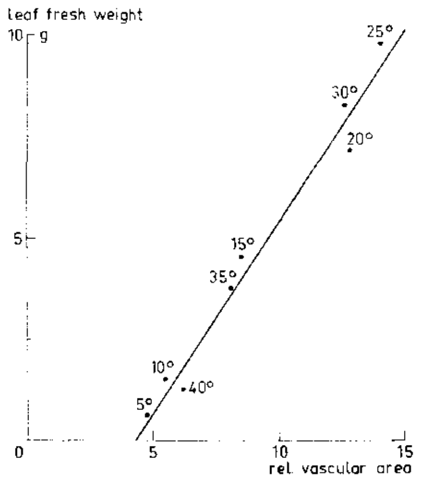


FIG. 9. Relation between leaf fresh weight and cross-sectional area of conducting tissue at the root temperatures indicated.

From the thickness of the various layers the area of the vascular zone can be calculated. This area is in a way a measure of the transport capacity of the system. Fig. 8 indicates that there was a close correlation between the areas of the vascular zone and the leaf surfaces (compare 4). In fig. 9 the leaf surface is plotted against the vascular area and the relation appears to be rectilinear with an intersection with the abscissa, possibly corresponding with the amount of tissue which is not essential for transport or the amount of conducting tissue present after germination before the unfolding of the leaves. If this is the case it means that the relation between transpiring surface and cross-sectional area of conducting tissue is independent of root temperature. As SINNOTT (6) states this relation, contrary to the assumptions made bij various investigators (6), may be without causal significance for the differentiation of vascular tissues and may therefore be simply another instance of a non-causal developmental correlation.

Leaves

The influence of root temperature on leaf development (2) is almost as great as the influence on root development. In fig. 10 photographs are shown of plants grown for 8 days at different root temperatures. The temperature effect was clear in the tap water series as well as with the use of nutrient solutions. Besides the differences in leaf area there were great differences in colour. At sub- and supra-optimal temperatures the leaves were deep-green, at optimal temperatures light green. As in the aeration experiments (1) in this case too a difference in elongation of leaf cells was the cause of these differences in colour. In fig. 11 some features are collected that give an impression of the relative dimension of leaf cells. The leaf surface followed an optimum curve (*c.f.* fig. 12), whereas the stomatal density showed just the reverse. It has been shown (7, 1) that stomatal density in bean leaves is negatively correlated with the size of the leaf cells. Multiplying the number of stomata per mm^2 by the leaf surface gives the total number of stomata per leaf. As was the case with growth inhibition due to lack of aeration it appeared that root temperature had hardly any influence on the number of stomata per leaf and therefore also on the number of cells per leaf. The influence of the various root temperatures on the leaf area therefore can be fully attributed to an influence on cell elongation.

As postulated elsewhere (3, 2) the influence of root temperature is mainly due to an

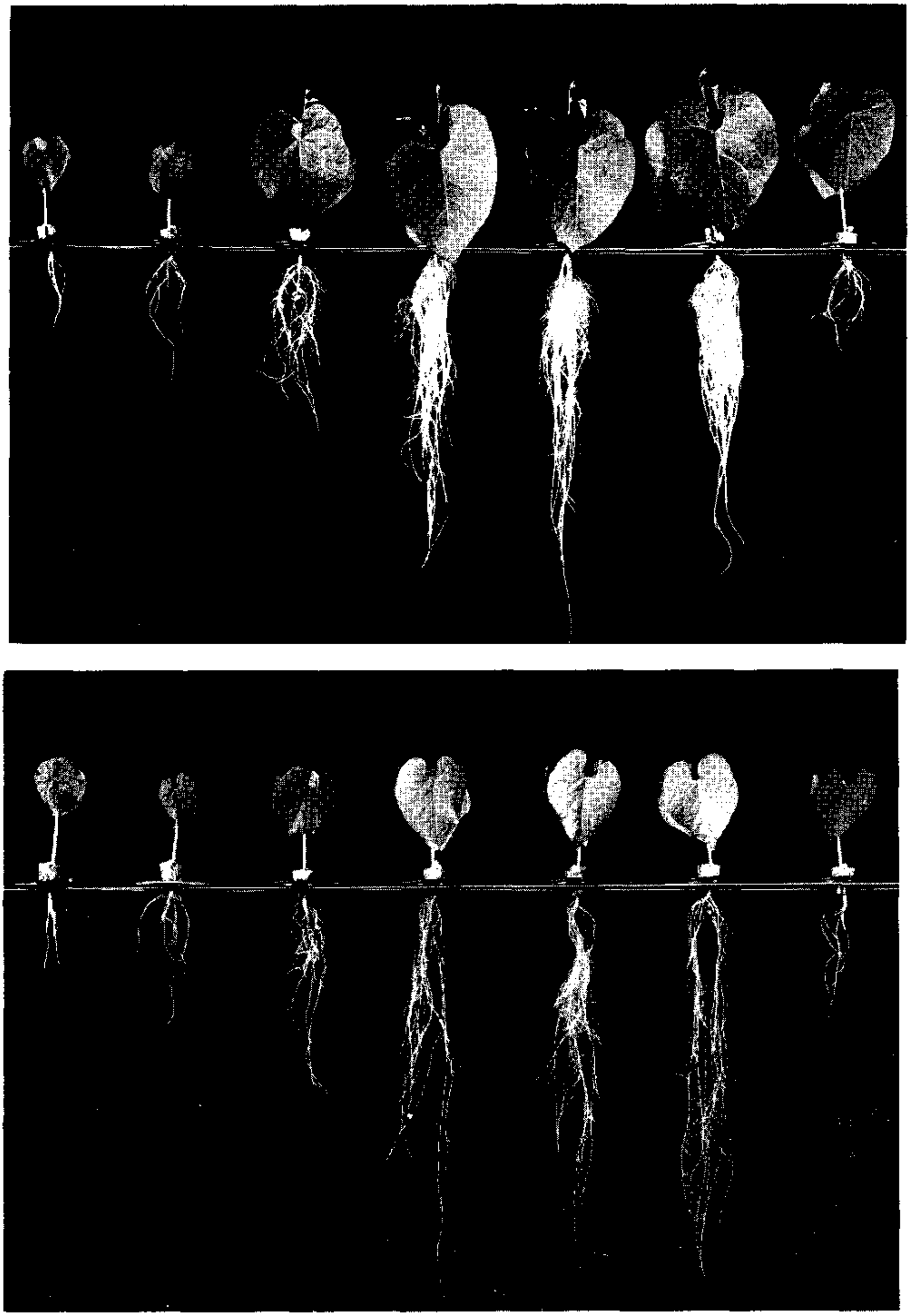


FIG. 10. Bean seedlings grown for 8 days (following germination at 20 °C) at the root temperatures 5, 10, 15, 20, 25, 30, and 35 °C (from left to right). Upper series nutrient solutions, lower series tap water.

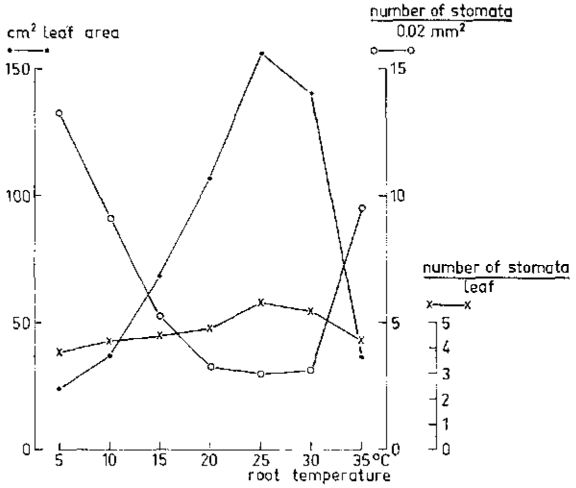


FIG. 11. Leaf area, stomatal density, and number of stomata per leaf of bean plants grown at the root temperatures indicated.

influence on the water balance of the plant. It may be expected therefore that plants grown at unfavourable root temperatures will show a more xeromorphic character. Colour and stomatal density confirmed this assumption. The leaves were thickest however at optimal growth conditions. This applies for both types of leaf parenchyma (fig. 12) and was due to the larger intercellular cavities. As far as the epidermis is concerned there are no differences. In preliminary experiments it was found that there were large differences in uptake of radio-active phosphorus and radio-active rubidium, applied to the leaves, due to root temperatures. At the same time it appeared that the cuticular transpiration of the leaves grown at high and low root temperatures was reduced (unpublished results). This may indicate that there was also an influence of root temperature on the development of the cuticular layer.

SUMMARY

Root temperature has a distinct influence on root growth and cell differentiation. Growth reduction in general is coupled with a reduction of the distance between the

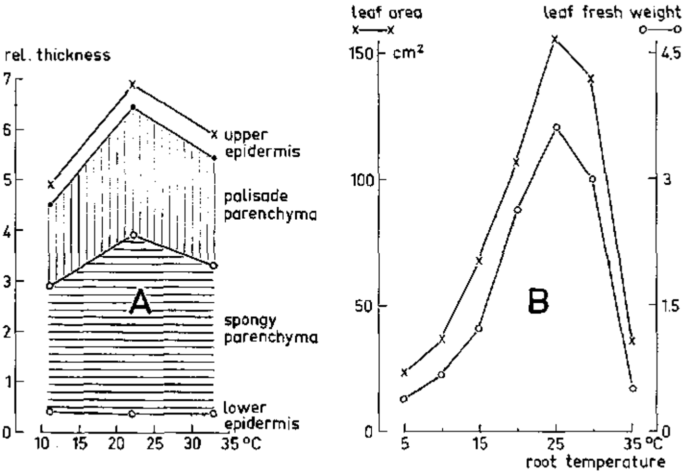


FIG. 12. A. Dimensions of various tissues in the leaf between the veins.
B. A comparison of leaf area and leaf-fresh weight of a primary leaf of bean plants grown at the root temperatures indicated.

root tip and the region where differentiation is completed. This is especially the case at 35°C. At this temperature the induction of branch roots penetrates into the apex proper. It is remarkable that although the growth of the tip meristem, including both cell elongation and cell division, has fully stopped, cell divisions continue in the centre of the roots.

Suberization of the walls of endodermal cells reduces the uptake strongly and leads to decreased leaf growth.

Similar as in literature for other treatments, a close relation between leaf growth and development of the cross sectional area of vascular tissue was found in these experiments as well. The leaves show xeromorphic characters at unfavourable root temperatures.

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INFLUENCE OF SOME ENVIRONMENTAL FACTORS ON GROWTH AND FIBRE FORMATION IN FLAX

A. G. HAVINGA-VAN DER MOLEN

INTRODUCTION

In the course of time many investigations have been published on flax. For the greater part they concern practical problems as manuring, methods of harvesting etc. Particularly in the older literature far less attention has been paid to the more fundamental problems of fibre formation in plants as hemp and flax.

In this respect work on the anatomy of flax and origin of fibres by TAMMES (12) and ESAU (4) has to be mentioned as well as that of Opitz *et al.* (8) and CROZIER (2) on the relation between anatomical properties of fibres and fibre quality. SCHILLING (9) tried to find some relation between the internode length of different flax varieties and their quality.

Nowadays JACQUEMART (5) and SZYMANEK (11) work on anatomical problems, LARSEN (6) on physiological problems and in Russia the effect of various climatical factors is under investigation (SIZOV 10).

Some years ago research on the effect of environmental factors on fibre formation in flax was started on the I.B.S. BLOK (unpublished results) tried to find a method for growing flax on a nutrient solution in climate rooms. The present publication deals with the effect of temperature and drought on plant development and fibre production in flax, using BLOK'S method of cultivation.

METHOD

Seeds of two varieties, Wiera and Fibra, were germinated in sand at 20°C. After emergence 120 seedlings were placed on 9 l of a Hoagland solution at half strength (pH 5.7–5.9). The solution was not aerated. BLOK found that alternation of normal Hoagland and Hoagland without nitrogen, repeated 3 times during the growth period, gave better results than plants grown on a complete Hoagland throughout. Under the last mentioned conditions the nitrogen supply was too liberal. In the nitrogen free solution the NO_3' was replaced by an aequivalent amount of Cl'. The volume of the nutrient solution was maintained by adding tap water when necessary. The plants were grown at a daylength of 16 hours and illumination was provided by 400 Watt HPL lamps. The light intensity amounted to 7.10^4 erg/cm^2/sec or more on the tops of the stems. When the plants reached the glass plate that separated them from the lamps they had to be placed on a lower level. This procedure was repeated several times during the experimental period.

The temperatures under investigation were 12.5°, 16.5°, 20.5° and 25°C.

Eight sets of 120 plants of Wiera and Fibra were placed at each temperature respectively. Three times a week the height of the longest stem of each set was measured and the average of those lengths gave an impression of the growth rate. When the average length of the variety Wiera, which grows more rapidly than Fibra, had reached 25 cm at a certain temperature, it was harvested for the first time, the second took place at 50 cm and so on.

At each harvest one set of Wiera and one of Fibra were reaped and fresh and dry weight of shoots and roots were determined. Of 20 stems the length was measured and the number of internodes counted and 10 of these were anatomically investigated on the 30th and on the middle internode. At the last harvest one set was used for chemical analysis and one was sent to the I.B.V.L.[1]) for the examination of fibre quality.

RESULTS AND DISCUSSION

The general appearance of the plants was very luxuriant as compared with field grown plants, leaves being dark bluish green and large, stems being thicker and the plants being far taller. Possibly the amount of nitrogen applied was still too large (MEYER and ANDERSON 7). The flax grown at 25°C had the most normal appearance.

At all temperatures development was irregular, partly due to the fact that during the growth period the plants had to be placed repeatedly at a greater distance from the light source; the slowest growing individuals became more and more retarded. This had an effect on duration of flowering as will be shown below.

Looking at the effects of temperature it can be seen that both Wiera and Fibra had the highest growth rate at 20.5°C, followed by 25°C and 16.5°C, whereas at 12.5°C the flax grew but very slowly (see fig. 1). Not only growth in length but also the in-

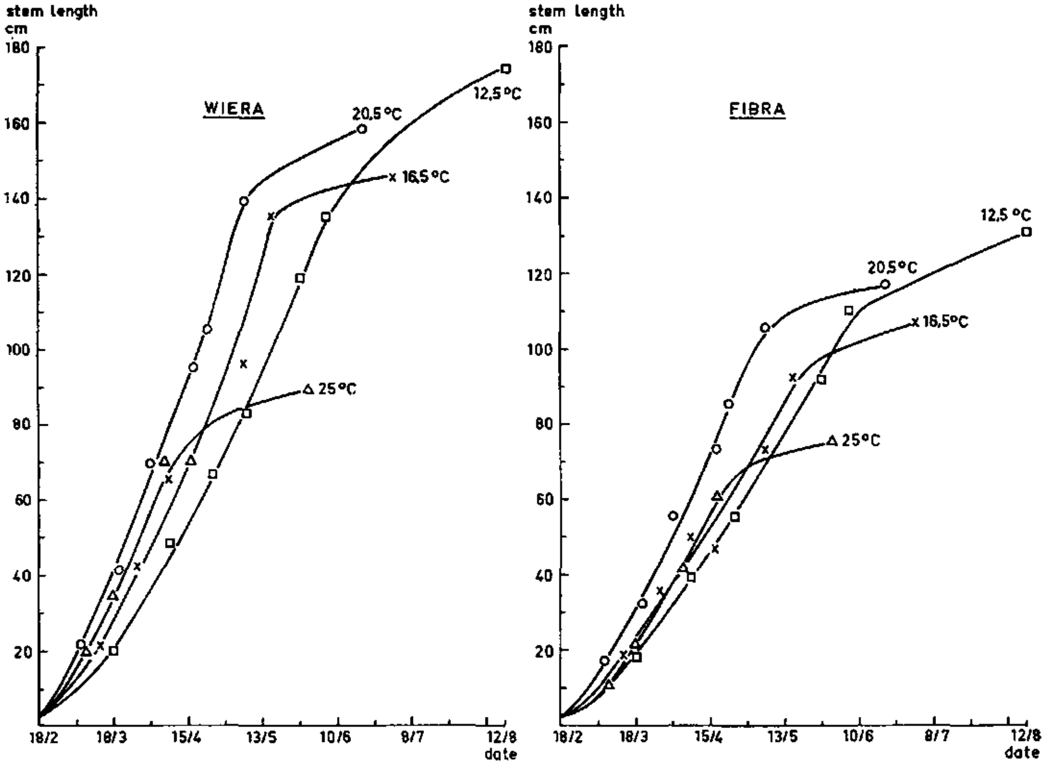

FIG. 1. Growth rate of Wiera and Fibra at various temperatures as increase in stemlength in the course of time from „emergence" to maturity.

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[1]) Institute for Research on Storage and Processing of Agricultural Produce.

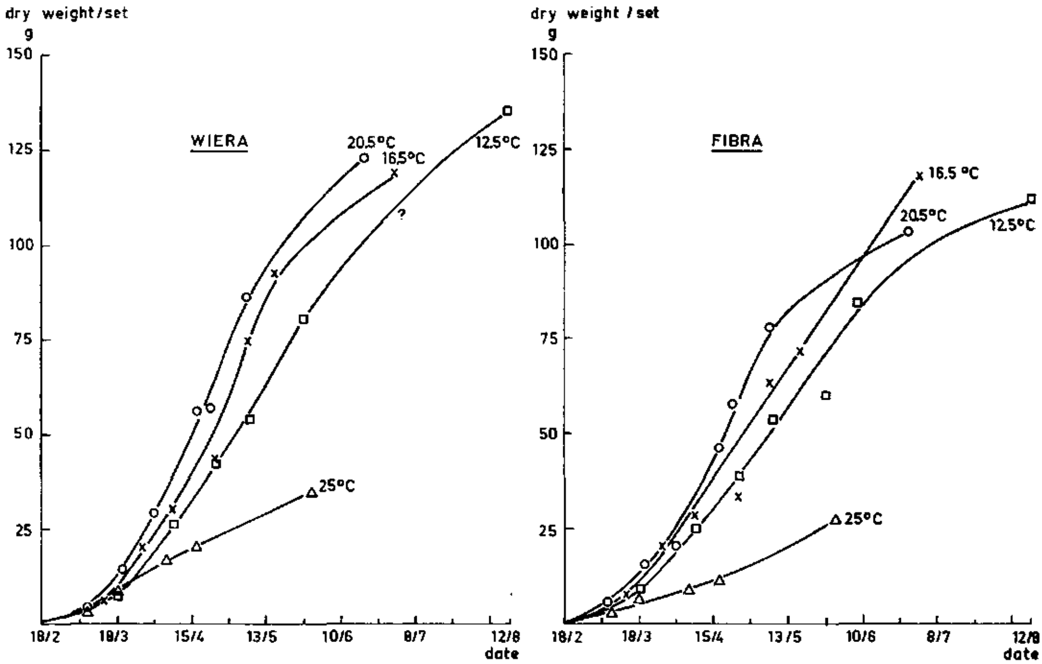

FIG. 2. Increase in total dry weight of Wiera and Fibra at various temperatures in the course of time.

crease in fresh and dry weight was the highest at 20.5°C, though the difference between the flax at 25°C and that at the other temperatures was far more pronounced (fig. 2). This is a consequence of the fact that the stem diameter at 25°C was quite normal but at the other temperatures very large. Comparing the ultimate lengths it can be seen that not 20.5°C gave the greatest length but 12.5°C (fig. 3). This may be explained as follows.

It may be assumed that there are two effects of temperature; one on growth rate and another on the rate of development of the plant (BROUWER 1, VAN DOBBEN 3). Fig. 4 shows the duration of different stages in the development of flax.

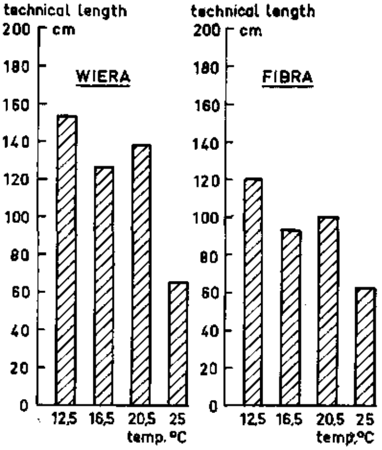

FIG. 3. Technical length measured from cotyledons to inflorescence, in cm at maturity.

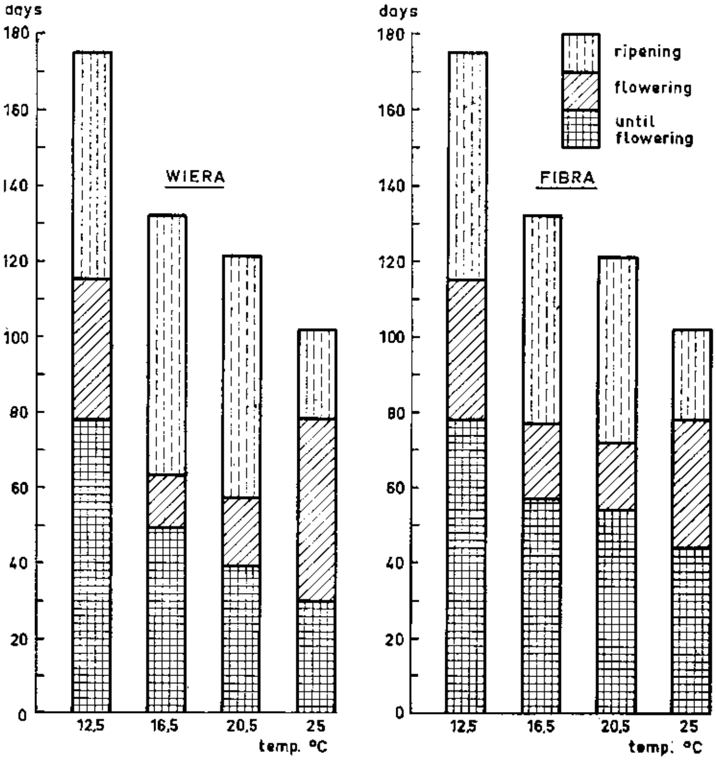

FIG. 4. Duration of subsequent stages in the development of Wiera and Fibra at different temperatures.

Apparently the vegetative stage was drastically shortened by increasing temperature. Now, plant height is the result of growth rate and of the time during which the plant may grow. In this experiment the conditions happened to be so that the result was a kind of two topped curve (see fig. 3).

SIZOV (10) showed in field experiments under various climatic conditions in different zones of Russia that a high temperature and also drought during the vegetative stage shortens that period in flax. The result was a shorter length. Fig. 4 also shows the long duration of flowering, especially at 25°C. This will partly be caused by the earlier mentioned irregularity in growth, but at 25°C there is another factor involved. The inflorescence was longer and more branched than at the other temperatures, so that there were more flowers per stem. Seeds were not produced, however. Possibly, the failing of the normal termination of the cyclus gave rise to abundant flowering.

The water uptake was measured in order to determine the transpiration coefficient. The results are shown in table 1.

TABLE 1. The transpiration coefficient of Wiera and Fibra expressed in ml water per gram dry matter; the vapour-pressure deficit (v.p.d.) is given in mm Hg.

Temp. °C	V.p.d.	Transpiration coefficient	
		Wiera	Fibra
12.5	2.9	330	364
16.5	4.6	444	512
20.5	7.9	528	586
25	8.9	700	880

The coefficients are very high and distinct differences occur. In how far this is caused by the difference in vapour pressure or by a specific temperature effect has not been determined, since the v.p.d. could not be maintained at the same level for the four temperatures. Larsen (6) found a transpiration coefficient of about 300–400 for Wiera in pot experiments under natural light and temperature conditions.

The relation between increase in dry weight and water uptake during the course of time is shown in fig. 5 for 20.5°C only.

The question rises in how far temperature affects fibre formation. The excessive nitrogen application must be considered as a factor of great importance. It is known that too much nitrogen lowers the yield of fibre and reduces fibre quality.

Fibre formation in flax takes place at an early stage (Tammes 12) in the protophloem (Esau 4, Jacquemart 5) together with the formation of sieve tubes and the companion cells. In *Linum perenne* (Esau) the ultimate number of fibres is present when the first sieve tubes of the protophloem are crushed but the fibres are in a different stage of development. In *Linum usitatissimum* the number is also fixed at an early stage (Tammes). The first fibres are longer than the later-formed ones (Jacquemart). According to Sizov fibre formation ceased at the end of flowering and 90% of the total amount of fibres should be present at that moment. The results of an earlier experiment gave the impression that thickening of the cell walls is also almost completed at that moment.

It is generally assumed that the thickening of cell walls occurs centripetally and usually begins after elongation has ceased but Esau showed for *Linum perenne* that formation of secondary walls already begins at the formation of secondary tissue. As it is known that several factors affect fibre content and quality the percentage of

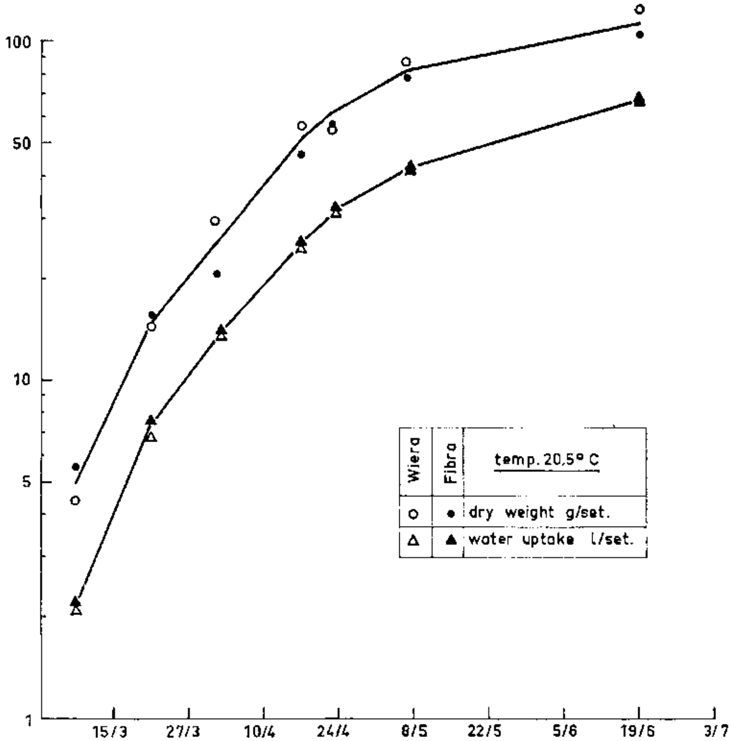

Fig. 5. Correspondence between the course of water uptake and dry matter production of Wiera and Fibra at 20.5°C. Logarithmic scale for ordinate.

fibre cells with thickened walls was determined by counting the total number of fibre cells and the number of thickened fibres in a transverse section of the middle internode of 10 stems. The results of the last harvest are given in table 2.

TABLE 2. Summary of various examinations. Quality-index number after CROZIER's method, determination of quality and fibre yield (fibre in % of straw weight under standard conditions). See further in text.

	Wiera							Fibra				
Nr. exp.	1363				1360	Blok	I.B.V.L.	1363				I.B.V.L.
Temp. °C	12.5	16.5	20.5	25	20	15–20	field grown	12.5	16.5	20.5	25	field grown
CROZIER	10	8	11.5	12	10–11	12	6	12–14	9.5	12.5	13	6
I.B.V.L.	6.5	7	6.5	3	–*	–*	9	5	5.5	5	3	9
% thickened fibres	72	77	70	63.4	83	66.8	85	66	77	70	65	84
Yield in %	16.4	16.3	13.2	11.6	17.9	9.2	24.7	14	16.2	15.4	8.9	27.7
Diam. stem } mm	2.2	2.38	2.15	1.15	±2	±2	1.5	2.2	2.2	2.35	1.35	1.5
Length intern.	6.9	6.7	6.4	6.3	5.5	6	9	6.0	6.0	4.9	5.0	8.5

* – examination was not possible.

In the same table the results are shown of an estimation of fibre quality by the method of CROZIER (depending on different anatomical properties of bundles and fibres) and a technical examination bij the I.B.V.L. As a comparison field-grown Wiera and Fibra were used as an example for good quality (highest mark according to the I.B.V.L. is a 10; in CROZIER's method a lower figure indicates a better quality). In the present experiment the flax at 16.5°C showed the best quality and the highest percentage of thickened cell walls. In comparison with the field-grown flax it appeared that the quality was low and the fibre yield was also abnormal. This is due to two factors at least: the low percentage of thickened fibres and the large stem diameter (not for the flax at 25°C). The amount of fibre does not increase proportionally with increasing stem diameter (TAMMES).

The fibres of the flax at 25°C had large lumina and were irregular of shape and size, the cell walls showed distinct layering. The flax at 12.5°C also had rather large lumina but this could have partly been caused by the fact that the last harvest occurred at a too early stage. In any case this was true for Fibra. The Wiera of an earlier experiment, 1360, had a high percentage of thickened cells but was of a poor quality. There was not enough material for an examination by the I.B.V.L.; according to CROZIER it was not too bad. It may be possible that the very short internode length played a role here. The field-grown flax had an internode length of about 9 mm, but all the flax grown in the climate rooms, 1360, the BLOK experiment included, had too short internodes. At higher temperatures this length decreased even more. Drought can also have this effect (LARSEN 6). SCHILLING (9) examined different varieties of flax and found that in general a short internode length was associated with poor quality.

For the moment it cannot be said by what factors these short internodes are caused.

SUMMARY

Two varieties of flax, Wiera and Fibra, were grown in climate rooms on nutrient solution at different temperatures: 12.5, 16.5, 20.5 and 25°C. The amount of nitrogen applied appeared to be too high, which may partly be the cause of the lowered fibre

yield and fibre quality. Growth rate and rate of development were influenced by temperature. Length, being the result of growth rate and duration of vegetative stage was ultimately greatest at the lowest temperature. Fibre quality was best at 16.5°C, though rather poor in comparison with field-grown flax. The rather short internodes shown by all the flax grown in climate rooms may partly be responsible for this difference.

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PRELIMINARY DATA ON THE TRANSPORT OF WATER, POTASSIUM AND NITRATE IN INTACT AND BLEEDING MAIZE PLANTS

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INTRODUCTION

In discussing the processes of water and ion absorption much attention has been paid to the exudation phenomenon. There are a number of aspects that are of special importance, *viz.*:

1. The rate of the bleeding transport in comparison with the rate of the transport in the transpiration stream.
2. The composition of the bleeding sap in comparison with the composition of the transpiration stream.
3. The mechanism of the exudation process.
4. The importance of the exudation process for the growth of the overground parts of the plant.

The ratio between the rate of transpiration and the rate of exudation has been investigated thoroughly. Some plants completely depend upon root pressure as far as their water balance is concerned (HUBER (7)), others only partially. So KRAMER (10) stated that the rate of exudation exceeded 5 per cent of the rate of transpiration only in exceptional cases. Experiments of RUFELT (12), however, indicate that the contribution of active transport to the total transport was much higher. His results with young wheat plants have earlier been confirmed by data of BROUWER (3) with barley and led to the assumption that in these plants about 50 per cent of the transpiration stream was due to an active process (*cf.* GRAÇANIN (4)).

Concerning the ion transport in bleeding stream and transpiration stream, very few data are available. The relation, found between salt and water uptake by intact plants, has led to most diverging results. VAN DEN HONERT *et al.* (6) demonstrated with maize plants that increasing the transpiration rate only had little influence on the ion uptake. KIHLMAN-FALK (8) found with wheat plants a distinct relation between potassium and nitrate absorption and transpiration.

Plotting ion absorption against water absorption usually results in a curve which, after extrapolation to zero water uptake, cuts the ordinate somewhere. This ion uptake at zero water uptake is considered by KYLIN and HYLMÖ (11) as the active component, whereas the increase, related to the water uptake, indicates the passive component. ALBERDA, LOUWERSE and BROUWER (1) showed however, that even a rectilinear relationship between ion uptake and water uptake is no proof of passive ion uptake. They demonstrated with tomatoes that the rate of water transport to the shoot during transpiration was about four times as high as the rate of exudation and that the rate of nitrate uptake showed the same ratio. In these plants, therefore, the amount of nitrate transport from the root system decreased after cutting to about 25 per cent of that, found in the intact transpiring plant. Preliminary experiments with maize plants, grown in climate rooms, however, indicated that water transport and ion transport did not show such a close relationship as found with tomatoes. A further investigation into these phenomena, therefore, seems worthwhile.

METHODS

For about 8 days after germination maize seedlings were placed on half strength Hoagland nutrient solution, based on the no. 1 formulation of HOAGLAND and ARNON (5). The composition of the solution is given in table 1. By adding sulfuric acid the pH of the nutrient solution was brought up to 4–4.5. $FeSO_4.7\ H_2O$ was added in applications of 2.8 p.p.m. Fe per litre nutrient solution. The solutions were refreshed 2–3 times a week. About one litre of solution was available per plant. Devations from this procedure are given at the experiments.

TABLE 1. Composition of the nutrient solution.

Salt	Concentration	Salt	Concentration
KNO_3	2.5 mM	H_3BO_3	0.1 p.p.m. B
$Ca(NO_3)_2.4\ H_2O$	2.5 mM	$MnCl_2.4\ H_2O$	0.1 p.p.m. Mn
KH_2PO_4	0.5 mM	$ZnSO_4.7\ H_2O$	0.01 ,, Zn
$MgSO_4.7\ H_2O$	1.0 mM	$CuSO_4.5\ H_2O$	0.004 ,, Cu
		$H_2MoO_4.H_2O$	0.01 ,, Mo

The plants were grown in climate rooms. Temperature was constant at 20°C. Sources of light were Philips HPL 400 W lamps or Philips TLF 65 W lamps, in a sequence of 16 hrs light and 8 hrs dark periods. Only during one experiment an 8 hrs light and 16 hrs dark sequence was used. Light intensities were as indicated at the experiments.

The uptake was measured by analysing the nutrient solutions. Nitrate was determined colorimetrically with the aid of a vitatron colorimeter, using the phenoldisulphonic acid method of SNELL and SNELL (14). Potassium was determined with the aid of a flame photometer. Transpiration was measured by the decrease of weight of the nutrient solution in the containers on which the plants were growing.

For the determination of exudation 100 ml beakers, filled with Kleenex tissue, were placed upside down over the cut stems to absorb the bleeding sap. The beakers were weighed before and after an exudation period. Afterwards a known quantity of demineralized water was added and intense stirring followed, in order to get a thorough distribution of the contents. Potassium and nitrate were determined in the way described above. With the dilution factor the potassium and nitrate concentration of the bleeding sap could be calculated, as well as the amounts of potassium and nitrate exuded.

RESULTS AND DISCUSSION

As stated in the introduction it may be expected that there are great differences in the ratio between transpiration rate and exudation rate for different plants and for the same plant under different conditions. In potometer experiments it has been established that with maize this ratio depends strongly on light intensity, relative humidity of the air and air temperature. The results of such an experiment are given in fig. 1. In this experiment the exudation rate was about 80 per cent of the transpiration rate. It will be shown further on, that in darkness the transpiration rate may be exceeded considerably by the exudation rate. In the experiment of fig. 1 the rate of exudation was rather constant during the first hour after cutting. If these experiments are continued for longer periods after cutting, the exudation rate decreases (fig. 2). The concentration of the exudate also decreases regularly, so that the amounts of potassium and nitrate exuded show a rapid decline. These experiments lead to the

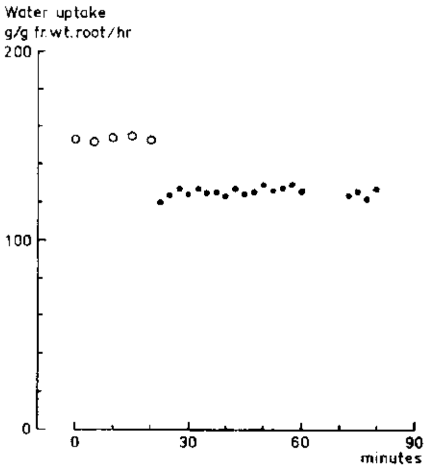

FIG. 1. A comparison of the transpiration rate (circles) and the exudation rate (dots) of maize in a potometer experiment.

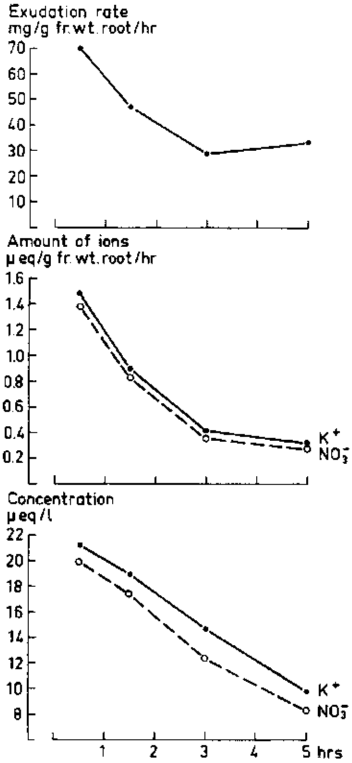

FIG. 2. Time course of the rate of exudation and the amount and concentration of potassium and nitrate in the exudate after cutting plants. (Means of 3 maize plants.)

conclusion that comparison between the transport of water and ions during transpiration and during exudation depends greatly on the time during which the exudation is measured.

The concentration of the exudate not being measured, it is impossible to establish whether the decrease in exudation rate is only caused by a decrease in salt secretion, leading to a reduced osmotic value in the xylem vessels, or whether it is also due to changes in water conductivity of the root tissue (comp. Formula of SABININ: Exudation rate = Conductivity × (osmotic value bleeding sap – osmotic value nutrient solution)). In the former case a change in the energy supply for the processes concerned seems one of the most probable reasons (9,2).

It is a fact that the decrease is strongly dependent on temperature (fig. 3). At a root temperature of 35°C the exudation rate is high at the beginning, but decreases very rapidly afterwards. The lower the temperature of the root medium, the smaller the initial rate of exudation and the smaller the decrease afterwards. The rapid decrease at 35°C seems to be in agreement with the assumption of the energy being the limiting factor. In table 2 potassium and nitrate concentrations of this experiment are given. Whereas the exudation rate (fig. 3) was measured in periods of 15 minutes, the concentrations were measured in 1 hr. periods. They were about the same at 23° and 35°C and distinctly higher at 11°C.

In fig. 4 the results of an experiment are given in which water, potassium and nitrate transport of intact plants in light and darkness were compared to those during exudation. The rate of water transport in the light was about three and a half time as great as in darkness. During the first and the second 2 hrs periods of the 6 hrs

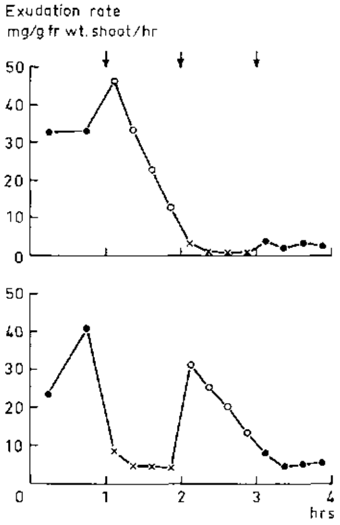

FIG. 3. Influence of the temperature of the root medium on the decrease of the exudation rate after cutting plants. Changes of temperature are indicated by arrows. Circles denote 35 °C, dots 23 °C and crosses 11 °C. (Means of 4 maize plants.)

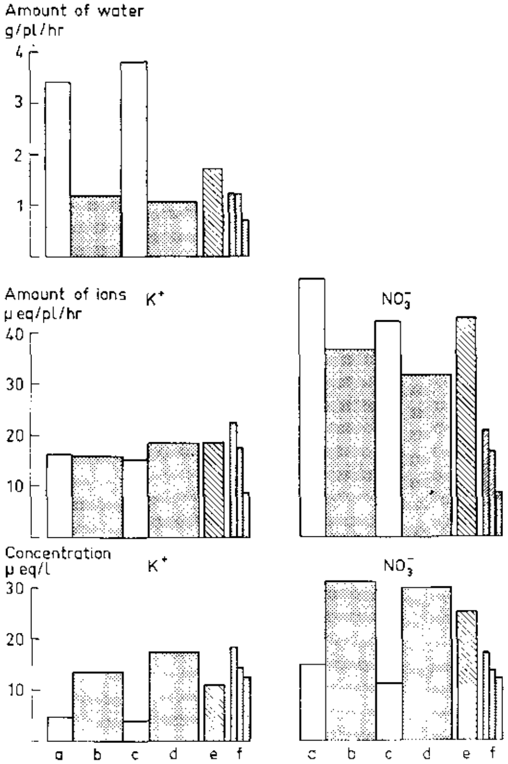

FIG. 4. Water uptake, potassium and nitrate uptake and concentrations during alternating 8 hrs light and 16 hrs dark periods (a, b, c, d). After the second dark period plants were cut and the uptake (e) was compared with the exudate (f) during a 6 hrs bleeding period. (Means of 3 maize plants.)

bleeding time the rate of exudation exceeded the rate of transpiration in the dark period. In column e the water loss of the containers with bleeding plants is shown. It appears that this water loss was higher than the amount of exuded sap. This may be due to evaporation and/or other errors in the technique.

The rate of potassium uptake was about the same in light, darkness and during exudation. The uptake of potassium during the exudation period was somewhat higher than the amount of potassium found in the bleeding sap. This may be due to accumulation of potassium in the root tissue. The picture of the nitrate uptake differed considerably from that found for potassium. There was a distinct difference between the uptake rates during the light and the dark period. The amount of nitrate taken up was about twice as high as the amount of potassium. This was even the case with the uptake during the exudation period. In contrast with the uptake, the amount of nitrate in the exudation stream equalled the amount of potassium. This is a general phenomenon with bleeding maize plants (*cf.* fig. 2 and table 2). The difference between the rate of nitrate uptake and nitrate transport through the cut surface may partly be due to accumulation of nitrate in the root tissue, as concluded for potassium. It stands to reason, however, that the great difference is also a consequence of the fact that part of the nitrogen, taken up by the root tissue, is transported to the shoot

TABLE 2. Potassium and nitrate concentrations, belonging to the exudates, plotted in fig. 3.

	2nd hr	3rd hr	4th hr
Temp. root medium	35°C	11°C	23°C
K^+ (meq/l)	13.8	22.3	14.4
NO_3^- (meq/l)	13.0	16.9	10.9
Temp. root medium	11°C	35°C	23°C
K^+ (meq/l)	21.6	17.3	15.4
NO_3^- (meq/l)	22.1	15.8	16.8

as organic compounds. In preliminary experiments it has been found that a considerable amount of organic nitrogen is present in the bleeding sap of maize plants (unpublished results). In fig. 4 (below) a comparison has been made between the concentration of the solutions entering the roots during the different periods a–e. In darkness and during the exudation period this concentration was considerably higher than in the light periods.

By plotting mineral uptake against water uptake (fig. 5) a clearer picture of the relation between these two can be shown. For nitrate this picture resembles the results of KIHLMAN-FALK (8). At the concentration used (7.5 meq/l) an influx coefficient (= ratio between the concentration of the transpiration stream and the concentration of the nutrient solution) of about 1 is found. The influx coefficient in this case is calculated for the solution entering the root. Due to the high value of the extrapolated uptake at zero water uptake, the transpiration stream concentration factor, as defined by SCOTT RUSSELL *et al.* (13), varies from ∞ at zero water uptake to about 2 at the highest water uptake found. As could be expected from fig. 4 there was no relation between the potassium uptake and the water uptake at the potassium concentration used (3 meq./l).

Comparing these results, obtained in short time experiments, with those of GROBBELAAR (5), a clear resemblance is to be found. GROBBELAAR showed that at a root temperature of 20°C the amount of nitrogen, found in the shoots, was about twice the amount of potassium. Using the transpiration ratio of 165 g water/1 g dry matter of shoots produced (= 110 g water/1 g total dry matter), an average concentration of

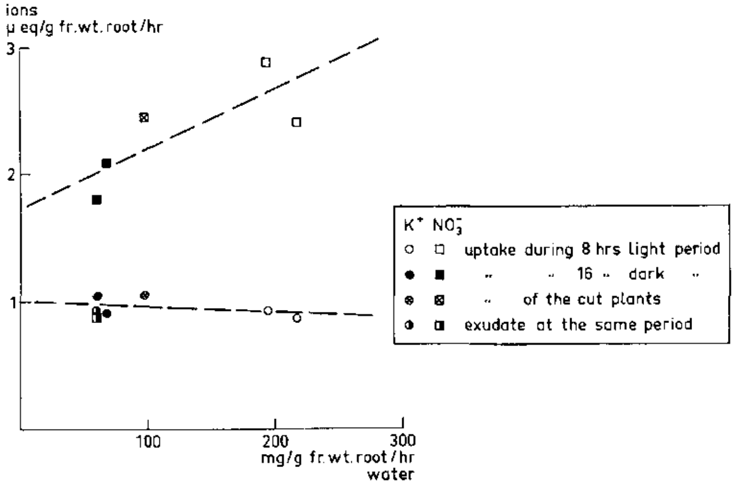

FIG. 5. Water uptake plotted against potassium and nitrate uptake. (Data of fig. 4.)

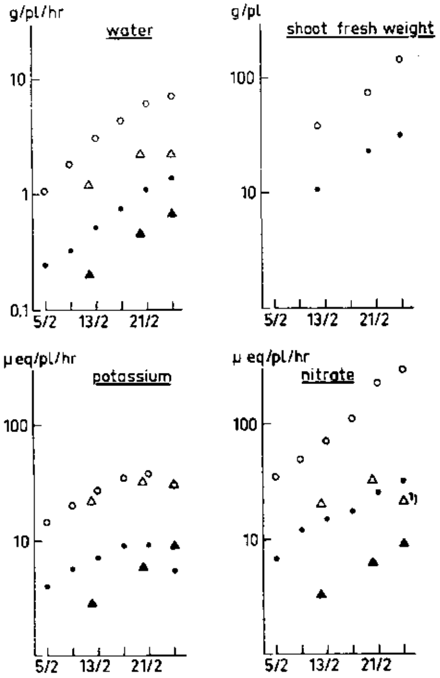

FIG. 6. Rates of the uptake of water, potassium and nitrate and the growth rate of maize plants, growing at 2 light intensities (TL; circles = 6.9×10^4 ergs·cm^{-2}·sec^{-1}; dots = 2.6×10^4 ergs·cm^{-2}·sec.$^{-1}$), in comparison with the rates of the exudation transport (4 hrs) (open triangles = high light intensity; closed triangles = low light intensity). Start of the experiment was at 20-1-'64.

[1] value too low, probably because of nitrate exhaustion in the nutrient solution before the last refreshment before bleeding.

the transpiration stream can be calculated. This value is about 10 meq. potassium/l and about 19 meq. nitrogen/l. These average concentrations are of the same order as those, expected from our experiment, if longer periods, consisting of alternating 16 hrs light and 8 hrs darkness, should have been used. It may be tentatively assumed, therefore, that in intact growing maize plants about one half of the nitrogen transport to the shoot takes place as organic nitrogen. The unequal amount of potassium and nitrogen, present in the tissue, in that case might be directly due to unequal uptake.

In various experiments the influence of plant age on the ratio between transpiration transport and exudation transport has been investigated. An example can be found in fig. 6. The plants were grown at two light intensities. The uptake rates were measured during a period of three weeks, starting at an age of 16 days after germination. During the experimental period the growth was about exponential for both light intensities. The same holds for the water uptake, showing a close relationship between water uptake and shoot-fresh weight. As for the mineral uptake deviations from the exponential curve occurred with potassium. The rate of nitrate uptake increased exponentially, but for potassium at both light intensities a flattening was found. It is probable that this phenomenon is due to a transition in growth stage (*cf.* GROBBELAAR (5)).

As far as the ratio between the transport rates in intact and bleeding plants is concerned, it appeared that at the higher light intensity both transport rates for potassium are about the same (*cf.* fig. 4 and 5). Nitrate showed the same differences, as described in the former experiments. At the lower light intensity the differences between intact and bleeding plants for potassium were relatively greater, which may be due to the less favourable energy supply. This seems the more reasonable, when the potassium transport is considered. The energy supply for the bleeding transport may be assumed to remain the same from 12/2–25/2 (exponential increase). The uptake by

TABLE 3. A comparison of the rates of water, potassium and nitrate uptake of intact maize plants (average of a 4 days' period containing periods of 16 hrs light alternating with 8 hrs darkness) with the subsequent rates of exudation (average of a 4 hrs period).
Plants grown at 5 different medium concentrations during pre-treatment and experiments. Light intensity (HPL) 6.1×10^4 ergs·cm^{-2}·sec^{-1}.

Concentration root medium	1/8 H	1/4 H	1/2 H	1/1 H normal conc.	2/1 H
H_2O (g/pl/hr)					
uptake	0.39	0.58	0.98	1.19	1.27
exudation	0.23	0.40	0.71	0.58	0.46
K^+ (μeq/pl/hr)					
uptake	3.0	4.6	7.8	13.0	17.6
exudation	3.3	5.9	10.2	8.4	8.1
NO_3^- (μeq/pl/hr)					
uptake	8.0	10.5	21.4	34.6	36.7
exudation	2.7	5.8	10.9	6.6	7.9
Fresh weight shoots (grams)	7.1	11.3	17.8	20.0	24.3
,, ,, roots ,,	5.8	6.0	7.8	9.9	9.5

the intact plant, however, decreased, possibly as a consequence of saturation, and the ratio between uptake and exudation became about 1.

In table 3 the transport rates of plants, grown at different concentrations of the nutrient solution, are shown. There is a distinct influence of the concentration on the growth rate of the plants. In this experiment exhaustion, even at the lowest concentrations, was prevented by regular refreshment. It remains possible, however, that since there was no aeration, some exhaustion occured in the vicinity of the root

TABLE 4. A comparison of the rates of water, potassium and nitrate uptake of maize (m), barley (b) and peas (p) (average of a 24 hrs period containing 16 hrs light and 8 hrs darkness) with the subsequent rates of exudation (average of 1 or 2 hrs period). Plants grown at 3 different light intensities (HPL) during pre-treatment and experiments.

Light intensity	9.1×10^4ergs·cm^{-2}·sec^{-1}			12.9×10^4ergs·cm^{-2}·sec^{-1}			16.8×10^4ergs·cm^{-2}·sec^{-1}		
Species	m	b	p	m	b	p	m	b	p
H_2O (g/pl/hr)									
uptake	1.63	1.10	2.0	4.33	2.28	3.83	10.96	4.85	8.2
exudation 1st hr	1.21	0.19	0.05	2.26	0.33	0.08	5.38	0.53	0.25
2nd hr	0.95			1.73			6.50		
K^+ (μeq/pl/hr)									
uptake	28.1	8.3	4.6	67.1	16.4	9.7	89.1[1]	24.5	19.3
exudation 1st hr	24.5	3.3	0.9	49.8	6.7	1.0	130.9	9.2	2.6
2nd hr	18.5			38.7			168.5		
NO_3^- (μeq/pl/hr)									
uptake	51.8	14.1	13.9	124.5	30.4	27.1	206[1]	44.0	58.8
exudation 1st hr	24.3	3.3	0.9	44.3	6.7	0.9	116.1	8.4	2.6
2nd hr	18.7			38.2			161.4		
Fresh weight shoots	26.1	8.5	9.1	58.1	16.8	17.3	104.8	25.2	27.3
,, ,, roots (grams)	12.3	2.7	4.0	25.0	6.3	8.0	50.5	10.6	13.3

[1] nutrient solution almost exhausted

surface. The ratio between water transport in intact and bleeding plants was about the same at the three lower concentrations used and increased at higher concentrations, due to reduced exudation rate. It appeared that the potassium and nitrate transport at the lower concentrations was about the same as found in the former experiments. At the higher concentrations, however, there were distinct differences, which may be due to the relatively reduced exudation rate as well. The uptake in the intact plant increased with the increasing concentrations to high values, the ratio between intact and bleeding plants changing from 3 to 5 for nitrate and from 1 to 2.2 for potassium.

In table 4 a comparison has been made of the uptake and the exudation of three plant species, grown at three light intensities. Whereas the water transport of exuding pea plants amounted to 3 per cent of the transpiration during a 24 hrs period (16 hrs light and 8 hrs darkness) and with barley amounted to 10–20 per cent (increasing with decreasing light intensity), it varied with maize plants between 50–75 per cent. In table 5 the percentages of the exudation transport, compared with the uptake by intact plants of the three crops, show that there were distinct differences between the values for water, potassium and nitrate. For each crop the nitrate concentration in the exudate equalled the potassium concentration. With intact plants the ratio between nitrate uptake and potassium uptake was about 3 for peas, independent of the light intensity in this range; for the other crops it amounted to about 2.

CONCLUDING REMARKS

The experiments with maize have proved that the uptake of potassium in intact and bleeding plants does not vary much within a wide range of conditions.

There are only some differences at higher concentrations (> 3 meq/l). As to nitrate distinct differences already exist at a concentration of about 1 meq/l. It is assumed that this is partly due to the nitrate fraction, which is assimilated in the root system and transported as organic nitrogen. The ratio of the water transport of intact and bleeding plants depends on the transpiration conditions (fig. 4) and on the concentration of the nutrient solution (table 3). At the various conditions, investigated here, the bleeding water transport amounts always to more than 40 per cent of the transpiration transport.

Since it may be expected that the secretion processes are even more pronounced in intact transpiring plants than after excision (2, 9), a considerable fraction of the upward water transport must be due to active processes (*cf.* 12).

With peas the share of osmotic water movement in the total water transport is less than 5 per cent (table 5) and this value agrees with many others in literature (10). Our experiments show that maize – and the same holds for tomatoes (1) – is an exception to the rule.

TABLE 5. Exudation transport as a percentage of the uptake by intact plants at a light intensity of $9.1\text{x}10^4$ ergs·cm^{-2}·sec^{-1} (table 4).

	m	b	p
H_2O	67	17	2.5
K^+	75	40	19
NO_3^-	40	23	6.5

SUMMARY

Experiments have been described in which the transport of water and ions in intact and bleeding maize plants have been compared. Whereas the potassium transport was rather independent of water transport, the nitrogen transport was greatly affected. The average concentration of the transpiration stream in growth experiments with maize (5) was the same as could be expected from these short time experiments.

Distinct differences as to the ratio between transpiration transport and bleeding transport in different crops have been shown.

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ERRATUM

Fig. 2 and 4, under Concentration: meq/l instead of μeq/l.

Mededeling 239 van het I.B.S.

THE SUSCEPTIBILITY OF STRAWBERRY PLANTS TO SIMAZINE

J. L. P. van Oorschot and J. G. Haker[1])

Introduction

The need for chemical weed control in strawberry growing is great, since mechanical control in this crop is difficult and handweeding involves considerable expenses (10). In order to select useful and safe herbicides for strawberries many products were tested in field trials, but only few proved to be useful (5, 10). One of them is simazine, of which low rates are sufficiently safe on established plantings during summer and autumn, whereas a treatment in spring may cause damage, if the soil is low in organic matter (11). In order to study the relative tolerance of strawberries to simazine a series of experiments was carried out, in which the factor soil could be excluded or standardized.

Material and methods

In preliminary experiments young strawberry plants of the variety Jucunda were taken from runner beds during April and planted in two types of soil, mixed with simazine and diuron at various concentrations.

In the next series of experiments plants of different varieties were taken from runner beds during August and September, placed on a Hoagland solution in a growth room at 15°C, and irradiated with high pressure mercury vapour lamps with fluorescent bulbs (HPL-400 Watt, Philips), yielding a light intensity of 0.1 cal $cm^{-2}min^{-1}$ ($\lambda < 0.7\ \mu$) at the height of the plants unless otherwise noted. A photoperiod of 16 hours was alternated with 8 hours of darkness. After 3–4 weeks of growth new leaves had developed. Then, the older leaves, developed during the growth on runner beds, were removed. Simazine was added to the culture solution in various concentrations, and the plants were grown during a period of 18–24 days. In some experiments also propazine and chloroxuron (3-(4-chlorophenoxy)-phenyl-1,1-dimethylurea) were studied. At the end of the growth period dry weights of the plants were determined. As in field experiments (10, 11) the variation between replicates appeared to be rather wide (see tables). Due to lack of space only 4–6 replicates could be used.

The effect of simazine and chloroxuron applied in the culture solution of intact strawberry plants on uptake of CO_2 was measured with an infrared gas analyser described earlier (7). For this purpose the plants were also grown on a Hoagland solution and placed in a growth room at 15°C under similar conditions as described before. During the experiments on uptake of CO_2 the light intensity was 0.35 cal $cm^{-2}min^{-1}$ ($\lambda < 0.7\ \mu$), obtained by two high pressure mercury vapour lamps of 700 Watt with reflectors.

Results

At first, experiments with the variety Jucunda were carried out in two types of soil,

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viz. a sandy soil with 4.5 per cent of humus, and a sandy loam with 23 per cent of clay and 2.4 per cent of humus. In April young plants from runner beds were transplanted into pots containing these soils, mixed with simazine and diuron in the following concentrations: 0.1, 0.3, 0.8, 2.0 and 5.0 mg per kg dry soil. Each treatment was replicated 5 times. These pots were placed under natural conditions and dug somewhat into moist peat in order to prevent drying out during the next 4 months.

Within one month after planting heavy symptoms (yellow to brownish leaves) developed at the highest concentration of both herbicides. These symptoms resulted in complete death within two months. At the concentration of 2 ppm these symptoms appeared more gradually, but the plants were also dead at the end of the experimental period. At the concentration of 0.8 ppm symptoms developed in general after 2 months. At 0.3 ppm some symptoms were observed towards the end of the experimental period, while the plants were generally somewhat smaller. At the lowest concentration (0.1 ppm) the plants were similar to the control plants. A series of representative plants at the end of the experimental period is shown in photograph 1, giving plants in sandy soil. The results with plants in sandy loam were similar. They indicate that both herbicides already affect the growth of strawberry plants at a concentration of 0.3 mg per kg of dry soil.

In a preliminary experiment with plants on culture solution the same variety was used. The plants were placed in a growth room at 20°C, so that both shoots and roots were at the same temperature. Under these conditions the root temperature of 20°C is in the optimum range for growth (2). A concentration range up to 2 mg of simazine per litre was applied, four replicates being used for each concentration. In this experiment attention was paid to visible symptoms only. After 4 days yellow to brown leaves had developed at 2 mg of simazine per litre, followed by complete death of the plants after 10 days. At a concentration of 0.8 mg of simazine per litre symptoms developed slower, resulting in complete death after 19 days. A concentration of 0.5 mg per litre showed only slightly better plants, while at a concentration of 0.3 mg per litre yellow leaves had developed. The plants at 0.1 mg per litre showed only very light symptoms (green-yellow young leaves). The daughter plants which had developed on runners during the experimental period showed similar symptoms as observed on the mother plants. A similar correlation was also found in the field.

In an experiment with the variety Regina a concentration range up to 0.5 mg per litre was used, while the experiment was carried out in three growth rooms at 10, 15 and 20°C respectively. After one week of growth flowering started at the highest temperature, while also a few runners were gradually formed. Most of the plants flowered at the end of the experimental period of 18 days, and had 6–8 leaves at 20°C, 6–7 leaves at 15°C, and 5–6 leaves at 10°C. At 20°C the first definite symptoms were observed after 5 days at the highest concentration (0.5 mg per litre); at the same time they were less pronounced at 15°C, and absent at 10°C. Such leaves showed a wilted appearance and gradually turned black. These symptoms developed much slower at lower concentration and temperature. Typical plants after 15 days of growth are shown in photograph 2.

The dry weights of the plants at the end of the experimental period, and the total transpiration are given in table 1. The variation between replicates is wide as is evident from the large mean errors. However, the results indicate that the dry weight of shoots (including runners and flowers) is reduced, especially at 20 and 15°C, as the concentration of simazine in the culture solution is higher. The effect on dry weight of the roots is comparatively less.

Since dry weights of comparable plants were determined at the beginning of the experiment (see table 1) the increase in dry weight of shoots and roots could be

PHOTOGRAPH 1. The effect of simazine and diuron in the soil upon strawberry plants (variety Jucunda). Concentrations of 0.1, 0.3, 0.8, 2.0 and 5.0 ppm. Top row: simazine, middle: untreated, bottom row: diuron. Planted: April 11, photograph: July 31.

TABLE 1. Dry weight and transpiration of strawberry plants (variety Regina) after 18 days at 20, 15 and 10°C, as affected by simazine in the culture solution. The values represent averages of 4 replicates.

Treatment		Dry weight (g)		Transpiration
Temperature	mg simazine/litre	Shoot	Root	(ml)
20°C	0	2.25 ± 0.29	0.58 ± 0.10	447 ± 65
	0.05	1.84 ± 0.31	0.52 ± 0.13	443 ± 25
	0.10	1.24 ± 0.56	0.47 ± 0.10	361 ± 72
	0.25	0.74 ± 0.13	0.38 ± 0.07	202 ± 22
	0.50	0.67 ± 0.26	0.38 ± 0.14	161 ± 42
15°C	0	2.24 ± 0.60	0.61 ± 0.14	326 ± 65
	0.05	1.66 ± 0.45	0.50 ± 0.18	299 ± 94
	0.10	1.24 ± 0.41	0.50 ± 0.16	307 ± 39
	0.25	0.80 ± 0.31	0.39 ± 0.07	176 ± 80
	0.50	0.57 ± 0.15	0.36 ± 0.16	120 ± 37
10°C	0	1.53 ± 0.47	0.70 ± 0.29	153 ± 31
	0.05	1.16 ± 0.05	0.63 ± 0.08	150 ± 10
	0.10	0.97 ± 0.24	0.48 ± 0.09	140 ± 15
	0.25	0.77 ± 0.19	0.41 ± 0.12	100 ± 33
	0.50	0.74 ± 0.17	0.41 ± 0.10	111 ± 26
At the beginning of the expt.		0.56 ± 0.14	0.36 ± 0.11	

calculated, and compared to the total transpiration during the experiment. The results are given in fig. 1. The effect of simazine on the increase in dry weight of the shoot at 20°C is almost identical to that at 15°C. At both temperatures reduction of shoot growth already occurred at 0.05 mg per litre, while almost complete inhibition is found at a concentration of 0.5 mg per litre. Root growth (expressed as increase in dry weight) was less affected by simazine at these temperatures. The effect of simazine on shoot growth at 10°C is much less pronounced.

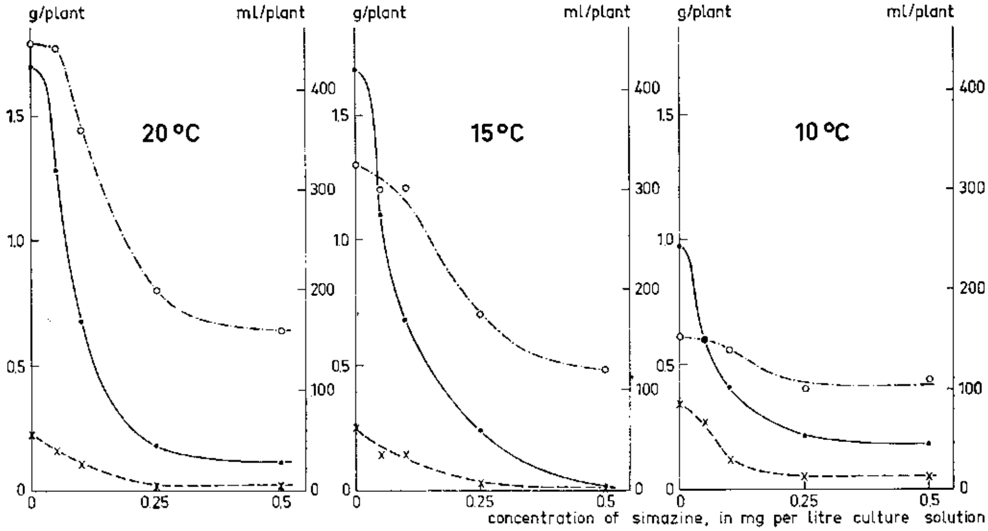
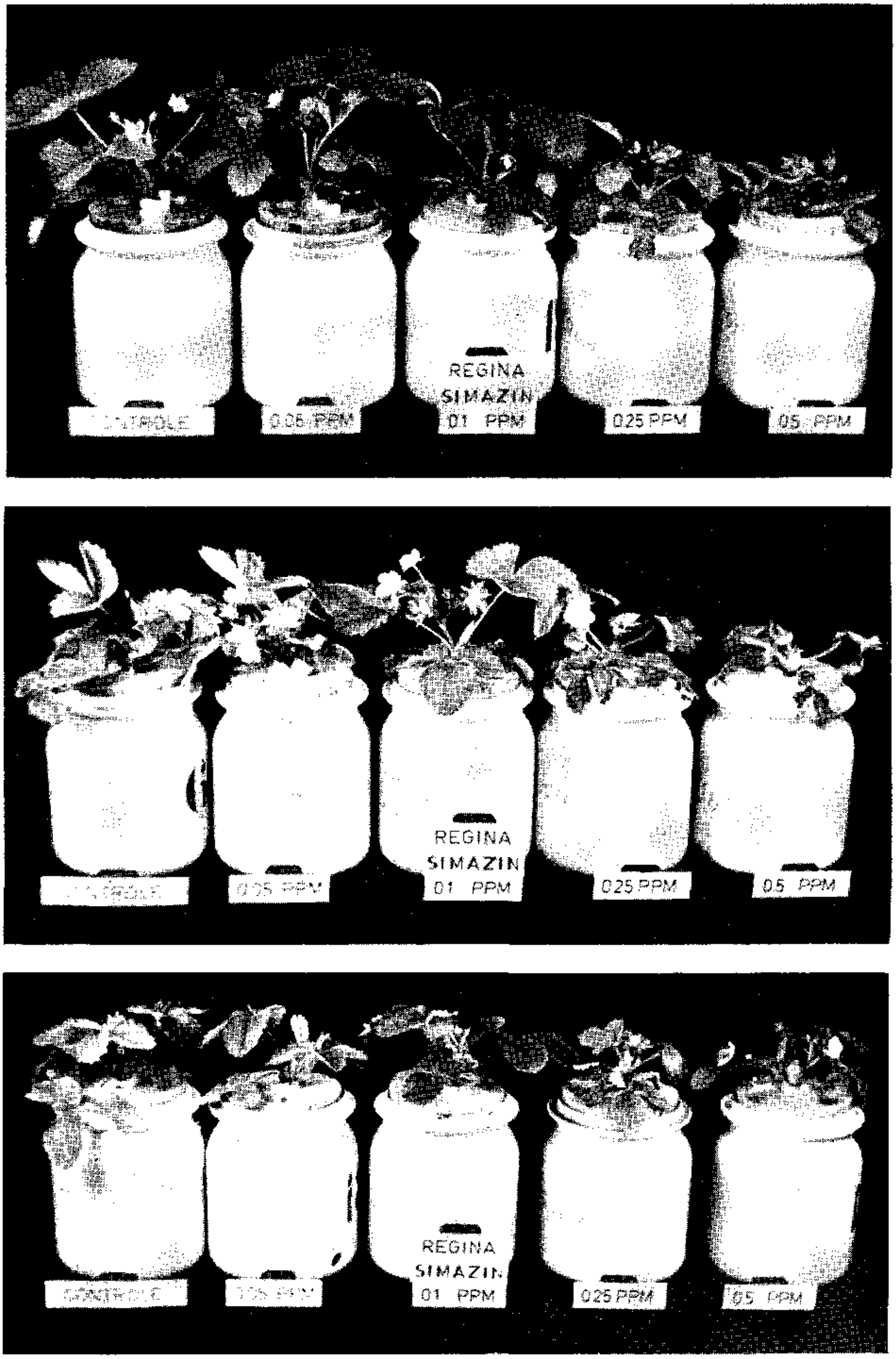


FIG. 1. Increase in dry weight (g/plant), and total transpiration (ml/plant) of strawberry plants (variety Regina) as influenced by simazine in the culture solution at 20°C, 15°C and 10°C during 18 days. Shoots: ●—●, roots: ×--×, and transpiration: ○-·-○.



PHOTOGRAPH 2. The effect of simazine in the culture solution upon strawberry plants (variety Regina) at 20°C (top), 15 C (middle), and 10 C (bottom). Photograph taken 15 days after beginning of treatment.

The effect of simazine in the culture solution upon dry weight of strawberry plants was also studied in an experiment in which the shoot/root-ratio was modified. At the beginning of an experiment with Regina the plants had 4 leaves. Two leaves were removed from some of these plants, while half of the roots were removed from others. In another group both leaves and roots were reduced to the half. This is, of course, rather roughly, since it is impossible to remove exactly the same part of leaves (being different in size) or roots from each plant. Then, 0.4 mg simazine was added to the culture solution, and after a period of 18 days the dry weights were compared to those of control plants grown under similar conditions. The results are given in table 2.

Table 2. Dry weight of strawberry plants (variety Regina) after 18 days at 20 °C, as affected by 0.4 mg simazine in the culture solution, and at different treatments of the plants. Averages of 4 replicates.

Plant treatment (see text)	Control		0.4 mg simazine per litre			
	Shoot	Root	Shoot		Root	
	g	g	g	%	g	%
Intact plants	4.02 ± 0.41	0.94 ± 0.18	2.14 ± 0.14	53	0.87 ± 0.21	93
Reduced roots	4.14 ± 0.63	0.67 ± 0.08	2.07 ± 0.37	50	0.51 ± 0.14	76
Reduced shoots	2.60 ± 0.28	0.93 ± 0.25	1.12 ± 0.18	43	0.74 ± 0.20	80
Reduced shoots and roots	2.58 ± 0.28	0.59 ± 0.06	1.32 ± 0.31	51	0.45 ± 0.09	76

In this experiment the reduction in dry weight of the shoot at 0.4 mg simazine in the culture solution is also much more pronounced than that of the roots, while this reduction is of the same order of magnitude (50%) for the different initial shoot/root-ratios. This suggests that the effect is mainly determined by transpiration of the shoot which is proportional to its size, and may result in almost equal concentrations of simazine in the shoot.

The response of different varieties to simazine was studied in some other experiments. In an experiment in growth rooms at 15 and 20 °C a concentration of 0.2 mg simazine per litre was added to the culture solution of the varieties Jucunda and Regina, and compared with untreated plants under the same conditions. These varieties reacted similarly to simazine. In some experiments more varieties were used in a greenhouse during autumn. The effect of a concentration of 0.3 mg per litre in the culture solution was compared to untreated plants. Only visible symptoms were recorded. The differences between the varieties seemed to be relatively small, and were mainly due to differences in fastness of response. In these experiments Deutsch Evern, Regina, Jucunda and Juspa showed the most rapid response, while Talisman, Senga sengana and Cambridge Vigour showed a less rapid response to simazine.

In subsequent experiments some other herbicides were used as well. A comparison between simazine and propazine in concentrations of 0.1, 0.2 and 0.3 mg per litre in the culture solution of the variety Jucunda was made in a growth room at 20 °C. The development of symptoms was similar for these herbicides. Also the reduction in dry weight as compared to control plants at the end of the experiment (24 days) was in the same order of magnitude for equal concentrations of both herbicides.

A comparison between simazine and chloroxuron was made in an experiment at 20 °C with the variety Talisman. At the beginning of the experiment dry weights of equivalent plants were determined. Then, in comparison with a concentration of 0.4 mg simazine, a concentration of 0.58 mg chloroxuron per litre was given so that an equal molar concentration of 2×10^{-6} M for both herbicides was applied. In addition also a higher concentration of 3.7 mg chloroxuron per litre was applied. In this experiment the plants were placed at a lower light intensity, *viz.* 0.06 cal cm^{-2} min^{-1} obtained by TLF-fluorescent tubes. The results are given in table 3. Again the variation between replicates is wide, but the results indicate that the plants better tolerate chloroxuron than simazine. Again the main effect is in the shoots. The shoot/root-ratio of treated plants is lower, especially with simazine and the higher concentration of chloroxuron.

TABLE 3. Effect of simazine on dry weight of strawberry plants (variety Talisman) as compared to that of chloroxuron. Averages of 6 replicates, duration of experiment: 24 days.

Treatment	Shoot total g	Shoot increase g	Root total g	Root increase g	Shoot/Root-ratio
control	6.37 ± 1.87	3.34	1.22 ± 0.40	0.72	5.20
0.4 mg simazine/litre	4.14 ± 1.22	1.21	1.46 ± 0.59	0.96	2.84
0.58 mg chloroxuron/litre	5.28 ± 1.06	2.35	1.24 ± 0.39	0.74	4.25
3.7 mg chloroxuron/litre	4.55 ± 1.16	1.62	1.31 ± 0.35	0.81	3.47

Simazine acts as an inhibitor of the Hill-reaction (3, 4, 6), and also reduces uptake of CO_2 by excised leaves (1) or intact plants (7, 8). Uptake of CO_2 and transpiration of intact strawberry plants (variety Talisman) was measured under influence of 2×10^{-5} M simazine (4 mg per litre) in the culture solution. In addition, the following technique (9) has been used in order to determine whether simazine is inactivated by plant species. When the rate of CO_2-uptake was reduced to 50 per cent of the original level, the culture solution containing simazine was removed and replaced by a normal culture solution. Under these conditions the rate of CO_2-uptake of maize gradually recovered, indicating that inactivation of simazine by maize takes place (9).

The results of similar experiments with strawberry plants are given in fig. 2. The variation between the three replicates was about 10 per cent of the average values. No recovery of the rate of CO_2-uptake is observed after removal of simazine from the root environment at the level of 50 per cent inhibition. Results with similar treatments of other plant species as sugar beets, chicory, cucumber and asparagus were the same (9). These results indicate that strawberry plants are unable to inactivate simazine, or only to such a small extent that it can not be measured in this way. Apparently, sufficient simazine has been taken up during the exposure to simazine to reduce uptake of CO_2 to less than 10 per cent after 19 hours. Assuming that uptake of simazine is

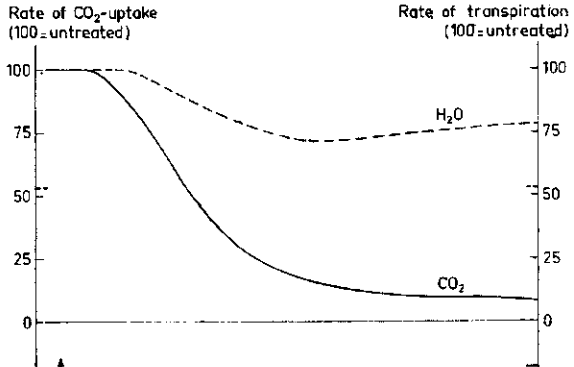


FIG. 2. The reaction of strawberry plants upon a temporary exposure to simazine. At ↑ a culture solution with 2×10^{-5} M simazine is applied until 50 per cent inhibition of the initial rate of CO_2-uptake occurs. Average of 3 experiments at 24.5°C leaf temperature during the light period, initial rate of CO_2-uptake (100) 355 μg CO_2 cm^{-2}hr^{-1}, and of transpiration (100) 14.8 mg H_2O cm^{-2}hr^{-1}. The horizontal dashes represent the values during the short dark periods.

proportional to transpiration rate and concentration in the culture solution the internal concentration in the water phase of the plants at the level of 50% inhibition was estimated at 2 × 10^{-5} M.

In an experiment with 1.3 × 10^{-5} M chloroxuron in the culture solution no effect on uptake of CO_2 by Talisman has been found. This effect was also negligible with carrots and maize, while the uptake of CO_2 by beans and peas was affected by chloroxuron to a rather small extent.

DISCUSSION

From the results of the experiments with soil (photograph 1), it is evident that a concentration of 0.3 ppm of simazine (or diuron) in the soil may reduce growth of strawberry plants. This susceptibility to simazine is almost comparable to that of a sensitive annual plant as rye in sandy soil, for which a toxicity curve was determined by VAN DER ZWEEP (13). The delay in final effect may have been caused by the larger amount of reserve materials of strawberry plants. In culture solution a concentration of 0.05 mg per litre already reduced dry matter production (table 1 and fig. 1), and especially that of shoots. This difference in level of toxicity can be explained by adsorption of the herbicide to the soil. The high toxicity of simazine to strawberry plants has also been demonstrated in experiments on uptake of CO_2, while the roots are in a culture solution containing simazine. Apparently, they cannot inactivate simazine taken up by the roots, or only to a very small extent.

In the field, low rates of simazine may be used on established plantings (5, 11, 12). Assuming a specific density of the soil of 1.00 the rate of 0.5 kg per hectare would result in a concentration of 1 ppm assuming an equal distribution in a layer of 5 cm, or 0.5 ppm in a layer of 10 cm. Obviously, the low penetration of simazine into the soil must protect strawberry plants against phytotoxic action of simazine, since contact of the roots with a low concentration of simazine reduces shoot growth. In contrast to the results of pot experiments with two types of soil in which the herbicides were equally distributed, in the field differences in soil constituents and especially in humus content are of great importance (11) since they determine penetration of a herbicide into the soil.

The reason for the higher sensitivity of strawberry plants during spring is not yet clear (5). At lower temperatures prevailing in early spring the shoot/root-ratio may be lower, as is evident from table 1 and in accordance with results of BROUWER (2). On the other hand a lower ratio does not seem to affect the susceptibility of the plants in culture solution (table 2). A possibly more shallow root system during this season together with a high transpiration rate of foliage in late spring could result in larger uptake and a higher concentration in the leaves. The differences between varieties in the field (5, 11) is probably mainly determined by edaphic factors, since varietal differences are relatively small, if simazine is applied in the culture solution. For instance, differences in root development in early spring may determine whether the roots of certain varieties escape from contact with simazine.

The results with chloroxuron (table 3) indicate that strawberry plants are less susceptible to this herbicide than to simazine which is in accordance with the field experience that chloroxuron is safer than simazine under the unfavourable conditions of spring. However, it should be noted that for equivalent weed control much higher rates of chloroxuron have to be applied.

SUMMARY

The growth of strawberry plants is reduced at a concentration of 0.3 ppm of simazine in the soil, and at 0.05 mg per litre in the culture solution. Also in experiments on uptake of CO_2 strawberry plants show a low tolerance to simazine. The applications in the field are discussed in relation to these results.

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Mededeling 240 van het I.B.S.

THE RELATION BETWEEN SALT AND WATER UPTAKE WITH INTACT AND DECAPITATED TOMATO PLANTS

TH. ALBERDA, W. LOUWERSE and R. BROUWER

INTRODUCTION

The relation between salt and water uptake has been the topic of many experiments and has led to a considerable diversity of opinion. Although in recent years VAN DEN HONERT *et al.* (7) were unable to demonstrate an influence of the transpiration on salt uptake, most people assume that the uptake of minerals from the root medium can be increased by an increase in transpiration. The difference of opinion centres around the question whether the ions are taken up mainly passively with the transpiration stream, or whether the relation is indirect in that both processes are related to one or more other factors which they have in common. The literature has recently been reviewed by RUSSEL and BARBER (15).

HYLMÖ and co-workers state that at least a part of the salt uptake is directly influenced by the transpiration stream, whereas BROUWER shared the opinion of HOAGLAND, ARISZ and many others that the salt uptake is essentially an active process, taking place at the expense of energy, while transpiration is not. When, nevertheless, the uptake of minerals is influenced by transpiration, this must be due to a common factor. According to BROUWER a high rate transpiration lessened the resistance of the protoplasm, both for water and for salts.

Although HYLMÖ (8) stated that by far the greatest part of the total uptake of calcium and chlorine could be ascribed to a 'mass flow' of solution from the medium into the vessels, later work of KIHLMAN-FALK (13) gave quantitatively different results. She wrote: 'In addition to the great transpiration-independent potassium and nitrate absorption there exists an uptake which is directly dependent on transpiration' (p. 431).

When the salt uptake is plotted against transpiration, a straight line is often obtained, which usually indicates some salt uptake when extrapolated to zero water uptake (a in fig. 1). To characterize the transpiration-dependent part of the salt uptake HYLMÖ calculated the *influx coefficient*, which is the salt concentration calculated from the water uptake and the transpiration-induced salt uptake, divided by the concentration of the external solution. Although the relation between transpiration and the water-induced salt uptake was said to be a direct one (8, 9) this has in fact never been demonstrated. On the other hand BROUWER (1, 2) could vary the rates of transpiration and salt uptake independently of each other, although he did not prove that there was no direct influence at all.

One could leave these differences of opinion with the different authors, were it not that JENSEN (11) recently published data, obtained with intact and bleeding high salt tomato plants, which pointed to a relation between water and salt uptake as given by b in fig. 1, thus indicating zero salt uptake when there is no transpiration. If such a relation indeed exists, this could be a good starting point for further research into this matter. In the following, some preliminary results on the relation between salt and water uptake with tomatoes are given.

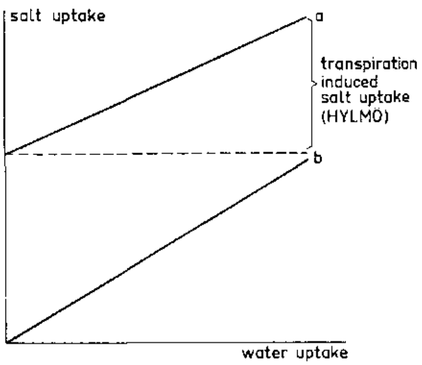


FIG. 1. Schematic representation of the relation between salt uptake and water uptake.

MATERIAL AND METHODS

Tomato seeds (variety Ailsa Craig) were germinated in fine gravel. The seedlings were placed on 1 litre pots filled with a half strength Hoagland solution, with iron added as Fe-EDTA. The pots were placed in a greenhouse at a temperature of about 23°C and aerated continuously. The nutrient solution was renewed twice a week. After the plants had grown for about six weeks they were transferred to plastic buckets of 8 liters capacity and again aerated continuously until they were used for the experiment. A few days before that, the plants were placed in a controlled room at a temperature of 20°C with artificial illumination by means of high pressure mercury vapour lamps with a fluorescent coating (Philips HPL 400 Watt). These lamps gave a light intensity of about 5×10^4 ergs $cm^{-2}sec^{-1}$. Where necessary, further details are given with the separate experiments.

During the experiment, and usually already some days before it, the nutrient solution was changed daily. The uptake of nitrate, and in some cases of potassium, and the uptake of water were determined over a period of 24 hours or shorter. With intact plants the uptake of water was determined by weighing the plastic bucket with solution and the plant at the beginning and the end of the experimental period. The rate of evaporation of the aerated solution proved to be small (see below). At both times a sample was taken from the solution for the nitrate determination. With these data the uptake of nitrate and water over the experimental period could be calculated. After the uptake was determined with intact plants the experiment was repeated for the same period of time and with the same plants, but after decapitation. This was done by cutting the stem about 5 cm above the roots with a sharp knife. Then a rubber tube of the appropriate size was slipped over the stump and fitted tightly to it by means of a copper wire when necessary. The other end of the tube was bent into a flask. The amount of bleeding sap and the concentration of nitrate in it were determined. In addition the uptake of water and nitrate from the solution was determined as mentioned above. The amount of water lost by evaporation could now be determined by subtracting the amount of bleeding sap from the total amount of water lost during the experimental period.

The determination of nitrate in the solution and in the bleeding sap was according to the method of SNELL and SNELL (16). The determination of total nitrogen in the plant was carried out with the method of DEIJS (5).

The method used for the potassium determination is as follows: The dried and ground material is ashed at 500°C. To the ash several drops of a 25 per cent HCl solution are added, after which it is evaporated to dryness on a boiling water bath.

Then again one drop of the 25 per cent HCl solution is added, after which the content is brought into a 100 ml measuring flask, made up to volume and then filtered. In the filtrate the potassium content is measured with a flame photometer.

RESULTS

Experiment 1

Tomato plants, approximately 10 weeks old, were brought into the growth room one day before the experiment. They were illuminated for 16 hours (from 4.00 till 20.00). The uptake of water and nitrate was determined over a 24 hours' period before and after decapitation. Table 1 and fig. 2 give the results. The concentration of the solution taken in by the plant was approximately the same before and after decapitation and was also very close to the nitrate concentration actually found in the bleeding sap. In fig. 2 the water uptake has been plotted against the nitrate uptake, both before and after decapitation. Through the two points a line can be drawn which meets the origin when extrapolated. Thus the findings of JENSEN (11) could be fully confirmed.

TABLE 1. Uptake of water and nitrate by intact and bleeding tomato plants

1	2	3	4	5	6	7	8
					NO_3-concentration		
NO_3-conc. in nutrient solution me.l^{-1}	Condition of plant	Water-uptake μl.g^{-1}.h^{-1}	Nitrate-uptake μe.g^{-1}.h^{-1}	Rate of bleeding μl.g^{-1}.h^{-1}	calc. from 3 or 5 and 4 me.l^{-1}	determined in bleeding sap me.l^{-1}	Influx coefficient
3.75	intact	340	8.87	–	26.1	–	7.1
	bleeding	–	2.04	84	24.3	26.4	

Experiment 2

For this experiment two groups of plants were used which were in low-salt and high-salt condition respectively.

The low-salt plants were 12 weeks old when they were brought into the growth room. The illumination was as in the foregoing experiment until the start of this experiment, which was carried out in continuous light. The plants were cultivated in plastic buckets of 5 litres capacity; the nutrient solution was changed twice a week. This

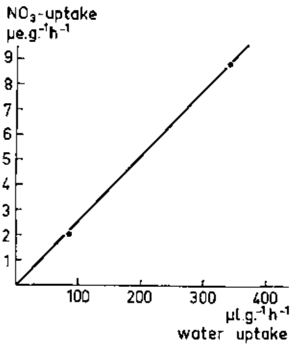


FIG. 2. The relation between the uptake of water and nitrate with intact and decapitated tomato plants.

appeared to be insufficient for plants of that size. When the solution was analyzed just before changing, no nitrate was present so that they were more or less in low-salt condition. At the start of the experiment four plants were placed on solutions with different nitrate concentrations as indicated in table 2. To avoid nitrate exhaustion at the lower concentrations the uptake periods were 18, 9, $4^1/_2$ and $2^1/_4$ hours respectively from the highest to the lowest concentration. The uptake of nitrate and water was determined before and after decapitation. The uptake periods were of the same length and carried out at the same time of the day. In between the two periods the intact plants were all placed on a nitrate concentration of 3.75 milli-equivalents per litre ($me.l^{-1}$).

The high-salt plants were 10 weeks old when they were brought into the growth room. They were cultivated in plastic buckets on 8 litres nutrient solution, which was first changed twice a week, but from the moment the plants were brought into the growth room this was done daily for one week, until the experiment proper began. For the rest, the conditions were the same as with the low-salt plants, except that the uptake periods were 24, 12, 6 and 3 hours respectively.

The results are given in table 2 and fig. 3 a and b. In fig. 3a the relation between water and nitrate uptake is given for the low-salt plants. When each set of points before and after decapitation is connected and extrapolated to zero water uptake, all four lines meet the ordinate at a point distinctly above zero. When the same experiment was carried out with high-salt plants the results differed (fig. 3b). Except for the 0.94 me./l concentration the lines went through the origin when extrapolated, thus giving the same result as in experiment 1.

Further differences between the two groups of plants become visible from table 2. The nitrate concentration of the bleeding sap was approximately the same for both high-salt and low-salt plants and differed not too much from the concentration calculated for the ingoing solution from the water and nitrate uptake data of the intact plants. With the bleeding plants, however, the calculated concentrations were much higher with the low-salt than with the high-salt plants. The latter values corresponded approximately with the other concentrations, but for the low-salt plants they were

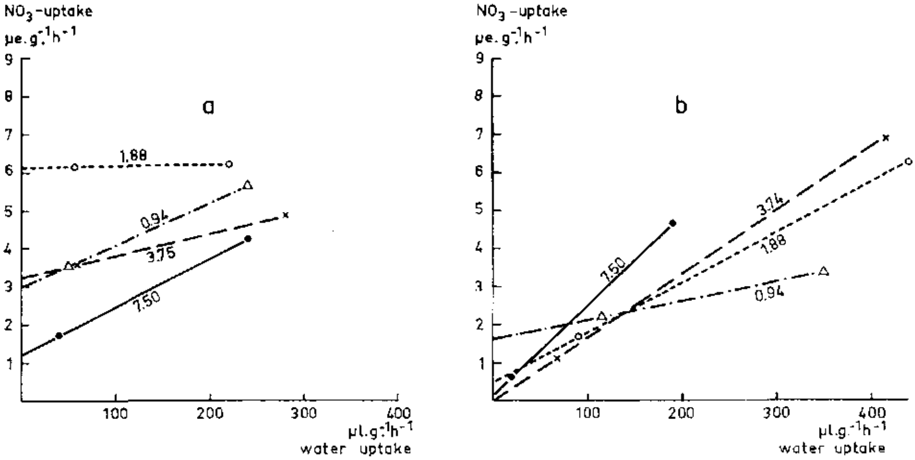


FIG. 3. The relation between the uptake of water and nitrate with intact and decapitated tomato plants. *a*. plants in low-salt condition; *b*. plants in high-salt condition.

TABLE 2. Uptake of water and nitrate by low-salt and high-salt tomato plants, both intact and decapitated.

	1	2	3	4	5	6	7	8
						NO_3-concentration		
Salt status of plants	NO_3-conc. in nutrient solution	Condition of plant	Water-uptake	Nitrate-uptake	Rate of bleeding	calc. from 3 or 5 and 4	determined in bleeding sap	Influx coefficient
	$me.l^{-1}$		$\mu l.g^{-1}.h^{-1}$	$\mu e.g^{-1}.h^{-1}$	$\mu l.g^{-1}.h^{-1}$	$me.l^{-1}$	$me.l^{-1}$	
low-salt	7.50	intact	240	4.27	–	17.8	–	1.72
		bleeding	–	1.69	40	42.3	23.5	
	3.75	intact	280	4.82	–	17.2	–	1.53
		bleeding	–	3.56	61	58.4	27.0	
	1.88	intact	220	6.21[1]	–	28.2	–	0.16
		bleeding	–	6.16[1]	57	108.1	27.6	
	0.94	intact	240	5.58	–	23.3	–	11.36
		bleeding	–	3.53	48	73.5	21.1	
high-salt	7.50	intact	190	4.65	–	24.5	–	3.15
		bleeding	–	0.59	19	32.0	21.2	
	3.75	intact	415	6.94	–	16.7	–	4.51
		bleeding	–	1.06	67	15.8	19.1	
	1.88	intact	440	6.27	–	14.3	–	7.13
		bleeding	–	1.58	90	17.5	26.6	
	0.94	intact	346	3.32	–	9.6	–	5.09
		bleeding	–	2.21	114	19.4	18.5	

[1] No nitrate present in the solution at the end of the uptake period.

exceptionally high. For the sake of simplicity the values, averaged over the concentrations, are given below in me./l NO_3:

			low-salt	high-salt
intact plants,	NO_3-conc.	calculated	21.6	16.3
cut plants,	,,	,,	70.6	21.2
bleeding sap,	,,	determined	24.8	21.4

With the high-salt plants the influx coefficient varied from 3–7. The values increased with decreasing nitrate concentrations in the nutrient solutions. With low-salt plants the results were far less regular. At the two highest concentrations the influx coefficient lay around 1.5; at the 1.88 me./l concentration it was very low, apparently since the nitrogen was exhausted at the end of the uptake period, and at the lowest concentration of 0.94 me./l it was very high. These irregularities can also be seen from the slope of the lines in fig. 3a.

Another conspicuous difference between the two groups of plants was that with low-salt plants there was no influence of the external concentration on the rate of bleeding, whereas with high-salt plants the rate of bleeding increased with decreasing external concentration.

Experiment 3

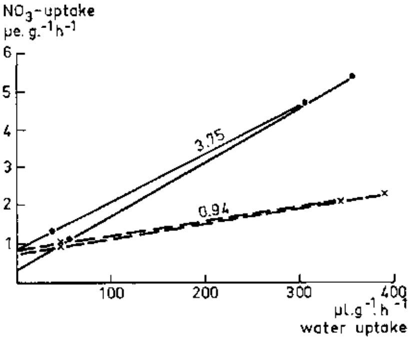
Four tomato plants were cultivated in the normal way on 8 litre plastic buckets in the greenhouse until they were 12 weeks old. Ten days before the experiment the plants were placed in the growth room and the nutrient solution was changed twice a day instead of twice a week. The daylength was 16 hours until the experiment began, when continuous light was given. At the start of the experiment two plants remained on the half strength Hoagland solution (nitrate concentration 3.75 me./l); the other two were placed on a nitrate concentration of 0.94 me./l. For the first two plants the water and nitrate uptake were determined over a 12 hours' period; for the other two plants the period was 5 hours. After each period the plants were set back on the normal half strength solution and the next day at the same time and for the same period of time the experiment was repeated with decapitated plants. The results are presented in table 3 and fig. 4.

TABLE 3. Uptake of water and nitrate by high-salt tomato plants at two different concentrations of the external solution.

1	2	3	4	5	6	7	8
					NO_3-concentration		
NO_3-conc. in nutrient solution me.l^{-1}	Condition of plant	Water-uptake $\mu l.g^{-1}.h^{-1}$	Nitrate-uptake $\mu e.g^{-1}.h^{-1}$	Rate of bleeding $\mu l.g^{-1}.h^{-1}$	calc. from 3 or 5 and 4 me.l^{-1}	determined in bleeding sap me.l^{-1}	Influx coefficient
3.75	intact	353	5.37	–	15.2	–	3.87
	bleeding	–	1.08	57	18.9	22.6	
3.75	intact	306	4.68	–	15.3	–	3.34
	bleeding	–	1.32	38	34.8	19.5	
0.94	intact	390	2.34	–	6.0	–	4.20
	bleeding	–	0.98	45	21.5	23.8	
0.94	intact	343	2.02	–	5.9	–	3.64
	bleeding	–	1.00	45	22.1	21.7	

All four extrapolated lines meet the ordinate close to the origin. Thus for all external concentrations it is possible to obtain with high salt plants approximately no salt uptake at zero water uptake. Just as in table 1 and to a lesser degree also in table 2, it appears from table 3 that there was a good agreement between the calculated nitrate concentration entering the bleeding plant and the nitrate concentration actually found in the bleeding sap; only in one case an excessively high concentration of 34.8 me./l was calculated. With the intact plants there was a considerable difference in the

FIG. 4. The relation between the uptake of water and nitrate from nutrient solutions of two different conditions measured with intact and decapitated high-salt plants.



calculated ingoing stream between the two external concentrations; the lower the external concentration, the lower the calculated concentration of the ingoing solution. This can also be seen in table 2. Comparing all three tables it is obvious that the nitrate concentration in the bleeding sap remained fairly constant. For both high-salt and low-salt plants, and with the whole range of external concentrations, its values were usually found between 20 and 25 me./l. The influx coefficient in all three tables was always greater than 1, which means that even for that part of the nitrate uptake that increased linearly with an increased uptake of water, the calculated concentration was always higher than that of the outer solution, usually 3 to 7 times as high. The only exception was the influx coefficient of the low-salt plant with an external concentration of 1.88 me./l, in which the nutrient solution was exhausted at the end of the experiment (table 2).

Experiment 4

Six tomato plants, 14 weeks old were brought into the growth room with a 16 hours' light period from 16.30 till 9.30. During a number of days the uptake of water and nitrate was determined during a 24 hours' period. After 13 days all plants were cut, during a following 24 hours the uptake was measured and the bleeding sap collected. The data are presented in table 4 and fig. 5. If all data for the uptake of water and nitrate are plotted against each other, they scatter reasonably well around a straight line through the origin as was found in the other experiments. The mean concentration of the ingoing stream was 18.3 me./l nitrate and the mean influx coefficient 4.87. In the lower half of the table the nitrate concentrations of the bleeding sap are given for the six plants, both determined and calculated from the uptake data. The agreement between the two values is fairly good; usually the actual values were slightly higher than the calculated ones.

Each plant had 8 litres of nutrient solution, so 8 × 3.75 = 30 me. NO_3 at its disposal. During 24 hours approximately 20 me. were taken up by the plants so that it was necessary to renew the solution at least once a day to keep them in a high-salt condition. On Sunday, 10 February, preceding the experimental period the solution was not renewed. Consequently no nitrate was present at the end of the first uptake period, and the nitrate uptake could not be calculated. During the following 24 hours' period (11–12 Febr.) the mean nitrate uptake was 21.4 me. and during the period thereafter 17.7 me. The first one was the highest uptake value in the whole series, the second one the lowest. From 18–19 February the aeration accidentally stopped with two plants. Consequently the uptake of nitrate and water was much lower during that period. The mean nitrate uptake values for this and the following periods were 9.5–19.1–14.6–15.0 respectively. These figures demonstrate that, when for some reason

TABLE 4. The uptake of water and nitrate of tomato plants on a number of days before and immediately after decapitation.
nd = nitrate could not be determined since all the nitrate present in the bucket was taken up: the data within the broken line are not used in fig. 5.

	Plant 1		Plant 2		Plant 3		Plant 4		Plant 5		Plant 6	
Uptake period Febr.	Water-uptake ml	Nitrate-uptake me.	Water-uptake ml	Nitrate-uptake me.	Water-uptake ml	Nitrate-uptake me.	Water-uptake ml	Nitrate-uptake me.	Water-uptake ml	Nitrate-uptake me.	Water-uptake ml	Nitrate-uptake me.
11–12	928	nd	974	nd	966	nd	690	nd	691	nd	908	nd
12–13	1098	22.1	1084	22.9	1145	24.8	840	12.4	870	24.8	965	27.4
13–14	1178	22.1	1122	21.1	1097	21.1	845	10.2	935	14.9	998	16.7
18–19	1430	24.1	1432	26.8	1535	24.8	778	10.0	709	9.4	799	9.7
19–20	1308	27.5	1124	24.4	1497	26.6	714	9.3	706	17.3	774	20.8
20–21	1672	29.8	1341	23.0	1381	25.3	640	9.9	794	13.6	805	15.6
21–22	1988	29.1	1601	21.4	1509	23.6	826	10.3	1016	14.4	1038	15.6

at 24 February all plants were cut; bleeding measured during 24 hours

	Bleeding-sap ml	Nitrate-uptake from bucket me.	Bleeding ml	Nitrate-uptake from bucket me.	Bleeding ml	Nitrate-uptake from bucket me.	Bleeding ml	Nitrate-uptake from bucket me.	Bleeding ml	Nitrate-uptake from bucket me.	Bleeding ml	Nitrate-uptake from bucket me.
24–25	337	4.93	305	4.64	342	5.35	222	2.57	428	7.14	516	7.35
NO_3-conc. calculated $me.l^{-1}$		14.6		15.2		15.7		11.6		16.7		14.2
Determined in sap $me.l^{-1}$		14.5		17.3		18.3		16.0		20.5		18.1

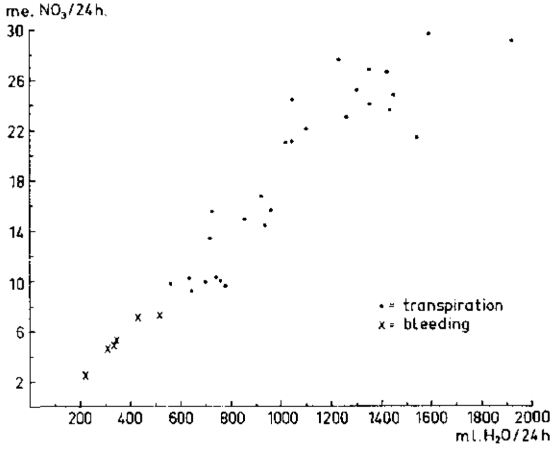


Fig. 5. Relation between the uptake of nitrate and water for intact plants during a number of days and for these plants decapitated thereafter.

the rate of uptake diminishes temporarily, it is resumed at an enhanced rate as soon as the normal conditions are restored.

This could be confirmed by an experiment similar to the one reported here, in which the nitrate and water uptake was measured for two groups of each three tomato plants. From the start of the experiment the culture solution was changed daily, except that the solution of one group was occasionally replaced by tap water during 24 hours.

Table 5 shows that after such a period on water the nitrate uptake was about twice as high as that of the plants remaining on the nutrient solution. Although the results were variable because of the fact that the plants were not in high-salt condition at the beginning of the experiment, the nitrate uptake differences at the other periods were never so great.

TABLE 5. The uptake of water and nitrate on a number of days (see text)

Uptake period June	Group 1		Group 2	
	Water uptake ml	Nitrate uptake me.	Water uptake ml	Nitrate uptake me.
15–16	550	21.8	618	24.6
16–17	541	on H_2O	549	10.1
17–18	590	8.96	606	4.72
18–19	510	7.72	574	6.08
19–20	nd	nd	nd	nd
20–21	nd	nd	nd	nd
21–22	nd	nd	nd	nd
22–23	476	9.95	511	11.9
23–24	510	5.77	533	on H_2O
24–25	464	4.60	522	8.66
25–26	476	4.54	493	5.89
26–27	nd	nd	nd	nd
27–28	nd	on H_2O	nd	nd
28–29	nd	on H_2O	nd	nd
29–30	557	13.3	525	7.00
30–1 juli	501	6.11	530	5.40

Experiment 5

Tomato plants, about six weeks old were divided into two groups of 12 plants each. One group was placed in a growth room with a high relative humidity (85 per cent). the other group got the same conditions except for a relative humidity of 67 per cent, At the beginning of the experiment the fresh weight of each plant was determined, and during the experimental period the water uptake was measured at each change of the nutrient solution. This occurred twice a week in the beginning and thereafter every second day. By regular nitrate determinations care was taken that no deficiency occurred. After the plants had grown under these conditions for 16 days the water and nitrate uptake were determined for a 24 hours' period. The six plants of each group were interchanged and the uptake was again determined over a 24 hours' period for the groups: high humidity throughout (H-H), high to low humidity (H-L), low to high humidity (L-H), and low humidity throughout (L-L). Then all plants were decapitated and the uptake and bleeding determined once more for a 24 hours' period, each group remaining at the same conditions as it was just before cutting. At the end of the experiment the fresh and dry weights of tops and roots were determined separately and both portions were analyzed for total nitrogen and potassium.

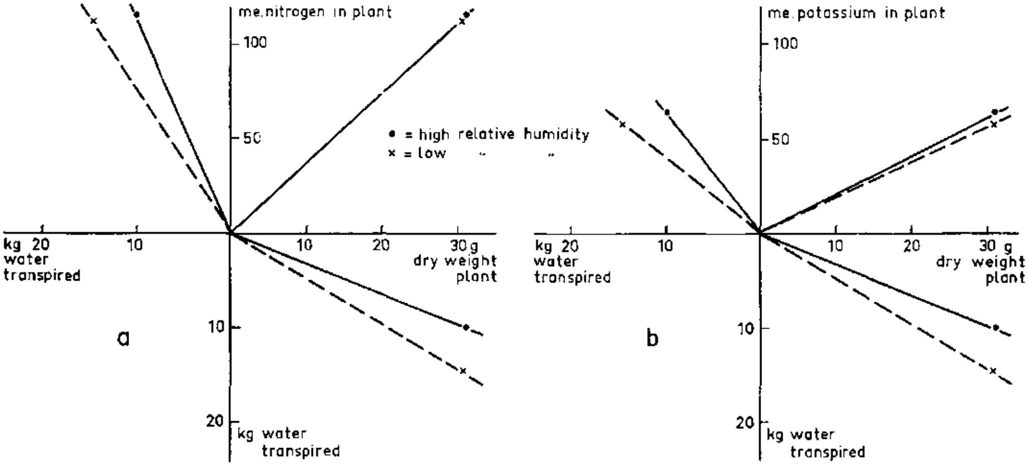


FIG. 6. The relation between plant dry weight, the amount of nitrogen (*a*) and potassium (*b*) taken up and the quantity of water transpired.

In fig. 6 the dry matter produced is plotted against the amount of water taken up during the same period (the transpiration coefficient is the line that connects the point so obtained with the origin) and against the amount of nitrogen (fig. 6a) and the amount of potassium (fig. 6b). Thus, from this figure can be read the transpiration coefficient, the percentage nitrogen and potassium of the dry weight and the concentration of the ingoing solution. The differences in relative humidity had no influence on the rate of dry matter production and neither on the rate of intake of both nitrogen and potassium. There were considerable differences in water uptake. The values were not corrected for evaporation. For the low humidity this amounted to about 60 ml per day but for the high humidity no determination was made. Since the differences were small in relation to the transpiration, this will not have much influence on the results. The transpiration coefficients (grams of water transpired per gram dry matter produced) for low and high humidity plants were 465 and 313 respectively. Since the amounts of water taken in were different, the influx coefficients were also different. From the results of experiment 4 it may be supposed that the line representing the relation between the uptake of water and salt goes through the origin. Then for low and high humidity plants the nitrate concentration amounted to 7.7 me./l and 11.4 me./l respectively with a corresponding influx coefficient of 2.05 and 3.04. For potassium the concentration values were 4.0 me./l and 6.4 me./l and the influx coefficient 2.67 and 4.27. It must be borne in mind that these values were not determined from actual salt uptake values but from an analysis of the plants at the end of the experiment. In addition, the nitrogen and potassium content of the plants at the beginning of the experiment was not known and has not been subtracted, so that the values given here are somewhat too high.

Table 6 presents the results of the actual measurement of the intake of water and nitrate of the four groups of intact plants, and the amount and nitrate content of the bleeding sap of the decapitated plants. The amount of water transpired corresponded with the humidity conditions in the chamber where the plants were growing; with the bleeding, however, the results were different. The quantity of bleeding sap of the plants which had beforehand grown under conditions of high humidity was twice as high as that of plants grown beforehand under conditions of low relative humidity.

TABLE 6. Uptake of water and nitrate at different humidity conditions, both intact and decapitated.

1	2	3	4	5	6	7	8
					NO_3-concentration		
Relative humidity	Condition of plant	Water-uptake ml.g^{-1}.day^{-1}	Nitrate-uptake µe.g^{-1}.day^{-1}	Rate of bleeding ml.g^{-1}.day^{-1}	calculated from 3 and 4 me.l^{-1}	determined in bleeding sap me.l^{-1}	Influx coefficient
H – H	intact	13.10	164	–	12.5	–	3.46
	bleeding	–	26[1]	2.45	–	10.7	
H – L	intact	21.16	178	–	8.4	–	2.16
	bleeding	–	23[1]	2.02	–	11.3	
L – H	intact	10.66	106	–	9.9	–	2.70
	bleeding	–	10[1]	1.17	–	8.8	
L – L	intact	19.56	133	–	6.8	–	1.77
	bleeding	–	11[1]	1.12	–	9.5	

[1] not determined but calculated from 5 and 7

In addition the amount of bleeding sap of the two plants at high humidity during the bleeding period was somewhat higher than that of the plants at low humidity (3.62 against 3.14), but these differences were only small and may not be of any significance. Since the nitrate concentration in the bleeding sap was also somewhat higher with the plants that had grown originally under high humidity conditions, the calculated nitrate uptake values of these plants were even more than twice as high. Unfortunately the nitrate uptake from the solution could not be determined with decapitated plants since the concentration differences before and after the uptake period were too small. The cause of the differences in rate of bleeding cannot be established at present. Apparently they cannot be explained by the difference in the nitrogen concentration in the vessels, but possibly the total osmotic differences may have been greater.

Finally fig. 7 shows the changes in water uptake and nitrate uptake when plants are brought from high to low humidity and vice versa. Fig. 7a shows the water and nitrate uptake of two groups of the same plants on two successive days; fig. 7b shows these

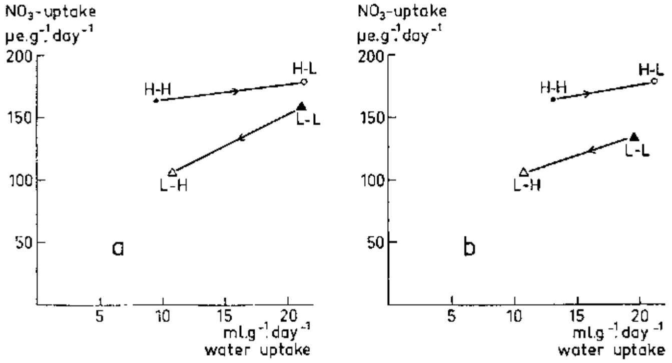


FIG. 7. The changes in the uptake of water and nitrate when plants are shifted from high to low relative humidity and vice versa. *a*. the same plants measured at two successive days, *b*. different groups of plants measured on the same day.

values for all four groups of plants on the same day. The results are approximately the same. When plants are brought from conditions of high relative humidity to low relative humidity and vice versa, it is not only the rate of transpiration that is affected, but also the rate of nitrate uptake, although to a somewhat less extent, for the lines all meet the ordinate above zero when extrapolated to zero water uptake. A comparison between fig. 6 and fig. 7 thus shows that in the long run the rate of transpiration has no influence on the uptake of nitrate but, in short time experiments the nitrate uptake is distinctly affected. Besides, the pre-treatment also had an influence on the rate of bleeding.

Experiment 6

Six tomato plants, about 6 weeks old, were placed at four different light intensities, *viz.* 8.1, 6.2, 4.3 and 1.6 × 10^4 ergs.cm^{-2}sec^{-1}, and for each plant the uptake of water was determined, besides the uptake of both nitrate and potassium. Then all the plants were decapitated and the uptake values were determined again. All data are put together in fig. 8 in which the water uptake is again given in relation to the uptake of both nitrate and potassium. This relation between water and salt uptake is principally the same for both ions. At low transpiration rate and when bleeding there is a linear relation which is given by a line through the origin. Above a transpiration rate of approximately 300 l H_2O per gram root tissue per hour the salt uptake still increases with increasing transpiration, but to a much less extent. This is in line with the results of experiment 5.

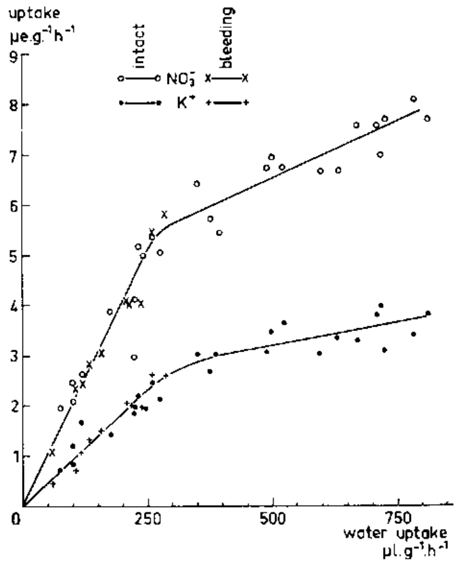


FIG. 8. The relation between the uptake of water and that of nitrate and potassium at large differences in transpiration.

DISCUSSION

From the results of the foregoing experiments the following conclusions or reasonable assumptions can be made:

1. The nitrate concentration of the bleeding sap is fairly constant under different external conditions and always considerably higher than that of the external solution.

2. There is a considerable difference between high-salt and low-salt plants as far as the uptake of nitrate and water is concerned. With high-salt plants the influx concentration calculated from the nitrate and water uptake values is close to the nitrate concentration actually measured in the bleeding sap, and there is not much difference between intact and decapitated plants. With intact low-salt plants values of the same order were also found, but after decapitation the calculated ingoing concentration was always much higher than the measured one.
3. There is still another difference between high-salt and low-salt plants. When the nitrate uptake is plotted against the water uptake for intact and decapitated plants, the line through these two points can be extrapolated to zero water uptake. With high-salt plants such a line goes then through the origin, or meets the ordinate very close to it, whereas with low-salt plants this line cuts the ordinate at a considerable distance above zero.
4. When plants grow at low and high relative humidity there is not much difference in dry weight and in the uptake of both nitrogen and potassium between these two groups of plants. However, the transpiration coefficient, *i.e.* the quantity of water transpired per gram of dry matter produced differs considerably.
5. When plants are shifted from low to high humidity and vice versa, it is not only the rate of transpiration that is affected, but also the rate of nitrate uptake.

It will now be shown in how far these data can be of help towards a better understanding of the relation between water and salt uptake. This relation has been interpreted as a direct influence of the transpiration on salt uptake. The directness of this relation was only derived from the fact that it was linear over a rather wide range of transpiration rates (HYLMÖ (8)). A pre-requisite for such a mass flow of solution from the medium into the vessels must be an influx coefficient equal to or smaller than one, which is not or only slightly influenced by the concentration of the nutrient solution.

This mass flow hypothesis was criticized by BROUWER (1, 2), who was able to vary either the water uptake or the salt uptake without influencing the other. He clearly recognized the relation between water and salt uptake, but he could not see it as a passive intake of ions with the transpiration stream, since the concentration of the ion studied was distinctly higher in the xylem vessels than in the external solution, and the uptake of ions was diminished by respiration poisons or lack of oxygen, whereas the water uptake was not. In later years PETTERSSON (14) and KIHLMAN-FALK (13) again studied the relation between water and salt uptake. PETTERSSON studied the sulphate uptake by sunflower plants. He found an influx coefficient smaller than one and explained this by a mass flow, but since this coefficient varied widely with the external concentration, he realized the 'complex nature of this (mass flow) component' and even stated that 'the transpiration-linked component is not of a purely passive nature'. KIHLMAN-FALK also encoutered difficulties. Studying the potassium and nitrate uptake of low-salt wheat plants she found the influx coefficient to vary considerably with the external concentration, it being higher than one at low concentration. Although she considered part of the salt uptake to be dependent on the transpiration, she had to admit active uptake and she stated that the difference of opinion with BROUWER was 'quantitative rather than qualitative'.

As has already been put forward in the introduction, JENSEN'S results pointed to a linear relation between the uptake of nitrate and water with intact and bleeding tomato plants, a relation with even zero nitrate uptake at zero water uptake. The influx coefficient, calculated from JENSEN'S (11) data amounted to 1.5. A repetition of these experiments confirmed his results in detail. The data indicate that with high-salt tomato plants the line representing the relation between water and nitrate uptake meets the origin. According to HYLMÖ this would point to a direct influence of the transpiration on salt uptake, but the high influx coefficients found and the fact that they are dependent on the external concentration clearly prove that this cannot be the case. Besides, as has also been found by JENSEN (11), the relation is not the same over the whole range of transpiration rates. Above a water uptake of approximately 300 μl H_2O per g root fresh weight per hour the ion uptake increased much less with increasing water uptake (NO_3^-) or hardly at all (K^+) (fig. 8). The results of experiment 5 (fig. 6) even indicate no difference in total potassium and nitrogen uptake and neither in dry matter production when plants were grown for a considerable time under conditions of different relative humidity. This proves very clearly that a direct causal influence of the transpiration on the uptake of nitrate and potassium does not exist. When plants were brought from low to high humidity or the reverse, the water uptake was far more affected than the nitrate uptake, although some influence was certainly present, especially when going from low to high humidity (fig. 7).

To explain his results, JENSEN (12) considered the uptake by bleeding plants to be of an active nature but the further uptake by intact plants was considered to occur passively with the transpiration stream. When the transpiration is increased a greater part of the roots takes part in it, and in these different parts the ratio of water uptake to salt uptake may be different. In the foregoing experiments, however, the nitrate uptake of intact plants, above what is taken up by a bleeding plant cannot be of a passive nature, since the calculated concentration of the ingoing solution is approximately the same with intact and decapitated plants, and well above that of the external solution.

It now has to be shown how such a correlation between water and salt uptake can exist, while both processes are of a different nature. A clue to this may be the fairly constant value of the concentration of the bleeding sap. In nearly all experiments this amounts of 20–25 me./l NO_3. It may be possible that ions cannot be 'pumped' into the vessels when the concentration difference is greater than let us say 20 me./l. With decapitated plants this concentration difference causes an osmotic suction of water into the vessels and a removal of the ions with the bleeding stream. With intact plants at low rates of transpiration the capacity of the nitrate uptake mechanism is such that the maximum concentration difference of 20 me./l can still be maintained. With increasing transpiration, however, a moment arises at which the uptake mechanism has reached its maximum capacity. Consequently a further increase in the rate of transpiration will have no more influence on the rate of uptake, and the concentration of the transpiration stream will be more diluted. This hypothesis agrees with the results presented in fig. 6, but from the fig. 7 and 8 there appears still to be some relation between transpiration and salt uptake. When from fig. 8 the concentration of the ingoing solution is calculated from the slope of the line above a water uptake of 300 $\mu g.g^{-1}.h^{-1}$, a value of 4.7 me./l can be calculated for nitrate and of 1.9 me./l for potassium. These values are fairly close to the concentration of the external solution, *viz.* 3.75 me./l for nitrate and 1.50 me./l for potassium. It may well be that some passive intake also occurs, which becomes visible when the active uptake has reached a constant value. The hypothesis is meant to show that a very close linear relation between water and salt uptake need not point to a passive uptake of ions with the transpiration stream, and in this case even cannot mean a passive intake on account of the high influx coefficient.

Up to now only high-salt plants have been considered. These plants take up the ions only because they grow, the full grown cells all being saturated in this respect. It can be seen from table 4 and 5 that a temporary shortage in the nitrate supply immediately resulted in an extra uptake during the following period. It can be assumed that the extra intake is caused by an ion uptake in full-grown root cells. Fig. 3a also shows a considerable nitrate uptake at zero water uptake for low-salt plants, whereas there was then practically no uptake with high-salt plants (fig. 3b). Consequently, one will always expect to find a fairly high apparent intake concentration calculated from the uptake data. Table 2 shows that this was indeed the case with the decapitated low-salt plants. The calculated concentration was several times higher than the actual concentration in the bleeding sap. For the low-salt intact plants the calculated concentration of the ingoing solution was for this reason expected to be higher than for high-salt intact plants. However, this was only the case for the lower external concentrations.

The same difference between the two groups of plants as found here was already found by BROYER and HOAGLAND (4), who also demonstrated that the influence of the transpiration was much greater with high-salt than with low-salt plants. They even refer to unpublished data with tomato plants, stating: 'the rapid removal of salt from the root system as accelerated by transpiration could promote the inward movement of salt through a disturbance of dynamic equilibria concerned with the active transportation of salt to the xylem conducting system'; in fact the same hypothesis as given above.

The great differences of opinion as to the relation between the water and salt uptake in the past are for a great part due to the salt status of the plant material. High-salt plants can be defined as such, and to obtain them one has to take great care that the concentration of the solution never drops below the value where it influences the rate of uptake. Low-salt plants, on the other hand, can be obtained in all degrees, and it is not astonishing that this degree of salt depletion influences the uptake, especially in relation to the uptake of water. Besides, there is the influence of the plant species. Results, as have been obtained here have up to now only been found with tomatoes. Other plant species have always shown some salt uptake at zero water uptake. This may be related to the fact that the roots of flowering tomato plants, as have been used in these experiments, do not show much growth, so that by far the greatest part of the ions taken up is transported to the shoot (KRAUS and KRAYBILL, cited after BROUWER, 3; HOFSTRA 6). The results of the experiments reported here clearly demonstrate that the linear relationship between water and salt uptake need not be a direct one since the nitrate and potassium uptake must have taken place at the expenditure of energy, which does not hold for the uptake of water. Consequently such a linear relationship can never be taken as a proof of a direct influence of the transpiration on salt uptake.

SUMMARY

The uptake of nitrate, potassium and water was measured with intact and decapitated tomato plants. With high-salt plants a straight line through the origin was found when the nitrate uptake values for intact and bleeding plants were plotted against the corresponding water uptake values. With low-salt plants this line still showed a considerable salt uptake when extrapolated to zero water uptake.

In considering the question whether the water-dependent part of the salt uptake is directly influenced by the transpiration stream, it was concluded that this could not be the case since the nitrate and potassium uptake proved to be the same at different relative humidities and since the concentration of the bleeding sap was always several times higher than that of the outside solution. The linear relationship between the two was explained by assuming a relation of both factors with the growth of the plant. With low-salt plants a shortage of ions in the full-grown cells is assumed to be responsible for the salt uptake at zero water uptake. It is supposed that the differences in results, obtained by the various authors, are caused by differences in the ion studied, the plant species used and, last but not least, by the salt status of the plant.

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INFLUENCE OF PHOTOPERIOD AND TEMPERATURE ON THE FLOWERING OF RED CLOVER

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In recent years much research work has been done to study the influence of light conditions on flower initiation in cultivated forms of red clover *(Trifolium pratense* L.).

It has been known for some time that red clover is a long day plant and in several investigations a rather high critical day-length has been established. A review of literature shows that extreme variability exists in this respect. In the first place the reaction depends on latitude. Northern forms generally require more light hours for flowering than southern ones. According to SCHULZE (16) the German variety Steinacher flowers in outdoor experiments already with a photoperiod of 15 hours, whereas the Norwegian Molstad requires at least 18 hours. Individual plants showed large variations. In Steinacher all plants flowered with 18 hours. Molstad required continuous illumination to get even the latest plants induced.

UMAERUS (19) describes similar relations for Swedish varieties from northern and southern parts of the country. STODDARD (18) working with English varieties, states that the critical day-length varies from 12 hours for early clones to 15 hours for late ones.

English, American and Canadian authors (STEPPLER and RAYMOND, 17; BULA, 3; WILLIAMS, 20; BIRD, 2; CUMMING, 4) distinguish several types within cultivated varieties, ranging from plants forming non-flowering rosettes in the year of sowing to strictly annual plants forming flowering stalks shortly after the seedling stage and no rosette.

Similar relations are reported from land varieties in Northern Russia (GUPALO, 10; DUSJETSKIN, 7). GUPALO distinguishes annuals, 'surviving annuals' and a group forming a flat rosette in the first year. Only in the last group winter-hardiness occurs. These 'winter types' are characterised by the formation of short petioles in short day.

A very important observation is made by CUMMING (4) who states that in perennial plants of red clover after flowering always a new cycle of vegetative growth begins with formation of vegetative sprouts even at the lower parts of flower stems irrespective of day-length. Apparently these sprouts must pass a juvenile stage before they can be induced to flowering. This gives an explanation for the fact that the influence of the photoperiod on red clover sprouts depends on their age (14).

There are few decisive statements about the occurrence of cold requirement in red clover. According to GORMAN (9) a treatment of 6 days (2–3°C) had no stimulating effect on 45 days old seedlings of English varieties, but it is a common experience that under short-day conditions, low temperatures accelerate development. BULA (3) supposed a relation between temperature and critical day-length.

In white clover low temperature is known to stimulate flowering under short-day conditions (LAUDE *et al.*, 13). EVANS (8) and MORLEY and EVANS (15) made extensive studies in flower initiation with *Trifolium subterraneum* L. It appears that some varieties have a genuine cold requirement. Others are exclusively stimulated by low temperature under short-day conditions as the result of an interaction between photoperiodicity and temperature. EVANS concluded that in *Trifolium subterraneum* as in some other species *(Beta, Raphanus, Spinacea)* adequate vernalisation confers complete independence of day-length, in contrast to winter cereals, many grasses and for instance the biennial form of *Hyoscyamus niger*, which after complete vernalisation still require long days.

PREVIOUS INVESTIGATIONS IN DUTCH RED CLOVER

According to previous investigations (VAN DOBBEN, 5, 6) wild forms of red clover from the Netherlands show the same interaction between photoperiodic reaction and temperature as reported by EVANS for subterranian clover and by LAUDE *et al.* for Ladino clover. Sprouts previously subjected to winter temperatures flower in any natural photoperiod, whereas plants grown at higher temperatures require 14–15 light hours daily. It could be shown that plants overwintering outside are gradually induced during the cold season. In spring at least early flowering types are completely induced and flower as soon as temperature permits. Cultivated early varieties show the same behaviour.

In one experiment plants flowered simultaneously in photoperiods of 15 and 24 hours at a temperature of 15°C, whereas at 25°C the shorter day caused a relative retardation. This indicates that the critical day-length moves up with temperature in the same sense as reported for *Hyoscyamus niger* by LANG and MELCHERS (12).

This dependence of photoperiodic reaction on temperature involves that in short day low temperature stimulates flowering. This phenomenon, however, must be distinguished clearly from the genuine cold requirement well known from winter cereals and the biennial form of *Hyoscyamus*. In the latter species the annual form has no cold requirement, but it shows an interaction between photoperiodicity and temperature.

The observations of EVANS with subterranean clover may be interpreted in the same way; in all varieties the photoperiodic reaction depends on temperature. Besides some varieties have a genuine cold requirement which must be met first and cannot be replaced by long day.

PRESENT INVESTIGATIONS

In the present investigations the following material has been used:
4 clones of wild red clover from motherplants collected in the natural pasture lands along the Rhine near Rhenen, numbered Grebbe I–IV;
2 clones of wild red clover from motherplants collected in the Norwegian mountains near Bövertun (latitude 61°43′, altitude 680 m);
2 clones of the cultivated Norwegian variety Molstad.

Experimental design 1962

The motherplants were devided into clones in the beginning of September 1961. They had about 5–10 short sprouts per plant at the end of October, when half the number of pots was removed from outside into a glasshouse (temp. 16°C). On March 1, 1962 plants from both groups were exposed to photoperiods of about 12 (natural day-length), 15, 17, 19 and 24 hours in the greenhouse at a temperature of 25°C (day) and 15°C (night). Several photoperiods were acquired by supplemental illumination in the evening with single fluorescent tubes of 40 Watt (Philips TL 32).

RESULTS

The dates of flowering are recorded in fig. 1.

The wild clones II and III showed the same reactions as those described in a previous paper (VAN DOBBEN, 5) for red clover from Dutch origin.

After wintering outside they flowered very soon after entrance into the glasshouse, almost irrespective of photoperiod. This can be explained as a completion of induction during winter conditions by lowering the critical day-length.

Plants of the same clone kept during winter at 16°C, appeared not to be induced and reacted strongly to day-length. In 15 hours flowering started readily although later than in the outside treatment. The controls flowered only in April, when the natural day surpassed 14 hours. This behaviour is normal for many forms of wild and cultivated red clover in the Netherlands. Clone I demonstrates that other reaction patterns occur. This clone did not show a reaction to photoperiod after any pre-treatment. It is apparently day-neutral. Under such conditions low temperature can only retard development and as a matter of fact plants overwintered outside flowered later than those kept at 16°C during the pre-treatment.

In the wild Norwegian clones the cold pre-treatment (outside) gave an enormous acceleration of flowering. In contrast to the clones from the Netherlands these treatments still showed a clear influence of day-length with the indication that 17 hours was about optimal.

The treatments overwintered at 16°C did not flower during the course of the experiment. After May 1 they were put at 25°C and natural day. The clone 'green' remained vegetative all summer. This indicates a genuine cold requirement which could be confirmed in a later experiment.

All replicates of the clone 'spot', however, flowered in the course of June. Flowering in June, much later than native plants was also observed in specimen of both Norwegian clones kept outside during the whole season. Plants entered into the glasshouse (16°C) already in September and kept in natural day remained vegetative.

The fact that the comparable treatment of the clone 'spot' behaved differently in the present experiment could be explained by the fact that these plants were kept outside

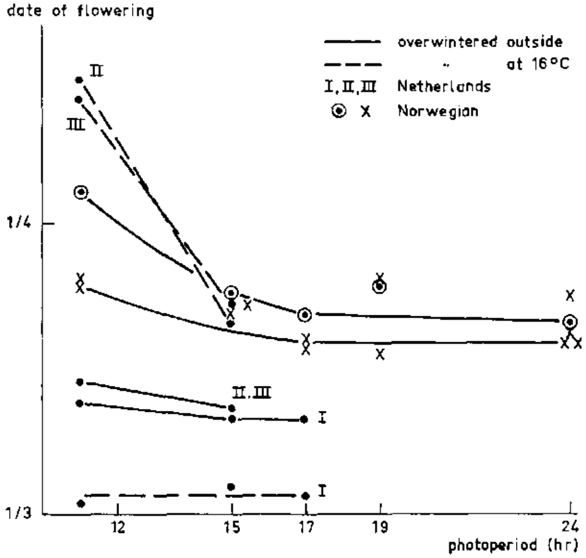

FIG. 1. Influence of the extension of natural photoperiod on some clones of wild red clover from the Netherlands and from Norway. Beginning of the treatment March 1. Temperature 25°C (day) and 15°C (night). Average dates of first 6 flowers.

until October 27. Later experiments have shown, however, that this clone grown at 16°C from Sept. 3 also may show an odd flower in summer.

In the same experiment some plants of the cultivated Norwegian form Molstad were added. These plants were sown on January 9 in the glasshouse. As could be expected for seedlings of a cross-fertilizing species the plants were heterogenous in respect to appearance and flowering behaviour. Flowering plants were observed only in the treatments 19 and 24 hours. Other plants remained vegetative during the following summer.

This result indicates that this northern variety has higher demands in respect to day-length than Dutch clovers. SCHULZE (16) came to the same conclusion during a comparison of Molstad with the German variety Steinacher. According to his results the critical day-length of these varieties under summer conditions is about 18 and 15 hours respectively.

Experimental design 1963

At the beginning of August 1962 the best clones were divided and planted in pots to obtain new material. On October 16 all plants were removed from outside into a glasshouse (16°C). Because of earlier planting plant size was much larger than in the preceding year. During December a short cold treatment was given in a glasshouse at a temperature of 6°C. The plants were divided into 4 groups receiving this temperature during 0, 4, 8 and 15 days. Afterwards the plants were moved back to 16°C. A number of pots with Norwegian clones received 2°C during one week instead of 6°C. During the cold treatment at 6°C a number of replicates received continuous day by supplemental illumination in the dark hours, whereas the majority received natural day.

From March 1 onward all plants were placed at 25°C and divided over two treatments, natural and continuous day.

Results

At the end of February it was clear that all treatments of the native clones (II, III) were going to shoot and flower in natural day. The application of artificial long day was too late to have any stimulating effect, there neither was any visible influence of the cold treatment in December.

The treatments '0 days at 6°C' are fully comparable to the treatment 'overwintered at 16°C' from the preceding year which flowered much later and reacted strongly to the photoperiod.

The different behaviour of the same clones in the two successive years may be attributed to the fact that in the first year the plants were much younger and presumably still in the pre-sensitive stage described by CUMMING (4), when taken into the glasshouse. In the second year the plants were much larger because of earlier planting. During October (average temperature in 1st–15th: 12°C) the sprouts seem to have been induced completely so that their reaction became comparable to the treatments 'overwintered outside' from the preceding year.

Although the Norwegian (wild) clones received the same pre-treatment as the Dutch ones no signs of generative development could be observed until April and reaction to photoperiod was strong.

In agreement with the results of the preceding year all specimens of the clone 'spot' flowered, though some were very late. In natural day flowering was delayed until June, while a slight stimulating effect of the cold treatment in December could be observed (fig. 2, left side).

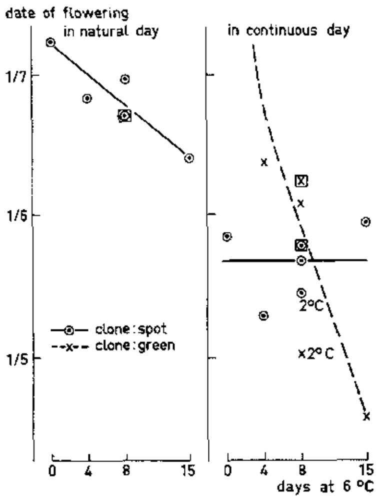

FIG. 2. Influence of short cold pre-treatments and the extension of natural photoperiod on the flowering dates of the wild Norwegian clones 'green' and 'spot' overwintered in the greenhouse at 16°C till March 1, at 25°C afterwards from the beginning of the long day treatment. Average dates of first 6 flowers.

The series 'continuous day-length' flowered much earlier without a clear after-effect of the December treatment. It may be masked by the much larger stimulating effect of the continuous day given from March 1 onward. The fact that in optimal day-length the preceding cold treatment has no clear effect indicates that the Norwegian clone 'spot', like the Dutch forms, has no genuine cold requirement.

The behaviour of the clone 'green' is quite different. Notwithstanding optimal day-length the influence of the cold pre-treatment is very clear. Without cold no flowering occurs at all, in agreement with the preceding year. With 15 days cold treatment flowering is earlier than in the clone 'spot'. Lengthening of the cold period and lowering of temperature to 2°C gave a marked acceleration of flowering. This points to a true vernalization effect, in accordance with previous conclusions (p. 79).

The clone 'green' did not flower when overwintered at 16°C in natural day. Under these conditions even 15 days pre-treatment at 6°C was not sufficient to cause flowering.

Experimental design 1964

Plants were divided on August 27 and the new specimens were placed in the greenhouse (16°C) on September 3.

In December a number of pots was treated in another compartment of the greenhouse at 6°C for 10 or 20 days (December 3–13 and 3–23 respectively). Some of these treatments received weak artificial light during the dark hours. Later on three different after-treatments were given. A first and second group remained at 16°C but received supplemental light in the dark hours from January 16 and February 3 respectively. The third group was placed into a glasshouse at a temperature of 25°C on February 27 and received supplemental light from this same data onward. Each group included controls receiving the natural day-length only.

Results

In table 1 the dates of first flowering are recorded. For a number of cases the average date of the first 8 flowers is also given. For the clones from Dutch origin the latter figures are plotted in fig. 3.

TABLE 1. Flowering dates of some red clover clones at several temperatures, photoperiods and short pre-treatments with cold (6 °C)
– = no flowering (observations ended on Aug. 1)

16 °C Clone	Natural day-length				17 hours			Continuous illumination			
	control	10 days 6 °C	20 days 6 °C	20 days 6 °C (LD)	control	10 days 6 °C	20 days 6 °C	control	10 days 6 °C	20 days 6 °C	20 days 6 °C (LD)
G II¹)	18–3	28–2	21–2	21–2				13–3	10–3	26–2	19–2
G II²)	1–4	15–3	28–2	2–3				21–3	15–3	28–2	26–2
G IV	18–5	23–4	24–3					25–3	18–3	14–3	
G IV	28–5	2–5	31–3					1–4	20–3	16–3	
N green	–	4–7			–	12–6	5–5	21–4	6–4	8–4	
N green								27–4	13–4	12–4	
N spot	28–7	25–7	–		16–7	25–6	14–7	27–4	4–5	17–4	
N spot								2–5		27–4	
Molstad I	–	–	–		–	–	–	29–5	19–5	8–5	
Molstad II	12–7	7–7	25–6		15–5	12–5	28–4	21–4	17–4	19–4	
25 °C											
G II¹)	24–3	4–3						10–3	5–3	15–2	
G II²)	27–3	5–3						16–3	8–3	28–2	
N green	–	–	–					–	2–5	14–4	
N green										1–5	
N spot	17–6	14–6						15–5	29–4	17–5	
N spot									8–5		
Molstad I	–	–	26–7					–	–		
Molstad II	30–7	4–7	31–5					21–4	14–4	10–4	
Molstad II								25–4	16–4	12–4	

¹) date of first flower. ²) average date of first 8 flowers.

It appears that the pre-treatment at 6 °C accelerated flowering substantially. Extension of the natural photoperiod in the after-treatment also had a stimulating effect especially in the controls without cold treatment.

In the clone G II the treatments with cold still reacted to photoperiod in January, but no more in February (16 °C) and March (25 °C) (fig. 3). Apparently the cold

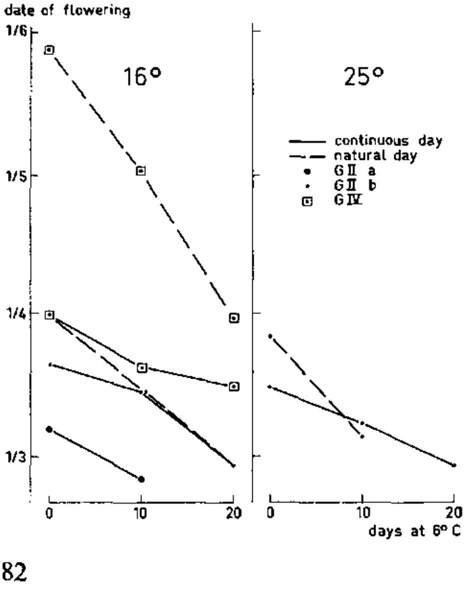

FIG. 3. Influence of short cold pre-treatments and the extension of natural photoperiod on the flowering dates of two Dutch wild clover clones overwintered in a greenhouse at 16 °C.

Average dates of first 8 flowers. G II a received long day already on Jan. 16, G II b and G IV on Febr. 3. A series in 25 °C was started on Febr. 27.

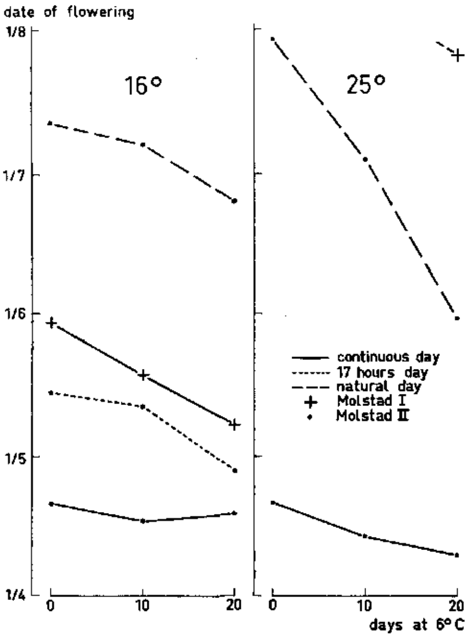

FIG. 4. As fig. 3 for the clones Molstad I and II. The 16°C-series was started on Febr. 3, the 25°C-series on Febr. 27. Dates of first flower.

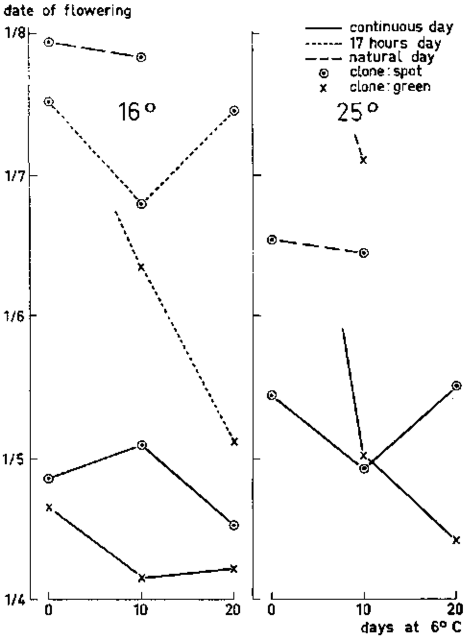

FIG. 5. As fig. 4 for the wild Norwegian clones 'green' and 'spot'.

treatment in December started a development leading to photoperiodic induction which was completed in February but not yet in January so that it could be still accelerated by long day at that time.

Clone G IV gives a somewhat different picture in this respect. It flowered later than G II and the treatments receiving cold in December still reacted in February to the extension of photoperiod although at a decreasing rate as cold had been applied for a longer time.

Apparently this clone was less easy to induce so that the short cold treatments in December were not sufficient to give complete induction in February.

With one exception (clone 'green' in 25°C) the Norwegian clovers (wild and cultivated) reacted far stronger to photoperiod in the after-treatment than to pre-treatment with cold (fig. 4 and 5). Apparently their demands in respect to photoperiodic induction are so high that a short cold period has relatively little influence.

THE LEVEL OF THE OPTIMAL PHOTOPERIOD IN THE NORWEGIAN CLONES

At 16°C a 17 hours' photoperiod was added to the experiment for the Norwegian clones.

Molstad I did not flower under these circumstances. Molstad II and the wild clone 'green' flowered with a clear retardation in comparison with the treatments continuous day; the clone 'spot' even showed a very great retardation. Apparently the optimal photoperiods for these clones lie above 17 hours.

At the latitude where the experiments were taken the natural length of day reaches this level in the course of June. According to expectation treatments flowering with 17 hours during May under artificial conditions did so during or after June in natural day.

INFLUENCE OF TEMPERATURE IN THE AFTER-TREATMENT

As stated already the 25°C-series was started 24 days later than the second experiment in 16°C. Nevertheless in the Dutch material and in the clone Molstad II flowering started earlier.

In Molstad I, however, flowering was retarded substantially at 25°C. Only in the course of July one odd flower was observed. In the wild clones flowering was also relatively retarded at 25°C with one exception, namely the clone 'spot' which flowered in natural day at 25°C only. Apart from this case 25°C appeared less favourable for flowering.

In the case of the clone 'green' flowering was even completely inhibited in the control, whereas this inhibition could be overcome by pre-treatment at 6°C.

This result agrees with a previous experiment at 25°C (fig. 2). As already explained this phenomenon closely resembles the classic cold requirement of the winter cereals. The only difference is that in the red clover clone 'green' the pre-treatment at low temperature looses its special accelerating effect already at a temperature level (16°C) at which it is still active in winter cereals. What remains is the slight acceleration resulting from the interaction between photoperiodism and temperature.

The Norwegian clone 'spot' widely differs from 'green' in so far that the retardation of flowering by high temperature is less and cannot be overcome by pre-treatment with cold.

INFLUENCE OF DAY-LENGTH DURING COLD TREATMENT

A number of replicates received continuous supplemental illumination with weak fluorescent light during the treatment at 6°C during December. There was no significant difference with natural day in the after-effect (fig. 2, table 1).

This agrees with the supposition that at low temperatures the critical day-length of red clover is lowered to a level at which natural photoperiod in December (about 8 hours) is already optimal. Under such conditions an extension cannot have any effect.

SUMMARY

The influence of photoperiod and temperature on the flowering of red clover was studied in clones from wild plants (originating from the Netherlands and from Norway) and in clones from the cultivated Norwegian variety Molstad.

Plants were grown during the winter season in a greenhouse and subjected to different photoperiods by suppletion of natural day by artificial illumination in early spring.

Substantial differences in reaction to photoperiod and temperature could be observed even between clones from the same origin. One clone from the Netherlands appeared to be day-neutral, all other clones proved to be long-day plants. Generally the Norwegian clones flowered much later, their long-day requirement being much stronger than in plants from Dutch origin.

In all clones reacting to photoperiod flowering was accelerated by short pre-treatments (even 4 days) at low temperature (6°C) at the beginning of the winter season. Overwintering outside until March even conferred complete independence of day-length in Dutch clones, but not in (wild) Norwegian ones.

This effect is explained by an interaction between photoperiodism and temperature which involves that at low temperatures the long-day requirement is lowered.

In the after-treatment with different photoperiods 25°C gave earlier flowering than

16°C in the Dutch clones and in the Norwegian Molstad II. In Molstad I and the wild Norwegian clones high temperature exerted a retarding influence. In one of them 25°C caused a complete inhibition of flowering which could be overcome, however, by pre-treatment at low temperature. This behaviour strongly resembles a genuine cold requirement as observed in winter cereals and in some varieties of *Trifolium subterraneum* (8.15).

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COMPARISON OF LAMP TYPES FOR LENGTHENING NATURAL PHOTOPERIODS IN WINTER

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Introduction

In experiments on photoperiodism it is useful to give *all* treatments the same basic period of illumination (for instance natural light) and to vary day-length by a different number of hours with supplemental weak artificial light. In this way all treatments receive about the same amount of light energy so that photoperiod will be the only factor responsible for differences in growth or development.

It is common experience, however, that the response of plants to supplemental light received in this way depends strongly on lamp type so that differences ascribed to photoperiodism may actually be caused by light colour. Especially in plant species in which flowering is preceded by stem elongation incandescent-filament lamps often have a much stronger effect than fluorescent ones, resulting in very early stem elongation and flowering.

The photoperiodic control of flowering and the control of stem elongation by low energy radiation are both reversible by red and far-red and thus appear regulated by the same photoreaction in this sense that far-red promotes stem elongation and inhibits flower initiation (4). A contradiction may be expected therefore in species that show a close relation between flowering and stem elongation; far-red inhibits flowering directly, but stimulates it indirectly by way of stem elongation.

Wassink and Stolwijk (7) supposed that strong effects of incandescent light on flowering could be explained by its high contents of red radiant energy which part of the spectrum is most effective in this respect.

Downs, Borthwick and Piringer (3) demonstrated, however, that the even greater share of far-red radiation emitted by this type of lamp was responsible for early flowering in these cases. According to these authors flower initiation which is normally promoted by red and inhibited by far-red may be stimulated indeed by far-red indirectly by way of stem elongation. This explanation agrees with conclusions drawn by de Lint (6) who worked with *Hyoscyamus niger*. In this species a basic short day in white light supplemented by near infra-red radiation gives an earlier stem elongation and flowering than by red or any other light colour.

Experimental methods

Spring rye (variety Petkuser) was grown in sandy soil with ample supply of water and minerals in a greenhouse at 16°C. Supplemental light was given during the whole dark period by a lamp 60–75 cm above the plants.

The following lamps have been used: incandescent-filament lamps 15 and 40 watt; fluorescent tubes Philips nr 29, 32, 33, 34, 55 and 57. The energy distribution spectra (for the continuous part of the spectra only) of these lamps are given in fig. 4.

The fluorescent lamps were rated at 40 watts and operated at 220 volts. They provided illumination of about 700–1000 lux at plant level when used uncovered and about 100 lux when covered by black paper leaving a split. The incandescent lamps were rated at 15 or 40 watts and provided an illumination of 100 and 200 lux respectively. Except in one case (TL nr. 57) the same specimens of lamps were used in all experiments.

Sowing began in November and was repeated during the winter months at intervals, so that results were obtained for several basic illuminations with natural light (table 7). The plants were harvested and measured periodically. Fresh and dry weights have been determined in tops, whereas for roots only dry weights are available. In some cases leaf counts were made and development stages assessed.

RESULTS

Rate of development

The prolonged photoperiod by supplemental artificial illumination accelerated ear initiation, shooting and heading. As the figures on development recorded in table 2 and 4 show, treatments under incandescent light gained a striking advantage over those supplied with fluorescent light (see also fig. 1). The successive emergence of leaves was speeded up accordingly (table 3-4).

A similar acceleration was obtained by the fluorescent tube Philips nr. 57, used at full intensity in December (table 1). Later in the season, as natural light increased, the effect of this lamp approached that of other fluorescent tubes (table 2). This effect could not be reproduced with another specimen of the same type in the following season, when the tube was used at low light intensity only.

TABLE 1. Influence of supplemental illumination with several lamp types during night hours on spring rye grown in a glasshouse at 16°C. Emergence Nov. 25, 1962. Average values for 10 plants.

Treatments		23 days after emergence				50 days after emergence				
		dry matter tops in mg	% dry matter tops	shoot/root ratio dry weight	plant length in cm	dry matter tops in mg	% dry matter tops	shoot/root ratio dry weight	plant length in cm	devel. stage
Controls		60	8.9	5.9	22.0	215	9.6	11.3	39	
Incandescent lamp (40 Watt)		72	7.2	13.5	33.3	296	11.1	19.8	66	10.1[1]
Fluorescent tubes:										
Philips TL nr. (40 Watt) 700–1000 lux	29	83	10.0	4.6	25.0	492	10.0	12.6	55	
	32	99	9.1	5.5	26.8	432	10.1	13.6	61	
	33	80	9.0	6.2	27.2	423	9.9	14.1	61	
	34	90	9.2	7.5	24.7	473	9.9	11.8	58	
	55	89	9.1	5.2	26.5	428	10.0	14.7	62	
	57	91	8.3	16.6	29.8	393	10.7	18.4	60	10.1

[1] heading

Relation between stem elongation and ear differentiation

In one case plants were dissected periodically to follow generative development. The plants were sown Dec. 18 and received supplemental illumination with incandescent lamp (40 Watt) or fluorescent tube (TL nr. 32, uncovered).

This prolonged photoperiod resulted in ear initiation. On Jan. 13 the plants treated with incandescent light had reached or already passed the 'double ridge' stage. The

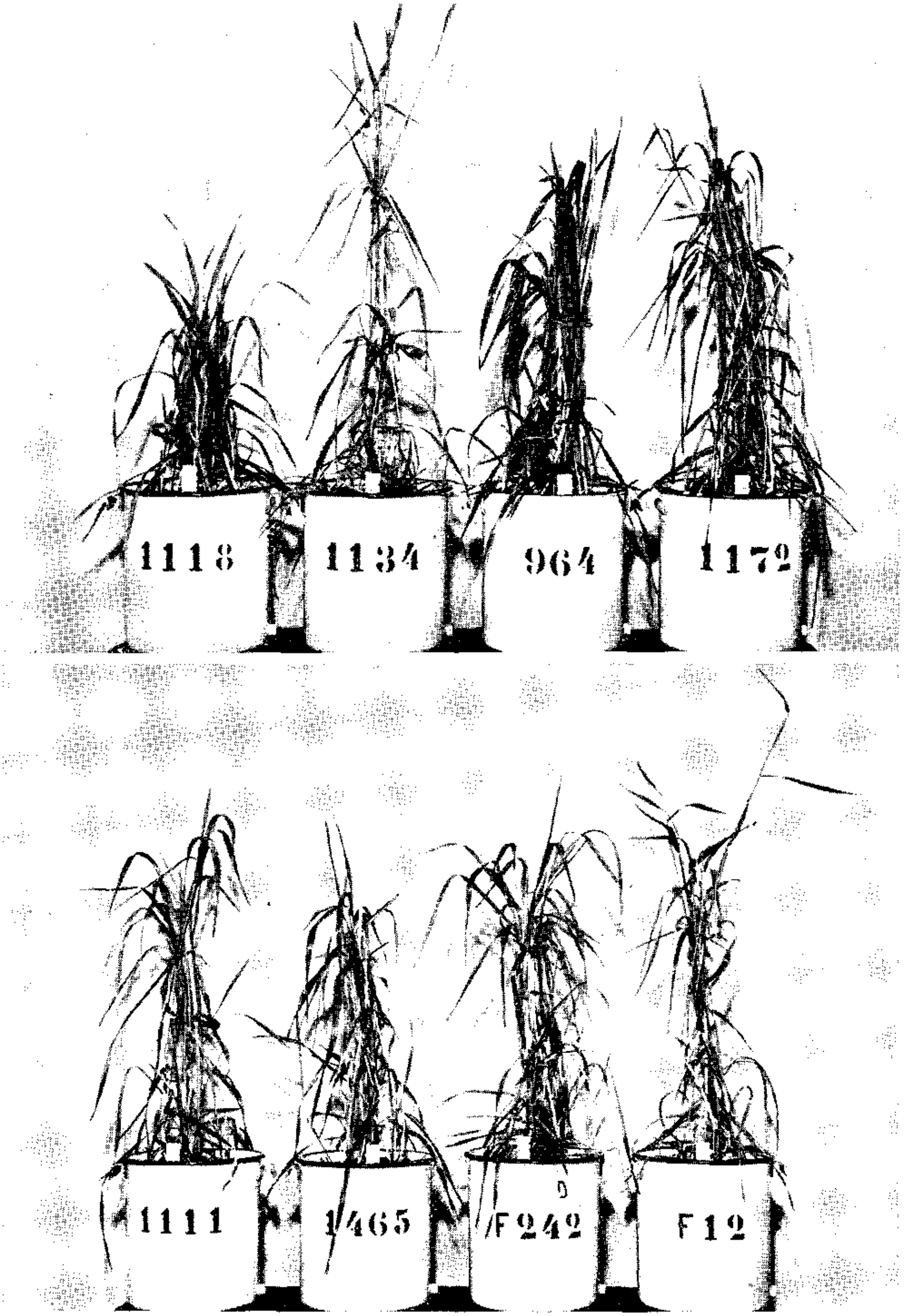

FIG. 1. Petkuser spring rye emerged at Nov. 25 1962 in the greenhouse (16 °C) and photographed Jan. 9 1963. The same plants as recorded in table 1.

nr. 1118 control
1134 incandescent light during the dark period
964 fluorescent light TL nr. 29
1172 „ TL 32
1111 „ TL 33
1465 „ TL 34
F 242 „ TL 55
F 12 „ TL 57

TABLE 2. Influence of supplemental illumination with several lamp types during night hours on spring rye grown in a glasshouse at 16 °C. Emergence Jan. 3, 1963. Average values for 10 plants.

Treatments		27 days after emergence					39 days after emergence				
		dry matter tops in mg	% dry matter tops	shoot/root ratio dry weight	plant length in cm	devel. stage	dry matter tops in mg	% dry matter tops	shoot/root ratio dry weight	plant length in cm	devel. stage
Controls		162	11.5	3.6	15	1[1]	341	9.2	5.6	22	
Incandescent lamp (40 Watt)		190	8.7	7.3	37	8–9[3]	486	11.4	12.2	60	10.1
Fluorescent tubes:											
Philips TL nr. (40 Watt) 700–1000 lux	29	190	10.8	4.0	17	2[2]	517	10.0	4.5	30	
	32	197	10.5	3.8	19	3	576	10.0	5.1	30	
	33	198	10.3	5.2	21	2	468	9.5	6.2	32	
	34	173	12.0	3.6	16.5	1	458	9.3	4.3	27	
	55	185	10.3	4.5	18.5	2+	556	9.7	6.2	31	
	57	164	9.2	5.4	23.0	4–5	630	9.6	6.0	41	8–9

[1] vegetative stage [2] 'double ridge' [3] flag leaf completely visible

plants under fluorescent light reached the same stage only after 7 more days (fig. 2). Stem elongation, however, showed a greater retardation, *viz.* about 12 days.

Under fluorescent light, stem elongation began when flower initials showed the first differentiation, which is the normal situation in natural light. Under incandescent light, however, a clear elongation of the lowest internode could already be observed at the double ridge stage. These observations indicate that in the combined

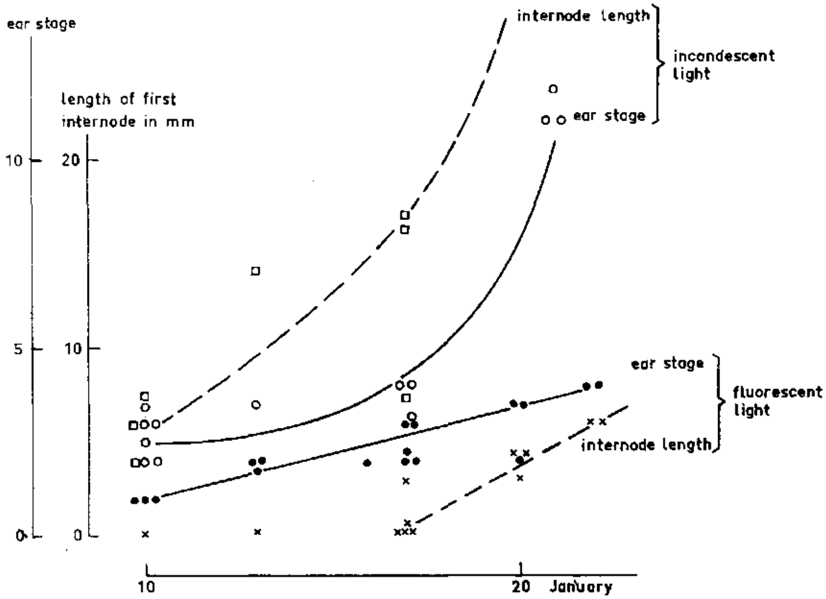

FIG. 2. Relations between ear stage and internode length for spring rye with supplemental incandescent or fluorescent light. Stages of development:
2 = ‚double ridge' stage
5 = differentiation of flower primordia
10 = tips of lemma bending; first indications of awns visible

processes of ear development and stem elongation under normal conditions ear development takes the lead, whereas under incandescent light relations may be reversed[1]).

This change in the relation between ear initiation and stem elongation supports the conclusion of DOWNS *et al.* (3) according to which the acceleration of flowering caused by supplemental incandescent light observed in plants in which stem elongation is an integral part of the flowering process must be explained as a secondary effect of far-red radiation. The primary effect is stem elongation and this process involves flower development.

Plant elongation in the vegetative stage

The results presented in table 1–4 show that supplemental light always increased plant length immediately after plant emergence in the juvenile stage when rye is not yet sensitive to the length of day, at least in respect to ear initiation (2). At this stage stem elongation has not yet begun and plant length is a matter of leaves only. Our observations do not permit to decide whether this leaf elongation in the youth stage is still a formative effect of long day or of light quality. The fact that incandescent lamps have a much stronger effect than fluorescent ones is in favour of the last supposition.

TABLE 3. Influence of supplemental illumination with several lamp types during night hours on spring rye grown in a glasshouse at 16°C. Emergence Jan. 22, 1963. Average values for 10 plants.

Treatments		29 days after emergence					35 days after emergence				
		dry matter tops in mg	% dry matter tops	shoot/root ratio dry weight	plant length in cm	nrs. of visible leaves	dry matter tops in mg	% dry matter tops	shoot/root ratio dry weight	plant length in cm	nrs. of visible leaves
Controls		153	10.4	3.0	12	4.5	350	11.5	3.0	25	5.7
Incandescent lamp (40 Watt)		204	8.9	9.3	33	5.5	446	11.2	9.7	60	7.0
Fluorescent tubes: Philips TL nr: (40 Watt)											
700–1000 lux	29	154	9.8	6.4	27	4.5	356	10.8		45	6.0
100 lux	29	160	10.4	4.0	19	4.6	474	11.4	3.8	36	5.8
	32	168	10.4	4.3	19	4.4	424	11.1	4.4	38	5.8
	33	149	9.3	5.3	20	4.5	365	10.8	4.3	45	5.8
	34	139	11.8	5.0	14	4.5	347	10.8	4.1	38	5.6
	55	180	10.6	4.0	18	4.6	412	11.0	3.8	38	6.0
	57	148	9.7	5.3	22	4.5	362	10.3	5.1	41	5.5

The fluorescent lamp TL nr. 57, when used at full intensity in December, was the only of its kind which caused reactions in the plants comparable to those observed under incandescent light.

These reactions resulted in an appearance closely resembling the features generally associated with etiolation. Table 5 gives details on the reactions of separate leaves.

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[1]) Comparable relations were observed by LANG (5) in *Hyoscyamus niger* in which, as a result of treatment with g.a. stem elongation occurred first, while flower initiation was delayed compared to normal inductive conditions (long day). According to LANG the primary effect of g.a. is stem elongation whereafter this process sets flower initiation in motion despite a non-inductive photoperiod. This view is supported by results of WELLENSIEK (8).

TABLE 4. Influence of supplemental illumination with several lamp types during night hours on spring rye grown in a glasshouse at 16 °C. Emergence Dec. 4, 1963.

Treatments	33 days after emergence					50 days after emergence					
	dry matter tops in mg	% dry matter tops	shoot/root ratio dry weight	plant length in cm	nrs. of visible leaves	dry matter tops in mg	% dry matter tops	shoot/root ratio dry weight	plant length in cm	nrs. of visible leaves	devel. stage
Controls nr. 1	78	9.3	2.8	25.3	4.2	197	11.7	3.7	33	5.5	5–6
Controls nr. 2	70	9.6	2.9	22.5	4.4	174	11.1	3.5	31	5.8	5–6
Incandescent lamps											
15 Watt (100 lux)	92	7.4	4.0	36.5	5.6	161	9.6	7.0	52	6.9	9–10
40 Watt (200 lux)	91	7.5	9.1	39.5	5.8	151	10.6	6.3	51	7.0	10
Fluorescent tubes:											
TL 32 (700 lux)	104	8.6	3.1	29.6	4.7	212	10.3	8.8	49	6.2	8
TL 32 (100 lux)	69	9.0	2.6	27.3	4.4	182	10.6	6.0	40	5.6	7
TL 55 (100 lux)	91	9.7	2.8	29.2	4.3	204	10.9	4.7	41	5.6	6
TL 57 (100 lux)	91	8.9	2.9	29.5	4.6	215	9.8	5.4	41	6.1	7–8

6 = 3rd last leaf visible
7 = 2nd last leaf visible
8 = last leaf visible
10 = ear almost emerging

TABLE 5. Average measurements of leaf blades and sheaths (in mm) under influence of supplemental incandescent light. Spring rye, sown Febr. 5 (1964). Temperature 16 °C.

	Controls in natural light		Natural light, in dark hours incandescent light	
	sheath	blade	sheath	blade
Length of first leaf	15	91	32	105
Length of second leaf	18	112	50	165
Width of second leaf blade		6.5		7.5

The greater length of leaf blades under incandescent light is mainly a matter of cell elongation. Microscopical examination of the leaf epidermis shows that distances between stomata are definitely larger under incandescent light, corresponding with larger sized neighbouring cells (table 6).

TABLE 6. Density of stomata on a second leaf of spring rye. Numbers counted in one row of 1.7 mm length.

	Controls (natural light)	Natural light, in dark hours incandescent light
Upper side leaf blade	15	10
Under side leaf blade	12	9
Outer side leaf sheath	8.5	7

The dry matter content of the plants under incandescent light is about as much lower as could be expected under these conditions (table 1–4). The yellowish green tinge of these leaves can also be ascribed to cell extension.

In leaf sheaths, elongation is relatively stronger than in leaf blades (table 5) and it is unlikely that elongation in this case can be wholly explained by cell extension.

Stomata are only present on the higher parts of sheaths and table 6 gives no indication of a stronger extension of sheath cells in comparison to those of blades.

Counts at the base of sheaths showed that cell measurements under incandescent light surpassed controls for 50% at the utmost which is not sufficient to explain a sheath elongation as recorded in table 5. It is most probable, therefore, that at least in leaf sheaths an illumination with incandescent light leads not only to a greater cell extension but also to an increase in cell number.

During shooting the relations concerning dry-matter content are reversed. In the juvenile stage plants under incandescent light (and under TL 57) showed lower contents but in later stages they surpassed all other treatments in this respect because of a rapid stem formation which process is always accompanied by a rise in dry-matter content (table 1–4).

Shoot/root-ratio

Under optimal conditions of nutrition shoot/root-ratio in cereals shows a rather constant average value from the seedling stage until heading (1). Individual deviations are rather large so that only clear and persistent differences can be accepted as significant. According to the results of several experiments the plants treated with incandescent light (and in some cases the treatments TL 57) show shoot/root-ratios far above the level of controls or the other treatments. It seems reasonable to suppose that this phenomenon is associated with the excessive elongation observed in the same cases. The distribution of dry matter over the whole plant is probably changed in favour of the shoot. Such a change would be in agreement with the observation that not only cell extension but also cell division is involved.

In the first experiment (table 1) treated plants as well as controls showed higher figures for shoot/root-ratio than in any other case, including the experiment recorded in table 4 which also took place in December under comparable light conditions. No explanation can be suggested for this deviation.

Growth rate

The supplied light energy by incandescent lamps or covered fluorescent tubes (100–200 lux) was too weak to expect any direct effect on growth rate. A direct increase in growth rate by a higher level of illumination can only be expected in the treatments with uncovered fluorescent tubes. Especially in December with its long nights a supplement with 700–1000 lux during all the dark hours means a considerable increase in total light energy. According to expectation the dry weights recorded for these cases exceeded the controls, especially in the first year (table 1, 50 days after emergence; compare fig. 1). In the second year (table 4) this tendency was less clear and the differences between yields under covered and uncovered tubes were not spectacular.

Next to direct influences on growth rate by supplemental irradiation indirect ones can be expected as a result of morphological changes caused in the plants. With a higher shoot/root-ratio an increased growth rate can be attained because a larger part of the available dry matter is converted to assimilating tissue. Moreover leaf elongation combined with a low dry matter content increases the leaf area per unit dry matter and in this way leads to better light interception. This explains why very weak supplemental illumination by incandescent light is able to increase growth rate substantially in most cases (table 1–4).

Influence of the intensity of supplemental light

In one case incandescent lamps of different strength (15 and 40 watt) have been used (table 4). The 40 watt lamp had a somewhat stronger formative effect but differences were significant only in the case of the shoot/root-ratio. In two experiments fluorescent tubes nr. 29 (table 3) and nr. 32 (table 4) used at full intensity (700–1000 lux) were compared to covered tubes of the same type emitting about 100 lux only. In this case the difference in light intensity was much greater and a more conspicuous elongation of leaves was observed under uncovered tubes.

The effect of the fluorescent tube Philips nr. 57 on seedling growth, which was much stronger than in the cases of nr. 29 and nr. 32, seemed to depend greatly on light intensity. This type was not used at different intensities in the same experiment, however, so that there is no decisive evidence.

TABLE 7. Measurements of spring-rye seedlings from successive sowings during winter in a greenhouse at 16 °C.

Date of emergence	Growth period in days	Average global radiation in cal/cm²/day during this period¹)	Controls		Plants treated with incandescent light in the dark hours	
			plant length in cm	nrs of leaves visible	plant length in cm	nrs of leaves visible
25-11-62	23	53	22.0		33.0	
4-12-63	33	46	23.6	4.4	38.0	5.6
3- 1-63	27	82	15.0	4.2	37.0	5.2
13- 1-64	23	59	19.9	3.1	32.1	3.6
22- 1-63	29	93	13.0	4.5	33.0	5.5
9- 2-64	24	111	19.4		39.0	
2- 3-64	18	206	22.0	4.0	32.0	4.0
29- 3-64	16	203	28.4	4.0	30.5	4.0

¹) The actual radiation in the greenhouse can be estimated at 75% of these values.

Influence of season

There are clear indications that the elongating effect of supplemental illumination on the young rye plants depends on the amount of natural light received.

In table 7 measurements are summarized of plants in the pre-shooting stage from experiments during the winter season. The figures concerning plant lengths are plotted in fig. 3. Plant age is different, but all plants are measured between the stage with 3 leaves fully grown and the beginning of shooting. During this period plant length does not change rapidly.

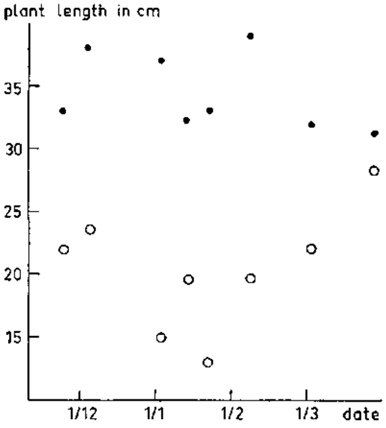

Fig. 3. Influence of the data of emergence on the length of spring rye plants grown in a greenhouse with (●) or without (○) supplemental weak illumination with incandescent light in the dark hours.

Plants receiving supplemental incandescent light do not show much difference in length during winter. There are differences in appearance, however. Whereas plants grown in the darkest part of the season show the morphological features associated to etiolation, later sowings more and more produce normally looking plants.

Controls grown in December also show some signs of etiolation as well as relatively low dry matter contents, though less extreme. Controls sown in January when natural light is definitely stronger, are shorter, have a quite normal appearance and dry matter content. In February and March control seedlings become sturdier and longer so that differences between the treatments disappear gradually.

This weakening of the effect of additional incandescent illumination may be related to the lengthening of the natural day or, more probable, to the increase in light energy which accompanies it. DOWNS *et al.* (4) working with Pinto beans found a clear influence of the daily duration of strong illumination (with fluorescent light of 1400 ft-c.) on the lengthening effect of following short treatments with incandescent light. For example: the length of the second internode was about 65 mm when strong illumination was given for 8 hours, whereas only 27 mm were measured after light periods of 12 hours. In the latter case the difference with the controls (which measured about 20 mm) was small. This result seems comparable to our experiment with rye.

Spectral emission of lamps

Fig. 4 gives the energy distribution spectra of some lamps used in the experiment according to data provided by the manufacturers. Radiation energy has been measured at a distance from the lamp where one lux is measured by the lux meter.

It is obvious that all fluorescent tubes have much in common in comparison with incandescent lamps. The latter emit the major part of their energy in the infra-red range and according to DOWNS *et al.* (3) it is this very wave length band which determines their elongating effect.

Some fluorescent lamps (for instance TL nr. 34) also emit a considerable amount of far-red (above 7000 Å) but the relation red/far-red is high, so that the elongating effect of the far-red may be antagonized by red.

TL nr. 57, the fluorescent lamp with the clearest elongating effect, emits less energy in the infra-red range than TL 34 but it is relatively low in red, so that the red/infra-red ratio is lower than in any other fluorescent tube. Moreover this lamp type emits a relatively high amount of blue radiaton. According to several authors this light colour also shows an elongating effect in some plants. DE LINT (6) has shown that pure blue light can produce a similar effect as near infra-red, only much weaker. This effect of blue is strongly reinforced by weak near infra-red admixtures. Such a combined action may be responsible for the rather strong elongating influence exerted by TL nr. 57.

In the following season, when another specimen of TL nr. 57 was used at low intensity only, no significant differences between this lamp and other fluorescent lamps could be observed.

EXPERIMENTS WITH OTHER SPECIES

In one experiment spring barley (var. Herta), spring wheat (var. Peko) and oats (var. Marne) were compared to spring rye (var. Petkuser). In all cases fluorescent light appeared to have a strong elongating effect only, whereas incandescent light caused leaf elongation, a significant increase in shoot/root-ratio and a drop in dry-matter content. Reactions to light colour seem to be about the same for these cereals.

With peas somewhat different results were obtained (table 8–9). Supplemental

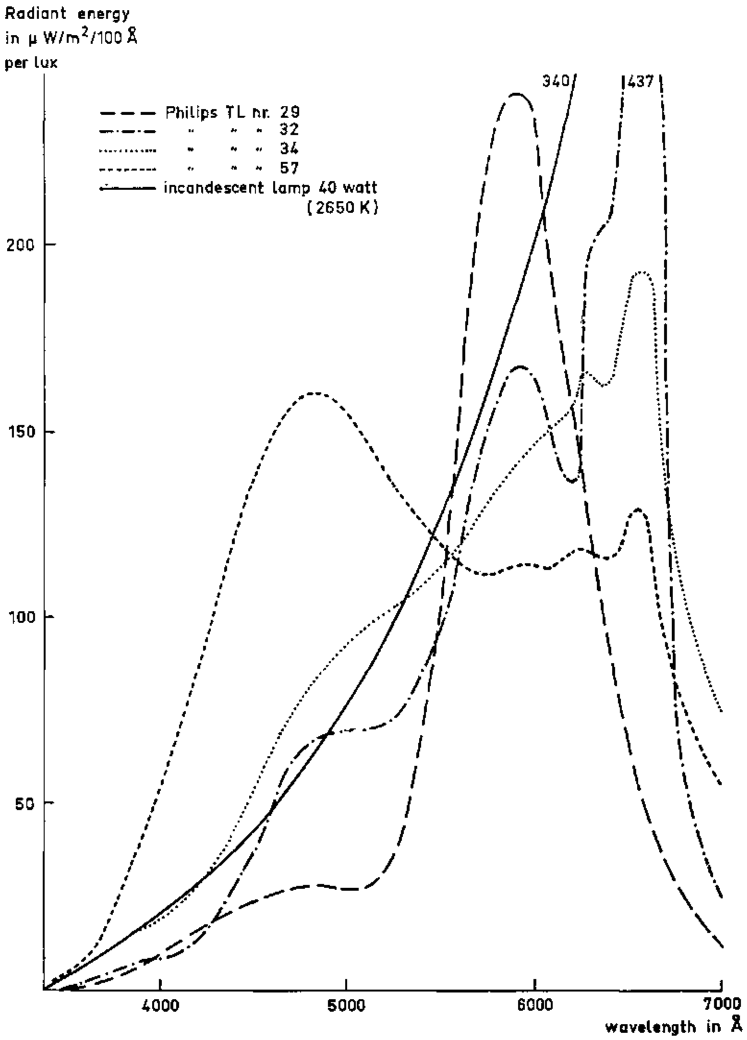

FIG. 4. Energy distribution of continuous spectra for some lamp types. Figures provided by the manufacturers.

TABLE 8. Influence of supplemental illumination with several lamp types during night hours on pea plants grown in a glasshouse at 16°C. Emergence Nov. 28, 1962. Average values for 10 plants.

Treatments	20 days after emergence			
	dry matter tops in mg	% dry matter tops	shoot/root ratio dry weight	plant length in cm
Controls	80	8.9	2.0	5.7
Incandescent lamp (40 watt)	79	8.8	1.7	7.9
Fluorescent tubes:				
Philips TL nr: (40 Watt) 700–1000 lux 29	86	9.4	2.4	4.6
32	99	9.8	2.2	5.1
33	90	9.2	2.5	5.6
34	99	9.7	2.1	4.8
55	94	9.6	2.3	5.3
57	87	9.2	2.6	5.9

TABLE 9. Influence of supplemental illumination during night hours with several lamp types on pea plants grown in a glasshouse at 16°C. Emergence Dec. 6, 1963. Average values for 10 plants.

Treatments	31 days after emergence				48 days after emergence				
	dry matter tops in mg	% dry matter tops	shoot/root ratio dry weight	plant length in cm	dry matter tops in mg	% dry matter tops	shoot/root ratio dry weight	plant length in cm	nrs. of visible leaves
Controls nr. 1	110	9.0	1.5	11.8	197	10.6	3.8	17.9	9
Controls nr. 2	128	9.0	1.8	11.6	208	10.0	2.4	15.5	10
Incandescent lamps									
15 Watt	135	8.0	2.2	18.6	168	9.2	2.0	25.4	10
40 Watt	123	8.0	2.0	18.4	152	9.0	2.5	27.6	10
Fluorescent tubes:									
TL 32 (700 lux)	124	8.9	1.8	13.0	210	11.4	3.4	21.6	11
TL 32 (100 lux)	124	8.9	1.5	11.4	212	11.0	4.2	17.8	10
TL 55 (100 lux)	145	9.0	1.7	12.2	239	10.7	3.0	19.0	10
TL 57 (100 lux)	140	8.8	1.7	13.6	197	10.1	2.3	17.4	9

illumination in the dark hours with weak fluorescent light had no significant influence on plant length or other relations. Incandescent light, however, gave a clear elongation and in one case (table 9) a slight drop in dry-matter content and a somewhat higher shoot/root-ratio. Elongation seemed to be restricted to internodes. Leaf area was not affected and leaf emergence nor flowering was accelerated.

Similar reactions to incandescent light are reported by DOWNS, HENDRICKS and BORTHWICK (4) for beans, sunflower and Ipomoea.

SUMMARY

Spring rye periodically sown in a glasshouse (temperature 16°C) during winter received supplemental weak light by several lamp types in the dark hours. Incandescent light appeared to accelerate development more than fluorescent light. This advantage is explained by the strong elongating effect of the high amount of far-red radiation produced by incandescent lamps. Whereas this wavelength inhibits flowering directly it can stimulate it indirectly in plants in which stem elongation and flower initiation are closely related (4,8).

This explanation is supported by the observation that in spring rye treated with incandescent light stem elongation shows a relative advantage over flower initiation compared with normal conditions (fig. 2).

The influence of supplemental light on young plants strongly resembled etiolation, especially in the case of incandescent lamps. Seedlings were much longer than controls, had a yellowish tinge and a low dry-matter content. The elongation of leaf blades seemed to be caused by cell extension only, whereas in leaf sheaths cell numbers also increased. Shoot/root ratio exceeded normal relations which indicates a change in dry-matter distribution.

The formative effect of supplemental illumination increased with its intensity, but was much more dependent on the strength of the natural light received during day time. In the course of the winter plants treated with incandescent light produced seedlings which did not differ much in length (fig. 3). The morphological features resembling etiolation, however, gradually disappeared and were absent in seedlings grown at the end of March.

Controls grown in the darkest part of the winter were also showing signs of

etiolation. Controls sown in January had a normal appearance and were shorter than their predecessors. Towards early spring seedling length of controls increased and all differences with the treated plants disappeared gradually.

Compared with incandescent light the effect of fluorescent tubes on leaf elongation was much weaker and in respect to shoot/root-ratio it was hardly significant. This can be ascribed to the much higher red/far-red ratio in the radiant energy which distinguishes fluorescent light. Only the type Philips TL nr. 57 when used at full intensity in the darkest part of the season gave results comparable to incandescent light. According to the manufactures the red/far-red ratio in this type is relatively high (fig. 4). Moreover it emits a high amount of blue which colour in combination with far-red stimulates plant elongation in some cases considerably (6).

Spring wheat, spring barley and oats grown in December reacted in about the same way as spring rye to supplemental light. In peas reactions were obtained by incandescent light only and these were almost exclusively restricted to elongation of internodes. Flowering dates were not affected.

Growth rate was generally accelerated by supplemental light. In the case of the uncovered fluorescent tubes providing 700–1000 lux this is mainly explained as a direct effect of increased illumination. In the case of incandescent light when only 100–200 lux was supplied a better interception of light by morphological changes in the plants may be responsible.

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VERTRAAGDE WATERONTTREKKING DOOR EEN GEWAS

with summary

G. F. MAKKINK en H. D. J. VAN HEEMST

INLEIDING

In het onderzoek van de waterhuishouding van de Schroewegpolder op Walcheren (3) werd de onttrekking van het water aan de grond door het gewas met een eenvoudig rekenmodel weergegeven. Hierbij werd aangenomen dat bij afwezigheid van neerslag al het voorhanden onttrekbare water kan worden opgemaakt. Later werd een wat ingewikkelder model ontworpen, dat in een programma voor automatische bewerking door de ZEBRA werd gebruikt en dat een verbetering bleek te zijn. Hierin werd het beginsel van een vertraagde onttrekking toegepast. De afleiding van de in dat model gebruikte formule was voor verbetering vatbaar en in het volgende is die op elegantere manier overgedaan.

DE WATERONTTREKKINGSFORMULE

We kiezen het geval van een gewas dat in de periode van waarneming niet verandert, noch bovengronds, noch ondergronds. We nemen een waarnemingsperiode van t dagen en onderstellen dat als in die periode de verdamping potentieel zou zijn, deze met constante snelheid zou verlopen en een waarde van E_p mm zou hebben in t dagen. We nemen verder aan dat het water in de grond homogeen verdeeld is, dat de grond homogeen is en dat in de laag die door de wortels tot de verwelkingscapaciteit kan worden geleegd, aan het begin van de periode, V mm onttrekbaar water voorhanden is. We nemen aan dat als de grond tot veldcapaciteit gevuld zou zijn, B mm water aan de grond zou kunnen worden onttrokken. De waarde B werd door ons vroeger (3) het bereik (de bereikcapaciteit) genoemd.

We willen nu berekenen hoeveel water op het eind van de periode in de grond aanwezig is en hoe groot de werkelijke verdamping E in die periode is geweest. We hebben te maken met een uitdroogproces, waarvan we onderstellen dat de snelheid, dV/dt, geleidelijk afneemt en steeds evenredig is met het vochtgehalte van de grond op het tijdstip van de onttrekking. Deze onderstelling is geoorloofd, omdat het droogproces van een grond door middel van een goed ontwikkeld wortelstelsel overeenstemt met het drogen van een dunne laag materiaal; de dikte van de laag is nl. die van de grondmantel rondom de wortels waaruit het water afkomstig is, de vorm ervan is die van het wortelstelsel. De vergelijking van de droogsnelheid van een dunne laag is nu (1, 2, 7)

$$-dV/dt = k \cdot V/B, \qquad [1]$$

waarin V/B het vochtgehalte van de grond aan het begin voorstelt. We verwaarlozen de vochtinhoud van het gewas, die in de regel slechts zeer gering is in verhouding tot die van de grond. De snelheid dV/dt is dezelfde als de snelheid van de werkelijke verdamping, dE/dt.

Is de grond op veldcapaciteit, waarbij het vochtgehalte V/B, de waarde 1 heeft, dan mogen we aannemen dat dan de werkelijke verdampingssnelheid gelijk is aan de potentiële, zodat k de waarde heeft van dE_P/dt. Dus is

$$-dV/dt = (dE_P/dt)\cdot V/B. \qquad [2]$$

Oplossing van deze differentiaalvergelijking levert

$$V_t = C\cdot e^{(-dE_p/dt)\cdot t/B}. \qquad [3]$$

Bij het begin van de onttrekking ($t = 0$) is de vochthoeveelheid nog niet geslonken en heeft nog de waarde V. De exponent wordt dan 0 en we vinden dat

$$C = V.$$

De algemene vergelijking wordt nu na vereenvoudiging van de exponent

$$V_t = V\cdot e^{-E_p/B}. \qquad [4]$$

Aan het begin van de periode was er V mm water in de grond, aan het eind V_t mm Er is dus verdwenen de hoeveelheid $V - V_t$, die gelijk is aan de werkelijke verdamping E. We vinden dus

$$E = V\cdot(1 - e^{-E_p/B}). \qquad [5]$$

Wanneer we de waarde van E_P uitdrukken in B vinden we de waarde van E uitgedrukt in V. Hoe de verhouding E/V varieert over het interval van de waarde van E_P/B dat van belang is, geven tabel 1 en fig. 1 weer.

Men ziet dat bij een grote waarde van E_P/B ten hoogste de gehele vochthoeveelheid kan verdampen. Als E_P 0 is, is ook de verdamping 0. Bij een geringe negatieve potentiële verdamping, die bij een surplus aan nachtelijke condensatie kan voorkomen, is ook de verdamping negatief en zwak. De formule geeft dus de voorkomende gevallen juist weer.

Hoewel de formule uitgaat van een niet groeiend gewas, mag deze in het veld toch worden gebruikt omdat in een reeks van opeenvolgende korte perioden, in elke periode de betreffende gewasfactor en bereikcapaciteit worden toegepast.

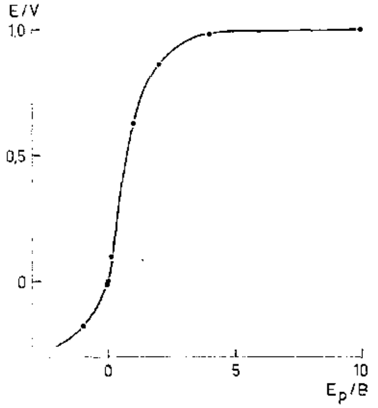

Fig. 1. De gegevens van tabel 1 in beeld gebracht.
The data of table 1 in a graph.

TABEL 1. De verhouding E/V berekend volgens vergelijking 5 en 6 bij gegeven verhouding Ep/B en twee waarden van B (zie tekst).
The ratio E/V calculated according to equation 5 and 6 at a given ratio Ep/B and two values of B (see text).

	E/V		
Ep/B	verg. 5	verg. 6	
		$B = 20$	$B = 200$
10	0,9995	0,9999	0,9999
4	0,9827	0,9836	0,9826
1	0,6320	0,6419	0,6369
0,1	0,0951	0,0977	0,0964
0	0	0	0
–0,1	–0,105	–0,108	–0,107
–1	–1,718	–1,792	–1,754

DE OUDE FORMULE

Vroeger werd een formule afgeleid door de wateronttrekking te berekenen wanneer de potentiële verdamping met stapjes van 1 mm vorderde. Daarbij werd stapje voor stapje de onttrekking berekend uitgaande van de voorafgaande vochtinhoud. De formule geeft de limiet van de som van een afdalende convergerende meetkundige reeks weer:

$$E = V\cdot\{1 - (1 - 1/B)^{E_p}\}. \tag{6}$$

Men ziet dat deze vergelijking met verg. 5 alleen verschilt in de laatste term, resp. $e^{-E_p/B}$ en B^{-E_p}. In tabel 1 is de uitkomst van E/V voor dezelfde verhoudingen van E_p/B vermeld, maar met toevoeging van 2 waarden van B.

De uitkomsten van de reeksvergelijking verschillen praktisch niet van die, verkregen met de differentiaalvergelijking.

Formule 6 werd eerder vermeld (4) en werd in een automatische berekening van de waterboekhouding gebruikt om voor de Schroewegpolder de afvoer te berekenen, die ook uit metingen bekend was. In 6 alternatieve berekeningen waarin de reeksformule was opgenomen werd voor de jaarafvoeren van 8 jaren een gemiddelde correlatiecoëfficiënt tussen berekende en gemeten cijfers gevonden van 0,964, terwijl in dezelfde 6 alternatieve berekeningen met het eenvoudige onttrekkingsmodel een coëfficiënt van 0,941 werd gevonden. Op grond hiervan mag dus worden besloten dat vertraagde onttrekking de werkelijkheid beter benadert.

BESPREKING

Uit figuur 1 volgt dat een grond alleen in verregaande mate wordt uitgeput, wanneer de potentiële verdamping tenminste 4 maal de maximaal onttrekbare hoeveelheid vocht in de grond overtreft. In het veld zal de grond dan ook meestal alleen tot het verwelkingspunt uitdrogen als de beworteling ondiep is, de verdampingspotentiaal hoog en regen uitblijft. In proeven met potten of kleine vaten daarentegen zal dit eerder het geval zijn.

De formule is afgeleid voor een plant of gewas waarvan de potentiële verdamping zich niet wijzigt. Tijdens de uitdroging verandert echter de plant wel, dus wellicht ook k in verg. [1]. De waarde ervan die bij een lage waarde van de osmotische potentiaal aan het begin gelijk is aan dE_p/dt, neemt vermoedelijk bij stijging der osmotische potentiaal toe, zodat de onttrekking wat verder zal voortschrijden dan formule [5] aangeeft.

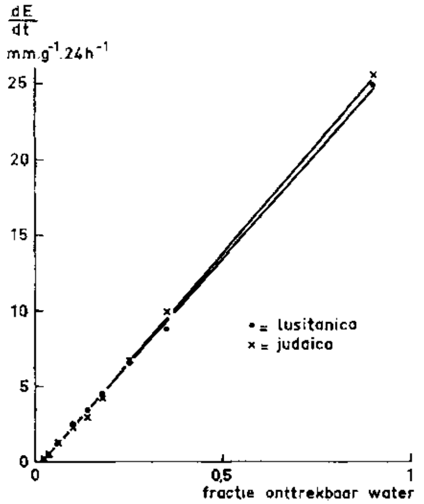

FIG. 2. De droogsnelheid in g water per g droge stof per etmaal als functie van de fractie van het onttrekbare water in de grond, bij de variëteiten lusitanica en judaica van kropaar. Samengesteld naar gegevens van PERRIER, MCKELL en DAVIDSON 1961.
The drying rate in g of water per g of dry matter per 24 hours, as a function of the fraction of total available water in the soil, with the varieties lusitanica and judaica of Dactylis glomerata L. *Plotted according to data of* PERRIER, MCKELL and DAVIDSON *1961.*

De uitdroging van de grond door een plant of gewas is een punt van controversen (8). Een deel hiervan kan op rekening worden gesteld van soortverschillen. Bomen en kruidachtige planten behoeven niet hetzelfde gedrag te vertonen tijdens de vochtonttrekking; evenmin kruidachtige planten uit semi-aride en uit humide gebieden. Voor ons doel beperken we ons tot het gedrag van planten van humide streken (granen, bieten, aardappels, bepaalde grassen). Voor twee rassen van *Dactylis glomerata* L. konden we aan de hand van gegevens van PERRIER, MCKELL en DAVIDSON (5) een droogsnelheidskromme berekenen. Hun proef werd genomen met pollen, die in vaten van ca. 4 liter met een zandige klei, bij optimale watervoorziening werden gekweekt. Op zeker ogenblik werden de pollen geknipt en werd de watervoorziening beëindigd. Tijdens het uitdroogproces bij een niveau van hoogstens 2,4 mm per dag werden bij verschillende vochtspanningen van de grond potten geoogst voor bepalingen. We hebben de uitkomsten gecorrigeerd voor de uiteenlopende gewasmassa en moesten daarbij de droge massa der stoppel schatten aan de hand van de kromme van de gewichtstoenamen. Het blijkt dat de droogsnelheid lineair afneemt met het vochtgehalte van de grond en dat beide rassen, die een zeer uiteenlopende habitus en opbrengst hadden, na correctie voor gewasmassa praktisch niet in gedrag bij uitdroging verschillen (fig. 2). De lijn is in goede overeenstemming met het uitgangspunt (verg. [1]) van de onttrekkingsformule. De fase van constante snelheid ontbreekt en de lijn gaat nagenoeg door de oorsprong. Hiermee is aangetoond, dat het gedrag van de huidmondjes waarvan de sluiting zich bij een aantal kruidachtige planten afspeelt tussen vochtgehalten van ca. 8 % tot ca. 24 % van het verse bladgewicht (6), niet tot uitdrukking behoeft te komen, wanneer men alleen de grond in aanmerking neemt. Blijkbaar bewerkt het regelmechanisme van de stomata dat de transpiratie tijdens de gehele uitdroging in overeenstemming blijft met de absorptie uit de grond. Dit betekent dat de weerstand van het watertransport in de grondmanteltjes rondom de wortels bepalend is voor de transpiratie.

Retarded water withdrawal by a crop

A formula (equation [5]) for the removal of water from a cropped soil is developed from equation [1] of drying materials in a shallow layer. It is almost identical with an equation of the sum of a convergent infinite progression [6]. The latter equation showed better results in calculating the run-off of a polder compared with a simpler model for removal of water from the soil (if there is water enough, potential use; if there is less, all available water taken up).

The equation for the rate of drying is a fair approximation of the drying in containers with *Dactylis glomerata* L. on a sandy clay soil in an experiment of PERRIER, McKELL and DAVIDSON 1961.

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DE VERDAMPING VAN WEILAND

with summary

G. F. MAKKINK[1]), C. KALISVAART[2]) en L. VEEGENS

INLEIDING

In de Noordoostpolder ligt bij Vollenhove een grasgebied van rond 300 ha dat door middel van drainreeksen wordt geïnfiltreerd. KALISVAART berekende daarvoor het waterverbruik (1). MAKKINK stelde dat wanneer een gewas potentieel verdampt de verdamping g maal zo groot is als de verdamping van een zeer korte gesloten grasvegetatie die optimaal van water wordt voorzien (3). De factor g noemde hij gewasfactor.

Is de vochttoestand van de grond niet optimaal en de verdamping subpotentieel, dan is de verdamping van het gewas r keer die bij optimale watervoorziening. De reductiefactor r kan men de beperkingsfactor noemen. Bij suboptimale vochtvoorziening is de verdamping van een gewas dus rg maal die van een korte grasvegetatie die optimaal van water wordt voorzien. In het veld treden r en g gecombineerd op. Het verdient daarom aanbeveling rg te gebruiken en deze b.v. veldfactor te noemen. Alleen bij optimale watervoorziening ($r = 1$) is g te bepalen. Het lag daarom voor de hand een gebied te kiezen waar het waterverbruik optimaal is en daarvoor het waterverbruik te berekenen. Dan kan nl. toegepast worden de vergelijking

$$E = rg \cdot l \cdot E_\pi \qquad [1]$$

waarin E de verdamping uit het gewas in het betreffende gebied voorstelt, E_π de potentiële verdamping uit een korte gesloten grasvegetatie die optimaal van water wordt voorzien te Wageningen en l een correctiefactor voor de betreffende plaats, nodig om zijn afstand tot Wageningen te overbruggen. Voor de locale factor wordt toegepast het quotiënt van de verdamping van vrij water (E_0) op plaats L en te Wageningen:

$$l = E_{0L} \cdot E_{0Wag}^{-1}. \qquad [2]$$

Als mag worden aangenomen dat bij de toegepaste infiltratie het gras potentieel water verbruikt, dan gaat [1] over in

$$E_P = g\, l \cdot E_\pi. \qquad [3]$$

De waarde E_π kan gemakkelijk worden berekend (2), terwijl E_P kan worden gevonden uit de waterbalansvergelijking

$$N + T = A + E + \Delta V.$$

Hierin stelt N de neerslag voor, T de toegevoerde hoeveelheid infiltratiewater, A de hoeveelheid afgevoerd water, E de verdamping, die in geval van potentieel verbruik E_P mag worden genoemd en ΔV de verandering van de vochtvoorraad in de grond.

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HET TERREIN EN DE METINGEN

Voor het gebied werden gegevens over 1948 tot en met 1959 gebruikt. Het heeft een klei- en humusarme zandgrond met een U-cijfer van 55, liggend op een vrijwel ondoorlatende kleigrond. In het infiltratiegebied werd de slootwaterstand op 35 cm beneden maaiveld gehouden, waardoor de grondwaterstand ongeveer 40 cm onder maaiveld lag. Na langdurige droogte is bij infiltratie de grond boven het grondwater naar schatting 35 mm water armer dan kort na langdurige regen. Zonder infiltratie werd geen kwel uit de nabije IJsselmeerboezem vastgesteld. Randverliezen werden niet waargenomen. Wegzijging van water naar de ondergrond is beperkt tot een gebied van ongeveer 13,5 ha. In 1956 en 1957 werd een deel van het gebied gediepploegd. Dit werd van 1956 af niet meer geïnfiltreerd. Hierdoor trad een belangrijke gebiedsverkleining op.

Het bleek dat de drainbuizen waardoor de infiltratie plaatshad, geleidelijk last van verstopping kregen. Hierdoor werd in droge jaren ondanks de infiltratie vooral op de beter doorlatende plek verdroging vastgesteld.

Het hele gebied was grasland, dat door boeren uit Vollenhove extensief werd beweid.

De meting van de regenval gebeurde met behulp van 1 of 2 regenmeters op 40 cm hoogte in en vlak bij het gebied. De meting van het toegevoerde en afgevoerde water gebeurde met behulp van meetschotten volgens CIPOLETTI, geplaatst respectievelijk in de enige toevoersloot en aan het eind van de 16 afvoersloten. Er werd dagelijks in de ochtenduren afgelezen; wanneer er aanleiding toe bestond ook later op de dag.

BEREKENINGEN

Omdat verdroging nu en dan werd waargenomen moet met de factor r worden rekening gehouden en zal worden gesproken van de veldfactor rg. De veranderingen van het vochtgehalte van de grond werden niet bepaald. Daarom zijn de perioden waarover de veldfactor werd berekend zo lang mogelijk genomen; een eventuele fout in de sluitpost E is dan zo klein mogelijk. Bij het berekenen van de gemiddelde jaarlijkse veldfactor werden de tijdstippen van het begin en eind der perioden zo gekozen dat de grond op veldcapaciteit mocht worden aangenomen, nl. kort na enige dagen met voldoende regenval.

De maandelijkse veldfactoren werden berekend door van alle gelijknamige maanden van de reeks jaren tezamen (behalve 1959) een gemeenschappelijke balansvergegelijking op te stellen. De fout ontstaan doordat de vochtvoorraad van de grond aan het begin en eind van de maand niet dezelfde was, wordt nu verkleind door het aantal jaren dat gebruikt is. Gegevens zijn beschikbaar over de periode van april of mei t/m oktober of de eerste helft van november van de jaren 1948 tot en met 1959.

Eerst werden de berekeningen gemaakt uitgaande van de onderstelling dat er geen wegzijging was. E werd uit vergelijking [4] berekend, waarbij ΔV werd verwaarloosd; daarna werd uit verg. [1] de veldfactor rg berekend. De gegevens en uitkomsten zijn in tabel 1 en 2 vermeld.

De gegeven waarden van rg spreiden van 1,52 tot 2,13 en van 1,55 tot 2,73 in resp. tabel 1 en 2. Bij potentiële verdamping van grasland kan rg echter slechts variëren tussen 1,0 (per definitie) en 1,55 (een grotere verdamping dan E_0 komt namelijk in de regel niet voor, E_0 bedraagt dan 1,55 E_π volgens de vergelijking (2):

$$E_\pi = 0,65\ E_{0Wag}. \qquad [5]$$

TABEL 1. De jaarlijkse veldfactoren en andere gegevens van het infiltratiegebied bij Vollenhove. Aangenomen werd dat geen water naar de ondergrond wegzeeg.
Annual field factors and other data on the infiltration area near Vollenhove. It is assumed that no water drained into the underground.

Jaar *year*	Opp. ha *Area ha*	Eerste dag *First day*	Laatste dag *Last day*	Aantal dagen *n* *Number of days*	$E = T+N-A$ mm	$l \cdot E_\pi$	rg $= E/l \cdot E_\pi$
1948	310	3/5	20/10	171	644	372	1,73
1949	310	10/4	29/10	203	691	381	1,82
1950	310	30/4	20/10	174	602	328	1,84
1951	305	1/5	31/10	184	604	379	1,65
1952	297	6/5	27/10	175	590	352	1,68
1953	291	1/5	25/9	148	550	338	1,63
1954	290	29/3	29/10	215	628	360	1,76
1955	290	21/5	25/10	158	490	322	1,52
1956	208	12/6	20/10	131	454	220	2,06
1957	186	7/5	23/10	170	638	356	1,79
1958	186	29/4	24/10	179	621	333	1,86
1959	176	6/5	25/10	173	860	403	2,13

TABEL 2. De maandelijkse veldfactoren als gemiddelden over de jaren 1948 tot en met 1958
Monthly field factors as averages of the years 1948 till 1958.

Maanden *Months*	Aantal dagen *n* *Number of days*	$E = T+N-A$ mm	$l \cdot E_\pi$ mm	$rg = E/l \cdot E_\pi$
april	243	768	433	1,77
mei	341	1502	780	1,93
juni	330	1360	874	1,55
juli	341	1407	837	1,69
aug.	341	1199	710	1,69
sept.	330	959	457	2,10
okt.	337	598	219	2,73

We zijn dus genoodzaakt te onderstellen dat er wel wegzijging (*W*) optrad. We nemen aan dat deze constant is in de tijd. Ten opzichte van de verdamping, die afneemt naar het eind van het jaar, stijgt de wegzijging dus en wanneer hij niet wordt afgetrokken, doet hij de veldfactor naar het eind van het jaar toenemen.

Omdat de doorlatende plek bij de gebiedsverkleining niet in omvang afnam moet de wegzijgsnelheid in liters in de reeks jaren eveneens constant worden genomen. Dit betekent dat de wegzijgsnelheid in liters per m^2 (d.i. in mm) omgekeerd evenredig moet worden gesteld aan de grootte van het infiltratiegebied.

Een berekening van de wegzijging *W* in mm in de verschillende maanden en jaren is nu uitgevoerd op grond van de onderstelling dat in oktober het gras kort is afgegraasd en de veldfactor gemiddeld de waarde 1,0 benaderde. Dan is in oktober *E* gelijk aan $1{,}0 \cdot l \cdot E_\pi$. Uit de vergelijking

$$N + T - A = rg\, l \cdot E_\pi + W \qquad [6]$$

werd nu berekend dat de wegzijgsnelheid in de 11 oktobermaanden gemiddeld 1,12 mm per etmaal bedroeg. Nu is ΣW_{ok} (ok = oktober) berekend over de jaren 1948 tot en met 1958, waarin het infiltratiegebied kleiner werd. Heeft $W_{ok,48}$ de waarde *p* mm per etmaal, dan heeft $W_{ok,51}$ de waarde (310/305) *p* mm per etmaal, $W_{ok,52}$ de waarde

(310/297) p mm per etmaal, enz. (zie kolom 2 tabel 1). Voor alle n oktoberwegzijgingen tezamen geldt nu

$$\sum^{n} W_{ok} = \sum^{n} \{(S_{48}/S_i)\cdot p\} \qquad [7]$$

waarin S de oppervlakte van het gebied voorstelt en i een van de betreffende jaren aanduidt.

Voert men deze berekening uit dan vindt men voor p 0,94 mm per etmaal. Hieruit laat zich voor elk jaar de wegzijging berekenen. Daarna kan men de gecorrigeerde verdamping en verder de bijbehorende waarde van rg berekenen (tabel 3). Zou men voor rg_{ok} 0,95 of 1,05 hebben genomen, dan zou men voor g_{48} de waarden 1,28 resp. 1,31 hebben gevonden. Dit betekent dat de waarde voor rg in het eerste jaar niet veel verandert wanneer men de waarde van rg voor oktober wat groter of kleiner kiest.

TABEL 3. De berekende wegzijging (W), de gecorrigeerde verdamping (E/W), de gecorrigeerde veldfactor (rg), de gemiddelde neerslag (in n dagen) (N/n) en het gemiddelde aantal dagen met meer dan 0,1 mm regen (m_N/n).
Calculated drainage (W), corrected evapotranspiration (E/W), corrected field factor (rg), average precipitation (in n days) (N/n) and average number of days with more than 0,1 mm of rain (m_N/n).

Jaar *Year*	W mm/etm.	W mm	$E-W$ mm	rg	N/n mm/etm.	m_N/n etm./etm.
1948	0,94	160	484	1,30	1,94	0,47
1949	0,94	191	500	1,31	1,64	0,43
1950	0,94	164	438	1,34	2,69	0,53
1951	0,96	176	464	1,23	1,95	0,45
1952	0,98	171	419	1,16	2,19	0,54
1953	0,99	146	404	1,19	2,31	0,47
1954	1,01	217	411	1,14	2,91	0,56
1955	1,01	160	330	1,02	1,51	0,39
1956	1,40	184	270	1,23	3,65	0,62
1957	1,57	266	372	1,04	3,08	0,56
1958	1,57	280	341	1,02	2,36	0,52
1959	1,66	287	573	1,43	0,88	0,22

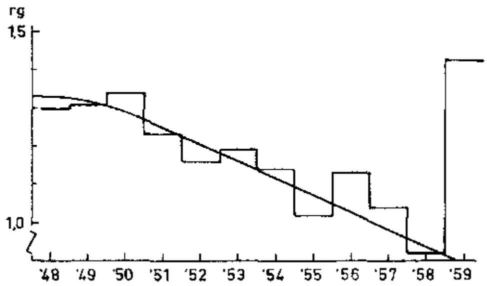
Het blijkt dat de veldfactor in de reeks jaren geleidelijk daalt (fig. 1). De afwijkingen van deze algemene trend hangen samen met de hoeveelheid neerslag in de betreffende periode (fig. 2).

Een soortgelijke positieve samenhang vindt men ook met het aantal regendagen in de betreffende periode (fig. 3). Het staat dus vast, dat neerslag de veldfactor verhoogt, al is het slechts 0,06 eenheid per gemiddelde mm regen per etmaal. Het droge jaar 1959 blijkt geheel buiten deze samenhang te vallen.

TABEL 4. Soortgelijke tabel als tabel 3, voor de maanden.
Similar as table 3, but for the months.

Maanden *Months*	W mm	$E-W$ mm	rg	N/n mm/etm.
april	295	473	1,09	1,30
mei	379	1123	1,44	1,60
juni	369	991	1,14	1,79
juli	379	1028	1,23	3,01
aug.	379	820	1,15	3,20
sept.	369	590	1,29	2,58
okt.	375	223	1,00	1,76

FIG. 1. Het verloop van de veldfactor *rg* in de reeks jaren. Er werd voor wegzijging gecorrigeerd uitgaande van *rg* in oktober = 1,0.
The field factor rg in the series of years. A correction has been made, assuming rg in October = 1,0.



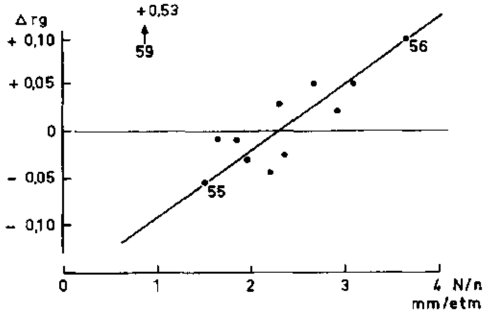
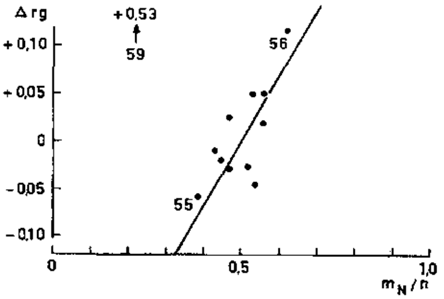


FIG. 2. De afwijkingen van de veldfactor t.o.v. de regressielijn in fig. 1 (Δ *rg*) uitgezet tegen de gemiddelde neerslag per etmaal (*N*/*n*). 55, 56 en 59 geven jaren aan.
Deviations of the field factor in relation to the regression line in fig. 1 (Δ rg) plotted against the average precipitation per day (N/n). 55, 56 and 59 indicate years.

FIG. 3. De afwijkingen van de veldfactor t.o.v. de regressielijn fig. in 1 (Δ *rg*) uitgezet tegen het aantal dagen met meer dan 0,1 mm neerslag (m_N) per etmaal (m_N/n).
Deviations of the field factor in relation to the regression line in fig. 1 (Δ rg) plotted against the number of days with more than 0.1 mm of precipitation (m_N) per day (m_N/n).



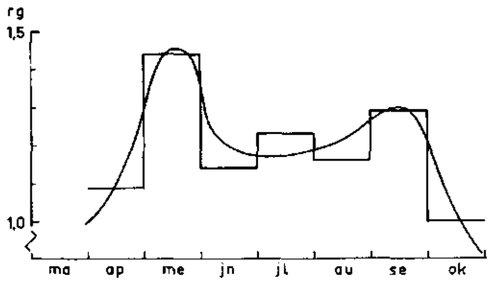


FIG. 4. Het verloop van de gemiddelde maandelijkse veldfactor (*rg*) in de loop van het groeiseizoen (histogram en kromme).
Average monthly field factor (rg) in the course of the growing season (histogram and curve).

Met behulp van de wegzijgsnelheid in elk jaar kunnen ook de gemiddelde maandelijkse veldfactoren berekend worden (tabel 4). Deze zijn in fig. 4 tegen de tijd uitgezet. Deze kromme heeft een top in mei en in september.

BESPREKING DER RESULTATEN

De geleidelijke daling van de veldfactor (fig. 1) is in overeenstemming met de waarneming dat de drainbuizen verstopt raakten. De uitkomsten wijzen erop dat dit proces in 1948 en 1949 waarschijnlijk nog geen invloed op de verdamping had. Het is geleidelijk voortgegaan en deed daarna de watervoorziening slechter worden, zodat de factor r in het produkt rg is gaan afnemen vanaf de waarde 1. In de periode van 12 jaren is hierdoor de veldfactor tot ca. 60% van zijn oorspronkelijke waarde in 1948 gedaald.

Het jaar 1959 valt geheel buiten de reeks. De verklaring moet gezocht worden in het feit dat in deze droge zomer veel verdamping gevolg was van aangevoerde energie. Deze verdamping wordt met de gebruikte formule niet berekend. De waarde van $l \cdot E_\pi$ is dus in dat jaar onderschat, waardoor een te hoge veldfactor is ontstaan. Om deze reden is 1959 van de verdere berekeningen uitgesloten.

De invloed van de regenval op de veldfactor is duidelijk, maar niet groot (fig. 2). De vele regen in 1956 was niet bij machte om de daling ten opzichte van 1948 op te heffen. Het is niet mogelijk vast te stellen of de hoeveelheid water die na regen op blad en grond achterblijft, tot verhoging van de veldfactor heeft bijgedragen.

Er moet op worden gewezen dat van de opeenvolgende jaren 1955 en 1956 het eerste droog was en het tweede regenrijk en dat de belangrijkste verkleining van het infiltratiegebied juist na 1955 plaatshad. Dat het neerslagverschil van deze twee jaren sterk uitkomt (fig. 2 en 3), maar de oppervlakverkleining niet (fig. 1), wijst erop dat voor de wegzijging op de juiste wijze is gecorrigeerd. Hiervoor werden de oktobermaanden als uitgangspunt gekozen, uitgaande van de gedachte dat dan de kans op een subpotentiële verdamping gering is en het gras meestal kort, zodat mag worden aangenomen dat zowel de beperkingsfactor r als de gewasfactor g niet veel van 1 verschilden.

Uit fig. 2 volgt ook dat bij een gemiddelde neerslag van ruim 2,3 mm per etmaal de veldfactor niet afwijkt van de waarden die door de lijn van fig. 1 worden weergegeven.

Behalve de neerslag verhoogt de hoogte van het gewas door zijn invloed op g de veldfactor (fig. 4), wat door een aantal onderzoekers werd gevonden en ook bleek uit het onderzoek met lysimeters (3, 5, 6). Deze lijn doet denken aan de tweetoppige kromme van de grasgroei, die dP/dt als functie van de tijd weergeeft (P produktie aan groene massa). De gewasfactor op een tijdstip is echter een functie van de totale hoeveelheid groene massa (P) die op dat tijdstip op het veld staat. Bij continue beweiding vanaf een bepaalde datum in het voorjaar, kan de groene massa op het veld beschouwd worden als een som van de dagelijkse hoeveelheden gras die overblijven, wanneer van de dagelijkse aanwas ($\Delta P/\Delta t$) de dagelijkse afgegraasde hoeveelheid gras ($\Delta G/\Delta t$) wordt afgetrokken. Deze rest $\Sigma\{(\Delta P - \Delta G)/\Delta t\}$ wordt groter in tijden dat het gras harder groeit dan het vee eet, dus van eind april tot begin juni, en kleiner in tijden dat het vee harder eet dan het gras groeit, dus in de tweede helft van juni en in juli. Of in de loop van augustus de hoeveelheid gras weer toeneemt hangt af van de groeisnelheid en de veebezetting. Uiteraard heeft ook het tijdstip van inscharen in het voorjaar invloed op de maximale hoeveelheid gras die er in de wei komt te staan en op het ogenblik dat dit maximum bereikt wordt.

In het infiltratiegebied werd het gras verpacht aan boeren te Vollenhove, die het vee over het algemeen lang laten grazen in een standweide. Deze gang van zaken komt met het geschetste beeld overeen. Het is dus aannemelijk dat de hoeveelheid groene massa een verloop heeft dat lijkt op dat van de vereffende lijn van de veldfactor in fig. 4. Dat de factor in mei hoog is kan erop wijzen dat er dan een overschot aan gras in de wei is, terwijl de lage factor van eind juli tot augustus en in oktober erop kan wijzen dat dan het gras zeer kort is. De waarde van rg is natuurlijk niet zeer nauwkeurig.

Wanneer men bedenkt dat de rg-kromme (fig. 4) een gemiddelde kromme voor de 11 jaren is, vraagt men zich af hoe de kromme is voor 1948 toen nog geen beperking optrad. Het is onwaarschijnlijk dat rg in oktober (rg_{ok}) in de reeks jaren veel verandert, dit geldt ook voor rg in het vroege voorjaar. De verdamping zal dan gewoonlijk potentieel zijn en het gras kort. Omdat een belemmerde watervoorziening zich vooral in de midzomermaanden uit, mag worden aangenomen dat rg van b.v. juli in 1948 belangrijk hoger zal zijn geweest dan het seizoengemiddelde van 1,30 (tabel 3), in juli 1958 moet rg dus belangrijk lager zijn geweest. Voor mei kan de variatie van rg in de reeks jaren slechts gering zijn geweest, omdat in 1948 een verhoging slechts mogelijk was tot weinig boven het maximum van 1,55, waarbij de verdamping van vrij water kan zijn bereikt. De waarde van rg in mei van de laatste jaren moet dan ook vrij hoog zijn geweest.

In 1948 mag de verdamping nog potentieel worden aangenomen. Om in dit jaar voor rg een gemiddelde waarde 1,30 te bereiken (fig. 1) bij een lage tot vrij lage waarde van g_{ap}, g_{se} en g_{ok} en een maximum in mei van 1,55, moeten ook de waarden van g_{jn}, g_{jl} en g_{au} vrij hoog worden genomen. Voor de potentiële verdamping van het extensief beweide grasland kunnen nu de maandwaarden van g volgens een aanvaardbaar verloop worden geschat zoals in tabel 5 is opgegeven.

TABEL 5. Maandwaarden van de gewasfactor g (bij potentiële verdamping) bij extensieve beweiding van grasland, geschat op grond van figuur 4, tussen de waarden 1,00 en 1,55 en bij een gemiddelde van 1,30 (1948).
Monthly values of the field factor at potential evapotranspiration with extensive grazing of grassland, estimated on the base of fig. 4, between the values of 1.0 and 1.55 and leading to an average of 1.30 (1948)

(maart)	(1,00)
april	1,25
mei	1,55
juni	1,45
juli	1,30
augustus	1,25
september	1,30
oktober	1,00
Gem. april/okt. *Mean*	1,30

Hierboven berekenden we de gemiddelde wegzijging in het gebied op 0,94 mm per etmaal. Dit bedrag heeft betrekking op een oppervlak van 310 ha. Omdat het lekkende gebied geschat wordt op 13,5 ha is daar de lek evenredig groter, nl. 310/13,5 maal 0,94, dat is 21 mm per etmaal. Hierbij is aangenomen dat er geen rand- of andere verliezen waren. De doorlaatsnelheid was bij de vigerende drukhoogte van het water dus 2,1 cm per etmaal. Een toetsing van onze berekeningen zou het best kunnen gebeuren door meting van deze wegzijgsnelheid.

Evapotranspiration of pasture

In an area irrigated by means of tile-drainage (*N.E.* polder), precipitation (N), supplied water (T) and run-off (A) were regularly measured. The area was about 300 ha, since 1956 190 ha and is covered with grass, extensively grazed. Evapotranspiration (E) can be calculated with equation 2 (changes of water content in the soil ΔV). The water balance periods were taken as long as possible every year; for the monthly averages the monthly values of 11 years were combined. Furthermore, eq. 1 was applied (r = reducing factor according to occasional sub-optimal water content of the soil, $r = 1$ with optimal water content, g = vegetational factor indicating the ratio of potential evapotranspiration of the vegetation (E_P) and that of a short, dense grassfield optimally provided with water in the same locality, E_π = evaporation of such a vegetation at Wageningen, rg = termed field factor, l = factor to correct the distance from the area to Wageningen). Calculation proved that rg was too high every month and year (table 1 and 2). The maximum cannot be much above 1.55 (which is the value for the evaporation of free water). Therefore water (W) was apparently lost to the underground. In calculating this averaged 1.12 mm per 24 hours, assuming that rg in October is 1.0. By correcting for a decrease in the irrigated area and water loss, new values for months and years were found (table 3 and 4). They are in the right interval.

Furthermore, the field factor decreased gradually with time (fig. 1), caused by obstruction of the drains, which was actually observed. The value of 1959 is much too high, probably due to much advective energy in this dry summer, which was not calculated in E_0. The deviations of the regression line (fig. 1) concern the influence of the amount of precipitation (fig. 2) and the number of rainy days (fig. 3).

The average monthly values of rg show a seasonal course similar to that of the growth of grass. The curve apparently shows the influence of the average grass height under grazing on the vegetational factor g. For 1948 (at potential evapotranspiration) the monthly values for factor g may be estimated observing an annual value of 1.30 (table 5).

The water loss to the subsoil was calculated at 21 mm per day in the concerning area of 13.5 ha.

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DE GEWASFACTOR VAN PLANTEN OP EEN POT

with summary

G. F. MAKKINK en H. D. J. VAN HEEMST

INLEIDING

Als gewasfactor (g_π of g_0) is gedefinieerd de verhouding tussen de potentiële verdamping van een gewas (E_P) en de verdamping van een standaardobject, waarvoor genomen kan worden een korte gesloten grasmat die optimaal van water wordt voorzien (index π) of een vrij watervlak (index 0) (2). Volgens deze definitie is

$$g_\pi = E_P / E_\pi \qquad [1]$$

en

$$g_0 = E_P / E_0 \qquad [2]$$

(E_π is de verdamping van de standaardgrasmat, E_0 die van een vrij watervlak). De waarde van g_π ligt voor situaties zonder advectieve energie tussen 1,0 en ruim 1,55, die van g_0 tussen ca. 0,65 en ruim 1,0.

Men kan zich afvragen hoe groot de overeenkomstige factor is voor een vrijstaande plant of voor planten op een pot. A priori zal deze ver boven 1,55 resp. 1,0 kunnen uitgaan, omdat de opgevangen straling meestal belangrijk groter is dan voor een plant die deel uitmaakt van een gewas en omdat ook de dampuitwisseling met de atmosfeer groter is.

Een vrijstaande plant is een grensgeval uit een reeks van uitgestrekte vegetaties met verschillende dichtheden, waarvoor geldt

$$g = f_1(D), \qquad [3]$$

wanneer D de dichtheid voorstelt (in planten per ha). Planten op een pot stellen een geval voor uit een reeks potten van verschillende wijdte met beplantingen van zekere dichtheid, waarvoor geldt

$$g = f_2(O), \qquad [4]$$

wanneer O de oppervlakte van de pot aangeeft. Deze reeksen zijn verschillend: bij de eerste komt men bij verkleining van de dichtheid in een situatie dat het verdampend oppervlak heterogeen over de ruimte is verdeeld (nl. in plantvormige constellaties); bij de tweede reeks komt men bij verkleining van de potoppervlakte tot een oppervlak waarop één plant staat binnen de ruimte boven dat oppervlak.

Dit artikeltje heeft voornamelijk op een geval uit de tweede reeks betrekking.

PROEFOPZET

In 1962 werd voor een ander doel een proef genomen met het haverras Marne op 20 buizen (diameter 19,5 cm). De gebruikte zandgrond bevatte van zichzelf weinig humus; het gehalte werd tot ca. 6% verhoogd door toevoeging van fijn, verteerd blad. De buis werd ermee gevuld tot 20 cm beneden de rand, tijdens het vullen aangestampt en ten slotte op veldcapaciteit gebracht. De bovengrond, vermengd met kunstmest (o.a. 0,23 g N per buis als Kas) werd toegevoegd en daarna op veldcapaciteit gebracht. Nadat de kiemplanten waren ingezet werd de grond met een laag fijn grind bedekt. Tien kolommen waren 1 m hoog, de andere tien 1,5 m. Ze stonden in een rij. Tegelijkertijd werd hetzelfde ras op Mitscherlich-potten gekweekt, die met hun bovenrand even hoog stonden als de kolommen van 1 m. De atmosferische verdamping werd met bakjes water (diameter 19,5 cm) gemeten op hetzelfde niveau. De proef werd buiten onder een glazen dak genomen. Het waterverbruik werd door geregelde weging vastgesteld. Tijdens de gehele proef ontvingen de kolommen geen water, de potten ontvingen elke 2 of 3 dagen de verbruikte hoeveelheid water. Wekelijks werden 2 kolommen en 2 potten geoogst, de kolommen van 1 m eerst, later die van 1,5 m. De laatste 6 kolommen en potten werden in enkelvoud geoogst.

RESULTATEN

Uit fig. 1, waarin het gesommeerde gemiddelde waterverbruik (transpiratie) van de kolommen tegen dat van de potten werd uitgezet, blijkt dat op ca. 7 juli de kolommen en de potten evenveel water hadden verdampt. Van ca. 13 juni tot ca. 4 juli was de transpiratiesnelheid van de kolommen even groot als die van de potten (de helling

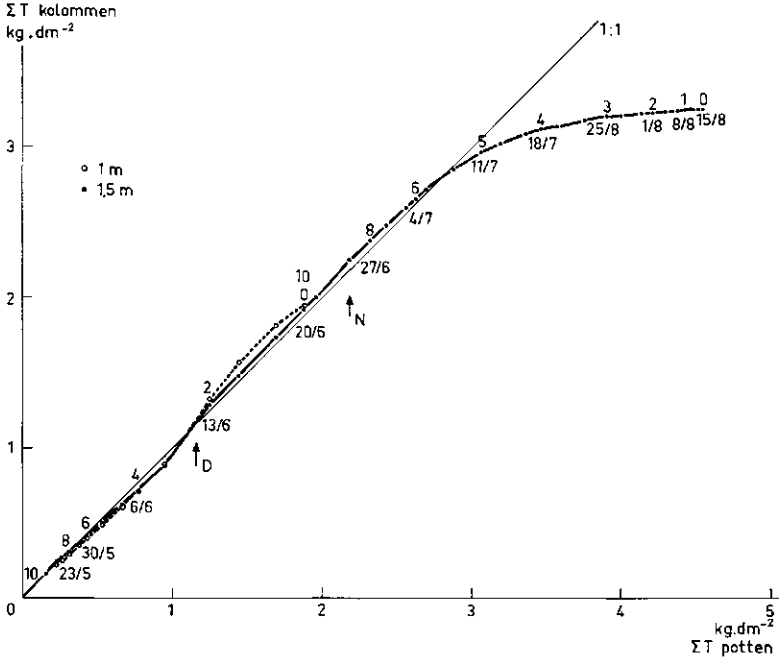

FIG. 1. De gesommeerde transpiratie (ΣT) van haver op kolommen grond uitgezet tegen dezelfde grootheid van haver op potten. Kolommen van 1 m ○, van 1,5 m ●. Aantallen nog niet geoogste kolommen aangegeven met cijfers boven de lijn (van 1 m bij het onderste deel van de lijn, van 1,5 m bij het bovenste deel). Oogstdata onder de lijn vermeld. D geeft een kortstondig vochttekort van de potten aan, N geeft toediening van extra stikstof bij de potten aan.

Accumulated transpiration (ΣT) of oats on columns of soil plotted against the same magnitude of oats on pots. Columns of 1 m ○, of 1.5 m ●. Number of columns not yet harvested are indicated by figures above the curve (of 1 m at lower part, of 1.5 m at upper part). Harvest data mentioned below the curve. D indicates a short waterdeficit of pots, N indicates additional nitrogen supply to pots.

van de lijn is 1), terwijl vóór 13 juni de transpiratiesnelheid gemiddeld ook gelijk was. Daar de transpiratie van de potten, met uitzondering van 11 of 11 én 10 juni steeds potentieel was, zou die op de kolommen bij dezelfde gewasontwikkeling ook potentieel kunnen zijn geweest. De planten op de kolommen hadden echter niet volkomen dezelfde ontwikkeling als die op de potten: op de kolommen waren ze donkerder groen en hun bladontwikkeling was niet volkomen gelijk verlopen, hetgeen zich weerspiegelt in de geringe richtingsveranderingen van de sommatielijn in figuur 1. Daar kolommen en potten echter tot ca. 7 juli evenveel water hadden verdampt, is het geoorloofd de transpiratie zeker tot 27 juni vrijwel potentieel te beschouwen. Bepalingen van het relatieve vochtgehalte van de onderste bladeren van de kolommen op enige datums midden overdag bevestigden dat op de kolommen pas na 4 juli een belangrijke daling van de transpiratie ging optreden: op 27 juni was het relatieve vochtgehalte nog 0,90, op 4 juli 0,96, op 11 juli 0,70, op 18 juli 0,59. (Het relatieve vochtgehalte is het quotiënt van de hoeveelheid water in het blad bij afsnijden en de hoeveelheid na herstel van het maximale vochtgehalte.)

De potgewas-factor

Als potgewas-factor, g_b, willen we definiëren de verhouding van de potentiële transpiratie van het gewas op de pot (T_p) tot de verdamping van water uit een even groot bakje, b, ter hoogte van de pot (E_b).

Voor de periode tot 27 juni (toen op kolommen en potten de aren juist waren verschenen) berekenen we voor potten en kolommen afzonderlijk de wekelijkse gemiddelde potgewas-factor. De hoogste van de twee benadert het best de juiste waarde in de betreffende week. Deze bereikte een hoogte van 3,1 (fig. 2) en is belangrijk hoger dan in het veld, waar het maximum 1,0 bedraagt (2) en misschien tot 1,1 komt. (Hier gebruiken we als gewasfactor g_0 bij een vrije waterverdamping E_0 volgens een gebruikelijke berekening.) We laten het geval dat advectieve energie de factor belangrijk verhoogt, buiten beschouwing. Dit geval deed zich ter plaatse in 1962 waarschijnlijk niet voor, te oordelen naar de omstandigheden en naar gegevens uit het veld vlakbij.

Hier moet opgemerkt worden, dat g_b groter is naarmate de planten meer zijdelings buiten de kolom of pot uitsteken. De gevolgen hiervan zijn moeilijk te scheiden van de gevolgen van het ontbreken van een omringend gewas. Wil men de transpiratie van

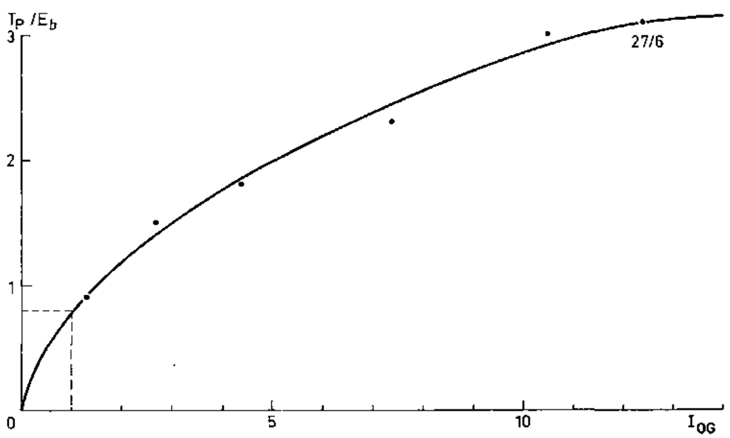

FIG. 2. De potgewas-factor (T_p/E_b) als functie van de groenoppervlakindex (I_{OG}) tot 27 juni.
The pot-crop factor (T_p/E_b) as a function of the greenarea index (I_{OG}) until June 27.

een potgewas vergelijken met die van een veldgewas, dan heeft men te maken met de relatie $g = f_2(O)$ en moet men daarom eisen dat de planten binnen de cilindervormige ruimte boven de pot blijven. De haver stak niet noemenswaard buiten de potcilinder uit, omdat hij bij het groter worden binnen stokjes werd gehouden.

Het transpirerend oppervlak

Gemakshalve werd de gemiddelde groene bladoppervlakte berekend als het produkt van gemiddelde lengte en gemiddelde breedte van de groene bladeren, vermenigvuldigd met 0,8. (Deze factor neemt bij grote bladeren een weinig af, nl. tot 0,765; met dit laatste is hier geen rekening gehouden[1].) De totale groene bladoppervlakte werd verkregen door dit getal met het aantal bladeren te vermenigvuldigen. Bij deze berekening werden alleen de groene bladeren of delen daarvan gebruikt. Ook transpireren echter de bladscheden, de halmen (3), de pluimassen en de kelkkafjes. De oppervlakte hiervan werd niet gemeten, maar met behulp van ongepubliceerde gegevens van haver berekend[1]). Zo kon voor onze periodieke oogsten de totale groene oppervlakte worden gevonden. Hieruit volgde de gemiddelde totale groene oppervlakte tijdens elke periode tussen twee oogsten (O_G). Deze werd uitgedrukt als veelvoud van de grondoppervlakte; deze verhouding noemen we de groenoppervlak-index (I_{OG}). Op 27 juni heeft I_{OG} de waarde 13, daarna is deze vermoedelijk slechts weinig toegenomen.

De ‚gewas'-factor van een standaardoppervlak

Het blijkt dat de potgewas-factor met de toeneming van het groenoppervlak steeds minder snel toeneemt (fig. 2). De maximale waarde van g_b (3,1) wordt vermoedelijk bereikt voordat I_{OG} maximaal was (al bij een waarde van ca. 13). Er stonden 6 planten per pot wat neerkomt op 2 miljoen per ha.

Uit figuur 2 volgt dat een groen haveroppervlak van de grootte van kolom of pot 0,8 van de hoeveelheid water verdampt die uit een even groot bakje verdampt: voor de waarde van I_{OG} van 1,0 is g_b ca. 0,8 De gewasfactor van een zeer korte gesloten grasmat is 0,65 (2), evenals die van een enkel haverblad in gelijke stand als een even groot stukje nat filtreerpapier (3). Hoewel de vrije watervlakken die in deze 3 gevallen als referentie-object dienden niet zonder meer gelijkgesteld mogen worden (ons bakje met een diameter van 19,5 cm, het theoretische watervlak van de Penman-formule (2) en een stukje nat filtreerpapier van 13,3 × 1,5 cm² (3)) lopen de waarden niet ver uiteen.

De effectiviteit van de transpiratie

Planten op een kolom of pot mogen zoals uit de kromming van de lijn in fig. 2 blijkt, niet als vrijstaand transpirerend materiaal worden beschouwd; ze beschaduwen en beluwen elkaar en vormen dus een ‚gewas', een potgewas. Om na te gaan hoe groot het effect van deze wederzijdse beïnvloeding is, zouden we de potentiële transpiratie van het potgewas moeten vergelijken met die van de afzonderlijke transpirerende plantendelen in vrije, maar overeenkomstige opstelling. De grootte van deze vrije potentiële transpiratie is echter niet zonder speciale waarnemingen te leren kennen. Wel kan de potentiële transpiratie van eenzelfde groen oppervlak in horizontale stand ter hoogte van het potniveau (T_{PHOG}) worden berekend. We willen de effectiviteit der transpiratie (e_T) met behulp hiervan als volgt definiëren:

$$e_T = T_P \,/\, T_{PHOG}. \qquad [5]$$

Nu is

$$T_{PHOG} = I_{OG} \,.\, (f_b \,.\, E_b), \qquad [6]$$

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[1]) Deze gegevens werden welwillend door H. D. GMELIG-MEYLING (I.B.S.) ter beschikking gesteld.

waarin f_b een specifieke factor is die de verhouding der potentiële transpiratie van het materiaal ten opzichte van de verdamping uit het bakje aangeeft. Nu volgt uit verg. 5 en 6

$$\begin{aligned} e_{T'} &= T_P \,/\, I_{OG} \,.\, f_b \,.\, E_b \\ &= (T_P \,/\, E_b) \,/\, I_{OG} \,.\, f_b \qquad [7] \\ &= g_b \,/\, I_{OG} \,.\, f_b \,. \end{aligned}$$

Voor ons potgewas in het stadium met een I_{OG} van 13 vinden we voor $e_{T'}$ 0,30. Voor een veldgewas met dezelfde waarde van I_{OG}, waarbij we in verg. 7 de grootheden g_O en f_O moeten toepassen en daarvoor resp. 1,1 en 0,65 moeten substitueren, vinden we voor e_T 0,13. Hierbij is aangenomen dat bij de bedekkingsgraad van dat gewas de grond niet belangrijk evaporeert.

Omdat de effectiviteiten van de transpiratie in het begin van de ontwikkeling bij een potgewas en een veldgewas 1 bedragen, zijn ze over de gehele vegetatieperiode hoger dan 0,30 en 0,13.

BESPREKING

In het voorgaande is gevonden dat een gewas haver bij goede watervoorziening ongeveer bij het maximum van zijn ontwikkeling van groen oppervlak nog niet half zo effectief transpireert als een potgewas met dezelfde groene oppervlakte. Dit laatste transpireert nog geen derde van eenzelfde groenoppervlak in horizontale stand op potniveau. Daar ons potgewas 57 g droge stof per pot opbracht en potopbrengsten van 162 g bij haver bekend zijn (1), rijst de vraag hoe de transpiratie-effectiviteit zich bij maximale opbrengst op pot en in het veld zich verhouden. Deze vergelijking is echter van een zekere opbrengst af niet meer mogelijk op basis van een gelijke groenoppervlak-index, omdat deze op het veld bij die op een pot achterblijft.

Bij het vergelijken van de potentiële transpiraties van pot en veld moet worden opgemerkt dat deze vergelijking alleen zuiver is wanneer het potgewas niet zijdelings buiten de pot uitsteekt. Deze eis verzuimt DILZ (1) te stellen met betrekking tot de pot/veldverhouding van opbrengsten, waarvoor deze beschouwing evenzeer van toepassing is.

Wil men voor de maximaal mogelijke opbrengsten de potentiële transpiratie van pot en veld vergelijken, dan kan dit alleen voor hun totalen over de gehele vegetatieperiode gebeuren, dus bij een uiteenlopende groenoppervlakontwikkeling. In dit extreme geval echter zal de verhouding, en dit geldt ook voor de assimilatie, nog kunnen uiteenlopen, omdat een potgewas vooral van horizontale lichtinval en luchtstroom profiteert, het veldgewas vooral van vertikale. De verhouding pot/veld zal het grootst zijn in perioden en streken met overwegend minder steile zonnestanden en in perioden of streken met krachtige wind. In jaren met veel directe straling zal een andere waarde van deze factor verwacht mogen worden dan in jaren met veel diffuus licht.

The vegetational factor of plants in pots

A comparison was made of potential transpiration (T_P) of oats on cilinders with soil and in pots, with evaporation of water from a container (E_b), on one hand and with the total green area (O_G) (leaves, stalks, peduncles, glumae) on the other hand. The ratio $T_P \,/\, E_b)$ shows (fig. 2) a curvilinear relationship with the green-area index $(O_G \,/\, O_{pot} = I_{OG})$. The potential transpiration of plants on a pot therefore is considerably reduced, according to shading and leeing. The effectivity of potential transpiration is defined as the ratio of the potential transpiration of the plants or crop and the potential transpiration of the total green area in a horizontal position at the level of the soil (T_P / T_{PHOG}). It was 0.30 at about maximal development of the plants in the experiment. For a field crop with the same green-area index it can be estimated at 0.13.

A pot with plants is considered to be a crop of certain density on the limited area of a pot. Therefore, the plants should not protrude beyond the pot area, if comparisons are to be made between pot and field.

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EXPERIMENTS ON COMPETITION AS A MEANS OF DETECTING POSSIBLE DAMAGE BY NEMATODES

L. Sibma, J. Kort[1]) and C. T. de Wit

Introduction

Van Dobben (1955) cultivated barley and oats in monoculture and in mixed culture on plots with different pH-values. Below a pH of about 4 the yield of barley was pH-dependent whereas the yield of oats was not. The yield of barley sown in a mixture with oats, however, was pH-dependent below a pH of 5. The adverse effect of low pH-values of the soil on the pH-sensitive barley is apparently increased by cultivating these plants in a mixture with non pH-sensitive oats.

De Wit (1960) showed that a similar effect occurs in mixtures of healthy and diseased plants, provided the disease occurs at an early stage.

Hence, to enlarge the effect of a disease on growth it may be helpful to cultivate beside monocultures, mixtures of varieties that are resistant and not resistant to that disease.

Experiments were carried out to determine whether the effect of nematodes on the growth of sensitive plants is increased by cultivating these plants in a mixture with nematode resistant plants.

Experiments with cereal root eelworm *(Heterodera avenae Woll.)* in 1961, 1962 and 1963

To exclude difficulties which might arise from using eelworm populations of unknown composition, a population of biotype A of the eelworm (Kort, Dantuma and van Essen, 1964) was used in the experiments with oats (cultivar Marne) as a sensitive host and barley 191 of the International Barley Disease Nursery as non-host.

Plastic buckets of 5 liter capacity were filled with 7 kg of nematode-free soil obtained from a depth of 50 cm below a permanent pasture. In half the containers cysts were added to the soil which resulted in an infestation of 500–600 larvae per 200 ml of soil.

The infested and non-infested containers were planted with 10 barley and oat plants in relative frequencies of 0, 0.2, 0.4, 0.6, 0.8 and 1 in three replicates.

The containers were placed in an open shed with glass roofing to ensure natural synchronisation of the life cycles of plants and nematodes. Limited water was given by watering the plants from above (sub-optimal) or a contineous supply by providing a constant water table (optimal).

After ripening, the plants were harvested and the total dry matter weight, seed and number of seeds were determined from each species per container.

Since oats ripens about two weeks later than barley, the number of seeds is a better yield measure in competition studies than the dry-matter weight (de Wit 1960).

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[1]) Plantenziektenkundige Dienst, Wageningen

The number of cysts and eggs at the end of the experiments were determined according to KORT (1960).

TREATMENT OF THE RESULTS OF EXPERIMENTS ON COMPETITION BETWEEN SIMILAR SPECIES

The method of treating competition experiments will be illustrated by means of the results of the 1962 experiment with barley and oats presented in fig. 2a.

The number of barley and oat plants per container is given along the horizontal axis. The fraction of both varies between 0 and 1, but the total plant number is 10 per container, throughout. The yields of barley and oats grown in monoculture are 210 and 360 kernels/container, respectively. If the yield of each species in the mixtures should be proportional to the number of plants of that species, the observations should be formed on the straight, broken line through these points and the origins. It is seen that the yields for oats are higher and those for barley lower than by these lines.

DE WIT (1960) showed that yields of both cereals in such a replacement series can be represented by

$$O_o = \frac{k_{ob}Z_o}{k_{ob}Z_o + Z_b} M_o \qquad [1]$$

$$O_b = \frac{k_{bo}Z_b}{k_{bo}Z_b + Z_o} M_b$$

O_o and O_b are the yields of oats and barley at plant-numbers Z_o and Z_b $(Z_o + Z_b = \text{constant})$ and M_o and M_b the yields of the monoculture. The constants k_{ob} and k_{bo} are the relative crowding coefficients of oats in barley and barley in oats. When the two species are sufficiently similar, they may crowd for the same space and then

$$k_{bo} \cdot k_{ob} = 1 \qquad [2]$$

This is always the case with barley and oats (DE WIT 1960).

The curves that fit the observations in fig. 2a are drawn according to equation [1] with $k_{ob} = 1/k_{bo} = 6$.

RESULTS

Experiment in 1961

Sowing took place on March 30; after one week 1 g of ammonium nitrate (NH_4NO_3) was given and an optimal water supply was maintained throughout the growing period. Both, oats and barley showed a rapid development and were harvested at the end of September.

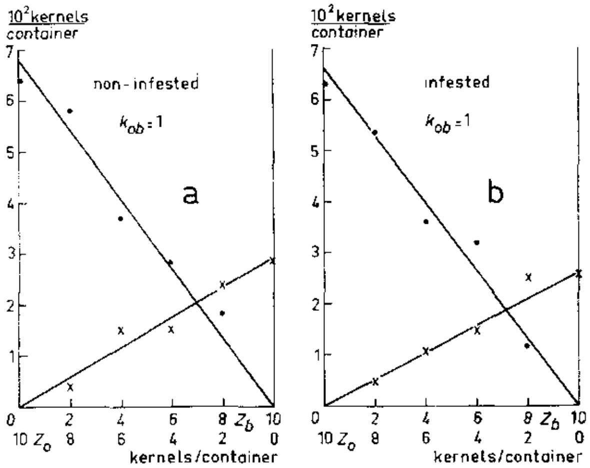
The yields in number of seeds per container are shown in fig. 1a for the non-infested soil and in fig. 1b for the infested soil. The values of the constants in equation [1] as calculated from the data are given in table 1.

TABLE 1. M_o, M_b and k_{ob} $(= 1/k_{bo})$ in 1961.

	non-infested	infested	
M_o	680	660	kernels per container
M_b	290	260	,, ,, ,,
k_{ob}	1.0	1.0	

Fig. 4 gives the number of eelworms per unit contents of soil for both treatments.

FIG. 1. Results of the experiment in 1961 with optimal supply, of water and nutrients and a succesful infestation.
● oats × barley



Experiment in 1962

Sowing was carried out on April 6; 1 g of ammonium nitrate was given 48 days later. A sub-optimal water supply was maintained throughout the growing period. Especially the early growth of both plant species was poor. The harvest took place in the middle of August. Yields are given in fig. 2a and 2b and the values of the constants in equation [1] are given in table 2.

TABLE 2. M_o, M_b and k_{ob} ($= 1/k_{bo}$) in 1962.

	non-infested	infested	
M_o	350	385	kernels per container
M_b	210	185	,, ,, ,,
k_{ob}	6.0	1.3	

The number of eelworms is also given in fig. 4.

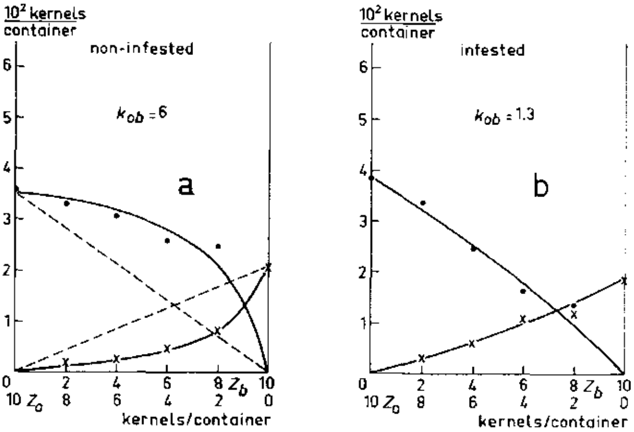


FIG. 2. Results of the experiment in 1962 with sub-optimal supply of water and a successful infestation. ● oats × barley

Experiment in 1963

Sowing was carried out on April 5; 1 g of ammonium nitrate was applied 14 days later and the plants were harvested at the end of August.

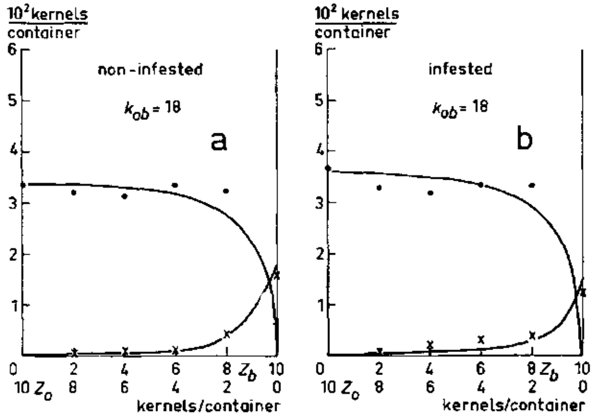


FIG. 3. Results of the experiment in 1963 with sub-optimal supply of water and an unsuccessful infestation.
● oats × barley

Half the containers were supplied with optimal and the other half with sub-optimal amounts of water. The yields of containers supplied with sub-optimal quantities of water are given in fig. 3a and 3b.

The constants in equation [1], as calculated for the sub-optimal and optimal water treatments are given in table 3.

TABLE 3. M_o, M_b, k_{ob} ($= 1/k_{bo}$) in 1963.

sub-optimal water

	non-infested	infested	
M_o	340	360	kernels/container
M_b	155	125	,, ,,
k_{ob}	18	18	
optimal water			
M_o	615	665	kernels/container
M_b	110	125	,, ,,
k_{ob}	18	18	

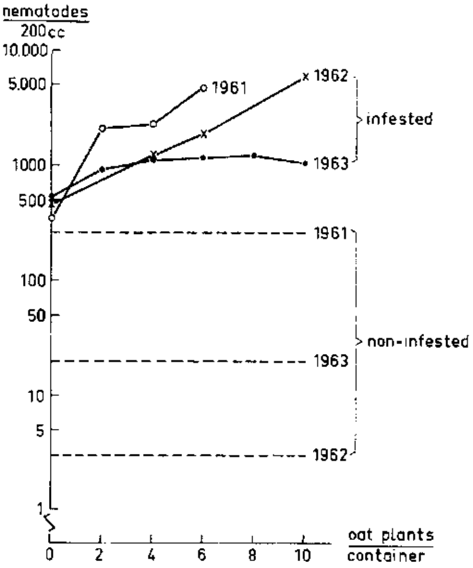
The number of nematodes at the time of harvest, averaged for both moisture regimes are given in fig. 4.

DISCUSSION

Fig. 4 shows that in 1962 nematodes were practically absent in the non-infested containers, but in the infested ones their number varied from 500 nematodes per 200 ml of soil for the monoculture of barley to 6000 nematodes per 200 ml for the monoculture of oats. The number of nematodes per oat plant increased with increasing relative frequency of oats in the containers. Hence the inoculation succeeded. In spite of a successful inoculation in 1962 the yields of oats as monoculture on infested and non-infested soil were the same (fig. 2). Experiments in which only monocultures on infested and non-infested soil are compared, will not show any differences in yield resulting from the presence of nematodes.

The absence of any influence of nematodes on yield in this kind of experiment may be due to the presence of sufficient roots to take up the limited amounts of water or nutrients or to the absence of damage by nematodes. These two possibilities can only be distinguished by comparing the yields of the mixed cultures.

FIG. 4. Density of the nematode population of infested and non-infested containers at the end of the experiment.



In the mixed culture on infested soil (fig. 2) the yield of oats is lower and that of barley higher in comparison to the non-infested soil. This effect of infestation is quantitatively accounted for by the value of the relative crowding coefficient k_{ob} which is 1.3 for the infested and 6 for the non-infested replacement series.

The effect on yield must be due to damage of the roots of oats by nematodes in an early stage. This damage prevented oats from occupying its share of the available soil and enabled barley to occupy more than its share.

Early activity of nematodes and a low supply of nutrients will enlarge the effect of infestation on the relative crowding coefficient.

In 1961 inoculation with nematodes succeeded (fig. 4) but in that year neither the yields in monoculture nor in mixed culture were affected. Supply with nutrients and water had been so liberal that even in the mixed cultures the yield of oats was not limited by a supply by the roots.

In 1963 there neither were differences between the replacement series with and without eelworms. This was true for both optimal and sub-optimal treatments. The data on the nematode numbers in fig. 4 show that the inoculation with nematodes for some reason was not successful.

The yield of oats with optimal water supply was twice that with sub-optimal water supply, but the yield of barley was the same for both. In spite of this, the relative crowding coefficient of oats was about the same in both cases (table 3). This is because the relative crowding coefficient is determined in an early stage and the effects of water supply on growth occur later.

The relative crowding coefficient of oats with respect to barley was 18 in the last year of experimentation. Since it is convenient to work with relative crowding coefficients closer to one, it would have been better if 1 seed of oats had been replaced by 2 seeds of barley, the mathematical treatment of a replacement series of this kind gives, of course, no difficulties (DE WIT, 1960).

SUMMARY

An experiment was designed to show whether the damage by eelworm infestation in oats could be enlarged by competition with a resistant barley variety.

Both cereals were grown in monoculture and as a mixed crop in containers filled with nematode-free soil and soil infested with cereal root eelworm. The infestation was successful in two out of three years, but the yield of oats in mixed culture was depressed by infestation.

In no case was the yield of oats affected, when grown in monoculture.

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COPPER FRACTIONS IN GRASS, RUMEN CONTENTS AND FAECES OF CATTLE

MARIA S. M. BOSMAN

INTRODUCTION

In the Netherlands copper deficiency in cattle occurs frequently during the grazing season (8). The copper-status of the animals often improves during the housing period, though the fodder often contains less copper than the fresh grass in summer.

DEIJS, BOSCH and WIND (3) found by statistical treatment of field data that at a given copper content in the grass the frequency of hypocuprosis increases at decreasing values of (Ca-S-P) me.per kg dry herbage. Recently, it has been found that this correlation does not always occur. Severe hypocuprosis was found in South-Limburg although both copper and Ca-S-P in the grass were relatively high.

Several authors mention an interaction of molybdenum and the sulphur from inorganic sulphate in the copper metabolism of animals (1, 7, 14), high molybdenum raising the frequency of copper deficiency. The generally low molybdenum content in Dutch pasture herbage and its rather high copper content render any importance of molybdenum in the Netherlands rather questionable. Different views on the effect of sulphate-S have been issued. In experiments with sheep, DICK (6) found that inorganic sulphate increases the influence of molybdenum on copper metabolism. MILLER (13) found that sulphate prevents the abnormal accumulation of copper in the liver of rats caused by molybdenum. MYLREA (17) mentions a decrease in liver-copper of steers after high doses of sulphate, but at these low copper levels in liver and blood the animals were in good condition and did not show clinical symptoms of copper deficiency. ALLCROFT (1) could find no differences in the average sulphate contents of pastures with copper deficiency symptoms in cattle and normal pastures. Addition of sulphate as aluminium sulphate gave no change in the copper content of blood and liver. As pastures with the same contents of copper, molybdenum and inorganic sulphate in the grass could give copper deficient animals and animals without symptoms it was concluded that other factors may interfere with the copper metabolism as well. From studies on cattle in Sout-Easterns Queensland, HARVEY *et al.* (10) also concluded that pastures contain factor(s) other than molybdenum in combination with inorganic sulphate which interfere with the copper metabolism of cattle. It is possible that, contrary to hay and other winter rations, fresh grass contains a larger quantity of copper-binding agents which decrease the availability of copper in the fodder. In earlier experiments (4) it was found that substances occur in fresh grass which can easily combine with copper (especially sulphur containing amino acids as cysteine, cystine and glutathione). PLOOY *et al.* (18) mentions a distinct negative copper balance in man after oral administration of 600–900 mg of the amino acid penicillamine to a patient suffering from hepatolenticular degeneration (Wilson's disease). In relation to these findings it seemed important to study the effect of oral administration of cystine to cattle. In collaboration with Dr. J. VAN DER GRIFT an experiment was carried out at the 'Instituut voor Veevoedingsonderzoek' at Hoorn in 1959. During

50 days each of three cows received 19.4 g of cystine daily (5). Liver copper was not affected by supplying cystine. In spite of the large fluctuations, the average copper content in the blood serum of the cystine-cows decreased distinctly relative to that of the control animals with constant blood serum copper. However, the between-animal variation and the fluctuations were too large and blood-sampling was not made frequently enough to obtain statistical significance.

It is known that copper porphyrins have a marked stability. Results of experiments on the formation of Cu-pheophytin in grassmeal suspensions to which copper ions were added in low concentrations (5) suggested an interference of chlorophyll with the copper utilization by grazing cattle. Though the availability of copper pheophytin for cattle is unknown, SCHULZE *et al.* (20) have shown, that copper from Cu-haematoporphyrin is not available to rats, contrary to the copper in Cu-proteins and Cu-amino acids.

Recent studies on fresh grass, rumen-contents and faeces of cattle, in which the samples were extracted with different solvents, have shown that only a small amount of copper is soluble in acetone (about 3.1, 3.6 and 2.9 % of the total amount of copper in fresh grass, rumen contents and faeces respectively). The conclusion is drawn that the products analysed contain only a small amount of Cu-pheophytin. The presence of other Cu-porphyrins from degradation products of chlorophyll remains possible.

EXPERIMENTAL PART

To study the solubility of copper in different solvents, fresh grass was extracted. 50 g of fresh grass were cut into small pieces (ca. 1–1.5 cm) and immediately covered with acetone. Taking into account the water present in the sample, acetone was added to a final concentration of 90 %. After about one hour extraction under frequent shaking the dark green acetone solution was filtered. The residue was ground in a porcelain mortar with 50 g of Cu-free sand. The ground material was transferred with some acetone (90 %) to a cylindrical separating funnel with a cottonwool plug and a small layer of sand on the bottom, percolated with acetone (90 %) until practically colourless and subsequently with acetic acid (0.1N), HCl(0.001N), HCl(0.01N), HCl(0.05N) and HCl(0.1N) in succession. Each liquid was evaporated and copper was colorimetrically determined in the residues, after destruction with sulphuric-nitric acid, with sodium diethyldithiocarbamate.

The results of the extractions of 5 samples of grass are given in table 1.

Since only a small fraction of total copper was soluble in acetone, only a small amount of Cu-pheophytin can be present in fresh grass if present at all.

The greater part of the coloured compounds were extracted by acetone. From the residues an important fraction of the total was extracted by 0.1N acetic acid. The amount of this fraction differed considerably in the individual herbage samples (30–58.5 %, average: 48 % of the total copper). With 0.001N hydrochloric acid only a small part of the copper was extracted. Using more concentrated hydrochloric acid (0.01N or 0.05N) the main part of the residual copper was dissolved. The acetic acid extracts showed an orange-yellow fluorescense, whereas the hydrochloric acid extracts were yellow.

The solubilities of the copper in grass, rumen contents and faeces from cattle were also compared. A sample of fresh grass from a pasture and samples of rumen contents and faeces from fistulated cows grazing this pasture, were obtained from Dr. J. VAN DER GRIFT ('Instituut voor Veevoedingsonderzoek Hoorn') in August 1963. As soon as possible 100 g of the material were extracted with acetone until a practically colourless filtrate was obtained. The residue was percolated with 250 ml of the acid solutions mentioned in table 2. The pH of the extracts was also determined.

TABLE 1. Extractions of copper from fresh grass.
The extracted copper is given in % of the total Cu.

Sample No.	I		II		III		IV		V	
Date	17/7 '63		17/7 '63		30/7 '63		30/7 '63		30/7 '63	
Total Cu(mg/kg d.m.)	13.9		12.9		9.5		7.9		10.9	
Solvent										
Acetone (90%)		*2.5*		*2.6*		*1.5*		*1.6*		*1.8*
Acetic acid (0.1N)	44.4		27.8		51.6		56.6		46.5	
,, ,,	3.5		2.2		2.4		1.9		1.8	
,, ,,	3.5									
Total		*51.4*		*30.0*		*54.0*		*58.5*		*48.3*
HCl (0.001N)	3.2		1.7		0.7		0.5		0.9	
,, ,,	2.5		0.2							
Total		*5.7*		*1.9*		*0.7*		*0.5*		*0.9*
HCl (0.01N)	19.8		26.3							
,, ,,	8.2		12.2							
Total		*28.0*		*38.5*						
HCl (0.05N)	6.0		11.2		32.2		28.6		37.6	
,, ,,	0.7		4.3		2.5		3.4		3.5	
Total		*6.7*		*15.5*		*34.7*		*32.0*		*41.1*
HCl (0.1N)	1.2		5.2							
,, ,,			3.2							
Total		*1.2*		*8.4*						
Extracted in total		*95.5*		*96.9*		*90.9*		*92.6*		*92.1*

TABLE 2. Extractions of copper from samples of fresh grass, solid rumen contents and faeces (I.v.V., Hoorn 12-8-1963, grazing cows). The extracted copper is given in % of the total Cu.

Sample No.	VI			VII			VIII			IX			X		
Material	Grass			Rumen contents			Rumen contents			Faeces			Faeces		
Total Cu (mg/kg d.m.)	15.7			14.3			16.0			35.9			38.7		
Solvent:	pH			pH			pH			pH			pH		
Acetone (90%)			*2.8*			*3.1*			*2.3*			*1.2*			*1.1*
Acetic acid (0.1N)	4.1	32.2		4.25	0.8		4.25	0.8		4.35	1.8		4.4	2.4	
,, ,,	3.55	2.2		3.5	0.8		3.6	0.1		3.7	0.3		3.7	0.1	
,, ,,	3.0	1.3		3.1			3.2			3.1			3.15		
Total			*35.7*			*1.6*			*0.9*			*2.1*			*2.5*
HCl (0.001N)	3.1	1.5		3.25	1.4		3.4	1.1			2.9		2.9	2.6	
,, ,,	3.0	0.9		3.2	0.6		3.4	0.4			1.4		3.0	1.1	
Total			*2.4*			*2.0*			*1.5*			*4.3*			*3.7*
HCl (0.05N)	2.0	34.9		1.9	24.7		1.9	25.7		1.8	13.3		1.8	11.0	
,, ,,	1.7	6.8		1.7	10.8		1.7	12.6		1.7	6.2		1.7	6.6	
,, ,,	1.65	2.9		1.65	5.2		1.65	4.5		1.65	4.5		1.65	4.1	
,, ,,	1.65	2.4		1.65	3.1		1.65	8.2		1.65	3.5		1.65	2.9	
Total			*47.0*			*43.8*			*51.0*			*27.0*			*24.6*
Extracted in total			*87.9*			*50.5*			*55.7*			*34.6*			*31.9*

The results given in table 2 show that from rumen contents and faeces only a small proportion of total copper is soluble in acetone. This indicates that a very small proportion of total copper can be present as Cu-pheophytin. The copper fraction, soluble in acetic acid (0.1N), being circ. 36% of the total copper in the fresh grass, had almost disappeared in rumen contents and faeces. As it is generally assumed that copper resorption by the animal takes place in the lumen of the small intestine (15), this indicates the formation in rumen and in faeces of less easily dissolving organic – or inorganic – copper compounds.

In rumen contents the total of the fractions extracted with HCl (0.05N) is about the same as in the fresh grass, but the figures in table II give the impression that the extraction is more difficult. It may be concluded that in rumen contents the copper is already distincly less soluble than in fresh grass. The quantities of total copper, remaining in the residue after the extractions are circ. 47% and 67% for rumen contents and faeces respectively.

Further experiments proved that the copper present in the different residues after extraction with acetone and acetic acid (0.1N) is more rapidly and more completely soluble in using acetone, containing 0.05 moles of HCl per litre instead of 0.05N HCl in water. This extraction has been applied to samples of fresh grass, rumen contents (fistulated cows) and faeces. These samples were also obtained from the 'Instituut voor Veevoedingsonderzoek' at Hoorn. They were analysed as follows. The fresh samples were treated with acetone (see table 1). After filtering the residue was air-dried and ground in a hammer-mill. This method is less difficult and tedious than rubbing in a mortar. The ground material (10 g of the residues from grass and rumen contents, 5 g of the faeces-residue) is subsequently percolated with acetone (90%) until practically colourless, with acetic acid 0.1N (2 × 250 ml) and with 500 ml of acetone (90%), containing 0.05 moles of HCl per litre. The analyses are shown in table 3.

TABLE 3. Extractions of copper from samples of fresh grass, solid rumen contents and faeces (I.v.V., Hoorn, 14-10-'63, grazing cows). The extracted copper is given in % of the total Cu.

Sample No. Material Total Cu (mg/kg d.m.)	XI Grass 9.8		XII Grass 9.6		XIII Rumen contents 14.7		XIV Faeces 27.2	
Solvent:	pH		pH		pH		pH	
Acetone (90%)		*6.6*		*5.7*		*5.5*		*4.6*
Acetic acid (0.1 N)	3.95	24.4	4.0	24.4	4.05	2.1	3.95	2.9
,, ,,	3.1	1.1	3.1	1.0	3.3	0.3	3.1	0.4
Total		*25.5*		*25.4*		*2.4*		*3.3*
HCl (0.05N) in acetone (90%)		*63.9*		*66.0*		*90.3*		*92.7*
Extracted in total		*96.0*		*97.1*		*98.2*		*100.6*

It is concluded that with the above method copper from grass as well as from rumen contents and from faeces was extracted completely.

In October 1963 samples of faeces from heifers and from a horse grazing the same pasture were analysed according to the same procedure. The results are summarized in table 4.

There is no significant difference between the amounts of copper in the faeces fractions of the heifers and of the cows (compare with table 3). In the horse's faeces the larger percentage of acetone-soluble copper suggests a difference in the copper metabolism of a horse as compared with that of cattle, although conclusive evidence cannot be drawn from this single observation.

In February 1964 samples were analysed of rumen contents and of faeces from fistulated-cows (I.v.V., Hoorn), fed on a winter-ration without silage or additional copper. The results (table 5) show only small differences in the copper-partition com-

Table 4. Extractions of copper from samples of faeces from heifers and from a horse (20-10-'63). The extracted copper is given in % of the total Cu.

Sample No.		XV			XVI	
Animals		Heifers			Horse	
Total Cu (mg/kg d.m.)		25.0			32.3	
Solvent:	pH			pH		
Acetone (90%)			*3.7*			*15.7*
Acetic acid (0.1N)	3.95	2.4		3.80	3.0	
" "	3.1	0.2		3.0	0.6	
Total			*2.6*			*3.6*
HCl (0.05 N) in acetone (90%)			*90.3*			*75.8*
Extracted in total			*96.6*			*95.1*

Table 5. Extractions of copper from samples of solid rumen contents and faeces (I.v.V., Hoorn, 17-2-'64), (cows on winter ration)

Sample No.	XVII	XVIII	XIX	XX
Material	Rumen contents	Rumen contents	Faeces	Faeces
Total Cu (mg/kg d.m.)	18.0	14.5	47.9	42.3
Solvent:				
Acetone (90%)	3.5	4.3	1.1	1.5
Acetic acid (0.1 N)	4.1	4.1	4.3	5.6
HCl (0.05 N) in acetone (90%)	95.8	90.6	94.2	94.3
Extracted in total	103.4	99.0	99.6	101.4

pared with the samples from the grazing period. There is a slight indication that the copper fraction soluble in acetic acid (0.1N) is somewhat higher in winter.

In order to trace whether copper porphyrins derived from chlorophyll other than Cu-pheophytin occur in rumen contents and in faeces, after the extractions with acetone (90%) and acetic acid (0.1N), the residu was subsequently extracted with a series of different organic solvents. Among these were benzene, chloroform, dioxane, ethyl acetate, ethyl alcohol and ethyl ether. In these extracts only negligible amounts of copper were found. From the above mentioned residue extractions were also made with acetone, to which HCl was added. Copper could be extracted easily with acetone containing 0.05 moles HCl per litre (see table 3, 4 and 5). These extracts had an orange red colour. Especially the extracts from the faeces thus obtained showed an intense fluorescence. After extraction of the acid-acetone fraction with chloroform, and washing the solution in chloroform several times with water, it showed a distinct porphyrin spectrum in faeces from grazing cows only. This spectrum corresponded closely to that of phylloerythrin-monomethylester (see also 2).

Marchlewski (11) already proved that this compound, derived from chlorophyll, is found in the animal only after a diet of fresh green fodder. This porphyrin could not be detected in rumen contents of the animals under grazing or winter conditions, nor in faeces from cows fed on winter rations.

DISCUSSION

The experiments reported prove that fresh grass contains copper, soluble in acetic acid (0.1N). In the rumen of cattle this copper is less soluble. There is an indication, that the percentage of total copper dissolving in acetic acid (0.1N) is somewhat higher in rumen contents and in faeces from cows fed on winter rations than from cows fed on fresh grass. Further practically no differences were found in the distribution of copper in the different extracts between rumen contents and faeces in summer and winter.

The generally accepted poor utilization of copper by cattle during the grazing period might be associated with the formation of copper compounds in the rumen that are more poorly soluble. The question remains why the copper-resorption by cattle seems to be higher during winter than during the grazing period. This difference in resorption probably is so small that until now it has not been clearly reflected in the solubilities of copper in the different solvents.

The poorly soluble copper compounds, formed in the rumen may be of organic and/or inorganic nature. Among the organic substances that may be formed are the Cu-porphyrins (5) which are formed from chlorophyll and its breakdown-products. However, our results show that in rumen contents as well as in faeces only a small proportion of the copper is soluble in acetone, so that only very little Cu-pheophytin can be present. In faeces from grazing cows copper is found in a fraction which also contains a porphyrin. The spectrum of a solution of this porphyrin in chloroform was very similar to that of phylloerythrin-monomethyl ester. It has not yet been proved that this copper is porphyrin bound in faeces. This porphyrin was not found in the samples of winterfaeces and in rumen contents from grass- or barn-fed cows.

Of the inorganic, insoluble copper-compounds the copper sulphide should be considered. SHERMAN *et al.* (19) showed that copper sulphide was unable to promote the haemoglobin formation in rats. They showed that CuS, administred to anaemic rats, could not improve haemoglobin formation, contrary to copper sulphate. Several other authors relate the presence of S-ions in the body to the copper metabolism of the animal (HALVERSON *et al.* (9), MILLS (16)). In this connection it should be noticed that MATSUMOTO and SHIMODA (12) found an increase of H_2S in the rumen of goats during grass-feeding. In our opinion, special attention should be paid to the presence of S-ions which may combine with copper forming the poorly utilizable CuS in the rumen of cattle. In our experiments almost the total amount of copper in rumen contents and in faeces is soluble in acetone (90%), containing 0.05 moles of HCl per litre. As it was found that from pure, freshly prepared CuS much more copper dissolved in 0.05N HCl in acetone (90%) than in 0.05N HCl in water this might indicate that the copper could be partially present as CuS.

The slight effect of the supply of cystine on the blood copper content of cattle, referred to earlier, may be due to the formation of CuS in the rumen, rather than to the complex binding of copper by cystine. SCHULZE *et al.* (21) also found that Cu as cysteine cupro-mercaptide was easily utilized by the rat, while Cu as CuS was a rather poor Cu-source.

SUMMARY

Samples of fresh grass, rumen contents and faeces of cattle were extracted with different solvents. In the fractions obtained copper was colorimetrically determined after destruction with sulphuric and nitric acid. Cu-pheophytin was found in very small amounts only. From faeces of grazing cows a fraction was obtained containing a *porphyrin* (probably phylloerythrin-monomethylester) and copper. This porphyrin

could not be detected in rumen contents from grazing animals, nor in rumen contents and faeces from barn-fed cows.

In fresh grass a part of total copper (average 48 %) is soluble in acetic acid 0.1 N. Already in the rumen this copper has been converted into a less soluble form, possibly CuS. The results of our extraction-experiments are not in disagreement with the supposition that a great proportion of total copper is present as CuS in the rumen.

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LE BILAN IONIQUE DANS LE DIAGNOSTIC FOLIAIRE

with summary

W. Dijkshoorn

Considérations biochimiques sur l'accumulation des ions salins

Pour la classification des substances salines contenues dan les tissus on s'accoutumerait d'exprimer les teneurs en équivalents électrochimiques, parce que cette façon de faire est nécessaire pour l'interprétation du bilan ionique. On devrait aussi se rendre compte de la présence ou de l'apparition des ions acidiques ou basiques pendant l'accumulation et l'utilisation des ions salins neutres du point de vue de l'acidité. L'application de la théorie de Brönsted sur les couples acides-bases apporte des simplifications importantes dans ce cas. Voir à ce sujet le résumé clair qu'a donné G. Charlot dans son livre 'Théorie et méthode nouvelle d'analyse qualitative', 2me Ed., Paris 1946.

L'expérience a conduit à supposer qu'il y a interférence des ions H^+ et OH^- de l'eau dans l'absorption des ions par la plante. On peut admettre, en effet, que les cations et les anions des sels nutritifs seront absorbés indépendamment les uns des autres, en introduisant OH^- et H^+ comme les ions compensateurs au point de vue de l'électroneutralité.

Il est commode de supposer que l'absorption d'un cation salin entraine l'absorption de OH^-, soit HCO^-_3, fourni par l'eau. Ainsi, l'absorption d'un anion salin se produit en association à l'absorption de son équivalent de H^+. (Il en est de même, si au lieu d'envisager cette absorption de OH^- et H^+, on admet l'échange de H^+ et de OH^- pour des ions salins du milieu.)

Dans le cas d'absorption d'un cation et d'un anion salin en quantités égales il en résulte que les ions H^+ et HO^- se neutralisent. Si on calcule la différence (cations salins – anions salins absorbés) on reconnait que si cette différence est positive, des ions OH^- sont absorbés en excès, et si elle est négative, les ions H^+ sont absorbés en excès.

Bien que l'absorption d'un excès des ions OH^- ou H^+ se produit souvent en quantités bien supérieures au pouvoir tampon du tissu, le pH du tissu n'est presque pas influencé. Cela est dû à ce que l'absorption des ions OH^- provoque une synthèse progressive des acides organiques qui neutralisent les ions OH^- absorbés, tandis qu'une absorption des ions H^+ libres provoque une décarboxylation progressive des sels organiques aux fonctions carboxyliques qui produit l'alcalinité pour la neutralisation des ions H^+ absorbés. Ainsi, la synthèse ou la dégradation des anions organiques aux fonctions carboxyliques a pour effet de s'opposer à une variation de pH des tissus lorsqu'il y a de différence entre l'absorption des cations et celle des anions des sels nutritifs (Böning et Böning-Seubert, 1932, Burström, 1945).

Voici maintenant un résumé des résultats expérimentaux d' Ulrich (1941) et des autres savants américains sur des racines isolées de l'orge.

Supposons un tissu végétal capable d'absorber des ions salins. En vertu même de cette faculté il disposerait de toutes ses facultés métaboliques pour la synthèse et la dégradation des anions organiques aux fonctions carboxyliques.

En plaçant le tissu dans l'eau exempte de sels, nous verrons que la respiration s'effectuera à une vitesse constante pendant plusieures heures, avec une dégradation complète des sucres à une quotient respiratoire égale à l'unité. Nous pourrons ensuite vérifier que la teneur en anions organiques et le pH du tissu demeurent constant pendant ces quelques heures de l'expérience.

Ce résultat s'explique par le fait que le tissu ne peut pas s'enrichir, ni s'appauvrir en anions carboxyliques, soit par carboxylation: $RH + CO_2 \rightarrow RCOO^- + H^+$ à un Q.R. inférieur à l'unité, soit par décarboxylation: $RCOO^- + H^+ \rightarrow RH + CO_2$ à un Q.R. supérieur à l'unité, sans que l'acidité libre H^+ au sein du tissu soit changé. L'expérience montre, en effet, que la réaction du tissu du point de vue de l'acidité ne change pas. Voyons maintenant ce qu'il advient lorsqu'un tissu soit placé dans une solution saline. En fournissant SO_4K_2, l'absorption de K se produit souvent en excès. Selon la règle de l'électroneutralité les equivalents de K^+ absorbés en excès apportent un nombre égale des ions OH^- ou HCO_3^-. Le tissu s'enrichit donc d'alcali libre égale à la différence entre les équivalents K^+ et les équivalents SO_4^- absorbés. D'autre part, une solution de Cl_2Ca peut donner lieu à une absorption des ions Cl^- en excès. Dans ce cas, l'électroneutralité nécessite une absorption des ions H^+ égale à l'excès des ions Cl^- dans les équivalents Cl^- et Ca^{++} absorbés. Dans le cas de l'absorption des ions salins, l'expérience montre encore que le pH du tissu reste le même par deux sortes de transformations. Dans le premier cas, la teneur en anions organiques s' augmente par carboxylation: $K^+ + OH^- + CO_2 + RH \rightarrow RCOO^- + K^+ + H_2O$ de sorte qu'elle reste égale à la différence entre les cations salins et les anions inorganiques au sein du tissu. La synthèse des fonctions carboxyliques s'annonce par une diminution du rapport CO_2 dégagé/O_2 consommé dans la respiration: Q.R. <1. Dans le deuxième cas, l'absorption des ions H^+ associés à l'excès des anions salins, la teneur en anions organiques se diminue par décarboxylation et les anions HCO_3^- libérés, comme base assez forte, neutralisent les ions H^+ absorbés, selon: $RCOO^- + K^+ + H^+ + Cl^- \rightarrow RH + K^+ + Cl^- + CO_2$. La décarboxylation s'annonce par un Q.R. > 1.

On a donc trouvé par l'expérience que l'absorption des ions $OH^-(HCO_3^-)$ associée à celle des cations salins K^+, Na^+ etc. ne signifie pas une accumulation d'alcalinité dans le tissu, parce que les anions basiques OH^- ou HCO_3^- sont transformés en des anions nonbasiques aux fonctions carboxyliques. En ce qui concerne l'absorption des ions H^+ associée à celle des anions salins, elle ne signifie pas une acidification interne, parce que les ions H^+ deviennent neutralisés par HCO_3^-, anion assez basique, produit par décarboxylation des anions carboxyliques.

1e Conclusion:

L'accumulation des ions salins régit le métabolisme anions organiques qui, en luttant contre l'acidité ou l'alcalinité absorbé, tend à diminuer l'amplitude des fluctuations de pH du tissu par une régulation de la teneur en anions organiques égale à la différence entre les cations salins (C^+) et les anions salins inorganiques (A^-) au sein du tissu. Lorsqu'un cation salin est absorbé la teneur en anions organiques s'augmente et lorsqu'un anion salin est absorbé la teneur en anions organiques se diminue. Le pH du tissu ne dépasse pas le domaine normal de pH 5 à 6 et la teneur en anions organiques demeure égale à ($C^+ — A^-$).

Vaccinium et *Rhododendron*, qui exhibitent un pH des tissus plus acides (pH 3 à 4) à une alimentation ammoniacale deviennent plus alcalines quand ($C^+ — A^-$) augmente par alimentation nitrique, et la croissance devient d'autant plus diminuée que le pH du tissu s'augmente vers pH 5 et la teneur en ($C^+ - A^-$) croit. (Cain, 1954; Colgrove et Roberts, 1956). Mais ce sont là, semble-t-il, des cas exceptionnels parmi les plantes de culture. De semblables phénomènes interviennent probablement dans le 'lime induced chlorosis', les *Ericaceae* en représentant des plantes extrêmement sensitives.

CONSIDÉRATIONS BIOCHIMIQUES SUR L'UTILISATION DES IONS SALINS

Pour la construction des bilans ioniques il n'est pas nécessaire de préciser les étapes parcourues pendant les transformations de NO_3^- et de $SO_4^=$ en des matériaux azotés et sulphurés organiques. Il suffit que l'on peut les schématiser par les équations générales: $NO_3^- + 8\,H \rightarrow NH_3 + 2\,H_2O + OH^-$ et $SO_4^= + 8\,H \rightarrow SH_2 + 2\,H_2O + 2\,OH^-$.

L'ensemble de ces réactions constituent les processus métaboliques de l'utilisation des nitrates et des sulphates, du point de vue du bilan ionique. Quelque soit la nature des composés N et S organiques, rappelons que leur caractéristique dominante est d'être dérivés de NH_3 et de SH_2.

Il en résulte la formation de la base NH_3, de l'acide assez faible SH_2 et de la base assez forte, l'anion OH^-, qui remplace les anions NO_3^- et $SO_4^=$ neutres du point de vue de l'acidité. Le nombre des équivalents OH^- libérés est nécessairement égal à cel des equivalents NO_3^- et $SO_4^=$ consommés par le métabolisme.

Nous avons déjà exposé que l'absorption de NO_3^- signifie l'absorption simultane d'un ion H^+ selon la règle de l'électroneutralité. Pendant l'accumulation des ions NO_3^- les ions H^+ deviennent neutralisés par l'alcali produit par décarboxylation. L'accumulation de NO_3^- se produit donc suivant: $H^+ + NO_3^- + RCOO^- + K^+ \rightarrow RH + CO_2 + K^+ + NO_3^-$, c'est-à-dire, elle provoque une diminution égale de la teneur en sels organiques, comme celle des autres anions inorganiques des acides forts inorganiques, Cl^-, $H_2PO_4^-$, $SO_4^=$.

Si l'accumulation de NO_3 est suivi par une complète réduction métabolique, les ions OH^- produits neutralisent les ions H^+ absorbés totalement et ce processus demeure donc sans une effet sur les anions organiques dans les tissus.

Les processus de l'accumulation et de l'utilisation des ions $SO_4^=$ peuvent être représentés par des réactions analogues.

2e Conclusion:

Bien que l'accumulation des ions NO_3^- et $SO_4^=$ comporte une diminution de la teneur en anions organiques dans les tissus, analogue à la diminution provoqué par les autres anions salins inorganiques, l'accumulation suivi par leur transformation complète en des composés N et S organiques se réalise sans aucune production ou consommation des anions organiques.

Il faut chercher maintenant si la transformation des premières étapes de l'élaboration des ions NO_3^- et $SO_4^=$, le NH_3 et le SH_2, en substances organiques azotées et sulphurées, pourrait influencer la teneur en anions organiques.

NH_3 est la base du couple acide-base $NH_4^+ \rightarrow NH_3 + H^+$, et la constante d'acidité corresponde à $pK = 9.2$. L'état d'acidité commun des tissus végétaux est de pH 5 à 6 et la base NH_3 n'existerait donc que sous forme de l'acide NH_4^+. Le passage de NH_3 à NH_4^+ demande donc la synthèse d'un acide organique qui donne naissance à un sel organique de NH_4^+ selon: $NH_3 + RCOO^- + H^+ \rightarrow RCOO^- + NH_4^+$. Une accumulation de sels ammoniacaux à une teneur plus élevée peut exister dans certaines plantes *(Begonia)*, mais plus souvent on trouve que la teneur en ions NH_4^+

demeure relativement faible, c'est-à-dire, $\leq$ 20 me. par kg de matière sèche. Cela résulte du fait que les sels organiques ammoniacaux sont transformés en amides: $RCOO^- + NH_4^+ \rightarrow RCONH_2 + H_2O$, et aminoacides: $RCH_2OHCOO^- + NH_4^+ \rightarrow RCH_2NH_3^+COO^- + H_2O$ qui donnent naissance à des 'zwitterions', grâce à leur pK's de 9 et 3 environ. La plupart des aminoacides est ensuite transformée en matières protéiques par condensations des fonctions RNH_3^+ et $RCOO^-$, en donnant des liaisons amidées et en dégageant de H_2O.

Le SH_2, formé par la réduction des ions $SO_4^=$, peut être consideré d'être transformé en thiol par condensation avec le fonction hydroxyl d'un hydroxyaminoacide, en donnant naissance à de cysteine etc.

L'assimilation de l'azote NH_3 et du soufre SH_2, premiers produits de la réduction métabolique des ions nitrate et sulphate, sous formation de diverses composés organiques de N et S procède donc sans gain ou perte en anions organiques.

On peut de façon analogue représenter l'utilisation du cation NH_4^+, absorbé dans le cas de l'alimentation ammoniacale.

L'absorption de NH_4^+, comme celle des autres cations salins, comporte une absorption d'un ion OH^-. L'accumulation de NH_4^+ dans le tissu se produit donc sous forme de sel organique selon: $NH_4^+ + OH^- + CO_2 + RH \rightarrow RCOO^- + NH_4^+ + H_2O$, et la transformation du sel organique d'ammonium se passe ensuite suivant les réactions que nous venons de discuter, au cours de sa transformation en aminoacides etc.

3e Conclusion:

L'accumulation de NH_4^+ donne naissance à un sel organique d'ammonium, qui, ensuite, donne par déshydration un aminoacide ou amide. L'utilisation de NH_4^+ se réalise donc sans gain ou perte en anions organiques.

CONSTITUTION DES BILANS IONIQUES

Occupons-nous maintenant avec la détermination du bilan ionique. Comme cations salins nous considérons les ions K^+, Na^+, Mg^{++} et Ca^{++}. En effet, l'indication de la présence de traces d'autres cations salins est sans valeur par leur ordre de grandeur relative.

Etant donnée la neutralité des tissus au point de vue de l'acidité, c'est-à-dire un pH des tissus dans la région normale de pH 5 à 6, la teneur en anions totales, inorganiques et organiques, serait donc numériquement égale à la somme des équivalents des cations salins $C^+ = K^+ + Na^+ + Mg^{++} + Ca^{++}$.

Pour obtenir une approximation de la teneur en sels inorganiques, il est nécessaire de connaître le nombre des équivalents de Cl^-, $H_2PO_4^-$, $SO_4^=$ et NO_3^-. En faisant la somme des équivalents indiqués ci-dessus, qui corresponde avec une bonne approximation aux états ioniques actuels, on obtient la teneur totale en sels inorganiques A^-. Il est facile d'introduire le phosphate total dans les sels inorganiques, l'estérification par le métabolisme n'intervienne pas dans la forme ionique: $H_2PO_4^-$, $RHPO_4^-$, $R_2PO_4^-$, et par cette façon de faire la teneur en sels organiques aux fonctions carboxyliques soit donnée par la différence ($C^+ - A^-$). Les règles précédentes ne sont qu'approchées. Par exemple, un pH des tissus de 7 indique qu'on doit se rendre compte de la présence des ions $HPO_4^=$, parce que le pK_2 de l'acide phosphorique est de 7 environ (COOIL 1948).

Les données analytiques qui se trouvent dans un bilan ionique de ce genre est donc restreint aux teneurs en cations salins totals K, Na, Mg et Ca, et à des teneurs en anions inorganiques Cl total, P total, $SO_4^=$ inorganique et NO_3^- inorganique, dans les tissus.

Nous avons reconnu que l'assimilation de soufre procède proportionellement. à celle de l'azote (Dijkshoorn, Lampe et van Burg 1960). Des données analytiques sur le soufre total (S_t), l'azote total (N_t) et le nitrate, permettent de calculer la teneur en $SO_4^=$ suivant: $SO_4^= = S_t - 0.054\ (N_t - NO_3^-)$, en exprimant toutes les valeurs comme d'équivalents des formes ioniques indiquées ci-dessus qui représentent les étapes ioniques primitives de l'utilisation des éléments.

4e Conclusion:

La construction d'un bilan ionique consiste à déterminer les teneurs en K, Na, Mg, Ca, Cl, P, $SO_4^=$ inorganique et NO_3^- dans les végétaux, à exprimer les résultats en équivalents ioniques et à faire la somme des cations salins C^+et des anions salins inorganiques A^-. Parce que les recherches de l'azote et du soufre total sont plus courantes dans les laboratoires que celles des ions sulphate inorganiques, il est utile de connaître la relation entre l'azote organique et le soufre organique qui permet de calculer le $SO_4^=$ des données sur l'azote total, le nitrate et le soufre total.

LA TENEUR EN SELS ORGANIQUES NORMALE

Les considérations qui précèdent nous montrent d'une façon tangible que l'on peut envisager une relation entre l'accumulation des ions inorganiques empruntés des sels nutritifs et le métabolisme acides organiques. D'ailleurs, elles nous intéressent à un autre point de vue. Notre but est de préciser comment les principes pourraient être applicable au diagnostic foliaire, des applications que nous allons nous efforcer de préciser. Des travaux effectués au cours de ces dernières années dans le domaine des bilans ioniques tendent à établir de plus en plus nettement que la teneur en sels organiques ($C^+ - A^-$) serait remarquablement caractéristique pour les diverses espèces de plantes.

Des valeurs trouvées par divers auteurs ou calculées de leur chiffres, et des résultats expérimentaux de notre laboratoire, viennent de montrer que, pour chacune des espèces de plantes, les diverses valeurs coincident d'une manière vraiment remarquable. Ces valeurs se rapportent aux échantillons de feuilles obtenues après la croissance à une alimentation normale et seront donc compatible avec la croissance optimale.

La signification que l'on peut donner à ces valeurs normales est, selon le théorème de de Wit, Dijkshoorn et Noggle (1963), d'une des conditions pour la croissance optimale.

Par une considération des données analytiques et des poids de récolte nous avons reconnu que, suivant les circonstances de l'alimentation, la teneur en ($C^+ - A^-$) peut varier d'une manière continue des valeurs plus basses aux valeurs plus hautes que la grandeur normale, mais cette continuité se manifeste seulement dans la zone des récoltes plus basses. Nous devons donc noter qu'une croissance retardée peut être associée avec une teneur en (C^+-A^-) normale, mais, en outre, des valeurs plus basses et plus hautes peuvent exister. Ceci nous amène à supposer qu'une croissance optimale n'est pas nécessaire pour que la teneur en (C^+-A^-) normale puisse se produire. Mais, d'autant que nous étendons notre enquête vers la zone de la croissance optimale, nous reconnaissons que les fluctuations de (C^+-A^-) autour de la valeur normale deviennent d'autant plus faibles. C'est donc en définitive la teneur normale en (C^+-A^-) qui permet l'apparition de la croissance optimale. Plus la teneur en (C^+-A^-) diffère de celle-ci, plus nombreuses aussi sont les possibilités d'apparition d'une croissance retardée. Il faudrait donc admettre que la coincidence de la croissance optimale et la teneur normale en (C^+-A^-) est obligatoire.

5e Conclusion:

Nous convenions d'appeler 'teneurs en sels organiques aux fonctions carboxyliques (C^+–A^-) normales' toutes celles qui se rapprochent de la teneur moyenne à croissance optimale. Il existe des teneurs en (C^+–A^-) infra et supranormales qui sont toujours associées à une croissance suboptimale. Une teneur normale peut être associée à une croissance suboptimale, mais plus que la croissance finira par attendre l'optimum, plus que les fluctuations possibles de la valeur normale deviennent faible. Une teneur normale en (C^+–A^-) est donc nécessaire pour que la croissance puisse se produire à sa vitesse optimale. La teneur en (C^+–A^-), parmi des autres facteurs, joue donc le rôle d'un facteur de croissance et sa mesure peut contribuer au progrès du diagnostic foliaire.

Cette interprétation elle-même reste toujours une tache fort délicate et on s'en acquittera d'autant mieux qu'on connaîtra plus complètement la façon dont se comporte l'espèce sur laquelle on porte son enquête. Nous croyons bien faire de prendre quelques exemples concrets.

EFFET DE LA FORME IONIQUE DE L'AZOTE DES SELS NUTRITIFS

On a très souvent comparé l'alimentation nitrique à l'alimentation ammoniacale. De telles expériences nous enseignent que l'alimentation ammoniacale est liée à une teneur en sels organiques (C^+–A^-) plus basse que sa valeur normale. En outre, la plupart des plantes de cultures exhibitent une croissance inférieure à celle obtenue à l'alimentation nitrique. On sait donc que si l'on fournit NH_4^+ au lieu de NO_3^-, la teneur en (C^+–A^-) se diminue a une valeur inférieure à sa valeur normale et, plus souvent, la croissance devient retardée.

Nous avons admis dans les considérations précédentes que l'utilisation complète de NH_4^+ et de NO_3^- se réalise sans gain ou perte en anions organiques. On aurait donc tort d'admettre que l'utilisation de NO_3^- serait une nécessité biochimique pour la synthèse des anions organiques au sein du tissu (CHOUTEAU 1960). La seule conclusion que nous serions en droit de tirer des expériences est que la plus faible teneur en anions organiques à l'alimentation ammoniacale résulte d'une diminution de la faculté d'accumuler les cations salins du genre C^+, c'est-à-dire K^+, Na^+, Mg^{++} et Ca^{++}. De ce que l'on observe, la quantité de l'azote absorbé est d'un ordre de grandeur d'une ou plusieurs fois celle des cations salins C^+.

Dans le cas de l'absorption des ions NH_4^+ en quantités relativement si grandes l'accumulation des cations C^+ ne s'effectue qu'en quantités beaucoup plus faibles par le phénomène de concurrence au cours de l'absorption, qui intervient au niveau du système d'absorption des cations salins, y compris les ions NH_4^+. En fournissant des ions NO_3^- au lieu de NH_4^+ cette concurrence n'existe plus que parmi les ions K^+, Na^+, Mg^{++} et Ca^{++} et, probablement, H^+.

Une série d'expériences, dont plusieurs sont assez récentes, vient donner à cette postulate la valeur d'une chose démontrée.

6e Conclusion:

Si on ajoute des ions NH_4^+ au lieu des ions NO_3^- aux sels nutritifs, la teneur en sels organiques (C^+–A^-) des tissues s'est abaissée, parce que le pouvoir d'absorber des cations K^+, Na^+, Mg^{++} et Ca^{++} du genre C^+, accumulés sous forme de sels organiques, décroît par une absorption concomitante des cations NH_4^+, qui ne laissent aucune place à l'accumulation des sels organiques grâce à leur transformation en azote organique.

LE BILAN IONIQUE CHEZ *Lolium perenne*

Nous avons conduit de nombreuses expériences sur le comportement de *Lolium perenne* (SAID 1959; DIJKSHOORN 1962, 1963; DE WIT, DIJKSHOORN et NOGGLE 1963; DIJKSHOORN et LAMPE 1962). Il ressort de ces expériences que la teneur normale en sels organiques (C^+–A^-) est de 1000 me. par kg de matière sèche environ.

En replaçant le K par Ca ou par Na dans les sels nutritifs, il s'établit au bout d'un certain temps de croissance une carence en K par dilution au niveau de besoin spécifique qui est de 200 me.K/kg m.s. environ. Dans le cas que Ca soit donné au lieu de K, cette carence est caractérisée par une teneur en sels organiques (C^+–A^-) de 500 me./kg m.s. environ. Cette teneur abaissée se produit par une absorption plus faible des cations salins car l'absorption de Ca ne se fait qu'à une vitesse beaucoup plus lente que celle de K, l'absorption des ions H^+ s'augmente et les sels organiques vont donc disparaître. En donnant Na au lieu de K, une carence en K se développe à une teneur en sels organiques (C^+–A^-) de 2000 me./kg m.s. environ. Cette valeur excessive de la teneur en (C^+–A^-) résulte d'une accumulation des ions Na en grand excès dans l'herbe carencée en K (VOSE 1963).

Bien que les teneurs en (C^+–A^-) soient assez différentes, les deux états sont des carences spécifiques en K, parce que l'un et l'autre exhibite une croissance retardée qu'on peut modifier en une croissance optimale par l'adjonction des ions K aux sels nutritifs. Dans le premier cas, cela effectue une diminution des ions H^+ absorbés, ions provoquant la dégradation des anions organiques. Dans le deuxième cas, le K donné diminue l'absorption des ions Na par concurrence, ions conduisant à une accumulation excessive des sels organiques de Na dans l'herbe de *Lolium perenne* carencée en K.

Nous pourrons donc réaliser le cycle suivant chez *Lolium perenne*. Commençons une culture à alimentation normale et à croissance optimale. La teneur en (C^+–A^-) serait de 1000 me./kg m.s., c'est-à-dire, d'une valeur normale. Pendant des récoltes successives ajoutons des sels nutritifs sans K et sans Na, en donnant le nitrate sous forme de nitrate de calcium. L'herbe deviendra carencée en K, par épuisement progressif de K, et la croissance s'arrêtera par déficience en K à une teneur en (C^+–A^-) abaissée parce que Ca est donné au lieu de K. Ensuite, donnons de nitrate de sodium et une faible quantité de K pour rendre possible la croissance progressive à une teneur en K au niveau de besoin spécifique (200 me./kg m.s.). Tandis que la croissance demeure suboptimale par carence en K, il nous sera possible d'arriver à une situation que la teneur en (C^+–A^-) va constamment en augmentant, elle croit jusqu'à la valeur normale de 1000 me./kg et, ensuite, dépasse cette valeur et tend vers la valeur excessive de 2000 me./kg., à une teneur tres élevée en Na. L'herbe serait donc passée de l'un à l'autre état de carence en K et, à croissance suboptimale, a parcouru toutes les valeurs de la domaine des teneurs en (C^+–A^-) possibles. Ensuite, nous pourrons augmenter la croissance en ajoutant de K et, pendant des récoltes successives, elle finira par attendre sa vitesse optimale à une teneur normale en (C^+–A^-) de 1000 me./kg de matière sèche. Nous avons donc obtenu l'état initial de (C^+–A^-) normale et d'une croissance optimale de l'herbe.

LE BILAN IONIQUE CHEZ *Nicotiana tabacum*

Voyons maintenant comment varie la teneur en sels organiques (C^+–A^-) chez le tabac. De nombreuses expériences sont effectuées sur le tabac (BÖNING et BÖNING-SEUBERT, 1932; PUCHER, VICKERY et WAKEMAN, 1938; VLADIRIMOV, 1945; HUTCHESON, WOLTZ et MCCALEB, 1959; CHOUTEAU, 1960; VICKERY, 1961) et on a trouvé que la carence en K se traduit souvent par un accroissement de la teneur en (C^+–A^-), annoncé par une augmentation de la pouvoir-tampon des liquides du tissu dans le domaine pH de 3 à 4, par l'alcalinité des cendres ou par des données analytiques sur les constituants ioniques inorganiques et organiques.

Le tabac diffère de *Lolium perenne* de la façon dont se comportent les ions Na, Mg et Ca pendant l'absence de K. Si au lieu de K nous apportons de Na avec Mg et Ca, *Lolium perenne* retient un excès de sels organiques de Na, tandis que les feuilles de tabac s'enrichissent d'un excès de sels organiques de Ca et Mg au dessus de la teneur normale.

En opérant sur ces deux espèces, élevées en terre carencée en K, nous trouverons donc plus souvent que la croissance s'arrête à une teneur en (C^+–A^-) abaissée chez *Lolium perenne* et augmentée chez le tabac, parce que les sols communs fournissent beaucoup de Ca et peu de Na.

Un cycle de l'allure que nous venons de discuter pour *Lolium perenne*, peut être effectué de la manière suivante chez le tabac. Les effects ne portent malheureusement pas sur des données expérimentales directes, mais montrent toutefois que l'on peut expecter des résultats expérimentaux sur l'effet des sels nutritifs différentes.

Commençons une culture à l'alimentation normale et à une croissance optimale. La teneur en (C^+–A^-) des feuilles serait de 3000 me./kg de m.s. qui correspond à la valeur normale chez le tabac¹).

Déplaçons le nitrate de K par $(NH_4)_2SO_4$ dans les sels nutritifs. Pendant la croissance, les feuilles deviennent carencées en K et la teneur en cations salins C^+ s'abaisse par l'effet de concurrence des ions NH_4^+ absorbés par le système d'accumulation des cations. Les ions NH_4^+ sont transformés en des composés azotés organiques sans accumulation des sels organiques ammoniacaux. La teneur en cations salins C^+ est diminuée d'où résulte une teneur en sels organiques (C^+–A^-) beaucoup plus faible que normale. La croissance s'arrête par carence en K et par l'alimentation ammoniacale au lieu de nitrate. Ensuite, deplaçons le $(NH_4)_2SO_4$ par $Ca(NO_3)_2$ dans les sels nutritifs et ajoutons des faibles quantités de K nécessaires pour qu'une croissance à une teneur en K égale à la besoin spécifique puisse se produire. Une croissance, retardée par carence en K, se produit et la teneur en (C^+–A^-) s'augmente, dépasse la valeur normale et finit par atteindre une valeur plus haute que normale, associée avec une accumulation excessive des ions Ca dans les feuilles. Enfin, ajoutons des ions K aux sels nutritifs en quantité suffisante pour que la carence en K disparaîsse et la croissance devienne optimale. Nous constatons que la teneur en (C^+–A^-) atteint sa valeur normale de 3000 me./kg de m.s., et l'état initial s'établit.

LA VITESSE RELATIVE DE LA RÉPARTITION INTERNE DES CATIONS SALINS

Bien que les expériences entreprises à cette fin soient effectuées avec de diverses espèces de plantes, les résultats ont montré toujours que la vitesse de rédistribution des ions Na, Mg et Ca demeure petite vis-à-vis de la prodigieuse messagère qu'est le K. RIPPEL (1921) a étudié l'épuisement des branches isolées de Salix par les feuilles et MCALISTER et KROBER (1951) celle des cotylédons par les jeunes plantes de *Glycine maxima*, et les résultats indiquent que les ions K sont transportés de préférence aux autres cations salins.

La répartition des cations salins parmi les feuilles de tabac (VICKERY, 1961) et les étages hautes et basses de l'herbe de prairie indique que les ions K sont distribués plus

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¹) La teneur en (C^+–A^-) varie de 2500 à 3000 me./kg de m.s., selon la position des feuilles, les feuilles basses en montrent une valeur plus élevée avec une plus forte teneur en Ca (BÖNING, HUTCHESON, CHOUTEAU, VICKERY).

également, tandis que le vieillissement des feuilles favorise l'accumulation des ions Ca.

7e Conclusion

Comme résultat des observations sur la rédistribution des cations salins dans la plante on a ce qu'on puisse appeler la gradation relative des mobilités ioniques, qui s'exprime par l'inégalite $K \gg Na, Mg > Ca$.

On a souvent observé une relation entre la teneur en Ca et celle en anions organiques chez les plantes (ILJIN, 1938,) qui doit avoir son origine dans l'immobilité des ions Ca au sein des tissus.

Dans le premier chapitre nous avons discuté que l'accumulation des cations salins du genre C^+ signifie l'accumulation sous forme de sels organiques de K, Na, Mg et Ca. Parmi ces derniers on doit distinguer ceux qui passent facilement à travers des tissus. Pendant la croissance et la différenciation des feuilles, tiges et racines ils pénètrent alors librement et rapidement dans toutes les parties qui se forment.

L'ascension et la descension dans les tissus conducteurs peut se produire facilement, de sorte qu'une teneur plus grande au niveaux des tissus adultes n'existe pas. Ainsi se comportent les ions K. Les ions Ca tendent à rester accumulés sur place, parce qu'ils s'opposent plus effectivement à la rédistribution. La teneur en Ca devient souvent beaucoup plus élevée par rapport à la matière sèche dans les feuilles adultes, par l'épuisement des tissus en matières organiques comme des glucides, des matières azotées etc., qui sont transportées vers les parties plus jeunes. Il en résulte que les feuilles adultes exhibitent souvent une plus haute teneur en Ca, associée avec une teneur élevée en cations salins balancés par des anions organiques (HUTCHESON, CHOUTEAU, VICKERY).

Il est donc inutile de vouloir discuter la relation entre Ca et les anions organiques du point de vue d'une toxicité des acides organiques, oxaliques etc. (TRUOG et MEACHUM, 1919 e.a.). Nous n'en retenons qu'un fait: l'immobilité des ions Ca se manifeste souvent par une accumulation augmentée dans les tissus plus agés, par rapport à la matière sèche, et cette accumulation procède souvent à une teneur plus haute des cations salins sous forme de sels organiques, avec une prépondérance relative des sels de Ca.

LE RÔLE DES IONS K DANS LA RÉGULATION DU BILAN

Nous venons de voir que la carence en K en présence de Na amène une teneur en $(C^+ - A^-)$ plus haute que normale sous forme de sels organiques de Na chez *Lolium perenne*. Cette règle n'est valable que pour les Graminées qui peuvent absorber des ions Na facilement en l'absence de K. Chez le maïs carencé en K la teneur en $(C^+ - A^-)$ demeure plus basse que normale même en présence de Na, parce que le maïs est incapable d'absorber Na. Grâce à leur sélectivité les Graminées sont incapable d'accumuler de plus grandes quantités de Ca et Mg et un excès en sels organiques des ions Mg et Ca n'existe pas, même quand on remplace les ions K par Ca ou Mg dans les sels nutritifs. En vertu même de leur sélectivité entièrement différente le tabac, et la majorité des Dicotyledones sont capable de remplacer une déficience en K par des ions Ca, et souvent des ions Mg, au niveau des feuilles.

L'augmentation de la teneur en $(C^+ - A^-)$ qui en résulte, proviendrait du fait que les ions déplaçant (Na chez *Loliume perenne*, Ca chez le tabac) ont une vitesse de rédistribution assez faible. Selon MASON and MASKELL (1931) les ions K peuvent être transportés par les tubes criblés des tissus conducteurs du niveau des feuilles aux racines chez le cottonier, tandis que les ions Ca résistent à ce transport. Cette comportement des ions K permet une circulation interne en deux sens, des racines aux feuilles et vice versa, et constituerait l'un des mécanismes regulateurs qui empêcherait l'accumulation d'un excès de sels organiques au niveau des feuilles.

8e Conclusion:

Si une plante est capable de remplacer K par des ions Na, Mg ou Ca au sein même du tissu, une déficience en K entrainerait une accumulation plus élevé que normale des cations salins sous forme de sels organiques. Si une plante, incapable de cette remplacement est soumise à une déficience en K, l'absorption des ions H^+ s'augmenterait et la teneur en sels organiques deviendrait plus basse que normale.

La régulation de la teneur en (C^+–A^-) à sa valeur normale dépend donc de la présence des ions K dans une quantité suffisante. Leur principe spécifique serait de rendre possible une récirculation des sels organiques de K, élaborés en excès au niveau des feuilles et destinés aux racines, d'où, après décarboxylation, ils peuvent émigrer au dehors sous forme de bicarbonate de potassium. La grande vitesse de récirculation des ions K permet donc une synthèse des sels organiques (C^+–A^-) assez grande et un drainage effectif d'un excès de sels organiques au niveau des feuilles.

9e Conclusion:

Les ions K se distinguent par une vitesse d'absorption et de drainage au niveau des feuilles assez grande pour la régulation de la teneur en (C^+–A^-) à sa valeur normale. L'adjonction des ions K serait donc suffisante pour empêcher une accumulation des sels organiques soit excessive, soit déficiente vis-à-vis de sa teneur optimale pour la croissance.

RÉSUMÉ

Les résultats donnés dans la présente communication sont fondés sur des considérations théoriques et des démonstrations d'ordre expérimental concernant l'accumulation et le métabolisme des ions salins pendant la croissance optimale et retardée. La lecture des conclusions, données dans les chapitres successifs, permet d'en tirer un résumé.

SUMMARY

The accumulation of salt ions governs the metabolic production of organic anions, which neutralizes any acidity or alkalinity of absorption and tends to diminish any fluctuations in tissue pH by regulating the organic anion content at a value equal to the difference between salt cation equivalents (C^+) and inorganic salt anion equivalents (A^-) in the tissues (ULRICH, 1941).

Accumulation of inorganic NO_3^- and of inorganic $SO_4^=$ (and of other inorganic salt anions) lowers the organic anion content (C^+–A^-). Accumulation, followed by a complete transformation into organic N and organic S, does not influence the organic anion content of the tissues (DIJKSHOORN, 1962). Accumulation of NH_4^+ occurs as an organic ammonium salt which is in most cases more or less completely transformed into amides and amino acids. Utilization of NH_4^+ therefore proceeds without effect on the organic anion content. The lower organic salt content of NH_4^+ fed plants, compared with those receiving NO_3^-, results from a lowered accumulation of the salt cations C^+ by competition with NH_4^+.

Construction of the complete ionic balance involves analyses on K, Na, Mg, Ca, Cl, P, inorganic $SO_4^=$ and NO_3^- in the plant material, expressed as equivalents, and calculation of the sum of the salt cation equivalents (C^+) and the inorganic salt anion equivalents (A^-) (DIJKSHOORN, 1963).

The organic salt content (C^+-A^-) is called normal, when it is near to the value observed at optimal growth. Higher and lower values occur at suboptimal growth only. The normal value may also occur at suboptimal growth, but when growth increases to its optimal rate, the possible deviations from its normal value are reduced. The normal (C^+-A^-) value is required for optimal growth and the (C^+-A^-) content is therefore a growth factor to be considered in foliar diagnosis (DE WIT, DIJKSHOORN and NOGGLE 1963).

The rates of internal redistribution of salt cations is reflected in the following relative gradation of internal mobilities: K $\gg$ Na, Mg $>$ Ca.

If a plant is capable of substituting Na, Mg, or Ca for K in the leaves, K deficiency may result in an organic salt content (C^+-A^-) higher than normal, because the exces of organic salts of the salt cations other than K resists to back translocation from the leaves. A K deficient plant incapable of this substitution by uptake will tend to suffer from an excessive uptake of H^+ with a subsequent decrease of the (C^+-A^-) content below the normal value. The occurrence of either type of K shortage depends on the plant species and on the salt cations supplied for K.

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THE EFFECT OF THE SULPHUR CONTENT IN THE FEED ON THE COPPER STATUS OF CATTLE

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INTRODUCTION

In the Netherlands hypocuprosis in cattle is most predominant at the end of the grazing season. The criteria mainly used for the evaluation of the copper status of animals are the copper contents in the liver and blood serum. When the copper level in the liver is sufficiently high, within a certain fluctuation a constant value is found in the blood. Only when the content in the liver falls below a certain level blood serum values will decrease below 'normal' (9, 10). Consequently the copper content in the liver gives more reliable and detailed information on the copper status of the animal than the blood value.

The copper contents in the livers of grazing cattle decrease under normal Dutch conditions; this is most striking in young animals (9, 10). Recently this finding has been confirmed in Canada (3) and in Queensland, Australia (12). On the other hand, the copper level in the liver is generally replenished during the indoor period when the animals are fed on winter rations with hay, though on an average the copper content in the feed during this period is lower than at pasture.

Whether hypocuprosis will occur or not is mainly determined by differences in the loss of copper from the liver during the grazing season.

In the Netherlands many attempts have already been made to establish some relation between the copper status of grazing cattle and the mineral composition of the herbage.

EARLIER WORK

In an early stage of research several data indicated that the copper content in the feed in itself is not deciding whether hypocuprosis in cattle will occur or not; this has been confirmed in all subsequent publications. High molybdenum contents in the feed are known to interfere with copper metabolism and to cause hypocuprosis elsewhere (1, 7); in the Netherlands the molybdenum content of the herbage is usually so low (up to 5 ppm) that this factor is considered to be of negligible importance.

WIND and DEIJS (13) studying the incidence of scouring attended with hypocupraemia in cattle found that to prevent scouring the copper content in the herbage should be higher as the base excess, expressed by the ratio (K + Na + Ca + Mg)/(Cl + S + P) (meq per kg of dry matter), decreases.

Afterwards there appeared to be many exceptions to this hypothesis. In an extensive investigation, mainly based on data on the copper-deficiency district in southwestern Friesland, the relation between the incidence of hypocupraemia and the mineral composition of the herbage has been studied again. The value of Ca-S-P

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(expressed as milli-equivalents per kg dry matter; P = 3 valences) was shown to be a better indicator of the copper supply to cattle. At low values of Ca-S-P more Cu was required in the feed to protect the animals from a low copper status than at higher values of this factor (4).

Continued research into the causes of hypocuprosis in other areas showed that the Ca-S-P-value in the herbage is not a general criterion for the utilization of copper by cattle (10).

In 1959 DEIJS and BOSMAN (5) suggested that sulphur containing amino acids as cysteine, cystine, glutathione and to a less extent methionine, which are present in fresh grass in a free state, might decrease the availability of Cu to the animal. They found that in very diluted solutions these amino acids combine rapidly with Cu to form complex compounds. An experiment was carried out in which a concentrate containing 19.4 g of cystine was fed daily to each of 3 cows in addition to freshly mown herbage for 50 days; 3 control animals were given concentrates without cystine, and freshly mown herbage. The copper status of the cows was examined by liver biopsies and blood samplings before, during and after the experimental period. The Cu-contents in the livers were not influenced by the administration of cystine. In spite of great fluctuations in the Cu-contents of the blood serum there was a general decrease of these values in the cystine administered animals compared with the controls. After terminating the cystine treatments there was a rise in the Cu-values of the blood serum in the experimental group. Between-animal variation and the fluctuations in the values however were so considerable in this experiment that the differences between groups were statistically not significant (2, 6).

Finally DEIJS and BOSMAN (6) drew attention to the possible role of porphyrins present in fresh grass, *e.g.* chlorophyll, in Cu-utilization by the grazing animal.

FIELD OBSERVATIONS

An attempt was made by statistical treatment of own field data to trace factors in the composition of the herbage in relation to the copper status of grazing cattle. The decrease in the Cu-content in the liver during the grazing season (approximately 6 months) has been used as a criterion for the copper supply to cattle. Previous experiments have proved that this can be done best by expressing the final Cu-content of the liver as a percentage of the initial value, as in this manner animals with diverging initial values react most uniformly.

From field experiments with grazing yearlings 4 farms were selected on which a very strong fall in the liver Cu-store was found during the grazing season: in autumn all animals (4 to 6 animals per farm) showed Cu-contents in the livers that were 10% or less of the values in spring with an average of 7%. On another group of farms the Cu-contents in the livers of the yearlings in autumn were more than 20% of the values in spring with an average of 28%.

The chemical composition of the herbage during the grazing season on both groups of farms is shown in table 1; represented are the average values, their variation and the results of a statistical treatment according to Wilcoxon's test. This test indicates a highly significant difference between groups in the (total) S-content of the herbage. Almost significant are the differences in the Ca-S-P-value, expressed in milli-equivalents per kg dry matter (4), and in inorganic S-content; the latter has been calculated according to data of DIJKSHOORN *et al.* (8) from the total S-content and the crude-protein content. Finally, there is an almost significant difference between groups in dry-matter content of fresh herbage.

As the average Ca, P and crude-protein contents in the groups show no or only

TABLE 1. Chemical composition of the herbage on 2 groups of farms with different falls in the Cu-content in the livers of grazing yearlings.

Average liver Cu-value in autumn expressed as % of spring value	Fall very fast 7		Fall relatively slow 28		
Factor (in sand-free dry matter)	Average	Variation	Average	Variation	Significance of difference (Wilcoxon's test)
Crude-protein %	20.5	12.4 –26.6	19.2	16.7 –20.6	
K %	2.85	1.97–3.69	2.75	2.36–3.00	
Na %	0.16	0.08–0.29	0.14	0.07–0.27	
Mg %	0.24	0.21–0.27	0.22	0.18–0.25	
Ca %	0.55	0.42–0.64	0.55	0.51–0.60	
Cl %	1.49	1.04–1.78	1.42	1.30–1.54	
S %	0.37	0.35–0.41	0.28	0.23–0.35	$P < 0.01$
P %	0.44	0.33–0.54	0.43	0.40–0.48	
Cu ppm	10.1	8.0 –11.3	10.2	8.0 –12.5	
Mo ppm	3.34	2.15–5.93	2.57	1.87–3.85	
Mn ppm	305	60–430	172	110–210	
Fe ppm	165	110–271	139	80–231	
(K+Na+Ca+Mg)/ (Cl+S+P)	1.26	1.09–1.30	1.30	1.06–1.46	
Ca-S-P meq	–399	–452-–352	–319	–410-–242	$P < 0.10$
Inorganic S %	0.155	0.094–0.205	0.091	0.024–0.119	$P < 0.10$
Dry matter in fresh herbage %	18.7	11.5–24.6	15.9	12.7–17.8	$P < 0.10$

slight differences there is reason to suggest that the differences in Ca-S-P-value and in inorganic S-content should be considered to have been derived from the more significant difference in total-S-content; no specific significance should be ascribed to these factors themselves.

Concerning the difference in dry-matter content it should be taken into account that attached water from rain, dew, *etc.* is included in the samples. The chance that due to accidental conditions the amount of attached water in the sample is not representative for the average situation is much greater than with respect to the mineral composition. The more so, as no further data are known indicating an influence of the dry-matter content in the feed on copper utilization by cattle, for the present no special attention has been paid to the differences in dry-matter content of the herbage between groups.

Though the data represented in table 1 are only based on a small number of farms, the highly significant differences in S-content of the herbage gave rise to set out a feeding experiment to test the influence of S-applications on the Cu-status of cattle.

EFFECT OF SULPHATE APPLICATION

19 female Friesian cattle, aged approximately 12 months, were divided into the following groups

I. control group (7 animals)
II. $CaSO_4$-group (6 animals)
III. Na_2SO_4-group (6 animals)

As hypocupraemia in the field in many cases is attended with persistent scouring it is obvious that the question arises whether this scouring in itself has a detrimental effect on the copper supply of the animal or not. An affirmative answer to this question would mean that under conditions of diarrhoea a low copper status in the animal would develop more rapidly. In order to get some information on this point two sulphate compounds have been compared, *viz.* the non-laxative $CaSO_4$ and Na_2SO_4 known as laxative.

During an experimental period of 73 days (January 5 up to March 19, 1962) the animals were fed on a basic ration consisting of 3.5 kg pasture hay, 8.0 kg grass silage, 4.0 kg potatoes and 0.85 kg of concentrates per day. The ration per group was weighed out daily. Only from the hay some residues remained sometimes; these have been weighed. The average daily dry-matter (DM) intake per animal was 6.13 kg; there were no differences between groups. The average mineral composition and the nutritive value are given in table 2.

TABLE 2. Mineral composition and nutritive value of the basic ration.

Factor	Unit	Value (in DM)	meq/kg DM
Na	%	0.32	138
K	%	2.16	552
Ca	%	0.56	280
P	%	0.34	329
S	%	0.27	171
Ca-S-P			-220
Cu	ppm	11.6	
Mo	ppm	0.71	
Mn	ppm	124	
vre (digestible crude protein)	g/kg	79	
ZW (starch equivalent)	g/kg	497	

The experimental animals received chemically pure $CaSO_4.2H_2O$ and Na_2SO_4 respectively in gelatin capsules by means of a dosing gun twice daily. Initially the dose was fixed on an increase in the S-content of the ration with 100 meq per kg DM. However when this dose did not decrease the DM-content in the faeces of the Na_2SO_4-group the dose was gradually increased to 300 meq per kg DM. Though this amount neither influenced the consistency of the faeces a higher dose was judged to be undesirable, because a very low pH (< 5.0) was measured in the urine of the $CaSO_4$-group. Meanwhile a considerable decrease in the Cu-content of the blood serum in the experimental groups occurred. In order to check whether a recovery of these blood values takes place if the S-dose is decreased the latter has temporarily been reduced to 200 meq per kg DM. During the entire experimental period the average S-supply was 22.0 g per animal per day; on a basis of the dry-matter content in the ration this means an increase of the S-content of 0.36% or 228 meq per kg.

Liver samples by means of liver biopsy were taken at the beginning and the end of the experimental period; samples of blood, urine and faeces were collected weekly during the experimental period and during two subsequent weeks; girth was measured every 2 weeks, including 2 weeks before and after the experimental period. The animals were weighed 3 times during and once after the experimental period.

On 8 collection dates individual blood samples were combined to group samples; liver and blood samples were analysed for Cu. The pH of urine was estimated with pH-indicator paper; group samples were analysed for minerals, creatinine and specific gravity at 3 sampling dates. Group samples of faeces were analysed for dry matter content; at 4 dates during the high S-dosing periods Cu was also determined.

Results are summarized in fig. 1 and in tables 3, 4 and 5.

TABLE 3. Cu-contents of liver and blood serum of the individual animals.

Group and animal	Liver-Cu mg/kg DM		Cu-content blood serum mg/l				
	3/1	19/3	3/1	1/2	1/3	19/3	2/4
I. *Control*							
2	176	255	0.92	0.93	1.00	1.09	0.92
3	392	321	0.67	0.62	0.69	0.72	0.61
9	123	89	0.75	0.72	0.71	0.81	0.71
10	142	73	0.86	0.77	0.73	0.91	0.82
16	311	180	0.69	0.62	0.71	0.75	0.69
17	308	282	0.75	0.75	0.86	0.83	0.70
18	262	159	0.92	0.94	0.86	0.77	0.70
Average	*226*	*171*	*0.79*	*0.76*	*0.79*	*0.84*	*0.75*
II. *$CaSO_4$*							
1	250	157	0.72	0.56	0.67	0.69	1.02
5	69	21	0.74	0.57	0.50	0.40	0.43
8	58	23	0.74	0.54	0.58	0.44	0.48
12	366	226	0.87	0.63	0.69	0.85	0.75
14	372	242	0.86	0.72	0.64	0.74	0.68
19	293	127	0.70	0.59	0.80	0.76	0.82
Average	*185*	*90*	*0.77*	*0.60*[1]	*0.65*[1]	*0.65*[2]	*0.70*
Average (5 and 8 disregarded)					0.70	0.76	0.82
III. *Na_2SO_4*							
4	179	143	0.73	0.78	0.81	0.78	0.86
6	88	38	0.73	0.56	0.71	0.61	0.64
7	141	69	0.70	0.67	0.60	0.63	0.85
11	58	16	0.87	0.76	0.67	0.56	0.67
13	187	88	0.78	0.70	0.76	0.64	0.93
15	238	125	0.79	0.61	0.69	0.63	0.69
Average	*134*	*64*	*0.78*	*0.68*	*0.71*	*0.64*[3]	*0.77*

Significance of difference in blood-Cu-value with control group at given sampling date:
[1] = $P < 0.05$; [2] = $P < 0.10$; [3] = $P < 0.01$.

TABLE 4. Alterations of the liver-Cu-contents during the experiment.

Group	Number of animals	Liver-Cu-values initial mg/kg DM	Liver-Cu-values final mg/kg DM	final in % of initial	Difference with control group (t test)
I : control	7	226	171	76	
II : $CaSO_4$	6	185	90	49	$P < 0.05$
III : Na_2SO_4	6	134	64	48	$P < 0.05$
II + III: SO_4	12	157	76	48	$P < 0.01$

THE COPPER STATUS OF THE LIVER

The Cu-contents in the livers of the individual animals at the beginning and at the end of the experiment confirm that animals of the same treatment react most uniformly when the change is expressed in percentages of the initial value. Therefore per group the geometric average has been calculated and not the arithmetic. Further the liver Cu-contents in fig. 1 are plotted on a logarithmic scale so that the alteration during the experiment may be represented by a straight line.

The Cu-contents in the livers of both experimental groups decreased considerably during the experiment, *viz.* to 49 and 48% of the initial value for the $CaSO_4$ - and Na_2SO_4 - group respectively. In the control group there is a decrease of 24% to 76% of the initial value. In housed animals of the same age a somewhat slighter decrease was found in earlier experiments (9). However, the fall in both experimental groups has been so sharp that each separate group shows a significant difference with the control group; when both groups are combined this difference is highly significant.

From table 4 it also follows that the effects of the $CaSO_4$- and Na_2SO_4-treatments are equal; this clearly suggests that the effects obtained are brought about by the sulphate and that the difference in cations has no effect.

THE COPPER CONTENT IN THE BLOOD SERUM

In the experimental groups there is a decrease in these values. This decrease seems to be related to the amount of sulphate dosed; however during the second period of the highest S-dose the decrease in the blood values is somewhat less, cf. fig. 1. Maybe some adaptation of the animal organism to the S-treatment has taken place.

As mentioned before, only part of the blood samples have been analysed individually; this concerns the sampling dates given in table 3.

There were significant differences in Cu-content of the blood serum between $CaSO_4$ and control group on February 1 and March 1 ($P < 0.05$, Wilcoxon's test); on March 19 the difference was almost significant ($P < 0.10$). On March 1, animals 5 and 8 of the $CaSO_4$-group showed obviously lower values; as this concerned animals with the lowest liver Cu-values at the beginning of the experiment it was to be expected that this decrease was due to Cu-exhaustion of the liver (10). The liver biopsy at the end of the experiment confirmed this supposition. From these 2 animals all blood samples after March 1 have been analysed individually; the decrease in the Cu-values proceeded during the experiment, afterwards they were fairly constant. The average Cu-content in the blood serum of the $CaSO_4$-group has also been calculated with these 2 animals excluded; this average is shown in fig. 1 by a dotted line. The result of disregarding these 2 animals was that the difference in blood Cu-values between the $CaSO_4$- and control group was no longer significant.

There was a statistically highly significant difference between the Na_2SO_4- and control group on March 19 ($P < 0.01$).

The SO_4-treated groups combined showed a significant difference with the control group on February 1 and on March 1 ($P < 0.05$); on March 19 the difference is highly significant ($P < 0.01$). The limits of significance do not change when disregarding animals 5 and 8, with lower Cu-levels.

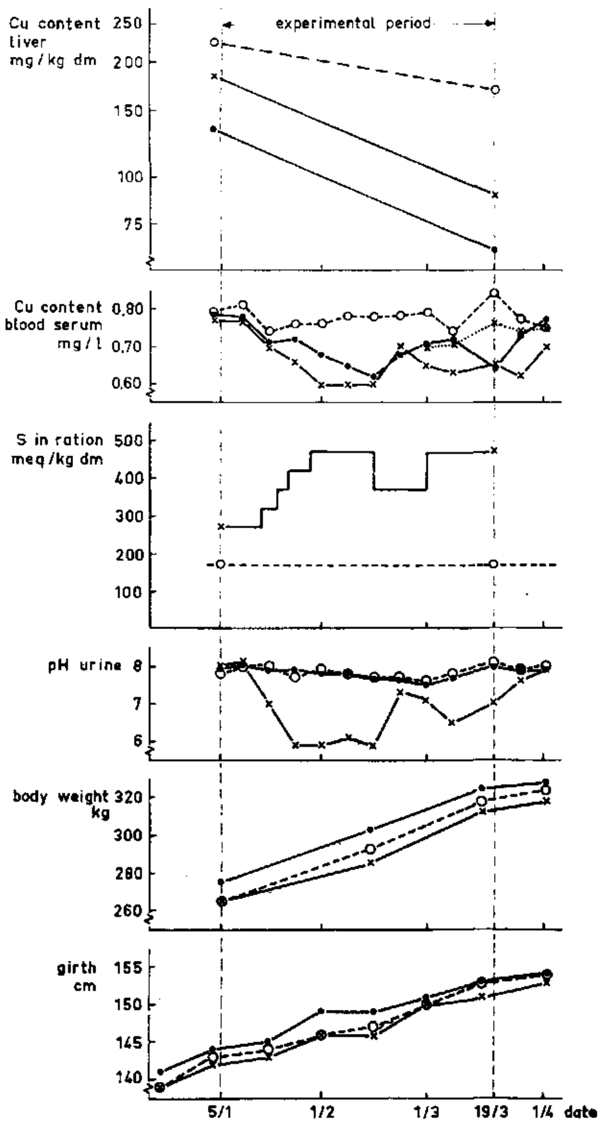
Comparing blood Cu-values of all sampling dates during the experimental period together, each of the experimental groups shows a highly significant difference compared with the control group ($P < 0.01$).

At the end of the after-period the blood Cu-values in all groups are essentially equal; this shows even more evidently that the differences mentioned are caused by the sulphate treatments.

FURTHER OBSERVATIONS

In the $CaSO_4$-group there is a response of the pH of the urine to the salt given, which is not present in the Na_2SO_4-group. Already 2 weeks after the beginning of the experiment the difference in pH of the urine compared with the other groups is highly significant ($P < 0.01$). This difference lasts until the dose is decreased in the middle of February; the pH of the urine rapidly increases and the difference with the

FIG. 1. Effect of various oral dosings of sulphate on the Cu-contents of liver and blood serum, pH of urine, body weight and girth of groups of yearlings.
○ – – – ○ control group
× —— × $CaSO_4.2H_2O$-dosed group
● —— ● Na_2SO_4-dosed group
× ... × $CaSO_4.2H_2O$-dosed group; animals 5 and 8 disregarded, see text.



other groups is no longer statistically significant. After increasing the dose again the highly significant difference returns ($P < 0.01$), though the fall in pH is smaller than before. During the after-period the difference rapidly diminishes and on April 2 the values in all groups are equal.

Undoubtedly the decrease in the pH of the urine in the $CaSO_4$-group is caused by the combination of a strong anion ($SO_4^{=}$) which is excreted by the animal for the greater part in the urine, and a weak cation (Ca^{++}) being mainly excreted in the faeces. The fact that the effect on the pH of the urine was not found in the Na_2SO_4-group is to be attributed to the combination of the strong ions Na^+ and $SO_4^{=}$ which both are mainly excreted in the urine, and thus counterbalance each others action.

Data on the excretion of several ions in the urine are given in table 5.

TABLE 5. pH, specific gravity and chemical composition of (group) samples of urine at 2 dates of high S-treatment and 1 date at the end of the after-period.

Group	pH			Specific gravity		
Date	15/2	19/3	2/4	15/2	19/3	2/4
Control	7.7 (7.0–8.0)	8.1 (8.0–8.2)	8.0 (7.2–8.2)	1.027	1.032	1.027
$CaSO_4$	5.9[1] (5.0–6.7)	7.0[1] (6.1–8.0)	7.9 (7.4–8.2)	1.030	1.028	1.033
Na_2SO_4	7.7 (7.4–7.8)	8.0 (7.8–8.0)	7.9 (7.7–8.2)	1.024	1.027	1.035

Group	Creatinine g/l			K g/l			Na g/l			Mg g/l		
Date	15/2	19/3	2/4	15/2	19/3	2/4	15/2	19/3	2/4	15/2	19/3	2/4
Control	0.90	0.88	1.15	11.62	12.70	16.19	1.81	1.95	0.10	0.10	0.12	0.10
$CaSO_4$	1.13	0.99	1.31	11.97	12.51	18.34	0.50	0.89	0.07	0.16	0.18	0.15
Na_2SO_4	0.90	0.77	1.42	10.29	10.29	18.01	4.87	3.25	0.42	0.10	0.08	0.11

Group	Ca g/l			S g/l			P g/l		
Date	15/2	19/3	2/4	15/2	19/3	2/4	15/2	19/3	2/4
Control	0.07	0.03	0.05	0.82	0.49	0.58	0.11	0.08	0.03
$CaSO_4$	0.49	0.17	0.05	1.60	1.16	0.69	0.29	0.15	0.24
Na_2SO_4	0.06	0.01	0.05	1.59	0.80	0.73	0.13	0.07	0.22

$P < 0.01$

There is little divergence in creatinine content of the urine between groups at any sampling date; this indicates that the differences in daily urine production per group are only slight as well. Consequently the concentrations of the various ions may be compared as a rough measure of the total daily excretion without applying corrections.

Taking into account a normal creatinine production and further considering the daily intake of the various minerals (cf. table 2), the mineral composition of the urine confirms the well-known fact that K, Na and S indeed are mainly excreted in the urine and Ca, Mg and P by other means (*viz.* in the faeces). More or less in contradiction to this conclusion the $CaSO_4$-group shows much lower Na-contents in the urine than the control group at the same Na-level in the ration; in the Na_2SO_4-group the Na-excretion is still considerably higher 2 weeks after the treatment has been stopped. At first there is increased excretion of the Ca^{++}-ion supplied in the urine of the $CaSO_4$-group as well; however at the end of the experimental period this is considerably less; 2 weeks later there are no differences with the other groups. Though the treatment has already terminated 2 weeks S still seems to be excreted in slightly higher amounts in urine. Striking are the increased P-excretions in the urine of the $CaSO_4$-group and in the Na_2SO_4-group on April 2.

Body weight and girth of the groups both show the same tendency, cf. fig. 1. During the experiment the average increase in body weight of the $CaSO_4$- and Na_2SO_4-group is 5 and 3 kg less respectively than in the control group; during the 2 week after-period these differences increase to 6 and 6 kg respectively. The measurements of the girth give the same indications, *viz.* a slight fall behind of the experimental groups. However none of the differences in body weight and girth has statistical significance.

In order to get some information whether differences in Cu-accumulation by the liver is reflected in the Cu-excretion in faeces and/or urine occasional samples of urine and faeces have been analysed for Cu. The Cu-contents in urine always were so low that they could not be determined accurately (approximately 0.01 mg/l); at an average daily Cu-intake per animal of 71 mg the excretion in urine may be neglected. In the faeces – which neither have quantitatively been collected – slight differences in Cu-content were found: 29.6, 30.5 and 30.7 mg per kg DM for control, $CaSO_4$- and Na_2SO_4-group respectively (average of 4 samples per group during the high S-dosing periods). Though these differences are not statistically significant they might indicate that the utilization of Cu by the sulphate-dosed animals is lower than by the control group.

It has already been mentioned that soft faeces were not obtained with Na_2SO_4-treatment; this has been confirmed by dry matter determinations in the faeces samples collected weekly. During the experimental period the average dry-matter contents were 15.5%, 15.2% and 14.9% for control, $CaSO_4$- and Na_2SO_4-group respectively; the differences are not statistically significant.

After terminating the experiment some animals of the Na_2SO_4-group received the same dose of Na_2SO_4 as a drench instead of in solid form. These animals showed diarrhoea within 24 hours. This proves that the laxative effect of Na_2SO_4 acts only when the salt passes over the rumen into the abomasum.

DISCUSSION

The question arises as to how far differences in S-content of the pasture grass can give an explanation for the differences occurring in Cu-status of the livers of cattle between farms. An approximate calculation will be made.

The average liver Cu-contents of the 2 groups in table 1 in autumn is 7% and 28% respectively of the spring values; accordingly a ratio of 1:4. This result was obtained in a grazing period of approximately 180 days; this is $2\frac{1}{2}$ times the duration of the S-treatments in the experiment. When this experiment should have been continued for 180 days the liver Cu-contents at the end could have roughly been estimated at $0.48^{2\frac{1}{2}} \times 100\% = 16\%$ and $0.76^{2\frac{1}{2}} \times 100\% = 50\%$ of the initial value for S-dosed groups and control group respectively, which is a ratio of 1:3. Though this ratio is in the same order of magnitude as that in table 1, it should be noticed that the difference in S-content of the feed in the experiment was 4 times as high as between the herbage of 'very Cu-deficient' and 'normal' farms in table 1. Assuming that an increased S-content in the feed will have the same effect on the Cu-status of cattle on winter rations or on fresh herbage, it may be concluded that other factors than the S-content of the feed must also have an influence on the decrease of the copper status of grazing cattle. In this respect we refer to earlier observations (10, 11) which indicate a highly significant relation between the incidence of any form of water stagnation in the top soil and a low Cu-status of yearlings at the end of the grazing season; this problem is still under investigation.

Another question that may rise is whether the higher S-content usually found in pasture grass compared with winter rations might give an explanation for the divergence in Cu-metabolism of cattle at pasture and housed. While the Cu-content in the livers is practically constant in housed cattle of approximately 1 year old (9) an average decrease takes place to 10, or 15% of the value in spring during the pasture period. In the experiment described it may roughly be expected that on a 180 days basis the liver Cu-values in the S-dosed animals will decrease to (16:50)× 100% = 32% of those of the control group. Moreover, the difference in S-content of the feed in this experiment was many times greater than is found in practice between pasture grass and winter rations. Therefore the difference in S-content of the feed can only partly explain the different Cu-utilization by young cattle at pasture and housed.

In the review of earlier work on feed composition in relation to copper status of cattle it has been mentioned that the factor Ca-S-P did not hold in all cases. The S-dosing experiment enables a further test of this value.

By application of $CaSO_4$ the value of Ca-S-P is not influenced when compared with the control group because the added equivalents of Ca and S are equal. Yet in the former group the Cu-contents of the livers decrease much faster than in the latter. On the other hand, there is no difference in Cu-utilization between $CaSO_4$- and Na_2SO_4-group though on an average the value of Ca-S-P is 228 meq per kg DM lower in the latter group. This indicates that the supposed influence of the factor Ca-S-P on the copper status of cattle does not hold. As our field data suggest any influence of P not to be likely (cf. table 1) the relation found earlier (4) should mainly if not entirely be attributed to the influence of S.

In the experimental groups there was a significant decrease in the Cu-contents of the blood serum. Still these animals did not diverge from the relation established earlier between Cu-contents in liver and blood of yearlings (10). The Cu-content of the blood serum varies between 0.60 and 1.00 mg per 1 as long as the Cu-contents in the liver do not decrease below 25 to 50 mg per kg DM. Only if contents in the liver are lower blood values may drop below 0.60 mg per 1. In this experiment the latter was the case in animals 5 and 8 of the $CaSO_4$-group, mentioned before.

Yet the Cu-contents in the blood serum of the animals of the experimental groups were lower than might have been expected according to the average relation between liver and blood values (0.80 mg/1 at sufficient liver Cu-values). This might indicate that the S-content of the feed is one of the factors that influence the Cu-content in the blood serum within the normally occurring deviations. There is no information by which means (lower utilization, higher excretion of endogenous Cu, immobilization or otherwise) this influence may be explained.

SUMMARY

The indication obtained by statistical treatment that the very strong decreases of the copper status of grazing cattle is related to the S-content of the herbage has been tested in a feeding experiment indoors. Groups of yearlings were given extra doses of S as $CaSO_4.2H_2O$ or Na_2SO_4 in solid form.

The Cu-contents of the livers in both groups decreased to the same extent and significantly more than in control animals not given supplementary S. The Cu-contents in the blood serum decreased significantly below the level normally occurring in animals with sufficient Cu-store in the liver. There were slight though not significant differences in body weight in favour of the control group.

However the reported influence of the S-content in the feed on the copper status of yearlings does not give a complete explanation for the differences in Cu-loss during the grazing season of yearlings on different farms as occurring in the field, nor for the differences in Cu-ultilization between grazing and housed animals.

Evidence has been found that the relationship between the factor Ca-S-P (meq/kg DM) in the feed and the copper status of cattle, as supposed by DEIJS *et al.* (4), is mainly if not entirely due to the effect of S.

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THE POSSIBILITY OF RELATING THE ASH ALKALINITY TO THE ORGANIC-SALT CONTENT

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INTRODUCTION

The tissue pH of the majority of agricultural plants varies from 5 to 7 (15) and, as a rule, the pH of shoots and roots does not differ more than one pH-unit (11). Since in this range the buffer capacity of the cell sap is low (2, 13, 14) relative to the large amount of alkalinity involved in salt utilization (9), maintenance of tissue pH during salt utilization and growth depends entirely on the synthesis and breakdown of organic salts (3). In this way larger changes in tissue pH, following alterations in ion utilization due to application or exhaustion of fertilizers, do not occur (1, 3). It has been found that some Ericaceous plants with, normally, a more acid reaction of the tissues, change their tissue pH to higher values when nitrate is applied instead of ammonia or when supplied with lime (4, 6). This change to a more alkaline salt utilization is associated with reduced growth and chlorosis. At present, this case seems rather exceptional and experience, gained with the more commonly cultivated plants, favours the view that tissue pH is only moderately affected by varying the salt supply (1), but that the organic-salt content may vary substantially with the nature and quantity of the salts supplied during growth.

One of the earlier studies on tobacco (2) reports titration curves of the expressed leaf sap of plants submitted to various salt treatments. The results show that the buffer capacity in the range of the normal pH is independent of the salts supplied and that pH remained within the range of 5.5 to 6.5 However, the sap, acidified to pH 4 to 3, shows considerable buffering and the magnitude of the buffer capacity in this acid range, which mainly operates through the soluble organic plant acids with pK's in the order of 4 to 2, depends to a great extent on the salt treatments of the plants. There appears to be a good agreement as to the effect of the different salts in the recent findings on the response of the organic-salt content of tobacco leaves to various salts (5).

Many other plant species have been investigated on various inorganic constituents in a manner suitable for a rough approximation of the ionic balance and organic-salt content. Other references, for instance (7), include complete data applied to accurate records of the balance-sheet of ion accumulation in growing plants.

From these records and from experiments made at this institute it was inferred that with adequate nutrition the organic-salt content, calculated as the difference between the equivalent sums C^+ (= $K^+ + Na^+ + Mg^{++} + Ca^{++}$) and A^- (= $NO_3^- + Cl^- + H_2PO_4^- + SO_4^=$), shows a magnitude characteristic for the plant species.
However, inadequate nutrition, associated with suboptimal growth and deficiencies, may result in values for (C^+–A^-) considerably different from the normal one. It appeared that these deviations are of diagnostic value for assessing the kind of malnutrition concerned and that a normal organic-salt content is one of the conditions required for optimal growth (18).

At present, it seems that constancy of the organic-salt content at optimal growth and nutrition should not be seen merely as a result of some determined capacity for synthesis of organic anions relative to dry weight production. It seems more probable that some regulatory process is involved and that abnormal organic-anion contents occur when the regulatory system fails to operate in a normal way, instead of being merely caused by a change in the capacity for synthesis of organic anions. It was suggested (19) that the presence of regulation might manifest itself by the occurrence of fluctuations in (C^+-A^-) during the time between change in nutrition and the establishment of a new steady state, associated with the phenomenon of 'overshoot'. Their presence in a measurable order of magnitude may serve as a useful tool in further studies on the nature of this regulation.

To test the presence of temporary fluctuations frequent sampling and a more rapid test on the organic-salt content of smaller samples are required. Therefore, a number of samples of different plant species with varying organic salt (C^+-A^-), Cl – and NO_3-contents were investigated on ash alkalinity. The values obtained were corrected for nitrate (16) and compared with the analytical figures for (C^+-A^-).

EXPERIMENT

The determination of ash alkalinity was made as follows: 0.5 grams of the air-dry, powdered sample are weighed and ashed in a porcelain crucible at 550°C during 3 hours. A free-flowing, grey ash is obtained which is moistened with a few drops of water and transferred with more water into a measuring flask.

Depending on the expected alkalinity either 10 or 20 ml of standard 0.1 N HCl are added. The ash dissolves and carbon dioxide is removed by boiling for a few minutes. After cooling the volume is made up to 100 ml, the liquid is filtered and 50 ml of the filtrate are transferred to a small beaker. Glass- and standard electrodes are inserted and the excess of HCl is titrated with standard 0.1 N NaOH under magnetic stirring up to pH 5 as indicated by a potentiometer.

In this way the first dissociation constant of phosphoric acid is included, as it is in the calculation of (C^+-A^-) from the analysis on the ionic constituents. Because phosphate in the samples is low compared to ash alkalinity in most plant samples, the buffering of phosphate is small and the titration curve is steep enough for accurate titration.

According to sample weight and the dilutions applied ash alkalinity is calculated as: 4(1/2 p. N (HCl) – q. N (NaOH)) me. per gram of sample, p and q representing ml and N the normality of the standard HCl and NaOH.

The determination of nitrate proceeds according to the xylenol-procedure described earlier (8), but with some modifications for serial work.

0.300 grams of the sample are transferred to a measuring flask and 10 ml of a solution of 17 grams of $CuSO_4.5$ aq. in 500 ml water, and 10 ml of a suspension of 17 grams CaO in 500 ml water are added in succession. The flask is shaken for 10 minutes, the foam is removed by adding a drop of octanol and the suspension is made up to 50 ml. After filtration 15 ml of the clear filtrate are transferred to a measuring flask. A small piece of litmus paper is added and the solution is acidified with a few drops of 4 N sulphuric acid. 10 ml of 0.02 N silver sulphate are added and the liquid is set aside for 30 minutes in the dark. The volume is made up to 50 ml and the liquid is filtered. 3 ml of the filtrate are transferred to a large test tube with glass stopper and 6 ml of a mixture of 3 volumes of 96 per cent sulphuric acid and 1 volume of water are added. After mixing a few drops of 0.5 per cent potassium permanganate are added until the liquid shows a more permanent pink colour, the excess permanganate is removed by adding small amounts of powdered, solid oxalic acid.

After adding 8 drops of a freshly prepared solution of 5 grams of 4,3-dimethyl-1-hydroxybenzene (Merck, m.p. 64–65°C), dissolved in 5 ml ethyl acetate, the tube is gently shaken to mix the liquids, closed and kept for 30 minutes in a thermostate at 50°C with occasional shaking.

Thereafter, the tube is cooled in running tap water and the liquid is transferred to a separatory funnel. The tube is washed with two 10 ml portions of isopropyl ether which are added to the liquid in the funnel. The isopropyl ether is freed of peroxydes and aldehydes in the usual way (8) before use; this purification is essential.

A set of separatory funnels for serial work was made and mounted on a support according to the instructions of WEYGAND (17) for the construction of a set for counter current distribution work. The capacity of the funnels was enlarged by using 18 mm glass tubing for the main compartment, and the closed end of Weygand's original funnel was provided with a glass tap for draining off the lower phase.

The nitroxylenol is extracted under slow mechanical oscillation at low frequency for 10 minutes and the lower phase is removed through the stop cock. The ethereal solution is washed twice with 10 ml water by a few minutes oscillation and the washings are drained off. Thereafter, 10 ml of 1 N KOH are added, the funnel is oscillated for 10 minutes and the yellow alkaline solution of the nitroxylenolate is separated, collected through the stop cock and measured for its extinction at 440 millimicron, or with a blue filter. Calibration is made by passing standard potassium nitrate solutions through the same procedure.

The figure obtained for nitrate, expressed as me. NO_3 per gram sample, is subtracted from the ash alkalinity in me. per gram sample. The value obtained is recalculated on an oven-dry basis after carrying out a separate moisture determination of the air-dry sample.

RESULTS

Ash alkalinity minus the original nitrate content of the material is sometimes used as a measure for the organic-salt content (16) of plant material. Our main purpose was to check on the relation between this value and the organic-salt content ($C^+ - A^-$), calculated from analysis on the main inorganic ionic constituents (9, 18). Interference was thought to be mainly due to uncontrollable losses of Cl, the behaviour of sulphur, analytical errors in the analysis of ionic constituents or incompleteness of the balance-sheets used.

A number of samples of birch leaves (0.5), of grass (1.0), of potato tops (2.0) and of sugar beet tops (3.0), obtained in experiments with varying nutrition and completely analysed on ionic constituents (the above figures refer to the average normal organic-salt contents in me./g) were submitted to the above procedure. According to the experimental treatments the plants differed appreciably in nitrate, nitrogen and chlorine content.

In figure 1a the values of ash alkalinity, corrected for nitrate, are plotted against the values for ($C^+ - A^-$) in me. per g d.m. It is seen that ash alkalinity is slightly too high at the lower values. At the higher values there is some spreading of the results around the 45° line. The graph shows, however, that there is no serious discrepancy between the values obtained. The deviations did not appear to depend on the nitrate and chloride contents of the samples. A replicate series of determinations of the ash alkalinity is given in figure 1b.

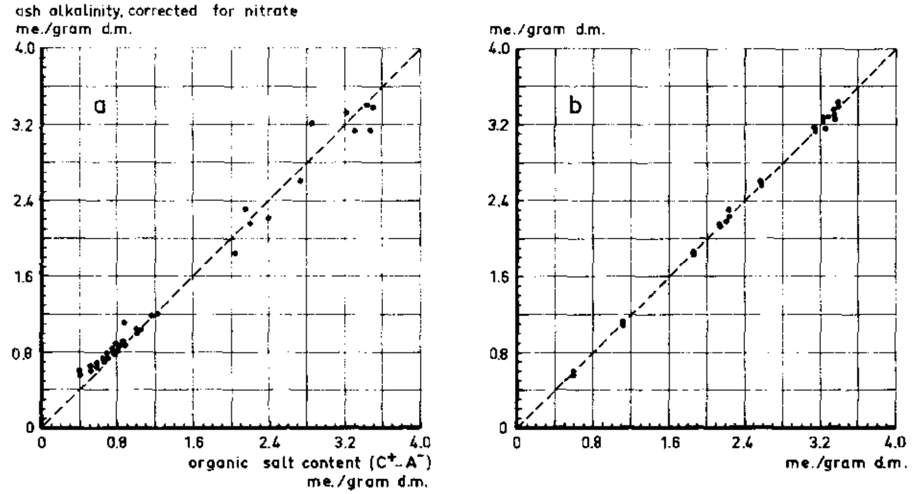

FIG. 1a. Relation of ash alkalinity, corrected for nitrate initially present in the samples (ordinates) and organic salt content ($C^+ - A^-$) found by analysis of the leaf samples on salt cations C^+ (= K + Na + Mg + Ca) and on inorganic salt anions A^- (= Cl^- + total P as $H_2PO_4^-$ + inorganic $SO_4^=$ + NO_3^-). Leaf samples of birch, grass, potato and sugar beet. All values in me. per gram of oven-dry material.
FIG. 1b. Results of two series of determinations of ash alkalinity, corrected for nitrate.

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Mededeling 251 van het I.B.S.

CONTROL OF NITRIFICATION BY 2-CHLORO-6-(TRICHLOROMETHYL) PYRIDINE

H. D. W. VAN TUIL and J. E. M. LAMPE

The rate of nitrification of NH_4^+ as influenced by temperature was studied by TYLER *et al.* (8). He found that in soils with pH 5.6–8.2 quantities from 100–800 mg N per kg soil (= 7–60 me. NH_4^+ per kg soil) were converted for 100–20 % within 2 weeks at 24°C. At 7°C nitrification was considerably delayed, only 20–5 % being converted, whereas at 3°C nitrification was completely inhibited. ANDERSON and PURVIS (1) studied the effect of temperature and time on NH_4^+-conversion to nitrate in the range from 3°C to 11°C, in relation to the possible advantage of using NH_4^+ instead of NO_3^- for applications in winter. They found a practically linear increase in the logarithm of the nitrate content in the soil with time. At 11°C 50 % of the NH_4^+ supplied was converted into NO_3^- in 42 days, while at 3°C only 5 % was converted to nitrate in the same period.

In greenhouse experiments temperature mostly fluctuates between 20°C and 30°C and the ammonium applied usually is rapidly converted to NO_3^-; at pH > 5 within a few weeks.

In previous work nitrification was controlled by acidifying the soil to below pH = 5 (2). Acidification of the soil to pH < 5 is known to inhibit nitrification completely as was shown among others, by ENO and BLUE (4). These authors demonstrated a marked acidification of the soil by the process of nitrification, the process itself inducing a drop of as much as 1.5 units of pH. This is conceivable from the ionic equation of the process: $NH_4^+ + 4\,O \rightarrow NO_3^- + 2\,H^+ + H_2O$.

Actually, the substantial decrease in pH is due to transformation of the very weakly acidic cation NH_4^+ (pK = 9.2) into the strongly acidic cation H^+. The quantities of NH_4^+ used were up to 780 ppm N_{NH4} which corresponds to about 50 me. per kg soil. The liberation of acidity in this amount in most soils is sufficient to reduce the pH of the soil by one unit.

In greenhouse experiments it is often desired to study the effect of NH_4^+-nutrition. However, at soil pH > 5 nitrification during growth may interfere to such an extent that the result obtained should be considered as nitrate nutrition.

The use of 2-chloro-6-(trichloromethyl)pyridine, marketed under the name of N-serve by the Dow Chemical Company (7), came to our attention by the work of GORING (5,6). In a greenhouse experiment in which temperatures were kept at about 18°C, a poor sandy soil was used with 1.5 % of organic matter, pH = 3.9 and a cation-exchange capacity of 140 me. per kg soil. Small vessels, each containing 160 g of soil were used. The treatments were 0; 3.12; 6.25 and 15.6 g $CaCO_3$ and 0; 4.7 and 9.4 me. $(NH_4)_2\,SO_4$ per kg soil. Each treatment was divided into two groups, one group receiving 10 ppm 2-chloro-6-(trichloromethyl)pyridine. The other group received no N-serve.

Each treatment had 6 replicates. The different amounts of $CaCO_3$ applied resulted in an increase in the pH from 3.9 to 7.5. Samples were collected 1, 9, 20, 42, 70 and 90 days after the experiment started. In these samples the nitrate content was measured

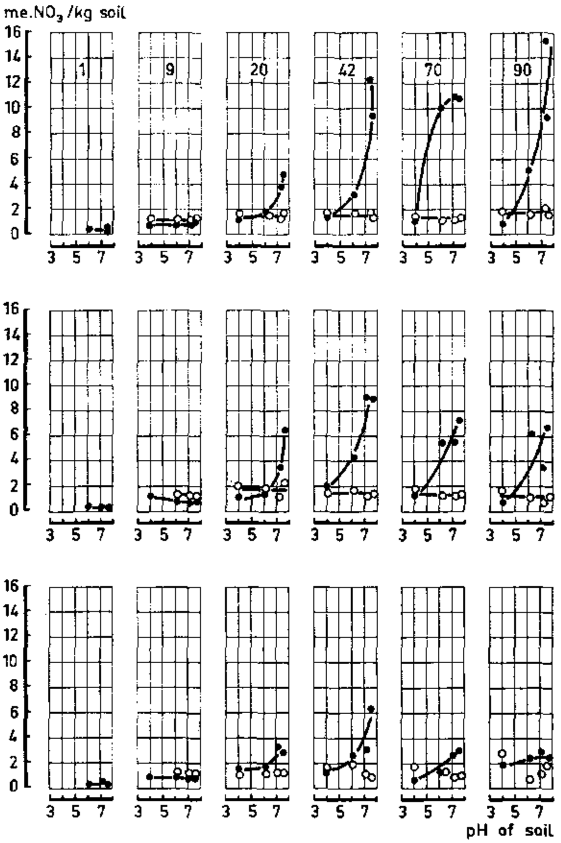

FIG. 1. Nitrate in the soil after incubation in the greenhouse. The soil was brought to different pH-levels with lime and fertilized with NH_4^+ at 3 levels before incubation. Open dots: received 10 ppm of 2-chloro-6-(trichloromethyl) pyridine. Filled dots: no N-serve.
NH_4 was applied at 9.4 me./kg (upper row), at 4.7 me./kg (middle row) and at 0 me./kg (lower row). The numbers at the top of the columns denote the time in days of incubation. Abscissae: pH of a (1:5) water suspension of the treated soils at the beginning of incubation.

(3), whereas the pH of the soil was measured in soil-water (1:5) suspensions at the beginning and at the end of the experiment. The change in pH with time was negligible for all treatments ($<$ 0.2 pH unit). The results of the experiment are given in figure 1, open dots representing treatments with N-serve, filled dots treatments without N-serve. It is seen that nitrification in the present experiment begins about 20 days after application of the treatments. In the vessels which received no ammonium fertilizer the nitrogen present in the soil leads to some nitrate production. In the unlimed soil nitrification is negligible, as in all portions treated with N-serve.

The increased application of $CaCO_3$ results in an increase in nitrate production in those treatments which did not receive 2-chloro-6-(trichloromethyl)pyridine.

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Mededeling 252 van het I.B.S.

DETERMINATION OF THE DISCOLOURATION OF RAW POTATOES

N. VERTREGT and ELISABETH G. PANNEBAKKER

INTRODUCTION

The discolouration of raw potatoes after cutting is a phenomenon that determines to a high degree the suitability for industrial utilization. Consequently, it is of importance to have a method to measure the discolouration.

The phenomenon can be described as follows: Damaged potato cells change colour from colourless through red to brownish-black. This discolouration is caused by the enzymatic oxidation of tyrosine by phenoloxidase. At first the red coloured dopachrome is formed. For this reaction 1,5 moles of oxygen are needed per mole of tyrosine. The dopachrome is oxidized by 1 mole of oxygen to the brownish-black melanin (1). It will be clear that this discolouration depends on the initial concentration of tyrosine in the tissue and on the phenoloxidase activity. To our purpose it is impractical to determine both quantities. It appeared sufficient to determine the coloured product formed. As the final product melanin is insoluble, it can only be estimated by measuring the reflectance of minced tissue. This method is not very accurate. It is more practical to measure the red intermediate, dopachrome, which is soluble. Moreover the formation of dopachrome occurs rapidly as compared with the much slower formation of melanin in the last stage of the oxidation process. Dopachrome can therefore be readily estimated before the formation of melanin has considerably proceeded.

METHOD

Potato juice is prepared by using a domestic juice press. 3 ml of the freshly prepared juice are pipetted within 30 seconds into a 150 ml beaker placed on a disk. The disk is adapted to support several of these beakers and is rotated with 60 r.p.m. in a slightly inclined position. Temperature is kept constant at 25°C by a thermostatically controlled waterbath (see fig. 1). The rotating movement brings about mixing of the juice and replacement of the air in the beakers to get an adequate oxygen supply without formation of foam on the surface of the juice. After exactly twenty minutes the proteins in the juice are precipitated by adding 5 ml phosphotungstic acid (6 g phosphotungstic acid and 1 g sodium acetate made up to 100 ml after adjusting the pH to 4.2 with sodium hydroxide or acetic acid). After filtration through a filterpaper of close texture the extinction in the clear solution can be measured with a colorimeter at a wavelength of 475mμ. In most cases extinction exceeds 0.6, above this value measuring proved to be less convenient and less accurate. In these cases the solutions have to be diluted to a suitable volume. After some experience this dilution can be performed before filtration after judging the colour. This is preferable as filtration proceeds more rapidly and the use of a measuring flask is avoided. The colour fades gradually. The extinction decreases about 5 per cent in 10 minutes. Therefore extinction has to be measured at a fixed time after precipitating the proteins. Finally the extinction has to be multiplied by the dilution factor of the juice.

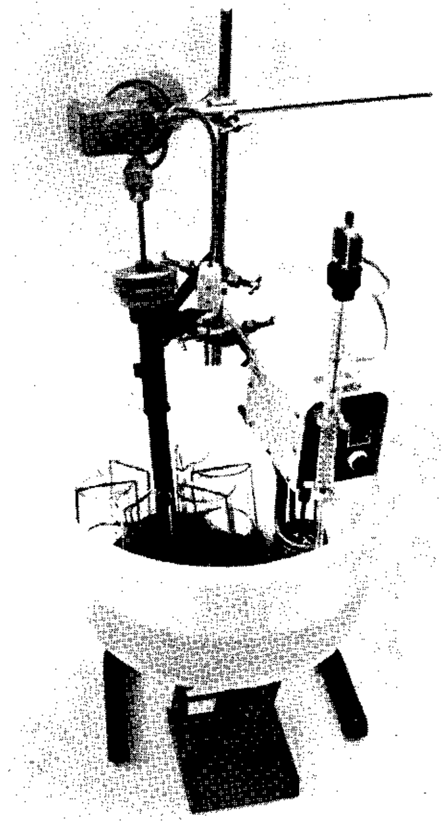

Fig. 1. Apparatus used in dopachrome formation.

DISCUSSION

The amount of dopachrome formed depends on the tyrosine concentration and the phenoloxidase activity in the juice. The tyrosine concentration can be determined by the method described by UDENFRIEND and COOPER (2). The phenoloxidase activity can be deduced from the 'discolouration number', determined as described above, only if the tyrosine content is known.

For the discrimination of potato varieties, with regard to raw discolouration, it is chiefly of practical importance to determine the amount of dopachrome formed in a fixed time. It is therefore not necessary to determine phenoloxidase activity or tyrosine content separately.

Results obtained from analysis of about a hundred potato varieties demonstrate that in twenty minutes 10 to 90 per cent of the available tyrosine is converted to dopachrome. Tyrosine content varies between 0.5 and 3.5 mmol per liter of juice. It will be clear from these figures that the amount of dopachrome formed in 20 minutes can vary from 0.05 to 3.0 mmol per liter of juice. The reaction time of the method described here is fixed at twenty minutes, because within this time no considerable amount of melanin is formed. No induction time, caused by reducing substances like e.g. ascorbic acid, was observed.

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THE NUMBER OF SPECIES IN GRASSLAND

A. A. KRUIJNE

INTRODUCTION

As a concomitant result of the correlative-ecological research, carried out under the direction of DE VRIES (6) for years on end, the number of species found in each field sampling has also been determined. This number is significantly lower than the total number of species that is to be found in a field, which can only be accurately approximated after hours of careful determination. The latter, however, is of little or no practical use, as a number of species would be recorded with a very low distribution density. Besides these, also species would be recorded, present by one individual only, which should be classified as migrants, as well as those found on places with deviating growth conditions, such as ditch sides, entrances and trenches.

Some idea of the total number of species in a grassland may be obtained from an investigation of NIELEN and DIRVEN (3). They determined the number of species in a field by taking composite samples, composed of an increasing number of sampling units. The sample with the largest number (*viz.* 1800 sampling units) was representative for the whole field. Furthermore, there are two experimental reports of KOOMANS (1) and LUEKS (2), they determined the increase in the number of species on increasing areas from 1 sq. cm onwards to the whole field. In view of these investigations it may be assumed that the number of species in a whole field, the deviating patches included, is 30–35% higher than that found in the normal sampling of a field.

Already in 1949 SANDERS and DE VRIES (4) estimated the influence of the use, degree of humidity and fertilization status on the number of species per field in the material available at that moment, from the above-mentioned correlative-ecological research. In this paper the results are reflected of a similar, though somewhat more elaborate examination of the material, meanwhile much increased. The results of SANDERS and DE VRIES could be confirmed in outline.

MATERIAL AND EXPLANATION OF THE EXPRESSIONS USED

The botanical analyses of 1577 fields of old permanent grassland distributed over the Netherlands were used. The fields were analysed according to the specific frequency- and order-method and every species present in about 100 sampling units of 25 sq. cm was recorded. Due to the surface and shape of the fields there were indeed small variations in the number of sampling units, which was reflected in the number of species found. However, it may be assumed on good grounds that the somewhat lower number of species found in less than 100 sampling units is almost compensated by a somewhat higher number in over 100 units. In any case there was no systematical deviation of the average number of sampling units.

The habitat factors have been classified as follows:

Type of use: pure hayfield (ph), hay pasture (hp), alternate pasture (ap) and pure pasture (pp);

Type of soil: sand (s), sandy clay (sc), clay (c), peaty soil (ps) and peat (p);

Alkalinity: very acid (va), moderately acid (ma), slightly acid (sa), almost neutral (an) and alkaline (a);
Degree of humidity: dry (d), normal moisture content (nm), moist (m) and wet (w);
Phosphate and potassium status (5): low (l), rather low (rl), moderate (m), rather high (rh) and high (h).

A more elaborate description of the classes mentioned is to be found in a publication[1] of DE VRIES and KOOPMANS (8). The term *'fertilization status'* used in this publication has been derived from the phosphate and potassium status and has the following 3 classes:
insufficient (i): all combinations of 'low' and 'rather low';
moderate (m): all combinations of 'rather low' and 'moderate' and 'rather high' as well as of 'moderate' and 'rather high' and the combinations 'moderate – moderate';
sufficient (s): all combinations of 'rather high' and 'high'.

It is quite feasible that not all data on a field are known and as a result not all averages concern the total 1577 fields. For each average number of species the concerning number of cases has been mentioned.

NUMBER OF SPECIES UNDER VARYING CONDITIONS

a. Number of species in the grade-of-quality-classes

The grade of quality (gQ) reflects the agricultural value of the herbage, estimated by the botanical composition (7). (The whole range of the gQ is divided into 6 classes, as is shown in table 1.)

TABLE 1. Number of species in the various grade-of-quality-classes.

gQ	poor 0–3.0	insufficient 3.1–5.0	moderate 5.1–6.0	sufficient 6.1–7.0	good 7.1–8.0	very good 8.1–10	av.
Average number of species	37	39	34	31	27	24	33
Number of samples	134	357	347	372	239	99	(1548)

The maximal number of species is found in the class 'insufficient' with a sharp decrease as the grade of quality increases, while less species are found in the class 'poor' as well.

This corresponds completely with the findings of SANDERS and DE VRIES (4), though they did find a somewhat lower number of species in both these classes. A sub-division of the two classes 0–3.0 and 3.1–5.0 finally gives the following averages (the number of samples in brackets). gQ $\leqslant$ 2.0: 36 (60); gQ 2.1–3.0: 38 (74); gQ 3.1–4.0: 41 (108); gQ 4.1–5.0: 38 (249). Evidently, the maximal number of species, *viz.* 41, is found in the gQ-class 3.1–4.0, decreasing with a lower as well as a higher gQ. This is due to the fact that the environment grows more and more extreme in both directions and as a result only a few specialized species are able to maintain themselves.

b. Number of species in the classes of the 6 habitat factors

Obvious differences, apparently related to the gradual change within the factor, are to be found with the type of use, the P- and K-status. A regular decrease in the

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[1] In the mentioned publication (8) the P- and K-status were classified as follows: poor, insufficient, moderate, sufficient and good. Meaning and order is the same as that of the classification used nowadays.

TABLE 2. Number of species in the 6 habitat-factor classes.

		Number of				Number of	
		species	samples			species	samples
Type of use	ph	41	130	Degree of humidity	d	34	158
	hp	35	395		nm	31	768
	ap	32	422		m	34	452
	pp	30	534		w	39	178
Soil type	s	32	385	P-status	l	41	212
	sc	33	433		rl	37	274
	c	35	290		m	33	359
	ps	32	168		rh	31	344
	p	33	271		h	28	346
Alkalinity	va	28	73	K-status	l	40	81
	ma	32	337		rl	38	277
	sa	34	526		m	35	472
	an	33	491		rh	31	349
	a	34	111		h	28	320

number of species is observed as grazing is more intensive and the nutrient status improves, respectively. This is due to a heavier competition of the better grasses.

Contrary to the preceding factors the number of species does not show a regular change in the subsequent classes of the degree of humidity. Presumably, this factor affects the number of species to a somewhat less extent. The relative minimal number of 31 species in the class 'normal moisture content' is again related to the heavy competition of the good grasses; proportionally, most of the pure pastures and fields with a better fertilization status are found in this class.

The soil type does not call for a closer inspection. This factor hardly shows any deviation from the average number of 33 species.

Finally, there is the alkalinity. Only the number of species in the class 'very acid' clearly deviates from the average. This proves that extremely low pH's often are associated with a low number of species. The average number of species in the samples of fields with a pH below 4.0 was only 14. Although the fertilization status of these fields is almost always 'poor' and consequently a high number of species is to be expected (see table 4), this proves to be of no significance in this case.

The small number of species is due to the fact that these extremely acid fields show a much deviating vegetation, somewhat resembling that of heaths. They have an open sod and can best be called 'grass-heaths'. These fields cannot pass as cultivated grasslands, which are used according to certain strict management rules.

c. Number of species in the (limited) type of use-classes

In order to gain a better insight into 4 type of use-classes, all fields with a disharmonious P- and K-status (*e.g.* P-status 'low', K-status 'rather high') as well as the fields with extreme pH's (below 5 and over 7) have been left out of consideration in table 3.

The number of species per class as well as the average number has remained almost the same by this treatment. The apparent influence of the type of use proves to be rather large. The fact that the type of use is often associated with a better fertilization status, *viz.* that more intensive grazing often goes together with a better fertilization status, has been left out of consideration. Under the heads *g.* and *h.* it will be shown, however, that the pure effect of the type of use is also important.

TABLE 3. Number of species in the four classes of the type of use, after eliminating the fields with a disharmonious P- and K-status or with an extreme pH (< 5.0 and > 7.0).

	Type of use				
	ph	hp	ap	pp	Average
Number of species	42	35	33	30	33
Number of samples	66	272	322	360	(1020)

d. Number of species in the (limited) fertilization-status classes

The same fields have been classed in table 4, according to the fertilization status, divided into 3 classes after the combination of the P-and K-status as described on page 168. A priori, the fields with a disharmonious fertilization status have already been eliminated.

TABLE 4. Number of species in the three classes of the fertilization status after eliminating the fields with an extreme pH (< 5.0 and > 7.0).

	Fertilization status			
	i	m	s	Average
Number of species	42	34	28	33
Number of samples	142	487	391	(1020)

Table 2 shows figures that are not completely comparable, but the numbers mentioned in the classes of the P- and K-status are of the same magnitude.

e. Number of species in the (limited) degree of humidity-classes

TABLE 5. Number of species in the 4 classes of the degree of humidity after eliminating the fields with a disharmonious P- and K-status or with an extreme pH (< 5.0 and > 7.0).

	Degree of humidity				
	d	nm	m	w	Average
Number of species	33	31	35	40	33
Number of samples	95	534	288	103	(1020)

The elimination of 500 odd fields, that deviate somewhat with regard to the fertilization status or (and) pH hardly influences the number of species as mentioned in table 2.

f. Number of species in all combinations of the fertilization status and alkalinity

In table 6 the two factors fertilization status and alkalinity have been separated. Similar as in table 3, 4 and 5 the fields with a disharmonious fertilization status have also been eliminated in this case, but the fields with a pH below 5.0 and over 7.0 have been included.

The total averages in the three classes of the fertilization status do not differ significantly from those in table 4. The number of fields with a pH of < 5.00 and > 7.00 added in table 6 is proportionally too small to affect the average significantly. In each pH-class, except for 'va', the number of species decreases as the fertilization status improves.

TABLE 6. Number of species in all combinations of the fertilization status and alkalinity, the concerning samples in brackets.

	Fertilization status			
	i	m	s	Average
va, pH < 5.00	26 (24)[1]	28 (23)	26 (5)	27 (52)
ma, pH 5.05–5.50	41 (53)	31 (150)	27 (60)	32 (263)
sa, pH 5.55–6.00	44 (60)	36 (210)	29 (141)	34 (411)
an, pH 6.05–7.00	45 (40)	37 (157)	28 (199)	33 (396)
a, pH > 7.00	40 (12)	39 (37)	26 (28)	34 (77)
average	41 (189)	35 (577)	28 (433)	33 (1199)

[1] Elimination of 4 samples consisting of a small number (± 55) of sampling units gives a value of 28 (20).

With an insufficient fertilization status the number of species in the class 'va' is far below that in the other pH-classes; the reason has already been explained under table 2.

The total averages in the 5 pH-classes do not differ much from those in table 2, in which the fields with a disharmonious fertilization status were also included.

g. Number of species with differing types of use and otherwise optimal conditions

Table 7 reflects the true influence of the type of use. Only those fields have been included of which the other ecological factors were optimal for grassland: a normal moisture retaining soil with a pH between 5.00 and 7.00 and a high P- and K-status.

TABLE 7. Number of species in the type of use-classes with otherwise optimal conditions.

	Type of use				
	ph	hp	ap	pp	Average
Number of species	–	29	27	23	25
Number of samples	–	8	25	51	(84)

There were no fields available in the class 'ph'. The number of species in the other classes is clearly below those in the tables 2 and 3. This is not surprising, because under optimal conditions the competition of certain species will be very heavy in the fields included. In this case *Lolium perenne* is dominant even in hay pastures in half the number of fields.

Although the number of fields is much smaller than that in table 3, the difference in the number of species, *e.g.* between 'hp' and 'pp' is of the same magnitude. Thus the true influence of the type of use is of importance as well.

h. Separation of the three factors with the greatest effect on the number of species

Figure 1 shows from top to bottom the three factors degree of humidity, type of use and fertilization status in groups of 3 or 4 squares (or so many less as data are lacking). Apparently, the decrease in the number of species in the degrees of humidity and in the types of use is less regular than that in the three classes of the fertilization status. Accordingly, it appears that the latter factor has the most important influence on the number of species. This is in complete correspondence with the findings of SANDERS and DE VRIES (4). The influence of the type of use is clearly less, but probably more important than that of the degree of humidity.

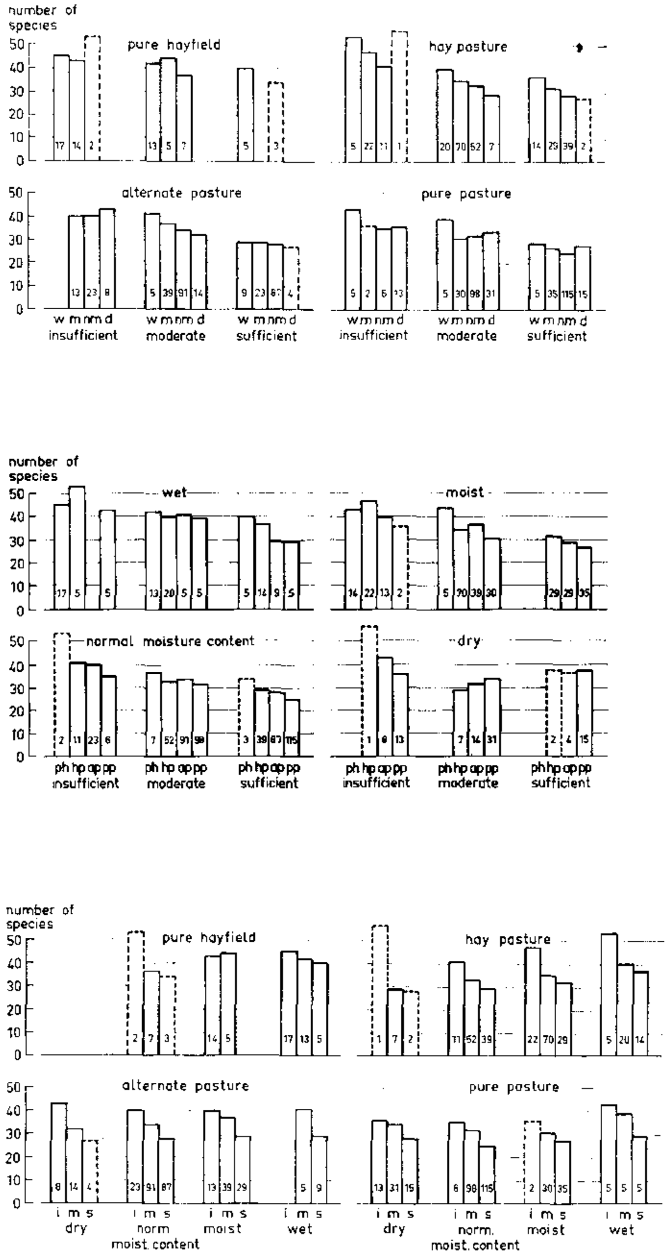

FIG. 1. Number of species in the separate classes of the type of use, degree of humidity and fertilization status. The number of samples has been indicated in the columns (below 5 the column has been dotted; see page 167-168 for abbreviations)

i. Number of species at varying degrees of dominance of the leading species

A species is dominant when its percentage by weight (W%) exceeds that of all separate other species. The degrees of dominance are: 'very pure', if the W% is over 50; 'pure', if the W% is at least 25, including 'very pure'; and finally 'impure', if the W% of the leading species is less than 25%.

TABLE 8. Number of species with increasing dominance of some leading species

	Degree of dominance		
	impure	pure	very pure
Lolium perenne	33 (79)	28 (296)	24 (68)
Festuca pratensis	36 (23)	34 (11)	–
Poa trivialis	31 (50)	29 (55)	–
Agrostis stolonifera	36 (58)	30 (100)	26 (26)
Holcus lanatus	40 (49)	35 (56)	–
Festuca rubra	35 (58)	34 (44)	27 (12)
Molinia caerulea	–	34 (33)	28 (8)

The figures clearly show that the number of species decreases as the dominance of the leading species increases. This is not at all surprising, because with impure dominancy the W % of the leading species remains below 25, but with very pure dominancy it will constitute half of the total herbage or even over. In the latter case the number of species consequently remains below the average of 33. With *Lolium perenne* and *Poa trivialis*, however, the number of species does not exceed the average, even with impure dominancy.

In considering the number of species primary and examining under what ecological conditions a certain number of species occurs, the high and the low numbers of species prove to be most interesting.

The above was examined for the pH, the P- and K-status, because these factors allow a division in a rather great number of subsequent classes. Thus the distribution in these classes of the fields with extreme numbers of species ($\geqslant 48$ and $\leqslant 22$) was investigated. It finally appeared that both groups of fields to a certain extent show contradictory ecological demands, as was to be expected from the preceding. Moreover, the samples with an extremely high number of species proved to be accumulated in a limited area, while the samples with few species covered a much wider range:

samples $\geqslant 48$ species: pH: 5.0–7.5; P-citric acid number: 0–60; K-value: 0–33.
samples $\leqslant 22$ species: pH: 3.5–8.0; P-citric acid number: 0–220; K-value: 0–100.

Figure 2 represents the K-status, clearly indicating the limited range in which high numbers of species occur as well as the percentage of fields present that shows extremely high and low numbers of species. It should be taken into consideration however, that it is not only the K-status that determines the number of species. Extreme numbers of species generally are found when certain ecological factors, influencing the number of species in the same sense, coincide.

Finally, an attempt has been made to show a general ecological picture of fields with exceedingly high numbers of species. In spite of the fact that extremely high ($\geqslant 60$) and extremely low ($\leqslant 15$) numbers of species were considered a distinctly outlined complex of environmental conditions could not be determined.

It may be stated that grasslands with over 60 species generally are fields not or slightly grazed with an ample moisture content and an insufficient fertilization status, especially with regard to the P-status. Fields with 15 species or less are generally found among the pure pastures on peat with a rather low pH.

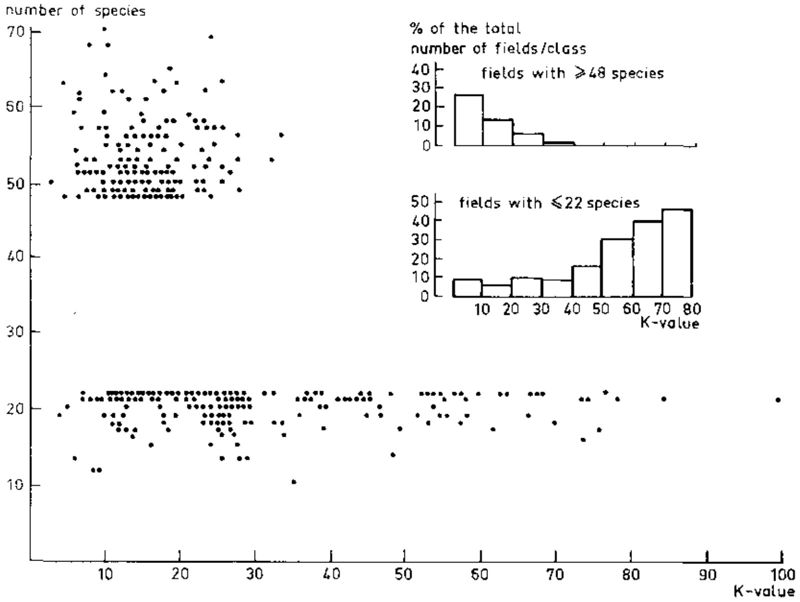

FIG. 2. Distribution of the fields with many (≥ 48) and with few (≤ 22) species over the range of the K-value.

SUMMARY

In the botanical research of 1577 grassland fields the average number of species per sample was estimated at 33. The total number of species in a whole field is about 30–35% higher.

With regard to the grade of quality-degrees (gQ) the number of species was maximal, *viz.* 41, with a gQ of 3.1–4.0, decreasing with a higher as well as with a lower gQ. More intensive grazing as well as an improving fertilization status also reduced the number of species regularly. As the fields concerned are drier the number of species will decrease, though not regularly. Separation of these three factors shows that the fertilization status is the factor with the greatest influence. The pure influence of grazing and the degree of humidity is clearly less.

With dominance communities the degree of dominance clearly affects the number of species, the 'very pure' communities remain far below the average number of species; the communities of *Lolium perenne* and *Poa trivialis*, the 'pure' and 'impure' included, do not exceed the average number of species.

Finally an examination was made under what ecological conditions (pH, P-, K-status) very high and very low numbers of species occur.

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Mededeling 254 van het I.B.S.

NOTES ON THE ACTIVITY OF EARTHWORMS

I. THE INFLUENCE OF *Rhododendron* AND *Pinus* ON EARTHWORMS

J. DOEKSEN

INTRODUCTION

In the well-known nursery district of Boskoop, hardly any earthworms are present in the peaty soil of nurseries.

The ground-water level in these soils has always been very high, but this only could not account for the absence of earthworms. The highest number of earthworms was found – up to 7 million per ha – in soils with comparable ground-water levels. Grasslands adjoining the nurseries show normal earthworm population densities.

At first we thought that the exposition of the soil surface to the sun, combined with the high ground-water level and the resulting anaerobic conditions, often even at a depth of 20 cm, might be the reason for the absence of earthworms.

Shortly after the experimental garden of the Boskoop Experimental Station for Arboriculture had been drained with a closed drainage system, 8000 earthworms were introduced in this garden, on a number of small experimental plots, to see whether earthworms would have any influence on the soil and on plant growth. The species introduced were *Lumbricus rubellus* and *Allolobophora caliginosa* in almost equal numbers.

After one year, however, not a single worm of these species could be found again, but very few specimens of *Octolasium lacteum* were found, a species which surely had not been introduced in the field. It lives at a depth of 60 cm where the peat is yellow by reduction.

The disappearance of the introduced worms seemed slightly mysterious, as the soil of the garden, brought into the laboratory, proved to be suitable for the keeping and breeding of earthworms.

Mr. H. EGBERTS, at the time Research Officer at the Experimental Station suggested that the intensive cultivation of *Rhododendron* spp. and/or coniferous trees might have to do with this phenomenon.

In order to check this suggestion some experiments were made, on which is reported in this paper.

EXPERIMENTS

First of all, we wanted to make sure, whether fallen leaves of the suspected plants do influence earthworm life in the soil.

The chief gardener of the Experimental Station, Mr. H. BOSCH, kindly provided a large quantity of fresh clean leaves of the locally mostly grown *Rhododendron* hybrides. We collected fresh needles of *Pinus sylvestris*, also taking care that they had not been in contact with soil.

The leaves and needles were dried at 70°C and finely ground in a fibratory ball-mill with stainless steel pot and balls.

Breeding cells ('flats') as described by DOEKSEN (1960) were filled with a good sandy garden soil in which, as far as could be ascertained, no *Rhododendron* nor coniferous trees had been grown for years.

To the soil were added 10% in dry weight of different materials as specified in table 1 and thoroughly mixed. With soil from each of the mixtures, 5 'flats' were filled to each of which 2 specimens of *Lumbricus rubellus* were added. The 'flats' were kept in the dark at 16°C, the moisture content of the soil being kept as constant as possible.

Observations on condition and activity of the worms were made twice a week. The experiment lasted for 72 days.

The 'average time of survival' per 10 earthworms was calculated, in which the day preceding that on which the death of an animal was observed, was taken as the last day of its life.

If at the end of the experimental period there were still living worms present, their 'time of survival' was taken as > 72 days and the average of the group of 10 to which it belonged, was indicated with a > sign. In table 1 the results of this experiment are given.

TABLE 1. Average time of survival of groups of 10 *L. rubellus* each, in garden soil with or without additions.

Garden soil	Average time of survival in days	Number of surviving worms
without any addition	> 63.1	8
+ 10% ground pine needles	> 57.6	3
+ id. extracted with ethanol	> 50.0	1
+ id. ,, ,, ether	> 67.4	5
+ id. ,, ,, benzene	> 65.0	4
+ id. ,, ,, acetone	44.7	–
without any addition	> 70.6	8
+ 10% ground *Rhododendron* leaves	18.1	–
+ id. extracted with ethanol	24.7	–
+ id. ,, ,, ether	> 21.8	1
+ id. ,, ,, benzene	13.7	–
+ id. ,, ,, acetone	> 54.1	7

Table 1 shows that pine needles may have but a slightly adverse effect on earthworms of the species used. The poor effect of extraction with acetone might result from remaining traces of acetone, though it was tried to prevent this.

The slight improvement when extracting the needles with ether or benzene might be an indication that resins or resinous products from the needles have some influence on earthworms. Therefore a cumulative effect might become evident if year after year so-called 'needle soil' from pinewoods is used to replace exported and oxidized material in the nurseries, but this will hardly ever cause the extermination of *L. rubellus* as this species can maintain itself in pinewoods, be it in small numbers.

It is quite evident however that *Rhododendron* leaves must contain a substance which is rather harmful to earthworms. This substance must be reasonably soluble in acetone, not however in ethanol, ether or benzene.

Another question was whether a living root system of a *Rhododendron* could influence worm life.

It is very difficult, however, to decide whether a certain effect is the result of the presence of living roots as it may be assumed that there will always be dead plant tissue present as well.

Therefore a relatively great quantity of dead plant material was added to the soil, many times the amount the plant could possibly add to the soil during the experimental period.

Four tubes of p.v.c. with a diameter of 30 cm and 1 m high were filled with a well-mixed garden soil. Four others were filled with the same soil up to 70 cm, whereas the top 30 cm were filled with the same soil in which 10% (as dry matter) of finely ground *Rhododendron* leaves had been mixed. The soil had not been sterilized, large worms were not observed but very likely some young ones and egg cocoons were present.

In October, in four tubes, two from each group, *Buxus sempervirens* was planted (2 per pot); in the other four one *Rhododendron* hybrid on *R. ponticum* rootstock each.

The tubes were dug in into the garden to their full depth. An artificial ground-water level of 95 cm was maintained to prevent aeration of the soil in the tubes from beneath.

In April 20 earthworms were added to each tube (10 each of *L. rubellus* and *A. caliginosa)*.

One year after planting and half a year after the introduction of the extra worms, the tubes were emptied. Root development of both plant species was excellent.

Worm counts showed great differences in number per pot; they were spread all over the pots; egg cocoons were even found at a depth of 90 cm. The possibility to be active at greater depths than normal may be the reason that in all tubes a relatively great number of earthworms was present.

In table 2 the data from this experiment are collected. Because of the great number of immature worms and the likeliness that other species than the introduced ones were present, no distinction between species has been made.

TABLE 2. Numbers of earthworms and earthworm cocoons in pots with two different plant species, with or without ground *Rhododendron* leaves added to the soil.

Plant species	Soil							
	with *Rhododendron* leaves				without *Rhododendron* leaves			
	worms		cocoons		worms		cocoons	
	per pot	mean	per pot	mean	per pot	mean	per pot	mean
Rhododendron hybrid	40	41	27	23	131	121	54	37
on *Rh. ponticum*	42		19		111		20	
Buxus sempervirens	61	63	4	7	167	181	40	45
	65		10		195		50	

Independent of plant species there is a decrease in number of earthworms as the result of the addition of *Rhododendron* leaves of 67 %. The decrease in number of earthworms under the influence of a living *Rhododendron* rootstock, independent of the addition of dead plant material is 33 %. Differences in number of cocoons are not conclusive.

CONCLUSIONS

The aim of the observations on which is reported here was an attempt to find an answer to the problem of the absence of the common earthworm species in the nurseries of Boskoop, Netherlands. Probably, this phenomenon is the result of the intensive culture of *Rhododendron* spp. Whether other *Ericaceae* have a similar influence remains to be studied. *Pinus sylvestris* hardly has any influence, but species of the genus *Pinus* are not the ones most commonly grown in Boskoop. It will be interesting to study the influence of *Juniperus*, *Thuya* and *Chamaecyparis* spp.

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Mededeling 255 van het I.B.S.

NOTES ON THE ACTIVITY OF EARTHWORMS

II. Observations on diapause in the earthworm *A. caliginosa*

J. Doeksen and C. G. van Wingerden

Introduction

In summer, in the field quite a number of earthworms are found coiled up in small rounded chambers, which are often plastered with faeces. The worms are pink, more transparent than normal, have an empty alimentary canal and are not easily roused to activity.

The tendency to go into this 'diapause' differs with the species. From the middle of May, up to the middle or end of September, hardly any active individuals of *Allolobophora longa* are to be found, whereas in most cases some active specimens of *A. caliginosa* can be found. It is a useful observation of Michon (5) that earthworms with red pigment never go into diapause; only species without red pigment do so. So far we did not meet with exceptions.

Stephenson (7) in discussing the sexual activity of earthworms writes: 'I think general experience in this country (England, *ed.*) agrees with Miss Pickford (6) that sexual worms may be found at all seasons, though they are on the whole much less common in winter than in summer.

Avel (2, 3) and Abeloos and Avel (1) state that in *A. terrestris (= longa)* and *A. caliginosa* the active portion of the sexual cycles takes place in winter, and that the worms are quiescent sexually during the warmer months from April onwards (locality not mentioned). This 'diapause' is not due to the desiccation of the soil, or to a rise of temperature, since it occurred in worms kept in a constant environment; it is not merely a state of rest after the sexual phase, since immature specimens, and castrated worms, undergo a similar period of inactivity. The gut is emptied, the worms retire deeply into the soil, and coil up within a cyst; the appetite for food ceases. The diapause is due to internal factors; it ends at the time of the autumn rains, and the worms then rouse and eat ravenously. It would seem that the modification of the usual cycle described by these authors is an adaptation to the climatic conditions of the particular region.'

The observations by Avel and Abeloos et Avel on diapause are very much to the point; it is therefore a step back if Michon (5) contributes diapause to drought only.

We do agree with most of the observations of Avel and Abeloos; however, that diapause should be due to internal factors (only) has not been proved. The fact that Stephenson at the time overlooked the importance of diapause in earthworms could be an indication that this phenomenon is less common in England than it is on the continent. This could equally well point to direct climatic influences and not necessarily to an adaptation of the worms to the different climates.

Since during our experiments with earthworms we always were confronted with this phenomenon of diapause which interfered in a most unpleasant way with our experiments, an attempt was made to find a way to overcome these difficulties. The use of red-pigmented earthworms which cannot go into diapause is not a solution of the problem, as these animals die under the circumstances in which the other ones go into diapause.

METHODS

A series of smaller experiments was carried out, in which specimens of *Allolobophora caliginosa* were kept in small breeding cells ('flats') as described by DOEKSEN (4). As a standard, a good sandy garden soil was used, after thorough mixing and sieving. The pH (water) of this soil was brought to 7.0 by the addition of $CaCO_3$. To study the influence of surface activity of the soil, 10% clay, nearly free from organic matter and 10% activated charcoal respectively were mixed with the standard soil.

Differences in pH were brought about by the addition of the necessary quantity of $CaCO_3$ or H_2SO_4. After thorough mixing, the soil was kept in the laboratory for some weeks, mixed again and the pH determined. We are well aware that, by these treatments, the different soils were not fully comparable, but at the time there were no better means to reach the pH-differences needed.

In all experiments, ten flats with two worms each were used per treatment. They were kept at constant temperatures; the moisture content was high and almost constant. Care was taken that demineralized water was always present in the boxes in which series of 10 flats were kept.

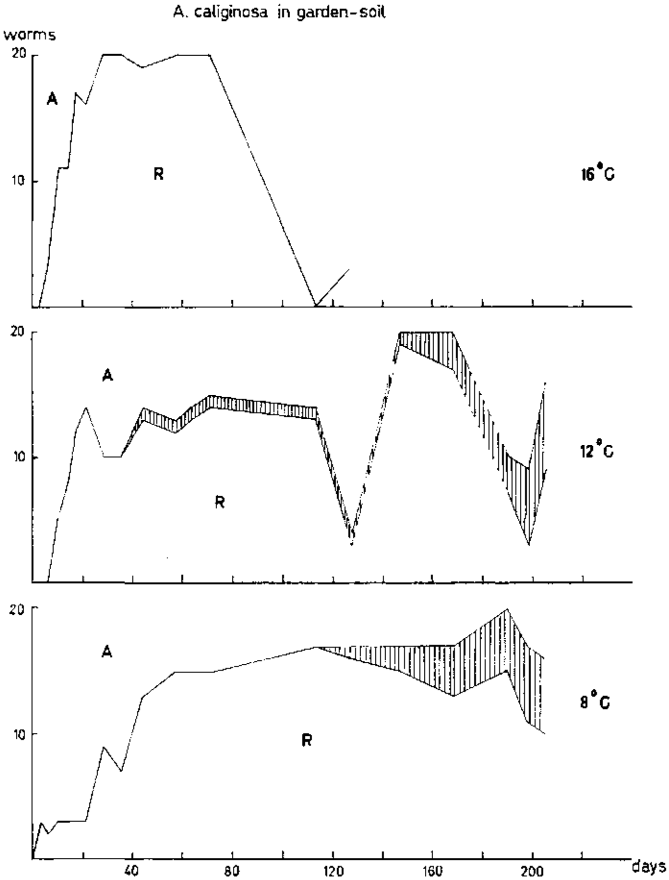


FIG. 1. The effect of temperature on *A. caliginosa* in a sandy garden soil. A: active worms. R: worms resting; Shading: dead worms.

INFLUENCE OF TEMPERATURE AND SURFACE ACTIVITY OF THE SOIL ON DIAPAUSE

In earlier experiments diapause was obtained under all conditions, but since STEPHENSON (7) suggested that differences in climate might be the reason for the supposed different behaviour of earthworms in England and in France, it seemed necessary to study the influence of temperature on diapause. We chose three temperatures, which are not abnormal for soil conditions, *viz.* 8°, 12° and 16°C.

As from the start diapause was suspected to be the result of some kind of poisoning, it was thought likely that the soil, expecially its buffering capacity, might have influence on the phenomenon. Clay and charcoal were therefore added to the soil as described.

In all, there were three 'soils' at three temperatures. The data from this experiment are brought together in the figures 1–3 incl.

Unfortunately, after 112 days, the 16°C-incubator broke down, the temperature for some time rising to over 30°C which of course proved lethal to the worms.

In the diagrams the lower part, marked R, represents the worms that are in diapause; the upper part A are the worms that are active, while shading indicates the dead specimens.

It is evident, that with rising temperature, diapause is induced quicker and is more general; this holds for the three soils. During periods in which only part of the worms

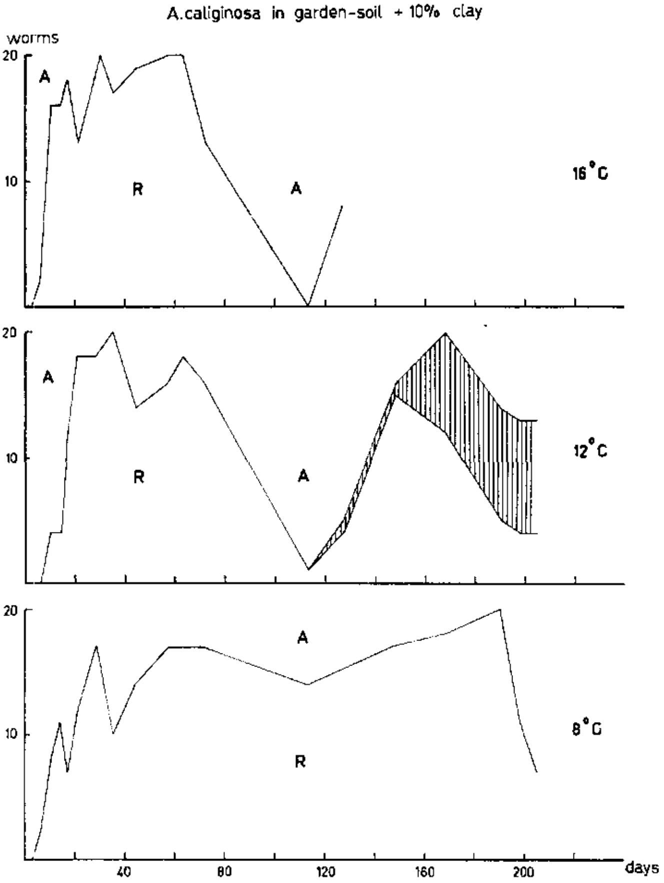


FIG. 2. The effect of temperature on *A. caliginosa* in a sandy garden soil + 10% clay. Explanations see fig. 1.

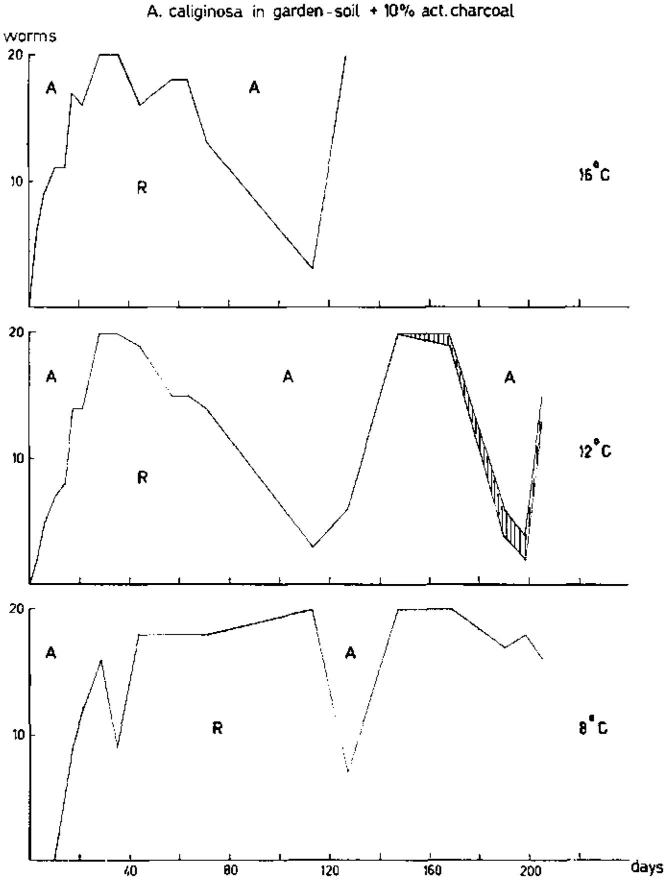


FIG. 3. The effect of temperature on *A. caliginosa* in a sandy garden soil + 10% activated charcoal. Explanations see fig. 1.

are in diapause, not always the same individuals are resting the whole time, but they may be active again while others go into diapause. This explains the small peaks in the diagrams.

It is interesting to note, that after a period in which a high percentage of the animals is at rest, almost general activity occurs, followed by a new period of diapause which is shorter than the first one, followed again by a period of activity.

This alternation of activity and rest is only slightly indicated at 8°C.

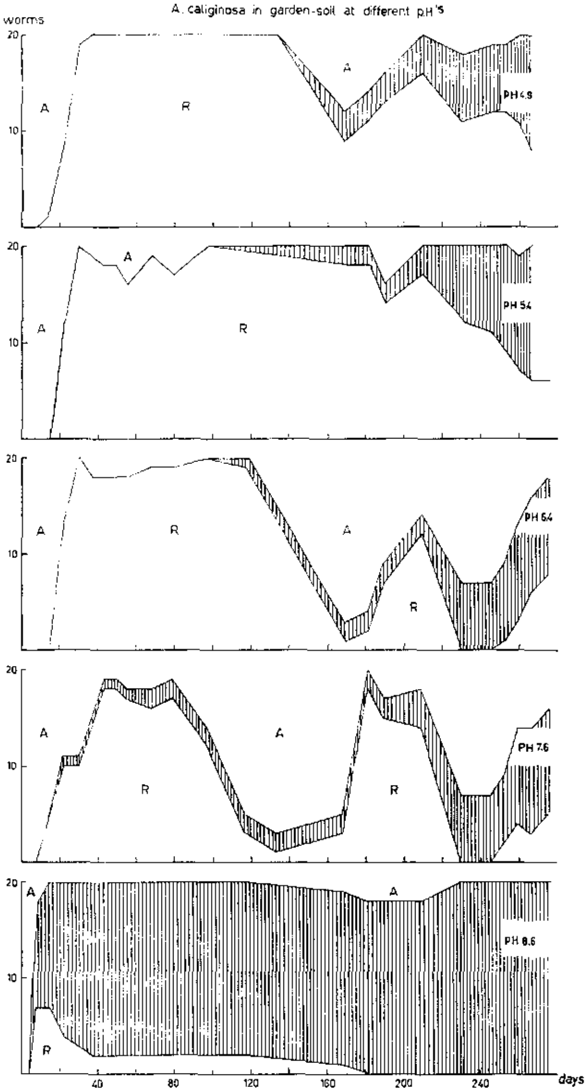
There is an indication that clay and charcoal shorten the time of diapause.

INFLUENCE OF THE pH OF THE SOIL ON DIAPAUSE

There is a general opinion that earthworms are sensitive to soils with a low pH-value or soils poor in calcium. As diapause often appears to be a way for the animal to escape and survive unfavourable conditions, it is indicated to study the influence of as many of these conditions as may be thought of. Therefore, five different pH-levels were chosen of nearly the same soil as described before, to study their effect on diapause, at 16°C; fig. 4 gives the result in diagrams.

It is evident that pH 8.6 is far too high for *A. caliginosa*. There is high mortality, and the living individuals go into diapause very soon, but most of them die after some weeks.

FIG. 4. The influence of pH of a sandy garden soil on diapause and death rate in *A. caliginosa*. Explanations see fig. 1.



The diagrams of pH 7.6 and 6.4 show the characteristics of those of fig. 1–3 incl., *viz.* an alternation of activity and diapause with a tendency of the following periods of diapause being shorter.

At pH 6.4 the first period of diapause is very long and intensive, but followed by a well-pronounced period of activity; at pH 5.4 and 4.9 there only is a faint indication of greater activity as is the case at lower temperatures.

DISCUSSION

From the data, given in the figures 1–4 incl. it is clear, that diapause is induced neither by abnormal temperatures, by changes in temperature nor by drought, as the moderate soil temperatures were kept constant and free water was always present in the flats. Often worms were lying coiled up, partly immersed in water.

It is neither likely that an internal rhythm should make the animals go into diapause, as all worms caught in the field at different times of the year behave in the same way.

Since in the field diapause is restricted to summer but is induced neither by temperature nor by drought, there must be conditions in our experimental procedure, which in the field are restricted to summer.

There are two possibilities: 1. The absence of water percolation, a washing out of the soil, both in the field in summer and in our flats. 2. The limited quantity of soil with which the worms come into contact. In the field, high temperatures and drought must influence worm activity adversely, which greatly restricts the amount of soil with which the animals come into contact, as is always the case in our flats, independent of temperature and moisture.

At this stage of our investigation we therefore assume that diapause must be induced by some substance which is primarily excreted by the worms themselves and may be changed by micro-organisms. The same or other micro-organisms are able to break it down under suitable conditions.

This would explain the changes from activity to diapause vice versa: During the active period a relatively great quantity of the supposed material is formed. Its concentration increases till the worms go into diapause after which the excretion decreases. Micro-organisms, which from the start must have been breaking down the material, now are able to lower its concentration to a level at which the worms can be active again. As the concentration of the active agent depends on the amount of soil with which it is diluted, to restrict the movements of the worms must result in locally high concentrations.

Presumably, the active agent is not easily broken down, but it is likely that the micro-organisms that are able to do so, will increase their numbers, so that a second period of diapause will have to be shorter than the first one.

As there is no great difference in the activity of earthworms at 8°C or 12°C, but as at 8°C hardly any re-activation of the worms occurs, it must be assumed, that the micro-organisms involved are more strongly influenced by temperature than the earthworms and that at lower temperatures they are not able to break down the supposed material fast enough to bring diapause to an end.

The influence of pH on the duration of diapause could equally well be an influence on the activity of micro-organisms, especially bacteria.

Adsorbents as clay and activated charcoal could partly inactivate the material which could explain the effect of these products on diapause.

Acting on the assumptions discussed here, we made further experiments on which will be reported in due course.

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Mededeling 256 van het I.B.S.

NOTES ON THE ACTIVITY OF EARTHWORMS

III. THE CONDITIONING EFFECT OF EARTHWORMS ON THE SURROUNDING SOIL

J. DOEKSEN

INTRODUCTION

In an earlier study on the influence of different factors on diapause in the earthworms *Allolobophora caliginosa* (2), the hypothesis was put forward that diapause in earthworms is induced by a product primarily excreted by the worms themselves, and most likely changed by micro-organisms.

Acting on this assumption, it was considered necessary to collect more evidence which might either confirm or contradict this hypothesis.

If a relatively great number of earthworms is kept together in soil (up to 200 in a 10 l pail), they do quite well for some weeks, dependent on temperature; afterwards they suddenly grow sluggish and the greater part of *Allolobophora caliginosa* goes into diapause, whereas most *Lumbricus rubellus* die.

If the worms are removed at the first signs of this change in behaviour, the remaining soil proves to be unhealthy for other, even freshly caught worms. This quality is conserved for months at 2°C, but disappears in 6 weeks at room temperature (20°–25°C), if the soil is kept moist. Heating the moist soil for three hours at 120°C increases the unfavourable effect on earthworms. Washing the soil by mixing it with an equal weight of distilled water and removing the water by a Buchner-funnel, has no effect.

In this paper soil, in which worms have been kept for as long as they could reasonably stand, is called 'conditioned' soil, while a difference is made between '*caliginosa* soil' and '*rubellus* soil', if it has been conditioned by *A. caliginosa* or *L. rubellus* resp.

OBSERVATIONS

A quantity of a good sandy garden-soil was thoroughly mixed and sieved. Parts of it were conditioned by *A. caliginosa* and *L. rubellus* resp., the remainder was kept under the same conditions as those under which conditioning took place.

As earthworms in a pail with soil are not regularly distributed in the soil, but show a pronounced preference for certain spots, only that part of the soil in which the worms aggregate will be fully conditioned. After mixing the total contents of the pail, the conditioned soil must have already been diluted, moreover it had been stored at 2°C for three weeks prior to the experiments. Nevertheless, in this paper, this soil is called 100% conditioned.

Mixtures of conditioned and original (fresh) soil were made, with increasing percentages of conditioned soil. Five breeding cells ('flats'), as described elsewhere (1), were filled with each of these mixtures. Per flat 2 specimens of *A. caliginosa* and *L. rubellus* resp. were added. The behaviour of the worms was regularly observed. The experiment lasted for 62 days. The average time of survival of the group of 10 worms per mixture was calculated. The day before that on which death of a certain individual was noted, was taken as its last day of life. The time of survival of the individuals still alive at the end of the experiment is > 62.

In the tables 1 and 2 the data have been collected on *caliginosa*-soil and *rubellus*-soil resp. However, figures obtained in experiments with earthworms always have a strong tendency to be irregular, unless a great number of individuals is involved, which demands much room, material and labour.

It is evident from table 1, that with increasing percentages of *caliginosa*-soil, the average time of survival of *rubellus* is very short. Table 2 shows that conditioning by *rubellus* itself has some influence on this worm, but the data are too irregular to allow further conclusions to be drawn.

More interesting is the behaviour of *A. caliginosa* in soils with increasing conditioning by the same species (table 1, fig. 1). The onset of diapause is earlier, but from 50%-caliginosa-soil onwards mortality is high, some worms dying without first going into diapause, others after they have already reached the resting state.

This high death-rate of freshly caught worms which were put into more or less conditioned soil was most remarkable. We did not notice any repellant effect of the conditioned soils, the worms burrowing into it just as they did into fresh soil. The rate of conditioning of the fresh and the 25%-conditioned soil (upper two diagrams of fig. 1) at the moment of general diapause, must have been at least as high, but possibly higher, as the conditioned soil with which the experiment was started. Yet, 30 days after almost general diapause had been reached in fresh- and in 25%-*caliginosa*-soil,

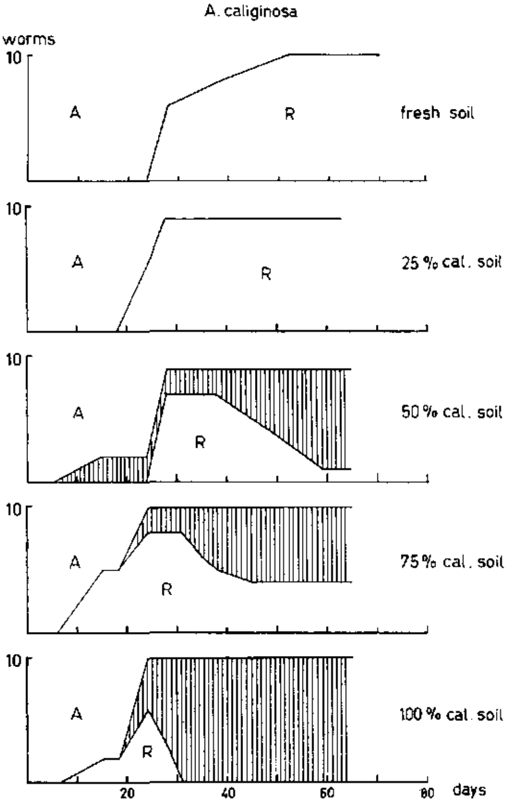

FIG. 1. The effect of different degrees of soil-conditioning produced by *A. caliginosa*, on diapause and death-rate of the same species.

no death had occurred, whereas 30 days after starting the experiment in 100 %-*caliginosa*-soil all animals were dead.

In *rubellus*-soil, mortality of *A. caliginosa* was low and irregularly distributed over the treatments (table 2). As for diapause, this was strongly influenced by the conditioning of the soil by *L. rubellus* (fig. 2), but the effect of conditioning by *L. rubellus* seems to be different from that by *A. caliginosa*.

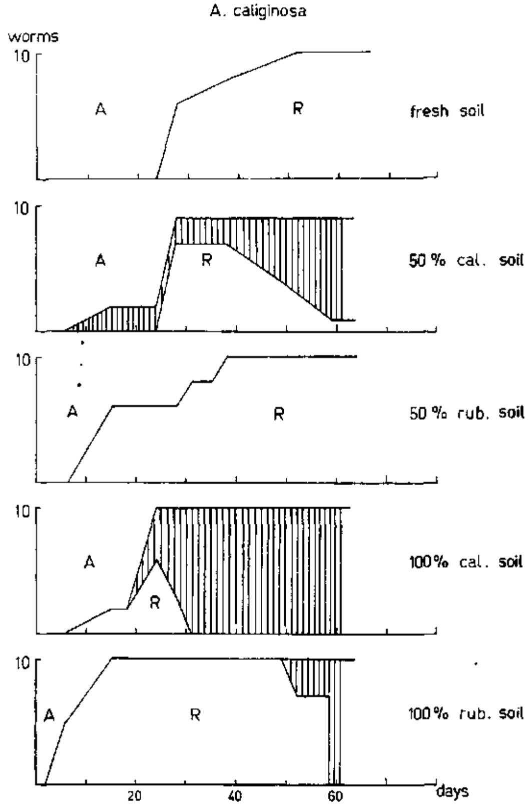

FIG. 2. The effect of different degrees of soil-conditioning produced by *A. caliginosa* and *L. rubellus* on diapause and death rate of *A. caliginosa*.

TABLE 1. Average time of survival in days of 10 specimens each of *A. caliginosa* and *L. rubellus* in a soil conditioned by *A. caliginosa*.

Caliginosa soil %	fresh soil %	*A. caliginosa*		*L. rubellus*	
		average time of survival	number surviving	average time of survival	number surviving
0	100	> 62.0	10	42.8	–
25	75	> 62.0	10	26.4	–
50	50	> 46.3	2	28.6	–
75	25	> 44.4	4	14.1	–
100	0	27.3	–	7.8	–

TABLE 2. Average time of survival of 10 specimens each of *L. rubellus* and *A. caliginosa* in a soil conditioned by *L. rubellus*.

Rubellus soil %	fresh soil %	*L. rubellus*		*A. caliginosa*	
		average time of survival	number surviving	average time of survival	number surviving
0	100	42.8	–	> 62.0	10
25	75	28.1	–	> 55.2	8
50	50	22.0	–	> 62.0	10
75	25	30.5	–	> 49.6	8
100	0	32.9	–	> 59.0	7

DISCUSSION

From the data given, it is likely that diapause primarily is induced by a substance excreted by the earthworms themselves. Whether this substance has to be changed by micro-organisms before it is harmful, remains to be studied. It is obvious that if the inducement of going into diapause is too strong or too sudden, *A. caliginosa* cannot react properly to it, but dies, either directly or shortly after the onset of diapause. In *L. rubellus* the same stimulant causes death quickly. It is not certain whether the active substances derived from *L. rubellus* and *A. caliginosa* are the same, or if the differences observed, depend on differences in concentration only.

Isolating possible diapause-inducing substances is complicated by the fact that a too strong stimulant causes death instead of diapause. Since death is not a very specific reaction, isolates have to be diluted before they can be tried on earthworms. So far, diapause could not be induced, or recognised as such, in vitro. Therefore diluted isolates have to be added to soil, which again, is complicating the procedure, as microbial activity cannot be excluded. Before any conclusions may be drawn and purification of the isolates can proceed, a normal diapause has to be observed, preferably both in the experiment and in the controls.

In the field, the alimentary tract of earthworms in diapause is always empty. During starvation in the laboratory it takes a worm 8–10 days to empty its gut. This shows, that in the field the inducement for going into diapause must be strong enough to make the animal stop feeding at least 10 days before the onset of diapause. Diapause in earthworms cannot simply be a prolonged resting state, as regeneration or the growth of new segments only takes place during diapause. Red-pigmented earthworm species which cannot go into diapause, do not regenerate nor grow new segments at the posterior end (3).

Considering all this, it is not surprising that a worm needs rather a long time to adapt itself to a stimulant before being able to go into diapause.

So far the only chemically pure substance with which a seemingly normal diapause could be induced, is lysine.

200 g of a garden soil with 8 g of organic matter in all was percolated daily for 5 days with 100 ml of a sterilised $1^0/_{00}$-solution of l-lysine dihydrochloride, brought to pH 6.8 with sodium hydroxide. Between percolations, the wet soil column was kept at 20–25°C in the percolation tubes. In all, 0.5 g lysine-dihydrochloride (= 0.335 g lysine) was used; the amount directly lost by the percolate was not determined.

Another 200 g of the same soil was percolated in the same way with distilled water only. The pH of the last percolates was 6.2 and 6.1 for lysine and water resp.

The soils were dried at room temperature for four days. After thorough mixing 5 flats were filled with each of the two soils. To each flat 2 specimens of *A. caliginosa* were added.

After 24, 27, 29 and 36 days, in the 'lysine-soil' 4, 6, 8 and 9 worms were in diapause resp., whereas in the 'distilled-water-soil.' only one was.

Evidently this experiment does not say much of the true character of the diapause-inducing agent. Experiments in this field are continued.

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