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VAN LANDBOUWGEWASSEN
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with summaries

INHOUDSOPGAVE/*CONTENTS*

WOORD VOORAF

Het lijkt misschien strijdig met de aard van ons toegepaste onderzoek, dat ook kennis en gegevens worden verkregen, waarvan men niet meteen kan zeggen of ze vruchtbaar toe te passen zijn of belangwekkend zonder meer. Toch komt dergelijk materiaal beschikbaar. Slechts de bekendmaking ervan kan uitwijzen of het nut zal afwerpen en of het wellicht aan werkers hier te lande of elders een nog ontbrekend stuk inlichting kan verschaffen.

Dit jaarboek bestaat weer grotendeels uit een bundeling van dit soort gegevens, met daarnaast bijdragen die meer directe toepasbaarheid lijken te hebben.

In het bijzonder voor buitenlandse contacten is weer veel taalkundig werk verzet door Mej. A. H. VAN ROSSEM (Engels) en drs. G. F. MAKKINK (Esperanto). Mej. E. H. ZEILER en de heer G. BEEKHOF hebben met de redactionele zorg, resp. het tekenwerk veel tot de vormgeving bijgedragen. Aan deze personen en aan de auteurs breng ik gaarne bijzondere dank! Moge hun werk vruchtdragend zijn.

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

Prof. dr. ir. G. J. VERVELDE

Wageningen, oktober 1965.

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 klarigo de la figuroj kaj tabeloj en Esperanto por landoj kie la angla ne ĝenerale estas komprenata. Ĝi estos alsendata post peto.

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1 AUGUSTUS 1965

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EFFECT OF ROOT EXCISION ON GROWTH PHENOMENA IN PERENNIAL RYEGRASS

R. BROUWER and A. KLEINENDORST

The gramineous plants accumulate a considerable proportion of the dry matter produced in their root system. For perennial ryegrass values up to 40 or 50% are regularly found. As in other plants, the share of the root weight in the total plant weight decreases after reaching the flowering stage. Since not all tillers are flowering, this decrease depends on the percentage of shooting tillers (7). At constant conditions there is a rather constant ratio between shoot growth and root growth during vegetative growth. Its value depends on the external conditions in the same way as described for other crops (2). BROUWER (3) showed the existence of a functional equilibrium between roots and shoots. The value of the shoot/root-ratio depends on external conditions. One of the main arguments in favour of a functional equilibrium was that a change in external conditions caused the value of the ratio, characteristic of the former conditions, to change rather rapidly into a value characteristic of the new conditions. In addition it was found that a removal of parts of the shoots or the roots was followed by a rapid re-establishment of the initial value.

To gain a more complete knowledge about these phenomena the effect of various root cuttings on perennial ryegrass has been investigated. Some of the data are described in the present paper.

METHODS

Uniform single tillers of a clone of perennial ryegrass were selected and cut to a length of 6 cm. All roots were removed. The tillers were mounted on hardboard discs and placed with their stem basis in aerated tap water for seven days. During this period adventitious roots emerged from the lower nodes. The plants were then placed in aerated nutrient solutions (two plants per 14 litres container in a climate room at a constant temperature of 20°C. The plants were illuminated during 16 hrs per 24 hrs by fluorescent tubes at a light intensity of about 7.2×10^4 ergs cm^{-2} sec^{-1}. One group of plants grew undisturbed and developed a great number of tillers and adventitious roots. Four other groups were subjected to repeated root excision so that plants were obtained with 1, 2, 3 or 4 adventitious roots respectively. Of each group 6 plants were harvested at 2 weeks' intervals and the fresh and dry weights, root lengths, root numbers, branching patterns, leaf lengths and leaf widths determined.

An additional number of plants of which all roots were excised but two was cultivated. The excision of newly formed roots was stopped at different times after starting the experiment. During subsequent periodical harvests the recovery was studied.

The plant material was also analysed for potassium, calcium, magnesium, nitrate, chloride, sulphate, phosphate, total nitrogen and carbohydrates.

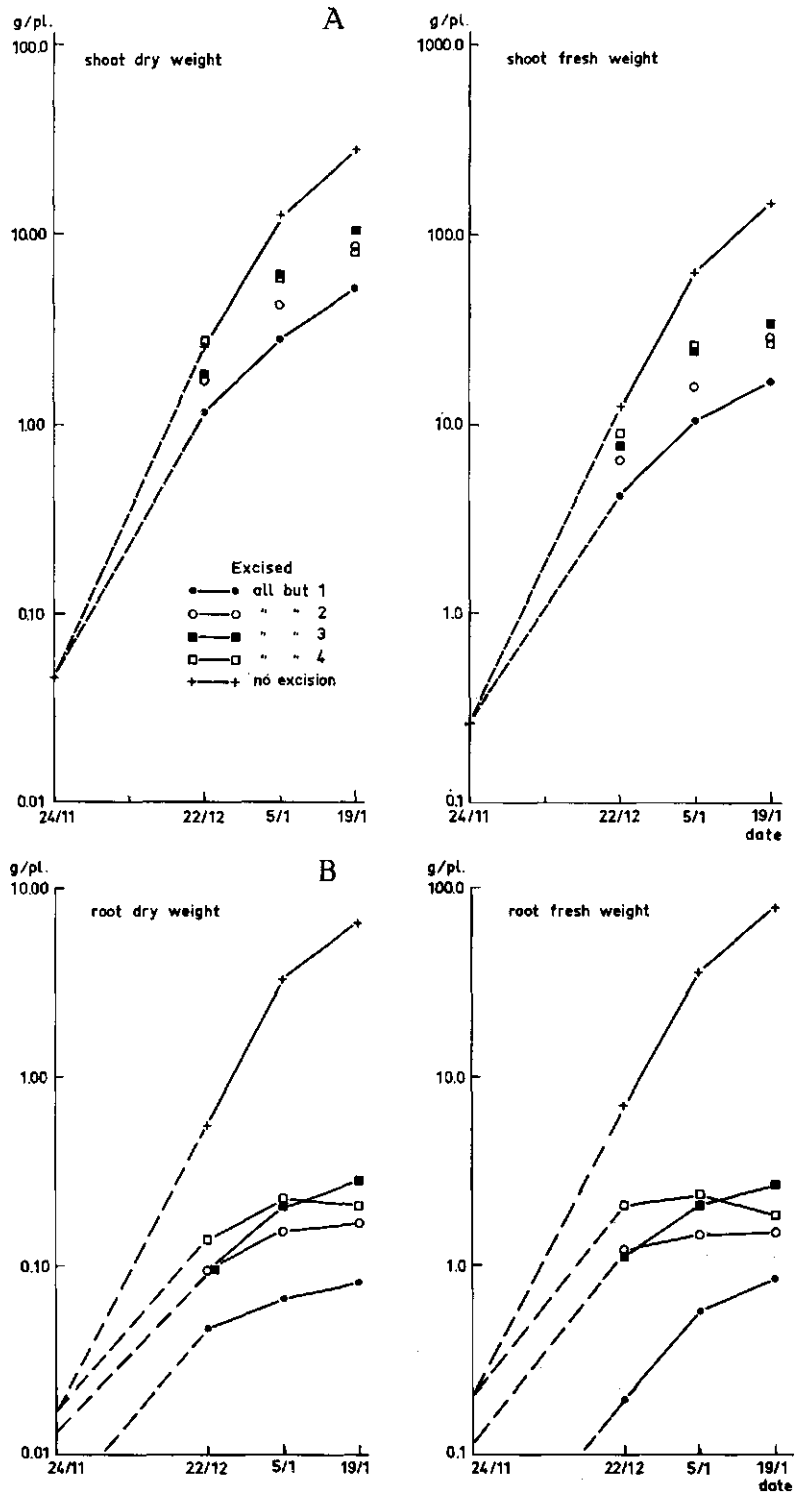

FIG. 1. The influence of root excision (as indicated) on the time course of the increase in dry and fresh weights of shoots (A) and roots (B) of perennial-ryegrass plants growing in climate rooms in nutrient solution.

Results

Growth features

Reducing the number of roots per plant drastically influenced shoot growth from the very beginning of the experiment. The control plants grew exponentially (Fig. 1A and B) during the first six weeks. Thereafter the relative growth rate decreased in shoot growth as well as in root growth. The growth of the treated plants was considerably behind that of the control plants. The growth rate of the shoots decreased continuously. The reduction in growth rate is considerably higher in the roots than in the shoots. The differences in weight between the plants with 2, 3 and 4 roots are probably not significant. Shoot and root weights of these plants are not proportional to the number of roots left to the plant. The same occurred in maize plants (4). Root growth of the treated plants decreased so much that there was hardly any growth in the later periods of the experiment. It seems that the growth of roots under these conditions is limited by age. In the intact plant new roots are steadily emerging. In this case the growth of the original roots terminated even somewhat earlier, probably as a result of reduced competitive power. Similar as in maize (4) newly developing roots inhibited the growth of older roots. Root initiation was not directly influenced by root excision. Up to six weeks there were no significant differences in the number of roots initiated (Table 1). Afterwards differences occurred, but it may be assumed that this is more or less an indirect effect of the reduced shoot growth and tiller number. The treatment considerably affected the morphology of the root system. The control plants showed a normal grass root system consisting of a great number of scantily branched threadlike roots (Fig. 2a). In contrast the treated plants formed a very fine and dense branching on the few roots left to the plant (Fig. 2b).

TABLE 1. Influence of root excision on root initiation by plants of *Lolium perenne* in water culture. L = number of roots left to the plant; E = number of excised roots; T = number of initiated roots (L + E).

Treatment	Harvest date											
	8/12			22/12			5/1			19/1		
	L	E	T	L	E	T	L	E	T	L	E	T
Intact plants	13	–	13	56	–	56	187	–	187	354	–	354
4 roots left to the plant	4	10	14	4	73	77	4	139	143	4	137	141
3 roots left to the plant	3	9	12	3	61	64	3	153	156	3	184	187
2 roots left to the plant	2	11	13	2	58	60	2	129	131	2	137	139
2 roots left to the plant after 8/12 no further excision	2	11	13	*46*	*12*	*58*	*215*	*11*	*226*	*197*	*9*	*206*
2 roots left to the plant after 22/12 no further excision	2	11	13	2	58	60	*118*	*48*	*166*	*221*	*50*	*271*
2 roots left to the plant after 5/1 no further excision	2	11	13	2	58	60	2	129	131	*62*	*91*	*153*
1 root left to the plant	1	13	14	1	60	61	1	109	110	1	110	111

As already shown in Fig. 1 the growth rate of the shoots diminished greatly by the excision of roots. The response was much more pronounced on a fresh weight basis than on a dry weight basis. Consequently, the dry-matter content was considerably higher in the treated plants.

Table 2 shows that the differences became more pronounced as the period was pro-

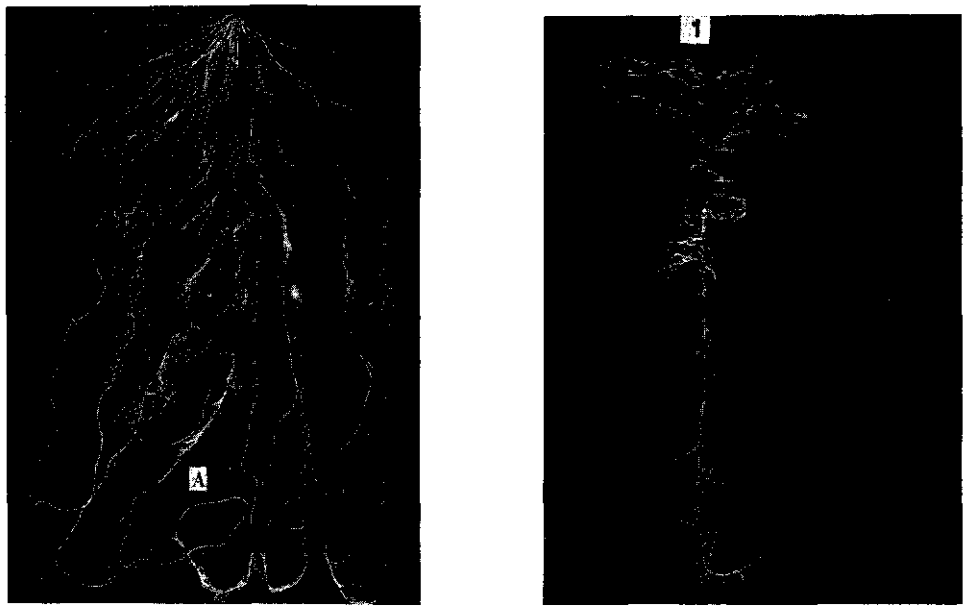

FIG. 2. The morphological structure of the root system of intact plants (left) and of a plant of which all the roots except one were regularly removed (right).

longed. The shoot/root-ratio shows a corresponding trend. Both factors reacted more heavily and rapidly as the number of roots left to the plant was smaller.

An analysis of various characteristics of the shoots grown under the different treatments reveals that tiller number and leaf number are affected in the same way as the weights, but much less (Fig. 3). The number of leaves per tiller was much the same for all treatments at all harvest times (Table 3). There are, however, great differences in leaf size, length and width. According to COOPER (5) leaf length depends on cell expansion and leaf width mainly on cell number. Since his data also concern perennial ryegrass, it seems likely that both cell expansion and cell division in the leaves are reduced by partial excision of the root system.

TABLE 2. The influence of root excision on shoot/root-ratios and on dry-matter content of the shoots.

	Harvest date	Number of roots left to the plant				
		1	2	3	4	all
shoot fr.wt. / root fr.wt.	22/12	22.6	5.5	6.9	4.5	1.8
	5/1	20.2	12.1	11.2	10.1	2.0
	19/1	21.5	19.9	13.1	17.2	2.0
shoot dr.wt. / root dr.wt.	22/12	26.3	18.5	21.4	18.6	4.1
	5/1	46.0	31.0	30.2	25.8	4.0
	19/1	67.6	53.1	37.5	44.0	4.6
dry matter content of shoots (%-age)	22/12	25.0	26.0	23.7	27.0	17.8
	5/1	26.7	27.3	27.4	25.8	19.0
	19/1	31.2	30.5	30.1	29.5	19.3

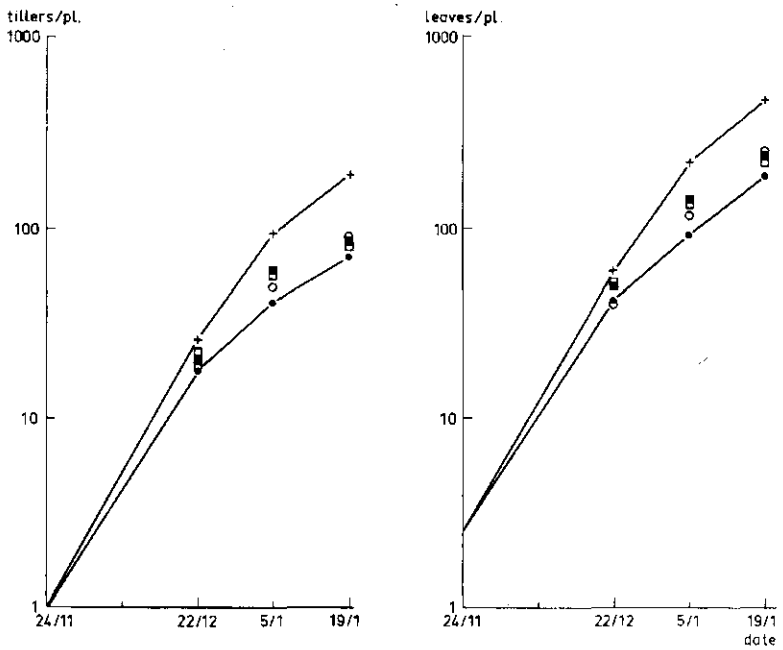

FIG. 3. Influence of root cutting on the time course of initiation of tillers and leaves.

Chemical analysis showed no differences in mineral composition between the various groups of plants. Therefore the reduced growth rate and the higher dry-matter content can hardly be caused by any effect of cutting on the mineral uptake. It seemed also desirable to examine the effect of cutting on the water balance. The water transport through the plants was measured during the 18-hours' period preceding the last harvest. The data listed in Table 4 show that great differences were induced by the cutting treatment.

For convenience the average values of all excised plants are compared with the average values of the control plants. It is striking that transpiration per gram of shoot fresh weight is considerably reduced by the cutting treatment. This indicates that the stomata are partly closed, and is in itself sufficient proof of the enhanced suction

TABLE 3. Effect of root excision on number of leaves per tiller and on leaf length and leaf width.

	Harvest date	Number of roots left to the plant				
		1	2	3	4	all
Number of leaves per tiller	22/12	2.4	2.3	2.4	2.3	2.3
	5/1	2.3	2.4	2.5	2.4	2.2
	19/1	2.7	2.9	2.9	2.7	2.4
Mean leaf length in cm	5/1	16.3	20.4	22.8	22.7	32.6
	19/1	16.9	16.5	16.3	22.8	36.5
Mean leaf width in mm	5/1	4.3	4.7	5.0	5.5	6.3
	19/1	4.0	4.7	4.8	5.2	7.2

TABLE 4. Effect of root excision on rate of water transport through the plant.

	Treated plants	Intact plants	
Transpiration per gram of fresh weight of shoots	0.87	2.86	g/g/18 hrs
Transpiration per gram of fresh weight of roots	17.6	5.4	g/g/18 hrs

tension in the treated plants. In addition the water transport per gram of root tissue is over 3 times higher in the treated plants than in the intact plants. As pointed out by BROUWER (1) this may be due to either one or both of the following facts. First of all the water transport per gram of root tissue is enhanced by increasing the suction tension in the xylem vessels. As stated above the transpiration rate per gram of root tissue shows that the suction tension is probably higher in the treated plants. This means that part of the increased water transport per gram of root tissue can be explained in this way. On the other hand the remaining roots on the treated plants show a much finer branching and therefore a higher water transport may be expected per gram of root tissue per unit of suction tension. The differences between the two groups of plants (Table 4) in this respect are most likely due to both factors. With only these data, however, it is impossible to estimate the share of each of these factors in the total process.

From these considerations it may be assumed that the enhanced suction tension in the plant may also be considered the direct cause of the reduced growth rate.

Recovery after a period of continual root cutting

The preceding shows that root cutting induces changes in the performance of the plants. These are: a reduced number of tillers, reduced number of leaves, reduced leaf size leading to an appreciably reduced shoot weight and a high dry-matter content. Concerning the dry-matter content it is likely that the reaction is much more pronounced on a fresh weight than on a dry weight basis. The plants with two roots per plant were divided into four groups. In one of these groups cutting was continued until the end of the experiment, whereas the other groups were allowed to grow undisturbed during two, four and six weeks after the beginning of the experiment. The results have been plotted in Figs. 4, 5 and 6. When cutting was stopped, the number of roots first of all increased very rapidly (Fig. 4) so that the retardation was largely compensated within two or three weeks. The same holds with regard to the fresh and dry weights of the root system (Figs. 5 and 6). However, this recovery was more rapid and more complete as the time during which the growth rate had been restricted by cutting was shorter. During the period of recovery the growth rate was considerably higher than the growth rate of the control plants (slope of the lines) in the corresponding periods.

As to shoot growth similar effects occurred. The number of tillers increased rapidly after stopping root cutting. A rapid increase in weight also occurred. However, the figures demonstrate clearly that compared with root growth shoot growth recovery was somewhat delayed. In how far this resulted from a more rapid establishment of new root primordia or only from more rapid growth of existing primordia is unknown. A comparison of shoot fresh weight with shoot dry weight reveals that during the early periods of recovery the fresh weight increased considerably more than the fresh weight of the plants with continued root cutting. The dry weight, however, did not. This means that during this period only the roots benefited by the additional amount

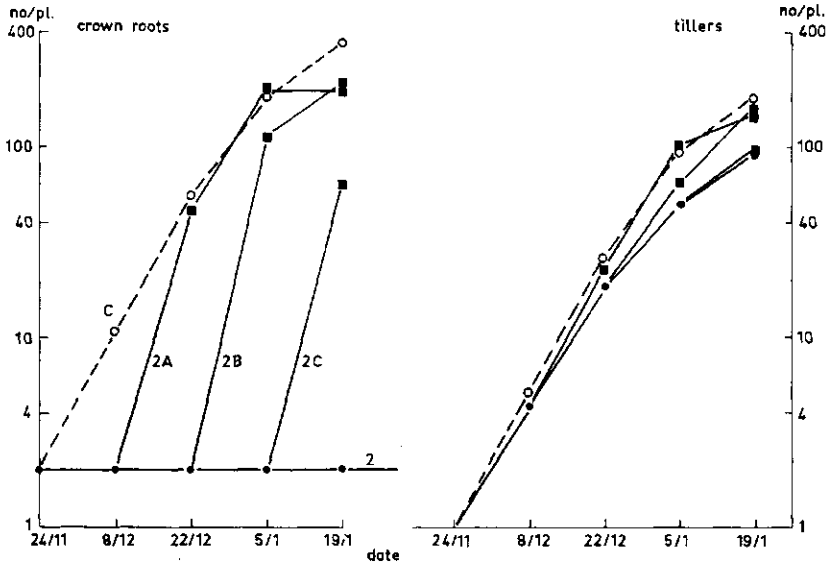

FIG. 4. Recovery of the number of roots (left) and tillers (right) after terminating root excision at subsequent dates (2A, 2B, 2C).

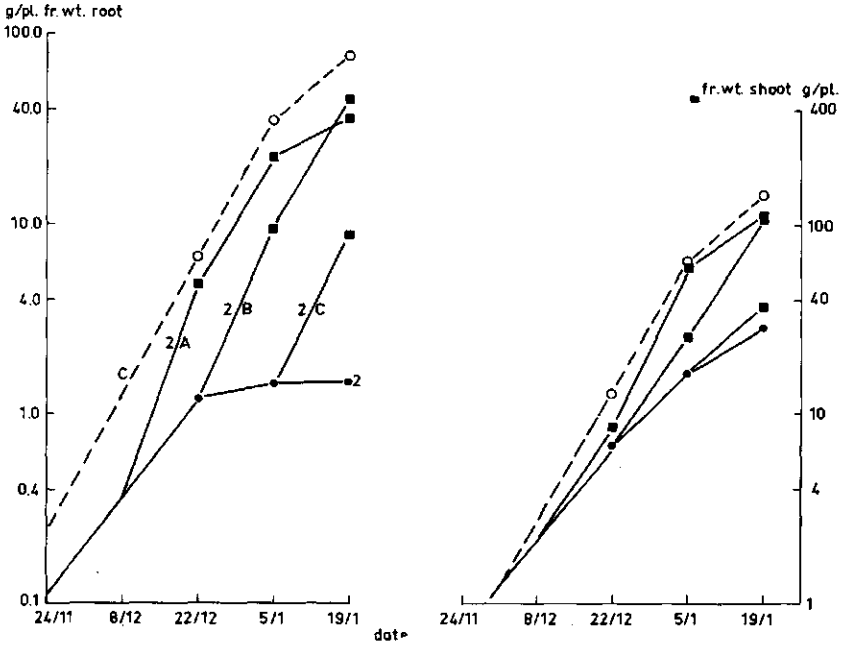

FIG. 5. As Fig. 4 for the fresh weights.

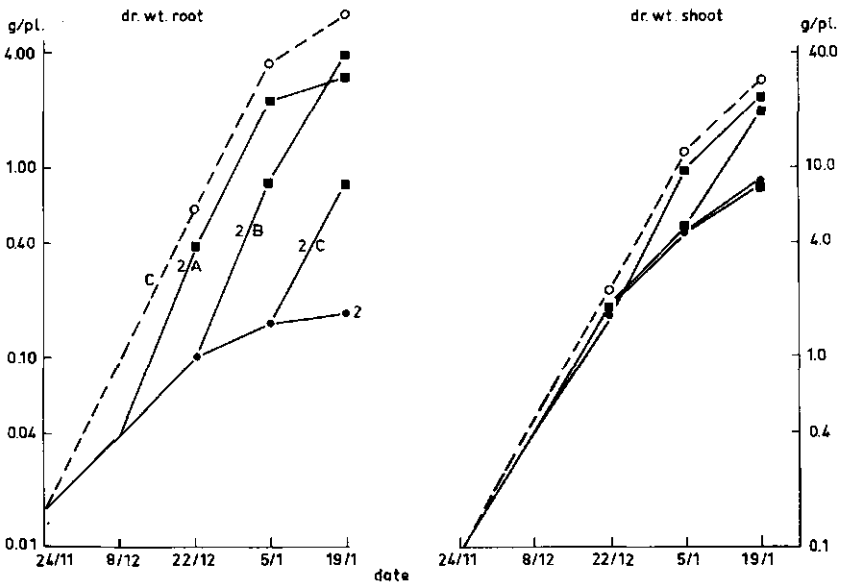

FIG. 6. As Fig. 4 for the dry weights.

of photosynthates. This growth pattern influenced shoot/root-ratios and dry-matter content in a very complicated way. Table 5 shows these effects.

The shoot/root-ratio which was high in the treated plants decreased immediately when root cutting was stopped as a result of the rapid root growth. Its value approached that in intact plants in a short time. The dry-matter content of the shoots decreased also very rapidly to the value in the control plants. Fresh weight of the shoot increased during the first fortnight of recovery without increased dry weight accumulation. It is evident that considerable morphological changes occurred at the same time. The habit of the plant changed from a dark green somewhat stunted and xeromorphous plant to that of a healthy thriving one. Even mean leaf length and leaf width approached that of the control plants within four weeks. This recovery to normal plants was also reflected in the transpiration rate per unit of shoot weight, which did not differ from the value of the intact plants.

TABLE 5. Effect of regrowth of roots on shoot/root-ratios and on dry matter percentage of the shoot tissue.

Harvest date	sh/r (fr. wt.)			sh/r (dr. wt.)			dry matter content (%-age)		
	22/12	5/1	19/1	22/12	5/1	19/1	22/12	5/1	19/1
Treatment									
Excised to 2 roots throughout	5.5	12.1	19.9	18.5	31.0	53.1	26.0	27.3	30.5
Excised to 2 roots until 8/12	*1.7*	*2.5*	*3.4*	*4.4*	*4.5*	*7.6*	*18.9*	*16.4*	*19.1*
Excised to 2 roots until 22/12	5.5	*2.8*	*2.4*	18.5	*5.5*	*5.6*	26.0	*17.6*	*19.8*
Excised to 2 roots until 5/1	5.5	12.1	*5.3*	18.5	31.0	*15.3*	26.0	27.3	*22.8*
Intact throughout	*1.8*	*2.0*	*2.0*	*4.1*	*4.0*	*4.6*	*17.8*	*19.0*	*19.3*

DISCUSSION

Continual excision of most of the adventitious roots arising on growing single tillers of perennial ryegrass resulted in a drastically reduced root weight. The remaining roots established a rather dense branch-root formation compared to the roots of the control plants which were growing and developing undisturbed. Because of these morphological changes the root length (main root + branches) per gram of fresh weight of roots differed considerably.

It stands to reason that these differences have led to important but quantitatively unknown differences in absorption capacity per gram of root tissue. Since the significance of the root system depends for a great deal on the various activities of the system, it may be assumed that an attempt to a quantitative evaluation meets considerable difficulties. In studying the significance of seminal and adventitious roots in *Phleum pratense*, WILLIAMS (8) found that the finely branched seminal root system was 50 times as effective in mineral absorption as the scarcely branched nodal roots. Since these deductions were made from the final harvest only, this ratio resulted from the integrated absorption over the whole life period. Since no data are available of the space of time, the mean duration of the period in which the activity was measured is unknown. It may be assumed that this is much greater for the seminal root system than for the adventitious roots. GOEDEWAAGEN (6) who made comparable experiments on the water uptake, found much smaller differences and if calculated from the present experiments the mineral absorption per gram of root tissue of the treated plants was about 5 times that of the control plants. More detailed data on this point will be published in a following paper.

The reduced root development due to root excision led to greatly reduced shoot growth. First of all a reduced rate of tiller initiation was established. The number of leaves per tiller was about the same, but the leaves were smaller in length and width. According to COOPER (5) this indicates that both cell division and cell extension were reduced. The quite normal mineral content of the treated plants shows that the reduced growth rate of the shoots was probably not induced by insufficient mineral uptake. It seems much more likely that the reduced water uptake was the direct cause of reduced shoot growth. Root cutting induced a considerably inhibited transpiration rate per gram of shoot fresh weight. This proves that the stomata are closed because of enhanced suction tension. The sequence of events can be summarized as follows.

Continual removal of roots emerging from the lower nodes of the stem before they reach the nutrient solution affects the number of roots that take part in the absorption process. This deficiency is partly overcome by an enlargement of the absorbing area in consequence of excessive branching of the remaining roots. If the cutting treatment is severe, as in these experiments, compensatory growth of the remaining roots is insufficient and a high stress on the activities of the root results. This stress mainly concerned the water balance. A direct effect was an increased suction tension in the plant giving rise to a reduced rate of cell extension and cell division. Since photosynthesis is less sensitive to adverse conditions than growth rate, the dry-matter content increased.

After the cutting treatment ended recovery was very rapid. Because of the high level of reserve carbohydrates, explosive growth of new roots and also of a great number of new tillers took place. This growth proceeded partly at the expense of the reserves so that recovery appeared to be especially rapid where fresh weight was concerned. Thereafter the dry weight also increased rather rapidly. The whole process of recovery, during which very high growth rates were observed, led to complete levelling

of all differences between treated and control plants: dry-matter content, shoot/root-ratios, transpiration rates, leaf lengths, leaf widths and number of roots and tillers.

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THE SIGNIFICANCE OF SEMINAL ROOTS IN GROWTH OF MAIZE

R. BROUWER and J. TH. LOCHER

INTRODUCTION

A great number of experiments has been done to explain the role of the various parts of the root system of *Gramineae* in the growth and establishment of the plants. These experiments led to considerable differences in opinion about the significance of the two types of roots, namely seminal roots and adventitious roots, for the water and mineral supply. Some authors found that considerable growth could be obtained even after continual excision of all the adventitious roots, others that this treatment resulted in very poor growth. OHLROGGE (9) demonstrated that growth of intact maize plants was fairly normal, even if only one of the crown roots had minerals at its disposal whereas all other roots grew in tap water. WILLIAMS (11) trained the seminal and adventitious roots of timothy in separate containers. All containers received water, but the nutrients were supplied either to the whole root system or to the seminal roots or to the adventitious roots only. Although the plants with the nutrients supplied to the seminal roots only, were considerably retarded, it was found that the seminal roots absorbed about fifty times as much minerals per gram of root weight as the adventitious roots. This difference may be caused, at least partly, by the fineness of the seminal roots compared to the nodal roots and this phenomenon, although less pronounced, has also been described by others. The great advantage of the seminal roots does not explain the phenomenon that single adventitious roots are able to meet the demands of the whole plant as well (9). This seems only possible by changing the morphology in such a way that a fine network of branches is established, or by an appreciably enhanced development of that particular root. Such compensatory growth of parts of the root system has been described by GOEDEWAAGEN (7), using oats grown in containers with sandy soil, in which dibasic calciumphosphate was mixed with the soil contents of half the container or with the entire amount. With a low rate of fertilization the amount of roots in the fertilized part exceeded several times the amount in the non-fertilized part. GOEDEWAAGEN's experiment confirmed earlier data of GILE and CARRERO (6) with rice and nitrogen fertilizer in water culture. BROUWER and LOEN (5) described results with maize plants, single crown roots of which were supplied with nitrogen and found that the development of the treated roots differed considerably from the roots which did not receive any nitrogen, if the entire plant was short of nitrogen. These differences gradually disappeared if the nitrogen supply as a whole was increased, a phenomenon also found by GOEDEWAAGEN (7). Only little attention has been paid so far to the quantitative aspects of these results for the shoot/root-ratio.

The present experiments attempt to gain more information on the way in which maize plants compensate for the partial excision of the adventitious root system.

Methods

Single maize seedlings were mounted directly after germination on 14 litre containers with Hoagland solution and cultivated in climate rooms. The temperature was kept constant at 20°C and fluorescent tubes were used as a light source. The light intensity was about 6×10^4 ergs cm^{-2} sec^{-1} on the tables and the plants were illuminated during 16 hours per 24 hours' period. The aerated nutrient solutions were renewed when necessary.

The plants were divided in several groups with various excision treatments. The lateral seminal roots were cut of all plants when starting the experiment, so that only the primary seminal root was left. During the experiment one group was not disturbed and of a second group all roots except the primary seminal root were excised. The other groups received a less severe treatment than the latter one. In one experiment the number of nodal roots per whorl was reduced to 2 or 4 and this was continued during the entire experiment. In another experiment only one or two whorls were reduced to 2 or 4 and the other whorls entirely excised. The roots were cut to the number required at least 3 times a week. The number of excised roots was recorded.

From each group 3–5 plants were harvested at regular intervals up to an age of 8 weeks. At each harvest the fresh and dry weights of shoots and roots were determined. With regard to the roots a separation was made between the primary seminal roots and the nodal roots of each whorl. In addition the density of branching of some roots and the length of the branch roots were recorded.

In some of the experiments the bleeding intensity was measured during 4 hours between shoot excision and harvesting the roots.

Results

Influence of root excision on shoot and root growth

In various preliminary experiments the partial excision of the established root system resulted in a corresponding reduction in shoot growth. Directly after partial excision water absorption, mineral absorption and leaf growth rate diminished. In addition this treatment invariably led to relatively increased root growth which led to almost complete recovery of the original shoot/root-ratio as described for other plants (3). In the experiments to be described here, a continual excision of all or a part of the adventitious roots in a very young stage resulted in a continuous additional growth of those parts of the root system which were left intact. Fig. 1 shows the results of an experiment which started on February 2. The four treatments are:

(a) all nodal roots continually excised throughout the experiment;
(b) all nodal roots continually excised until March 3;
(c) all nodal roots continually excised until February 24;
(d) undisturbed growth.

These data show that the total mass of shoots and roots was not much influenced by the treatments. Although at the end of the experiment the weight of the adventitious roots of the undisturbed plants (Fig. 1d) was about 4 times as high as that of the primary seminal root of these plants, the weight of the single seminal root of the most severe treatment (Fig. 1a) was only slightly lower than that of all the roots of the former plants. The percentage of the primary root weight in the total weight decreased regularly as the time increased during which the adventitious roots were permitted to grow. Since the total root weight did not differ much between the treatments, this decrease also holds for the weight of the primary root itself. Fig. 2 shows the root and shoot fresh weights at the final harvest presented in such a way that the time during

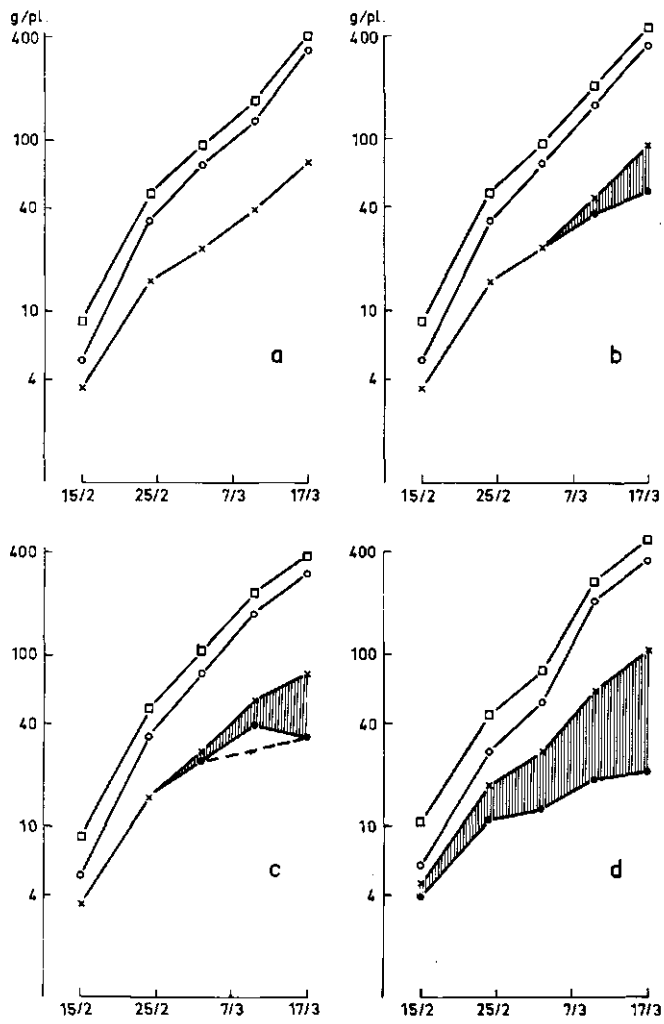

FIG. 1. Time course of weight increase of primary seminal roots (●——●), all roots (×——×), shoots (o——o) and total plants (□——□) of maize from which parts of the adventitious root system were excised during different periods: (a) throughout; (b) until March 3; (c) until February 24; (d) not at all.

which only the primary seminal root system was present decreases from left to right (see Fig. 1a, b, c, d). The share of the seminal root system in the total weight decreases in the same direction. This means that the growing nodal roots considerably inhibit the increase in weight of the seminal system. This may be due to a competition for the essentials available and most likely for carbohydrates.

If the various whorls of crown roots are further separated, the same tendency applies to the competition between the successive whorls (Fig. 3A). In this experiment 2 or 4 crown roots are left to the plant on each of the successive whorls (c_1, c_2 and c_3). Here the total root fresh weight increases if more whorls are left, but the seminal root weight not. Considering one whorl Fig. 3B and C show that a set of 4 crown roots is more successful in the competition with the seminal root than a set of 2 crown roots per whorl. This holds for the entire whorl, whereas the weight per crown root within a whorl decreases with increasing number. Competition therefore is more successful with an increasing number of 'sinks', but it is not proportional to it.

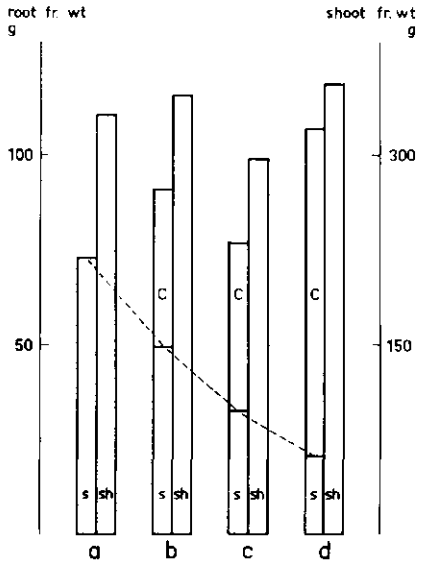

FIG. 2. Weights of primary seminal roots (s), nodal roots (c) and shoots (sh) at the final harvest time. (Same plant series and excision as in Fig. 1.)

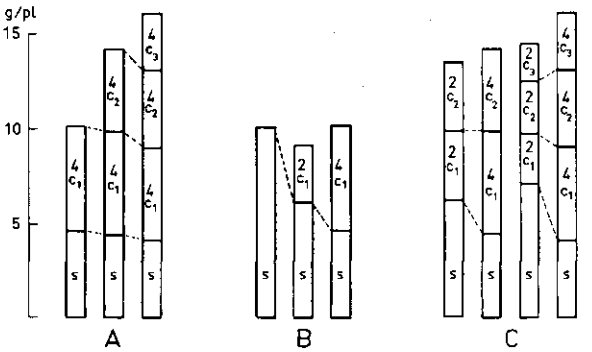

FIG. 3. Weights of primary roots (s) and 1–3 whorls of 2 or 4 crown roots (c_1–c_3). Rest of the root system is excised.

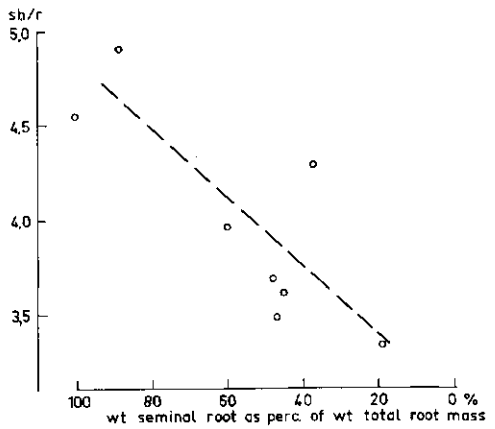

FIG. 4. The relation between shoot/root-ratio and the percentage of weight of the primary seminal root in the total root mass.

The excision treatments described above did not influence the total root weight much. It is not surprising therefore that they neither interfered much with shoot growth. It is clear from Figs. 1 and 2 that shoot weights were not significantly influenced. If however the shoot/root-ratio is considered, there appears to be a slight decrease in this ratio with a decreasing share of the seminal roots in the total weight (Fig. 4). Accepting a functional equilibrium between shoots and roots (3) this may indicate that the seminal root system per gram of root weight is slightly more effective in supplying essential substances for shoot growth. This may be due to a greater absorption area per gram of root weight. It seems worthwhile therefore to look somewhat closer into the morphology of the various parts of the root systems.

Morphology of the root system

The preceding pages show that the seminal root system is able to compensate for the partial removal of the adventitious system. In investigating how this compensation is realized it was found that the extension growth of the primary seminal root itself was almost similar in all cases. The mean length of all five harvests of the intact plants was 92 cm and of the plants with complete excision of the nodal roots 88 cm. Fig. 5 shows some characteristics of the various roots. The distribution pattern of the branches on the seminal roots and on the crown roots, counted per 5 cm, is rather irregular, even in aerated water culture where conditions may be expected to be similar for all parts of the root. The primary seminal roots have a high density of branches at their base, followed by a region of a rather constant number and a second peak near the tip. The time series show that the number of branch roots steadily increases even in regions where the bearing root is full-grown. The length of the branch roots decreases more regularly towards the tip of the main root. The difference between the primary seminal roots of the series with complete excision (Se) and those of the intact plants (Si) concerns the number of branches per unit length and the length of the branches. Both quantities are about 20–30% higher in the former treatment. Besides this 20–30% increase in number and length the second order branching also increases. The weight ratio of the two kinds of seminal roots at the fourth harvest (Fig. 1a and d) is about 2.

A calculation of the total root length per gram of fresh weight of roots reveals that in both cases the same values are found, *viz.* c. 600 cm/gram fresh weight. This means that the length ratios are rather accurately reflected in the weight ratios of the primary seminal root and that the latter are adapted by a somewhat greater branch density and increased length of the branches.

The branching pattern of the crown roots deviates somewhat from that of the primary seminal roots. The deviation increases with the higher number of whorls. This may partly be due to only the crown roots of the first whorl being fully grown, even at the fourth harvest. The thickness of the crown root itself, however, increases considerably, the higher the node on which it is developing. The branch density decreases in the same direction as the ultimate length of the branch roots. In the experiment of Fig. 5 the respective root lengths per gram fresh weight are:

primary seminal root	excised crown roots	637 cm/g
	intact plant	629 cm/g
crown roots	1st whorl	607 cm/g
	2nd whorl	468 cm/g
	3rd whorl	253 cm/g

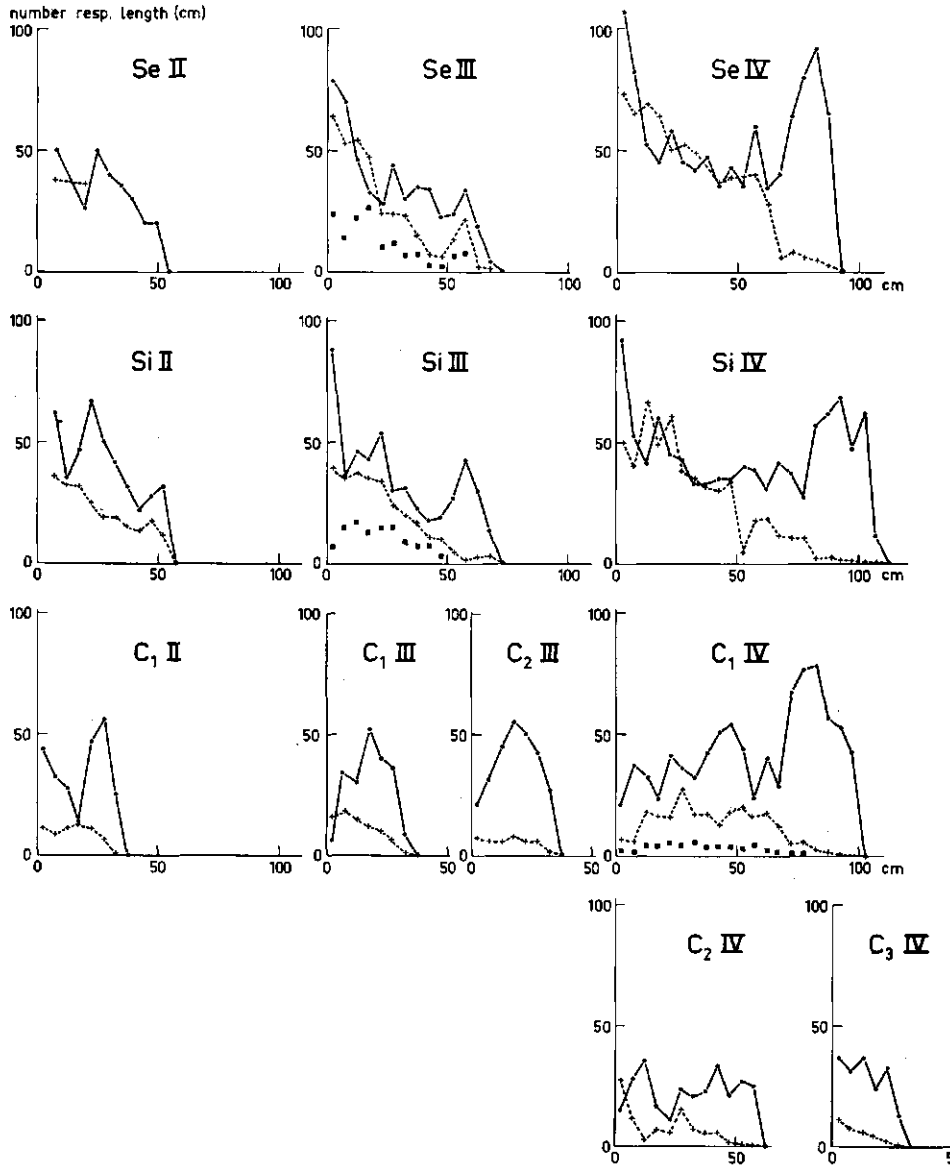

FIG. 5. Distribution pattern of branches along the primary seminal roots and crown roots, counted per 5 cm (•——•), together with the maximum length (+---+) and average length (■ ■) of the branches.
Se = primary seminal root of plants with completely excised adventitious root system;
Si = primary seminal root of plants with completely intact adventitious root system;
$c_{1\,2\,3}$ = crown roots of 3 whorls at the latter plants.
II→IV = different harvest data (24/2, 3/3, 10/3).

These data show that the first whorl of crown roots is more or less identical with the seminal roots in most of its aspects. The length per unit of weight decreases in the successive whorls. It may be expected, therefore, that the activity per gram of root weight also decreases in the same direction. Since it may be expected that shoot growth depends on root activities (3) it seems likely that the amount of shoot growth per gram of root tissue decreases in the same way. This may be the background of the decreasing shoot/root-ratio at a decreasing share of seminal roots in the total root weight (Fig. 4).

In contrast to the changes induced by root excision this treatment does not significantly influence the rate of initiation of root primordia on the nodes.

TABLE 1. Influence of root excision on root initiation.

Number of roots left to the plant	1	3	5	5	7	9	13
Number of excised roots	21	15	13	14	11	9	4
Number of roots initiated	22	18	18	19	18	18	17

DISCUSSION

In many papers dealing with shoot/root-ratios one is at a loss what to do with this quantity. BOONSTRA (1) introduced the indication 'root value' and assumed that the amount of shoot weight grown per unit of root weight gives information on the efficiency of the root system. Since the activity of the root system not only depends on the root itself, but also on the external conditions, BOONSTRA's statement seems justified when root systems are compared, growing in comparable media. Using various peas' varieties under similar cultural conditions, BOONSTRA showed that these varieties differed considerably in root value and found that the absorptive capacity per gram of root tissue of water as well as nutrients varied in the same direction as the root value. These differences are mainly caused by differences in the morphology of the root system. The pronounced influence of the morphology, as for instance the fineness of branching, has often been shown. The most striking differences have been reported between seminal and nodal roots of grasses (8, 10). Here the differences exist even between parts of the root system of individual plants. In previous papers BROUWER (2, 3) showed that the shoot/root-ratio is a fixed and regulated quantity, the value of which depends indeed on the external conditions, but in a predictable way. The prediction should be based on the activities of the various organs and the performance of the shoot/root-ratio is considered as a functional equilibrium between shoots and roots. Since the shoot/root-ratio on the one hand is expressed as a weight ratio and on the other hand is based on an activity ratio, differences in morphology leading to differences in activity per unit of weight will complicate a right understanding. The aim of a series of experiments was to gain more information on the quantitative aspects of morphological changes on the shoot/root-ratio. BROUWER and KLEINENDORST (4) showed that root excision in perennial ryegrass induced abundant branching in the remaining roots, which resulted in a highly increased root length and absorption per unit of root weight. This weight, however, was drastically reduced by the cutting treatment.

The comparable experiments with maize in this paper show that no generalizations can be made. Root cutting in maize increases branch-root density and branch-root lengths in the remaining roots, but the effect of this on root length per unit of weight was much less pronounced. This indicates that compensatory growth in maize roots follows much the same growth pattern as in normal roots. As a consequence the

shoot/root-ratio, increasing five times in grass, increased only 30% in maize. Another difference between these two species is that the maize root system compensates the roots removed by cutting almost completely, whereas the compensation in grass is much smaller. It is quite reasonable, therefore, that shoot growth in maize is influenced very little, whereas it is considerably reduced in perennial ryegrass. The effect of root cutting, so much discussed in the literature, greatly depends on the rate of and the way in which the compensatory growth takes place.

Summary

In these experiments parts were excised of the adventitious root system of young maize plants grown in nutrient solutions. The influence of these excisions on shoot growth a nd on growth of the remaining primary seminal root was examined. The growth of the latter compensated the loss of the adventitious root system for the greater part. Shoot growth was not much influenced.

Some morphological observations were made on primary seminal roots, the adventitious root system being present or not, and also on crown roots. The branching pattern of the primary seminal root did not change very much during the compensatory growth. The root length per gram of root fresh weight of the successive whorls of crown roots decreased and consequently the shoot/root-ratio of the plants with an excised adventitious root system was highest.

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THE EFFECT OF TEMPERATURE ON TWO DIFFERENT CLONES OF PERENNIAL RYEGRASS

A. KLEINENDORST and R. BROUWER

INTRODUCTION

It is a well-known fact that the response to temperature depends greatly on the species used. This holds for rather simple well-defined processes as water absorption (9) and for much more complicated phenomena as growth and development (7). Even within the species large differences have been described (1) and it might be expected that a very widely-spread species as *Lolium perenne*, which in other respects also shows considerable heterogeneity (10), should show this heterogeneity with regard to temperature response. Before these experiments ryegrass plants were collected from various parts of the Netherlands. These showed large differences with regard to time of flowering and type of growth. The response of two clones, an early one (C 3) and a late one (C 12), to air and root temperature will be described in the following pages.

METHODS

Plants of two clones of perennial ryegrass, C 3 and C 12, were divided into single tillers. The tillers were cut to a length of 7 cm and the roots were removed. Thereafter the tillers were mounted on hardboard discs and placed in nutrient solution. In some experiments the individual tillers were planted in containers with sandy soil.

The plants were grown in climate rooms, each with a different air temperature. A series of various root temperature was usually applied at the same air temperature. The relative humidity was maintained at about 65%. Various light sources were used with an intensity of about 7.10^4 ergs cm^{-2} sec^{-1} and a duration of the light period of 16 hours per 24 hours' period.

Further details will be given in the description of the various experiments.

At each harvest various growth factors were determined *viz.* fresh weights of shoots and roots, dry weights, tiller number, leaf number, shoot/root-ratios, dry-matter content and sugar content.

Besides in some experiments the rate of water and mineral absorption during a period directly preceding harvest was measured.

RESULTS

Comparison of C 3 and C 12 at 15°C and at 25°C in soil culture

The particular experiment was performed in climate rooms of 15 and 25°C. Single tillers were planted in pots with sandy soil. At each temperature one half of the pots, referred to as 'wet', were maintained at a water content of 25–30%, the other half, referred to as 'dry', at a water content of 12–15%. In previous experiments it was observed that C 3 was much more sensitive to drought at high temperature than C 12.

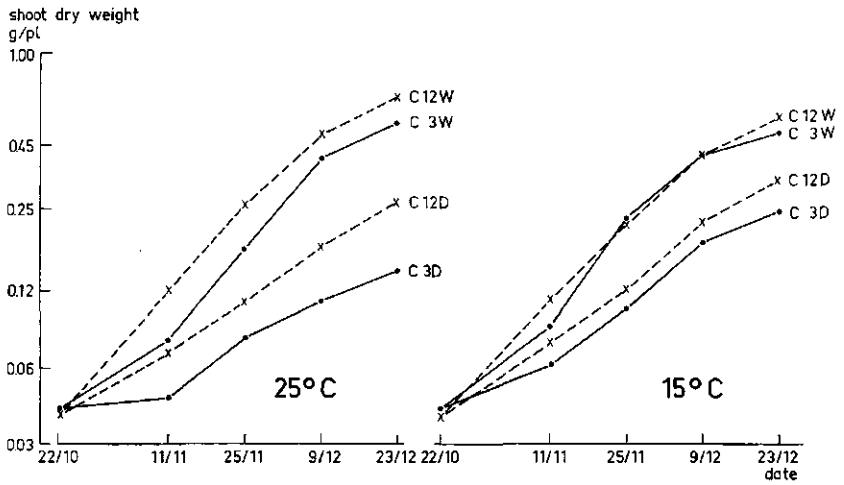

FIG. 1. Increase in weight of single tillers of two clones (C 3 and C 12) of perennial ryegrass grown in soil at two levels of moisture content – D(ry) and W(et) – and two temperatures.

Fig. 1 shows the shoot dry weight of the plants at subsequent harvests on a logarithmic scale. It appears that in 'wet' soil the differences in growth rates between both temperatures are rather small. There are, however, consistent differences since at each harvest time till 23 December the dry weight of C 3 was higher at 15 °C than at 25 °C. The dry weight of C 12 shows the reverse picture. In 'dry' soil this could not be observed because the plants at 25 °C suffered to a greater extent from drought than those at 15 °C. All these observations indicate that the optimum temperature of the early clone (C 3) is lower than that of the late clone (C 12).

There is a remarkable difference in response to the water supply of both varieties. At both temperatures, and especially at 25 °C, C 3 is much more affected by a limited water supply than C 12.

The pots in this experiment were watered regularly by sprinkling from above. During the time between sprinklings the surface layers were rather dry, especially in the 'dry' treatment. As will be shown the root development of C 3 is very scanty at high temperatures, resulting in a very superficial root system of these plants. This may be one of the reasons of the more pronounced response.

Growth in water culture at 5, 15 and 25 °C respectively

In the former experiment air temperature and soil temperature varied at the same time. Since it seemed likely that the rooting behaviour was influenced by temperature, especially in the 'dry' pots, the experiments were continued in water culture.

In this experiment three different root temperatures (5, 15 and 25°C) were used whereas the air temperature was 15°C. Differences in growth rate between treatments and between clones were obvious from the very start of the experiment. A photograph of the plants (Fig. 2) shows a clear picture of the habit of the plants at an age of 57 days. Fig. 3 shows the height of the crop after 46 days of growth. There were only minor differences between the clones at 5 and 15 °C. The root temperature of 25 °C caused a reduced growth rate of C 3 compared to 15 °C and an increased growth rate of C 12. The plants were harvested at an age of 60 days. Various growth factors are shown in the following figures and tables. The number of tillers (Table 1), growing

FIG. 2. Photograph of perennial-ryegrass plants (clone 12 above and clone 3 below) grown during 57 days in nutrient solution of the temperatures indicated. Air temperature 15°C.

TABLE 1. Effect of root temperature on number of tillers and leaves per plant.

	Root temperature (°C)	C 3	C 12
Number of tillers	5	32.2	32.5
	15	56.0	44.8
	25	38.2	59.8
Number of leaves	5	109.5	96.8
	15	191.7	144.7
	25	154.7	185.2
Number of leaves per tiller	5	3.4	3.0
	15	3.4	3.2
	25	4.1	3.1

from the original single tiller, shows the same picture as the heights in Fig. 3, increasing in C 12 to 25°C and with a clear optimum at 15°C in C 3. The number of leaves per tiller is somewhat higher in plants of C 3. This number is not much influenced by the root temperature. Only C 3 shows an increase at 25°C which may be due to the reduced tillering causing a relatively small number of young tillers. The dry weights of shoots and roots (Fig. 4) show a similar picture as the other graphs. With regard to the shoot/root-ratio (Table 2) the data seem more or less equivalent for both clones. This ratio increases steadily with temperature. It could be shown (2) that in a sense the shoot/root-ratio is a resultant of a functional equilibrium between these two organs. This indicates that fewer roots meet the requirements per gram of shoot as the temperature in the root medium within the limits of the experiment is higher. This seems quite logical since it may be expected that various activities of the roots, such as water absorption and ion absorption show an increasing rate at increasing temperature. Besides the temperature of the root environment may have some influence on the morphology of the root system. The higher the temperature, up to 25°C, the finer the branching. This can already be seen from the photograph (Fig. 2) and may be another reason for the higher efficiency on a shoot growth basis. The transpiration rates per unit of shoot weight are about the same, indicating that

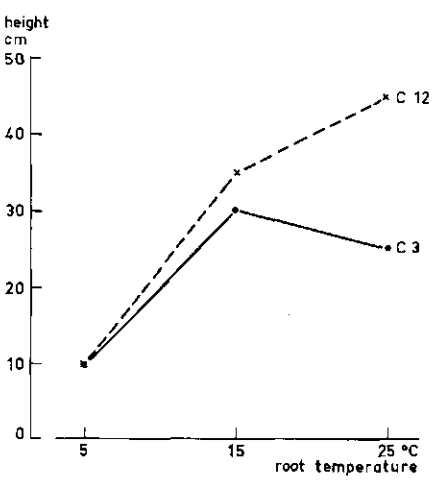

FIG. 3. Height of the plants of Fig. 2 at an age of 46 days.

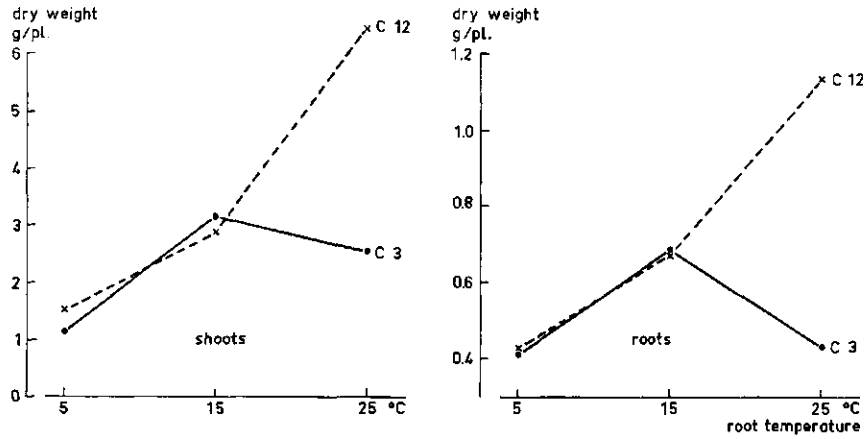

FIG. 4. Dry weights of shoots and roots of the plants of Fig. 2 at the age of 60 days.

TABLE 2. Shoot/root-ratio at various root temperatures (dry weight basis).

Root temperature (°C)	C 3	C 12
5	2.8	3.6
15	4.7	4.2
25	6.1	5.6

the water supply varies between very narrow limits and cannot be considered to limit shoot growth to a large extent (Table 3). Possibly mineral supply does, but the results are irregular in this respect (Table 3).

In some of the experiments the roots of the original single tiller were only partly removed leaving the basal parts of the root to a length of 2 cm on the plants. In

TABLE 3. Water movement (on shoot weight and root weight basis) and absorption of nitrate and potassium during 48 hours before harvest.

	Root temperature (°C)	C 3	C 12
Transpiration	5	2.62	2.38
g/g of fr. wt. shoot	15	2.41	2.23
	25	2.84	2.08
Transpiration	5	5.9	6.5
g/g of fr. wt. root	15	9.8	9.5
	25	16.9	10.3
Nitrate absorption	5	0.04	0.11
m mol/g of fr. wt. root	15	0.18	0.10
	25	0.12	0.12
Potassium absorption	5	0.05	0.08
m mol/g of fr. wt. root	15	0.11	0.07
	25	0.06	0.09

those cases the lag phase at the start of the experiment could be prevented, since the root 'stumps' branched at once rather elaborately. The advantage of this treatment lasted throughout the whole experiment. The C 3 as well as the C 12 plants respond favourably to the presence of the root stumps. This concerns number of tillers and number of leaves (Table 4) as well as the dry weights (Fig. 5).

The differences are more important in clone 12 than in clone 3. Since the effect of the root stumps is probably caused by the fact that the root base is more ready for branching than the stem nodes, this difference is smaller in clone 3 than in clone 12.

TABLE 4. Comparison of the influence of partial or complete excision of the roots on number of tillers and leaves.

	Excision	C 3	C 12
Number of tillers	partial	45.4	57.0
	complete	38.8	34.4
Number of leaves	partial	160.6	174.5
	complete	143.3	109.9
Number of leaves per tiller	partial	3.6	3.1
	complete	3.7	3.2

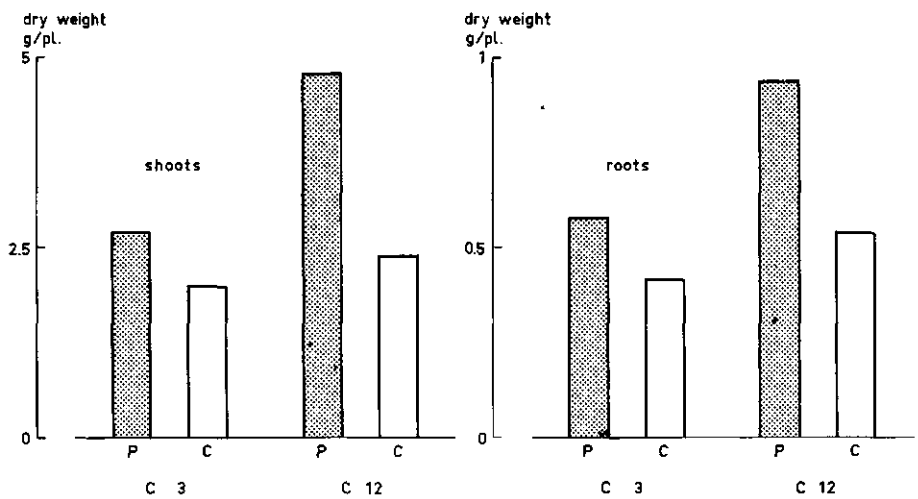

FIG. 5. Dry weights of shoots and roots of perennial-ryegrass plants grown during 60 days in nutrient solution. Mean of three root temperatures (5, 15 and 25°C). Air temperature 15°C. The experiment was started with single tillers from which the roots were completely removed (C) or excised to about 2 cm from the stem base (P).

Growth in water culture at 5 up to 40°C and air temperatures of 15 and 25°C.

From the above it is clear that root temperature has a distinct influence on root as well as shoot growth. The differences between the two clones are convincing. The early clone 3 shows a lower optimum temperature than the late clone 12. The larger range of root temperatures revealed quite interesting results (Fig. 6). First of all it is rather astonishing that neither clone responds to the prevailing air temperature. In

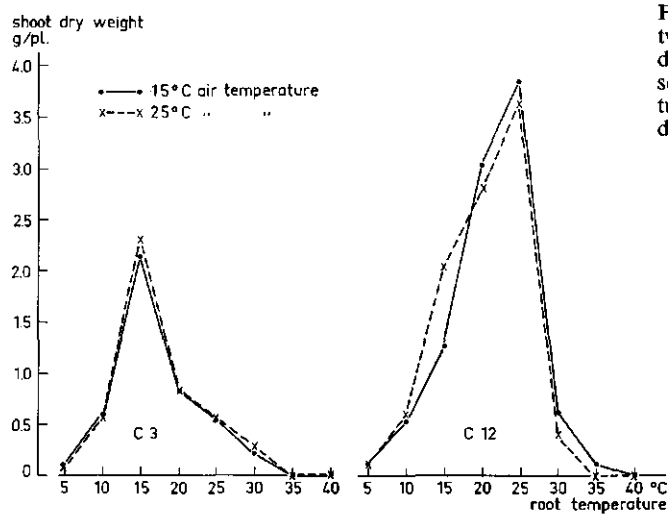

FIG. 6. Dry weights of shoots of two ryegrass clones grown during 9 weeks in nutrient solutions at the root temperatures indicated and at two different air temperatures.

addition, and in agreement with the former experiment, the yield at root temperatures of 5 and 10°C is similar for both clones. The further increase in the yield of clone 12 at increasing temperature up to 25°C could also be found in this experiment as well as the decline in the yield of clone 3 at the same temperature range. The maximal yield of clone 12 is much higher (about twice as high) than that of clone 3. Both clones show a very sharp optimum.

TABLE 5. Dry-matter percentage of shoots and roots at different root temperatures.

	Root temperature (°C)	C 3	C 12
Shoots	5	17.3	18.9
	15	13.8	12.6
	25	15.6	13.5
Roots	5	13.9	13.9
	15	12.0	12.6
	25	15.5	12.0

Influence of root temperature on spatial orientation of the shoots

Considerable differences exist between grass species with regard to the direction of the shoots in relation to gravity. Prostrate growing species from the pastures interchange with erect species from the hay-fields. Even within the same species both growing types may be found. It is generally accepted that the transition from the prostrate form to the erect form is related with the transition from the vegetative phase to the generative phase. The nature of this relation, however, is very obscure and the evidence is insufficient. In experiments in which vegetative ryegrass plants were grown in the greenhouse at temperatures of 10 up to 32°C the plants at 10°C grew prostrate, whereas those at 32°C were growing completely erect, although no bolting was observed. Transference from one temperature to another induced a rapid

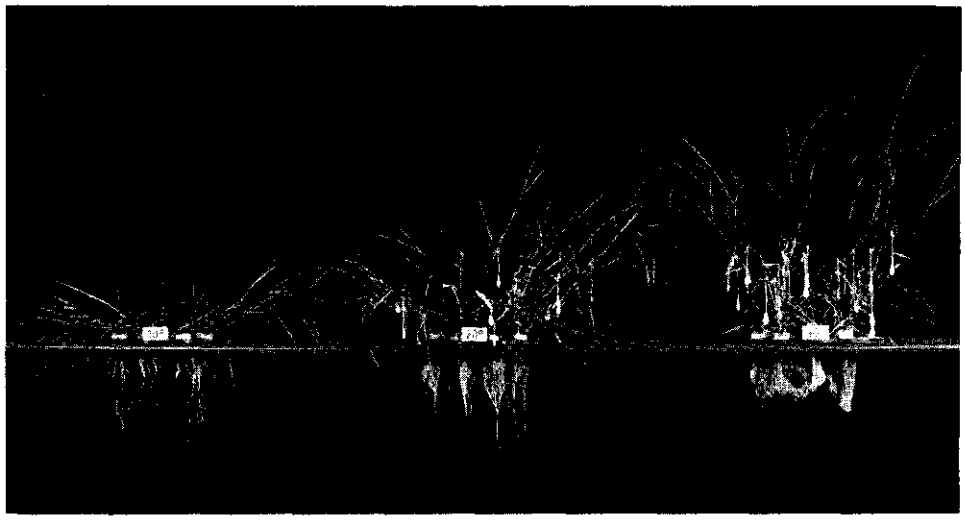

FIG. 7. A photograph showing the difference in orientation of stems of ryegrass plants grown at root temperatures of 10, 20 and 30°C.

change in growth habit and within 3 or 5 days the direction fitting to the new condition was achieved.

In the current experiment vernalized ryegrass plants were grown in nutrient solutions at 10, 20 and 30°C respectively and at an air temperature of 15°C. Fig. 7 shows that even flowering shoots grew completely prostrate at a root temperature of 10°C. At 30°C the shoots grew erect and at 20°C the direction was somewhat intermediate. Measuring the angles between the horizontal level and the direction of the various internodes showed that after shooting the declination of the subsequent internodes co-operated in defining the ultimate direction (Fig. 8). It may be stated that this result demonstrates that the orientation of the shoot is not caused by flowering conditions, but depends completely on temperature. The fact that higher temperature

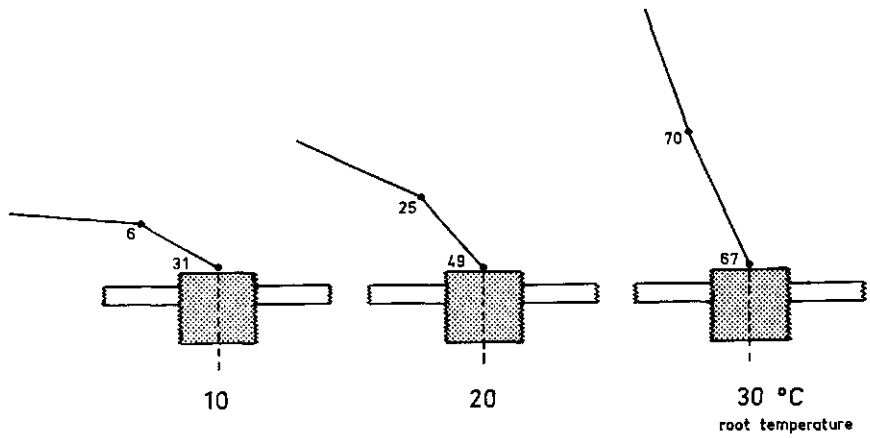

FIG. 8. A schematic representation of the angles with the horizontal line of two lower internodes of ryegrass plants grown at the root temperatures indicated.

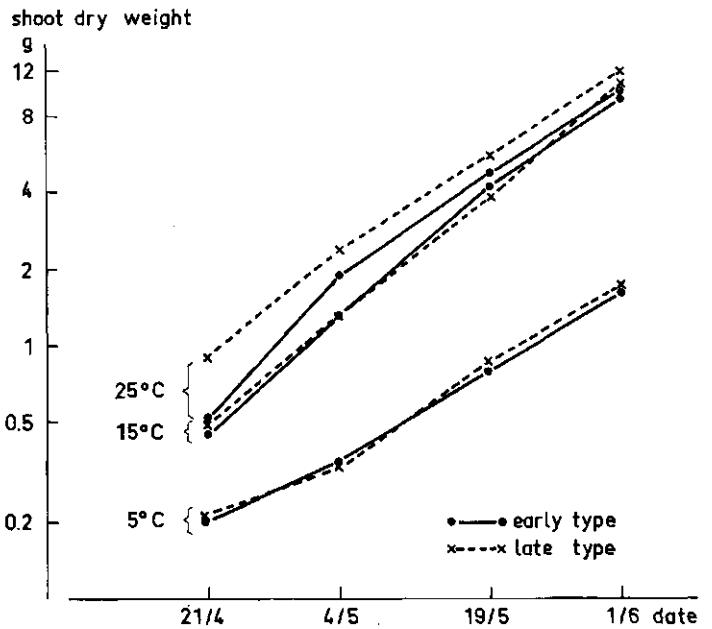

FIG. 9. Growth rate of shoots of two types of perennial ryegrass seedlings (early and late) at the root temperatures indicated.

and transition to the generative phase normally coincide in the field may be responsible for the misleading assumption mentioned above.

Response of two types of perennial ryegrass

In addition to the clones 3 and 12 two other varieties of perennial ryegrass have been taken from normal commercial sources, *viz.* the early type 'Barenza' and the late variety 'Melle'. Seedlings of these types were grown at three root temperatures (5, 15 and 25°C). The result given in Fig. 9 shows that at 5 and at 15°C there are no differences at all.

There seems to be an indication that at 25°C the early variety shows some disadvantage, but the differences are much less pronounced than in the clones 3 and 12.

DISCUSSION

Many of the phenomena described in this paper require a more detailed investigation before satisfactory explanations can be found. There are, however, a number of aspects that are worth discussing.

GROBBELAAR (8) and BROUWER (3) described the influence of root temperature on various growth phenomena of maize and beans respectively. Both concluded that the influence of root temperature on shoot growth was caused by the influence on the water absorption by the roots and via this on the water balance of the shoots. They assumed that a resulting increased suction tension in the shoot tissue leads to reduced leaf growth. In the case of maize the growing point of the shoot (8), situated in the direct vicinity of the root medium, may have reacted directly to the root temperature, but the effect was quite similar to that with beans (3) where the growing point of the shoot was situated well above the root area. Both beans and maize show a greatly reduced transpiration rate per gram of shoot fresh weight below and above optimum root temperature (6) which proves to be a justification of the assumption

given. In the current experiment, however, the transpiration rate per unit of fresh weight of shoots was not influenced whatsoever (Table 3). This indicates that there are no true difficulties about the water balance. Although the rate of water absorption per gram of root fresh weight increased with temperature, this cannot be used as an indication that the suction tension was increased in this case. It should have been if the morphology of the roots had been the same at the temperatures investigated. However, even a first glance at the root systems (Fig. 2) is convincing of the large differences in this respect. This means that the water absorption per gram of root tissue per atmosphere of suction tension may be expected to follow the intensity of branching. Experiments with maize (4) have given substantial evidence that finer branching results in a considerably increased water conductivity per gram of root tissue. Since detailed data are not available of perennial ryegrass the quantitative aspect of this has to be further investigated. Nevertheless it may be assumed that the growth response to root temperature is not caused by an unfavourable water balance in these experiments. In looking for the cause of decreased shoot growth it may tentatively be assumed that in the current experiment the influence of the root temperature is exerted mainly as a direct influence on the initiation of nodal roots. The lasting effect of root stumps on the subsequent growth throughout the experiment is a further indication of the importance of a rapid root development. If the initiation is delayed, the lag period cannot be overcome during the first 8 or 9 weeks of the experiment. The small differences in growth rates (slope of the lines in Fig. 1 and 9) confirm this assumption and are completely different from those in maize and beans. The relatively small differences in dry-matter content (Table 5) compared to the differences described by BROUWER and KLEINENDORST (5) in the same species by more severe treatments may be additional evidence to this statement. It may be concluded therefore that in this particular experiment growth reductions of the shoots at unfavourable root temperatures are mainly due to a reduced rate of root initiation resulting in a lag period that cannot be overcome during the relatively short duration of the experiment.

Besides, the situation of the growing point near the root medium may also be an important factor. Since both leaf initiation and tillering are affected to the same extent as dry-weight increase, the effect of this situation cannot be underestimated. The observation that the root temperature is completely responsible for the differences in growth rate irrespective of the air temperature points to the importance of this. More detailed studies, in which a spatial separation of roots and shoot growing point should be obtained, are required before an estimation can be made of the relative importance of root initiation and leaf and tiller initiation in the growth response. The differences between the two clones are quite clear, but the physiological background is as yet unknown.

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INFLUENCE OF THE NUMBER OF DAYS IN CONTINUOUS LIGHT, AFTER VERNALIZATION, ON SHOOTING AND MORPHOLOGY OF THE INFLORESCENCES OF TWO TYPES OF PERENNIAL RYEGRASS *(LOLIUM PERENNE* L.*)*

A. KLEINENDORST and A. SONNEVELD

INTRODUCTION

A great number of perennial-ryegrass plants were collected from old permanent pastures throughout the Netherlands during an investigation into the ecotypes of this species. Some of these plants were vegetatively propagated. Two of these clones were studied more intensively. One was striking by the combination of early shooting and prostrate habit (clone 3), while the other combined late shooting with an erect habit (clone 12). These clones were also used in other investigations (8, 10, 11) and it was found that their reaction to external conditions was completely different.

In the present experiment an investigation was made on the influence of a varying number of days in continuous light, after vernalization, on the development and morphology of the inflorescences.

METHOD

Plants of both clones were placed in a nutrient solution in autumn. Each plant (unit) consisted of 4 separate tillers at the beginning of the experiment. During winter these plants remained at a temperature of about 5°C.

In February (clone 3 on 7 Feb. and clone 12 on 26 Feb.) the plants were transferred to a climate room with a daylength of 7 hours (TL-tubes) and a temperature of 18–20°C. Earlier research had already shown that the clones remained vegetative at this daylength. At the moment of transference the clones were completely vegetative. The growing points had 4–5 vegetative primordia. After the plants had been in this climate room for a few days they were transferred to a climate room with continuous light (TL) at a similar temperature. The plants remained here for 0, 1, 2, 3, 4, 7 and 8 days, after which they were returned to the climate room with 7 hours of daylight. Each treatment consisted of 4 units (= 16 tillers). The date on which the ear was visible was noted as the shooting date.

RESULTS

Ear formation

The plants of clone 3 were harvested on 28 April and the ears were analysed (Table 1). The plants receiving continuous light for less than 3 days did not show ear formation and consisted solely of vegetative tillers (Fig. 1).

Examination of the growing points (Table 2) showed that the main tillers achieved the double-ridge stage after 3 days, while the initiation of the inflorescence began

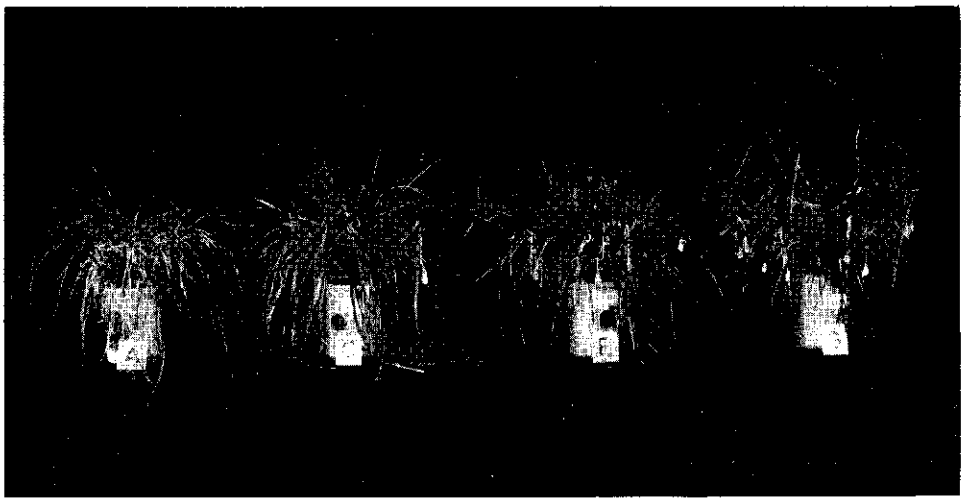

FIG. 1. Influence of the number of days in continuous light after vernalization on the shooting of perennial ryegrass in short day.
A = 0 days in continuous light after vernalization
C = 2 days „ „ „ „ „
E = 4 days „ „ „ „ „
G = 8 days „ „ „ „ „

after 7 days. It may be stated, therefore, that 2 days or less in continuous light is not sufficient to induce the growing point, so the plants remain vegetative. Once the beginning of the double-ridge stage was achieved (after 3 days), the plants could form ears in short day.

RYLE and LANGER (16) found in timothy S 48 that 50% of the plants were shooting in short day after a preliminary period of 2–3 days in long day and 100% after 4–5 days in long day. GARDNER and LOOMIS (5) found that 10% of *Dactylis glomerata* L. was shooting after 3 days in long day and 90% after 12 days in long day.

Not a single tiller of clone 12 was shooting on 5 June. Examination of the growing points showed that these were still completely vegetative (Table 1). Possibly, 8 days of continuous light is insufficient to induce head formation in this clone in the vernalization condition in which it was. Plants in the same vernalization condition which were not returned to short-day conditions did shoot after about 40 days in continuous light. The findings of WYCHERLEY (25) in *Cynosurus cristatus* L. were similar; three clones received 0 to 16 days of continuous light after which they were transferred to short day. The induction of 16 days did not have any influence on one of the clones, so that these plants did not shoot. The other two clones, however, did after they had received continuous light for about 10 days or longer. Here too, the growing point was in the double-ridge stage after about 10 days.

Number of ears

The number of ears in the shooting plants was not the same (Table 1). This table shows that the number of ears increases as the number of days in continuous light increases. This greater number of ears with 7 and 8 days in continuous light is caused by more shooting laterals in these plants (each unit has 4 main tillers). Perhaps the laterals formed during the late autumn and early winter were not completely vernalized. The double-ridge stage is achieved earlier as the plants have been vernalized

TABLE 1. Shooting date, number of ears per plant (unit) and morphology of the inflorescence of perennial ryegrass in 7 hours of daylength, after a pre-treatment with a varying number of days in continuous light.

Clone 3 (28 April)	Number of days in continuous light						
	8	7	4	3	2	1	0
Av. number of ears per plant (unit)	9.3	15.0	7.5	7.0	0	0	0
Av. shooting date	22/3	22/3	1/4	3/4			
Av. number of spikelets per ear	14.9	14.7	15.5	16.8			
a. % of normal spikelets	43.5	36.0	3.3	6.3			
b. % of normal spikelets partley filled with flowers	15.8	29.9	24.6	16.2			
c. % of spikelets without flowers	33.8	26.4	49.0	54.0			
d. % of spikelets grown into leaflet or tiller	6.9	7.6	18.5	22.7			
e. % of rudimentary spikelets	0	0	4.6	0.9			
Clone 12 (5 June)							
Number of ears	0	0	0	0	0	0	0
	All vegetative with 2–3 vegetative primordia						

more completely (10), so that after 3–4 days in continuous light these laterals were not yet in the double-ridge stage and did not shoot. After 7–8 days they had achieved this stage and more laterals were shooting.

Date of shooting

The date of shooting was considerably earlier after 7–8 days in continuous light than after 3–4 days, *viz.* averaging 11 days (Table 1). According to Table 2 the plants were in the double-ridge stage after 3–4 days and in the secondary stage after 7–8 days. In the treatments 7–8 days and 3–4 days in continuous light, the development from the secondary stage to shooting took place in short day, so it may be assumed that the time needed for this process is the same for both treatments. The period from the vegetative stage to the double-ridge stage was passed by both treatments in continuous light and lasted 3 days. The treatment 7–8 days in continuous light took 4 days to achieve the secondary stage from the beginning of the double-ridge stage in continuous light (Table 2). This shows that the treatment 3–4 days in continuous light, which passed the period from the beginning of the double-ridge stage to the secondary stage in short day, should have taken 15 days (11 + 4) to achieve this stage. Apparently, continuous light accelerates the development of the growing point more than short day. There is in fact little difference in the number of hours of light received, *viz.* 15 days of short day (7 hours) is 105 hours of light and 4 days of continuous light is 96 hours. More detailed analysis of the growing point could confirm the preceding statement.

TABLE 2. Development of the growing point (9) of perennial ryegrass (clone 3) in continuous light. Temperature 18–20°C.

After 0 days in continuous light	5 vegetative primordia.
After 3 days „ „ „	8 vegetative primordia. In the centre of the apex the double-ridge stage begins.
After 5 days „ „ „	11–12 vegetative primordia. Double-ridge stage.
After 7 days „ „ „	Beginning of the secondary stage – secondary stage.

Number of spikelets per ear

It seems that the number of spikelets initiated per ear is greater as the plant receives fewer days of continuous light (Table 1). However, no correlation was found between the number of days in continuous light and the number of spikelets initiated by the inflorescences of the main tillers. This was not expected because other research has shown a close correlation between the number of vegetative primordia at the moment of transition to the reproductive stage and the ultimate spikelet number (9, 11, 17) and it cannot be assumed that in 3–4 days of continuous light more vegetative primordia are formed than in 7–8 days of continuous light. Moreover, the plants are beginning to change to the reproductive stage after 3 days in continuous light (Table 2). This apparent correlation is caused by more laterals showing ear formation after a greater number of days in continuous light, but the inflorescences of these laterals initiate fewer spikelets than those of the main tillers (7).

Morphology of the inflorescence

The ears in the different treatments showed considerable morphological variation. The spikelets varied as follows:

a. normal spikelets;
b. normal spikelets, but only partly filled with flowers;
c. spikelets without flowers, or with rudimentary flowers; these only have a clearly visible glume;
d. spikelets in which the flowers or parts thereof are shaped like a leaflet or a young tiller;

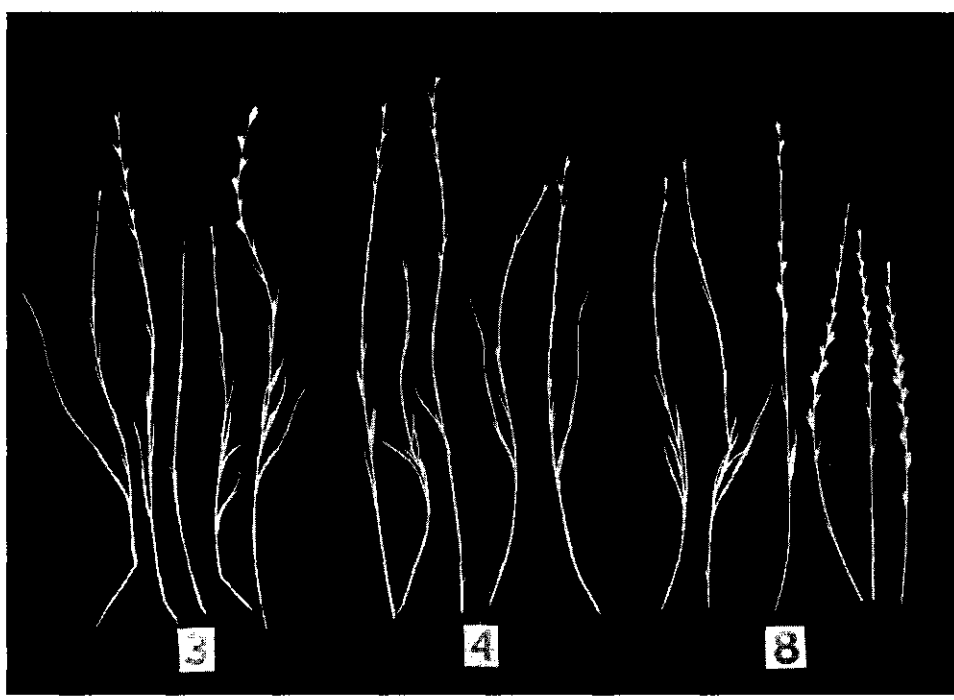

FIG. 2. Influence of the number of days in continuous light (3, 4 and 8 days respectively) after vernalization on the morphology of the inflorescence of perennial ryegrass in short day.

e. rudimentary spikelets; there is only a slight thickening of the stem, without a visible glume.

All these variations may occur in one ear. Table 1 and Fig. 2 show that if the plant has received more days of continuous light the percentage of normal spikelets will be higher. Spikelets developing into a leaflet or even into a tiller, mostly occur in the treatments with a low number of days in continuous light. This is plausible, because the less advanced the formation of spikelets is at the return to short day, the greater the chance will be that these spikelets do not form complete flowers or none at all (incomplete differentiation caused by incomplete induction). This mostly occurred in the lower spikelets. The spikelets mentioned under c and d were difficult to distinguish, because it could not be established during the harvest whether a c shape might have developed into a d-shape if it could have grown longer.

Vivipary

The above mentioned leaflet and tiller formation in the inflorescence without seed is called vivipary and vegetative proliferation (1, 12, 21, 22). Fig. 3 clearly shows that if such ears are brought into moist conditions separate plants will develop.

WYCHERLEY (23, 24) divides this proliferation into two groups: Firstly, those grasses in which it occurs frequently under natural conditions and where it is transmitted by the plant, external conditions being of secondary importance only. Plants of this type belong to the viviparous races. Secondly, in grasses in which proliferations seldom occur, external conditions are primary and there is no question about heridity; these are the ephemeral or vegetative proliferations. These proliferated spikelets have been observed in various grasses (1, 4, 6, 18, 19, 21), but mainly in the viviparous races.

FIG. 3. Development of an inflorescence with proliferating spikelets into a series of separate plants.

A possible influence of daylength was mentioned by RAZUMOV (15) and LAWRENCE (13). Under experimental conditions the occurrence of vegetative proliferations was first mentioned by TINCKER (20). His cocksfoot plants flowered normally in natural summer daylength, but remained vegetative in 6 or 9 hours of daylength. In 12 hours of daylength vegetative proliferations were formed. NYGREN's (14) findings in *Deschampsia cespitosa* P.B. were similar. WYCHERLEY (22) also found these spikelets in *Cynosurus cristatus* L. as a result of partial induction in continuous day followed by short day. Most plants which had received over 10 days of continuous light formed normal ears. After a shorter period of continuous light the plants remained vegetative or showed highly proliferated flowers. In this experiment WYCHERLEY considered 10 days the critical period. He stated that proliferation of spikelets occurs if the induction period for ear initiation is minimal.

LANGER and RYLE (12) found proliferating spikelets in timothy S 48 when the plants had been subjected to an unfavourable daylength during spikelet formation.

MARGADANT found that *Lolium*-plants remained vegetative if they experienced natural winter conditions for a very short period. Very few plants were shooting and these showed proliferating spikelets. Incomplete vernalization could therefore be another cause of proliferation (25). This was also found in experiments we started for another purpose, *viz.* an experiment in which perennial-ryegrass plants (clone 12) were regularly transferred from the field to the greenhouse and thus were more or less vernalized. The plants transferred to the greenhouse very early in winter, did not show ear formation. A few plants had one or two ears with vegetative proliferations. Perennial-ryegrass plants (clone 3), which were meant to remain vegetative and were placed in a greenhouse at 16°C and 17 hours of daylength, also formed a few ears with proliferating spikelets. Perhaps these plants had experienced some cold before they were brought into the greenhouse. It would thus appear that very incomplete vernalization may stimulate vegetative proliferation.

In all previous cases vegetative proliferation occurred in long day plants. GALINAT and NAYLOR (4) found in the short-day species maize that strain C 31 was prone to proliferation of the male inflorescences. Daylengths of $10\frac{1}{2}$–$11\frac{1}{2}$ hours achieved normal vigorous flowers. In daylengths of $11\frac{1}{2}$–$12\frac{1}{2}$ hours, however, the flowers were sterile and proliferations were observed after 15 hours. In longer daylengths the maize remained vegetative. Clearly, marginal daylength resulted in proliferation.

EVANS (2) could not find any influence of incomplete vernalization on the occurrence of vegetative proliferations in perennial ryegrass. Neither did he observe vegetative proliferation when after vernalization, perennial-ryegrass plants received a few days of long day and were then placed in short day. Vegetative proliferation (50%) did occur in perennial ryegrass from New Zealand which received a few weeks of short day after vernalization before the plants were transferred to continuous light.

The occurrence of vegetative proliferations in the plants of clone 3 used in this experiment, however, does not conclusively prove that under these conditions this is a general phenomenon in all types of perennial ryegrass.

SUMMARY

The influence of a varying number of days in continuous light, after vernalization, on the shooting and morphology of the inflorescences in two clones of perennial ryegrass was investigated.

The plants of one clone only (No. 3) were shooting after 3 or more days in continuous light. The growing point had achieved the beginning of the double-ridge stage after 3 days. Shooting only occurred if the period in continuous light had been long

enough to achieve the double-ridge stage. Clone 12 remained completely vegetative, even after 8 days in continous light. Perhaps, these 8 days were insufficient for the growing point to achieve the double-ridge stage. Consequently, a difference was found between the two clones with regard to the influence of continuous light on shooting (on the development of the growing point).

In clone 3 a greater number of days in continuous light proved to accelerate the development of the growing point compared with short day, which resulted in earlier shooting of the plants. The number of tillers shooting in clone 3 was greatly influenced by the number of days in continuous light. This may have been caused by incompletely vernalized laterals achieving the double-ridge stage after a longer period in continuous light. The number of spikelets does not seem to be directly influenced by a longer period in continuous light. However, the average number of spikelets decreases in longer periods of continuous light, because more laterals which have fewer spikelets will shoot thus decreasing the average number. The longer the plants of clone 3 received continuous light, the greater the number of normal inflorescences formed. With a smaller number of days in continuous light more deviating spikelets were formed: *e.g.* poorly filled spikelets; abortive flowers in spikelets; glumes, flowers or spikelets growing into a leaflet or tiller (vegetative proliferation).

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INFLUENCE OF THE VERNALIZATION PERIOD AND LIGHT INTENSITY ON SHOOTING AND COMPOSITION OF THE INFLORESCENCE OF PERENNIAL RYEGRASS *(LOLIUM PERENNE* L.)

A. KLEINENDORST and A. SONNEVELD

INTRODUCTION

In the scope of an investigation into the ecotypes from old permanent pastures a great number of perennial ryegrass plants were collected throughout the Netherlands. Of these plants two were strikingly different and these were vegetatively propagated.

One was very early flowering and its growth was prostrate, the other grew erect and was very late shooting. These differences are quite in contrast with the general rule that early flowering varieties (ecotypes) are erect and late flowering ones are prostrate.

In the autumn of 1963 both clones were used in an informative investigation into the vernalization requirement.

METHODS

In the late summer of 1963 the plants of the concerning clones all in the vegetative stage were divided into separate tillers. These tillers were cultivated in a greenhouse in nutrient solution at a constant temperature of 16 °C. On 16 September, 16 October and 15 November a number of them was cut to about 5 cm. All visible laterals were removed and the roots over 1 to 2 cm long were shortened to that length. The thus treated tillers were placed in a darkroom at 2 °C with the shoot base and roots in water. On 16 December all the tillers were transferred from the darkroom to a climate room at 20 °C and placed in a modified nutrient solution of HOAGLAND and ARNON, generally used at our institute (LOCHER and BROUWER (1)). The medium was aerated. Half of the tillers were placed at a light intensity of about 4.7×10^4 ergs cm^{-2} sec^{-1} and the other half at about 2.4×10^4 ergs cm^{-2} sec^{-1}. Daylength was 17 hours. The date of ear emergence of the vernalized tillers was recorded as the shooting date. Each treatment comprised 24 individual tillers.

RESULTS

Table 1 shows the average shooting date and various other data on the ears formed on the early shooting and prostrate growing clone.

Shooting was later as the 2 °C-treatment was shorter. By checking a number of control plants it was established that the growing points were completely vegetative when the tillers were placed in 17 hours daylength at 20 °C. The tillers placed in the high light intensity clearly showed that one month at 2 °C was already sufficient to shoot, even if much retarded because the vernalization period had not been optimal. This was also not yet reached after 2 months. Shooting occurred with almost all tillers in the high light intensity. This was not the case in the low light intensity; there

TABLE 1. Average date of shooting and further data on the ear formation of these plants.

Treatment		Number of shooting plants (max. 24)	Average date of shooting	Number of days between 16 December and average shooting date	Average number of spikelets per ear	Average number of flowers per spikelet	Average length of the ear in cm	Average distance between the spikelets in cm	Average dry weight per ear in g	Total number of shooting laterals
2 °C-treatment	Light intensity									
3 months	high	24	7 Jan.	22	16.4	8.3	21.2	1.4	0.126	1
3 months	low	19	13 Jan.	28	16.5	4.7	14.8	1.0	0.046	0
2 months	high	23	13 Jan.	28	19.4	8.3	23.6	1.3	0.154	4
2 months	low	17	18 Jan.	33	18.6	4.8	16.7	1.0	0.054	0
1 month	high	24	27 Jan.	42	24.3	7.1	24.8	1.1	0.150	8
1 month	low	8	7 Feb.	53	24.9	4.3	20.1	0.8	0.074	0

the percentage of tillers forming a visible ear was lower as the period at 2 °C was shorter.

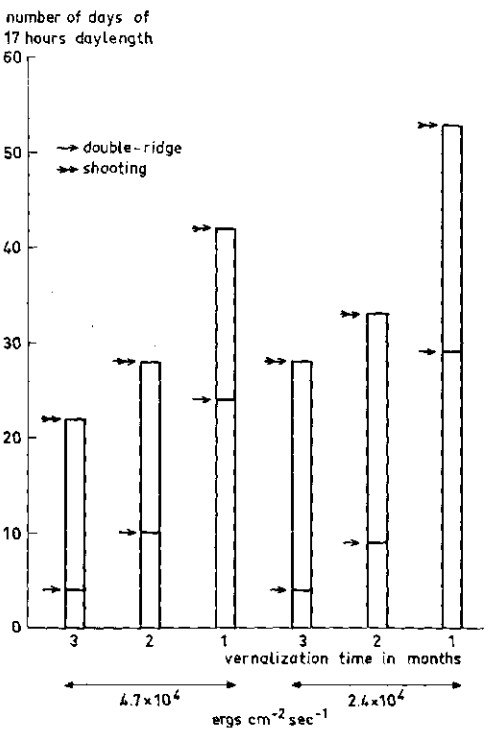
It was striking that the average number of spikelets per ear was larger as the period at 2 °C was shorter and, consequently, the time interval between transference and shooting longer. The length of the cold period affected the number of flowers per spikelet only to a very slight extent but the light intensity did appreciably. The average length of the whole inflorescence was influenced by the period at 2 °C as well as by the light intensity. With a decreasing length of the cold treatment the inflorescence length increased less than the number of spikelets, thus decreasing the average distance between the spikelets. The total length of the inflorescence as well as the average distance between the spikelets was decreased by the low light intensity. The average dry weights per ear clearly show that the ears formed under the low light intensity were less developed.

After transference to 20 °C and 17 hours' daylength laterals were developed by the experimental tillers. The tillers under the high light intensity approximately formed four times as many laterals as the plants under the low light intensity but unfortunately, the numbers were not exactly recorded. It was however found that some of these showed ear formation with the high light intensity only and their number was higher as the period at 2 °C was shorter.

Besides the experimental tillers an additional number of tillers was included; these tillers received the same treatment, but some of them were analysed every 2 or 3 days to follow the development of the growing point. In this way it could be accurately established how many days after transference the double-ridge stage was attained by the tillers of the different treatments. This number as well as the number of days between transference and shooting have been summarized in Fig. 1. The vernalization period had much influence on the time interval between transference and attaining the double-ridge stage, but did not affect the period elapsing between attaining the double-ridge stage and shooting. The light intensity did not have any or only a very slight influence (with 1 month vernalization) on the time interval between transference and attaining the double-ridge stage, but it did appreciably lengthen the period between the double-ridge stage and shooting. To illustrate this some observations on the growing points of tillers with the highest vernalization level have been summarized in Table 2.

The rapid change to the reproductive phase as shown in Table 2 indicates that at

FIG. 1. Number of days between transference to 20°C and 17 hours daylength and attaining the double-ridge stage, shooting respectively.



2°C a vernalization period of 3 months is nearly optimal. However, one wonders if a temperature of 2°C is optimal for this purpose and if the period could be shortened by a somewhat higher temperature. This could not be investigated, however, at that time, since only a darkroom at 2°C was available but the effect of the temperature on the vernalization period is one of the next items to be investigated. From Table 1 it was already striking that the average number of spikelets per ear was larger as the period at 2°C was shorter and, consequently, the time interval between transference and shooting longer. Since it can be seen from Fig. 1 that the period at 2°C does not affect the time interval between the double-ridge stage and shooting, the increase in number of spikelets must be the result of the difference in time between transference and attaining the double-ridge stage. This is confirmed by the fact that the light inten-

TABLE 2. Some observations on the growing points of tillers with the highest vernalization level.

	High light intensity	Low light intensity
On the moment of transfer	4–5 vegetative primordia	4–5 vegetative primordia
After 3 days at 20°C and 17 hours daylength	8 vegetative primordia, (in the beginning of the double-ridge stage)	8 vegetative primordia, (in the beginning of the double-ridge stage)
Ditto after 5 days	11–12 primordia, double-ridge stage	10–11 primordia, double-ridge stage
Ditto after 7 days	13–14 primordia, beginning secondary-secondary stage	13–14 primordia, beginning secondary-secondary stage

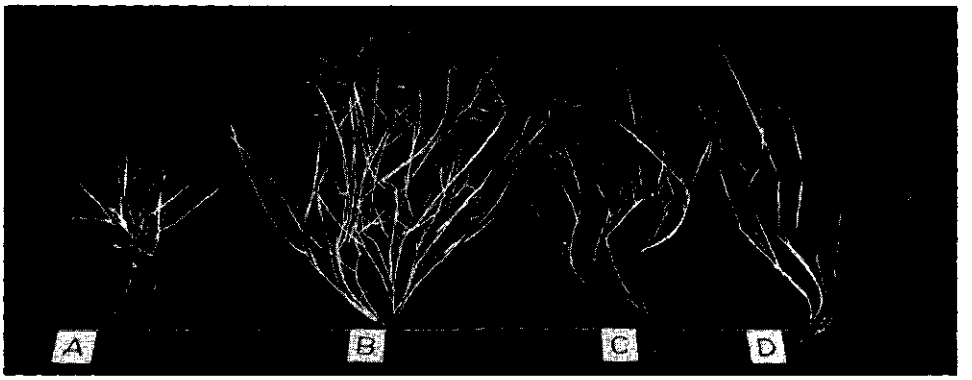


FIG. 2. Tillers of an insufficiently vernalized clone after various 2 °C-treatments, followed by various light intensities at the high temperature and long day.
A. 3 months at 2 °C, afterwards high light intensity
B. 2 months at 2 °C, the same
C. 1 month at 2 °C, the same
D. 3 months at 2 °C, afterwards low light intensity.

sity, which did influence the period from the double-ridge stage to shooting, did not affect the period from transfer to the double-ridge stage, neither influenced the number of spikelets per ear. However, the light intensity appreciably affected the number of flowers per spikelet which was influenced only to a very slight extent by the length of the cold treatment. A period of one month at this temperature affected the number of flowers per spikelet somewhat unfavourably.

The experimental tillers of the clone with late shooting, showing relatively erect growth in the field, remained vegetative under all conditions, as shown by the regular inspection of the growing point. The vernalization requirement of this clone is apparently so high that a period of 3 months at 2 °C did not result in an inflorescence when the tillers were placed at a high temperature and long day. The plants receiving a 2 °C-treatment for 3 months and afterwards placed under the high light intensity did after some time show extension of the lowest internodes on the oldest shoots. The laterals formed afterwards were consequently situated at a certain distance from the base (Fig. 2 A). The plants receiving a 2 °C-treatment for 1 or 2 months did not show this effect after they had been transferred to the high light intensity (Fig. 2 C

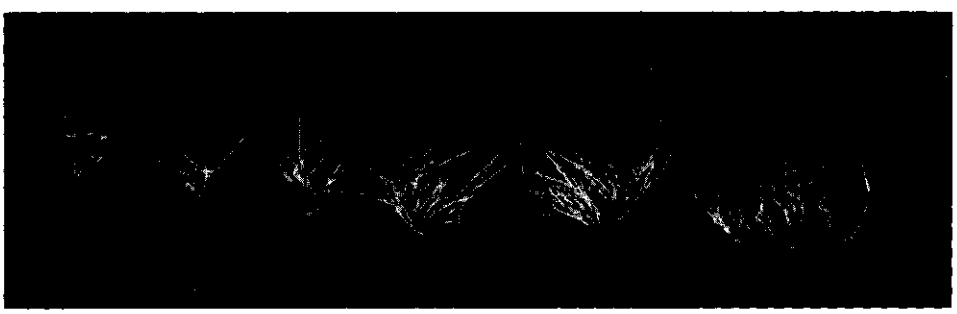


FIG. 3. Various tiller formations on the plants of an insufficiently vernalized clone, subjected to 3 months of the 2 °C-treatment, placed afterwards at 20 °C and 17 hours daylength under a relatively high light intensity (4.7×10^4 ergs cm^{-2} sec^{-1}).

and 2 B). The plants placed under the low light intensity did not show the phenomenon at all, neither after a period of 3 months at 2 °C (Fig. 2 D).

The shoots showing this internode elongation displayed slight differences dependent on the place of the elongating tillers within the plant. The laterals of the tillers on the outside of the plants, which tillers grew almost horizontally, were not united to a group, but distributed all over the main tiller. Fig. 3 shows a survey of these forms.

Discussion

The influence of the period at 2°C on the time interval between transference to 20 °C under 17 hours of daylength and attaining the double-ridge stage as well as the effect on the composition of the inflorescence are an interesting aspect. In order to show this process clearly the number of days between transference and the double-ridge stage and the average number of spikelets per ear have been plotted against each other in Fig. 4. The relation found suggests that the number of spikelets cannot be increased unlimited by increasing the number of days between transference and the double-ridge stage, but that the maximum lies at about 30 days. Attempts to lengthen the time between transference and the double-ridge stage by giving the plants a still shorter 2 °C-treatment, will obviously have the result, that the plants stop shooting altogether more or less soon. The concerning clone needs a certain amount of cold as it remained in a vegetative stage in a greenhouse at 16 °C for rather a long time, also under long-day conditions. The greater number of spikelets ultimately formed was already indicated before the double-ridge stage. After the longest 2 °C-treatment the tillers changed very rapidly to the reproductive phase after transference to 20 °C and 17 hours daylength. The vernalization of these tillers had been nearly optimal. After a shorter 2 °C-treatment a distinct lengthening of the growing point and an increase in the number of vegetative primordia could clearly be established after transference to 20 °C and 17 hours daylength. Kleinendorst and ten Hove (2) and Kleinendorst and Sonneveld (3) working with perennial ryegrass as well as Ryle and Langer (5) experimenting with timothy showed a positive correlation between the number of visible primordia at the change to the reproductive phase (beginning of the double-ridge stage) and the ultimate spikelet number. No

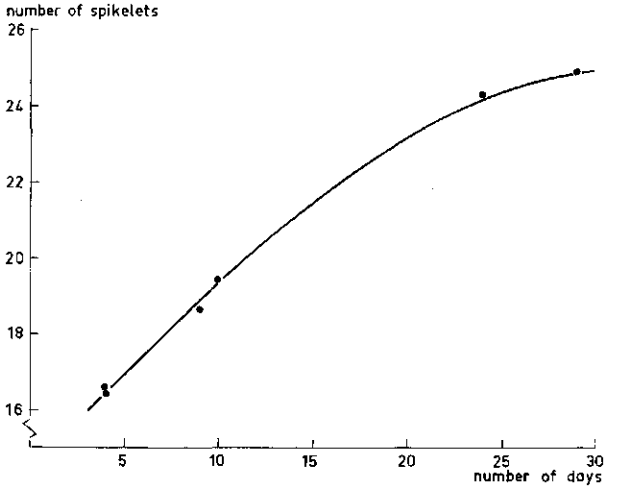


Fig. 4. Relation between the number of days between the beginning of the treatment at 20 °C and 17 hours daylength and attaining the double-ridge stage and the average number of spikelets per ear.

effect of light intensity was observed on this phenomenon. Another investigation (KLEINENDORST and SONNEVELD (3)), however, showed that there was an influence of the light intensity on the size of the growing point of vegetatively growing tillers and of tillers kept in short day after vernalization. Transference of such tillers to a higher temperature also caused extension of the growing point and an increase in the number of vegetative primordia. This number, however, decreased again to normal under these conditions, while the formation of new leaves was continued. In this case the temporarily higher number of vegetative primordia came to a higher level at higher light intensities.

In the present investigation in which more or less vernalized plants were placed at a higher temperature and long day a similar effect of the light intensity could not be observed. Apparently, it is completely outdone by the formative influence of the daylength. The light intensity in such cases can only influence the realization of the possibilities offered by higher temperature and long day. With inadequate assimilation the inflorescence will not emerge at all or later on and its size will be much smaller. The axis will be thinner, the spikelets closer together (Fig. 5) and within each spikelet less primordia will develop into a full-grown flower.

Moreover, a low light intensity appears to hamper the plants in forming inflorescences on the laterals formed after vernalization, while the total number of laterals formed by the plants under the low light intensity was appreciably smaller too.

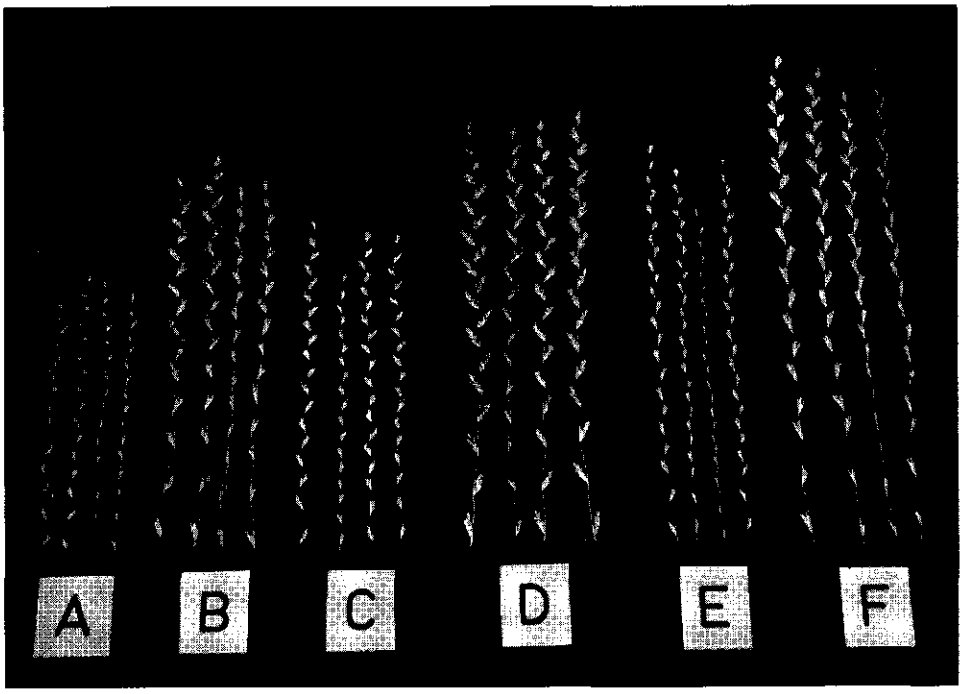


FIG. 5. Composition and size of the ears formed.
A. 3 months at 2°C, afterwards low light intensity
B. the same, afterwards high light intensity
C. 2 months at 2°C, afterwards low light intensity
D. the same, afterwards high light intensity
E. 1 month at 2°C, afterwards low light intensity
F. the same, afterwards high light intensity

RYLE (4) also found with timothy that reduced light intensity resulted in reduced vegetative growth as well as a smaller number of emerging inflorescences and a smaller number of full-grown flowers.

SUMMARY

Tillers of an early shooting clone of perennial ryegrass appeared to be almost optimally vernalized after 3 months at 2°C. A shorter period at this temperature resulted in retarded shooting, but also in a larger number of spikelets per ear. The latter is due to a longer interval between transference to a higher temperature and long day and attaining the double-ridge stage. A lower light intensity during the period at higher temperature and long day caused decreased and also retarded ear formation. Moreover, the ears were smaller and the number of flowers per spikelet as well.

A late shooting clone of the same species remained completely vegetative under the same conditions.

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INFLUENCE OF DIFFERENT ROOT TEMPERATURES ON TRANSPIRATION AND EXUDATION OF YOUNG MAIZE PLANTS

J. TH. LOCHER and R. BROUWER

INTRODUCTION

Temperature of the root medium has been found to affect the water permeability of plant roots seriously. KRAMER (6) distinguished two important causes: changes in water viscosity and changes in permeability of the cytoplasm. Root temperature also has a considerable influence on the transpiration rate of the plants, because the changing permeability of the root tissue has a secondary effect on the suction tension in the plants and as a consequence on stomatal width (2).

Some investigations suggest that the growth reductions, found at high and low root temperatures, are wholly due to the inhibited water transport and the increased suction tension (5, 3). Prolonged exposure of the plants to extreme root temperatures also causes impermeable layers in the walls of the endodermal cells, leading to an unfavourable water balance (4).

In this paper some experiments are described on the influence of root temperatures on the transpiration and exudation of young maize plants, especially during the first 24 hours after exposure of the plants to these different root temperatures.

METHODS

A description of rearing the plants before use in the experiments and of the determination technique has been given elsewhere (8). The light intensity at the surface of the tables was $4-6 \times 10^4$ ergs cm^{-2} sec^{-1}. Other conditions varied and are mentioned in the description of the experiments.

RESULTS

In fig. 3 in a previous paper (8) an example was given of the influence of root temperature on the exudation of 6 weeks old maize plants, grown in aerated Hoagland nutrient solution at 20 °C. Exudation of the cut stems was measured every quarter of an hour during a couple of hours, the root temperature being changed each hour. If the temperature of the root medium was lowered from 23 or 35 °C to 11 °C, the exudation rate at once decreased to a very low value. Transference to 35 °C caused a high initial exudation rate, immediately followed by a rapid decrease. It was suggested that this decrease was caused by a lack of energy after cutting the shoots. The potassium and nitrate concentrations, determined for one hour' periods, were much higher in the small quantities of exudation sap at 11 °C than in the larger quantities at 35 °C. It seems likely that the water transport is rapidly and directly influenced by changes in root temperature and the transport of ions as well, though the latter does not react as sharply and as rapidly as the water transport.

In Fig. 1 A and B exudation rates are shown of maize plants which, after growing

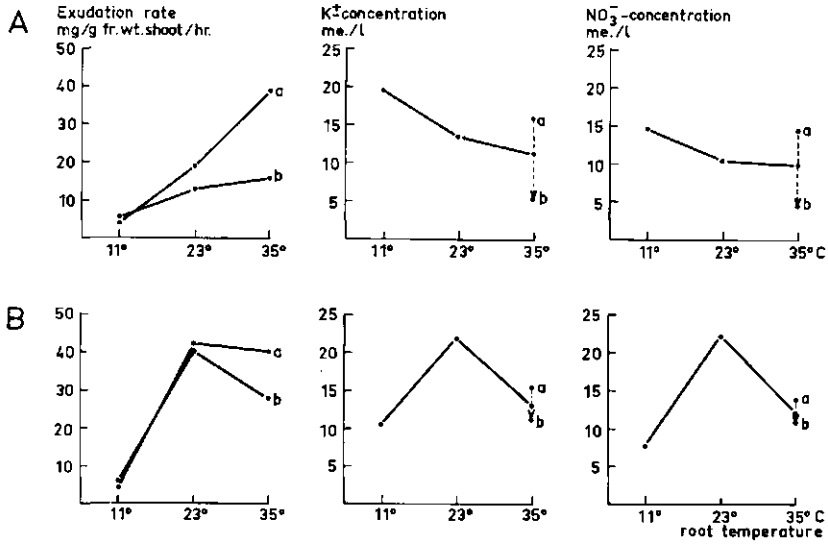

FIG. 1. Exudation rate together with potassium and nitrate concentrations in the exudate, directly (A) and 24 hours (B) after exposing plants to different root temperatures of 11, 23 and 35 °C. a = 1st hour; b = 2nd + 3rd hour of bleeding period.

in aerated Hoagland solution at 20 °C, were exposed to different root temperatures and then treated in the following way: Half the number of the plants was decapitated immediately. Fig. 1 A shows the exudation rates of these plants during the first hour and during the subsequent two hours after decapitation. Fig. 1B gives the exudation rates of the remaining plants which, before decapitation, were exposed to different root temperatures for 24 hours. Here again the exudation rates of the first and of the subsequent two hours were given separately. Potassium and nitrate concentrations of the bleeding sap were determined over the whole exudation period of three hours. Only at the root temperature of 35 °C these concentrations were determined separately over the first (a) and second (b) period. In the figures both these dots are connected by an interrupted line.

In this experiment, as well as in the former one, the plants show a high initial exudation rate at the highest root temperatures, which decreases rapidly, and a low initial exudation rate at the lowest root temperatures, which does not further decrease. The potassium and nitrate concentrations are lowest at the higher root temperatures.

The plants already exposed to the different root temperatures for 24 hours show another picture. With these the exudation rate at 35 °C was not any higher than at 23 °C, if measured during the first hour after cutting. A few hours later it was even considerably lower at 35 °C than at 23 °C.

The potassium and nitrate concentrations in the exudate of the latter group of plants were highest at 23 °C and at this temperature higher than in the exudate of the plants cut immediately after exposure to the different root temperatures. At 11 °C concentrations were lower in the second group of plants, while the two groups did not differ much at 35 °C.

To examine transpiration and subsequent exudation of maize plants, exposed to different root temperatures, experiments were carried out in which water uptake was determined during a 16 hours' period after exposure to 7 or 8 different root tempera-

tures from 5 to 40°C. After this period the plants were decapitated and the exudation rates were determined during a 4 hours' period. Before the experiments the plants were grown in non-aerated Hoagland nutrient solutions at 20°C. Plant ages differed. During the experiments the plants were placed in one litre pots, filled with non-aerated solutions of different temperatures. Transpiration of the plants was measured by weighing the solutions (with corrections for free evaporation). Light sources were TLF tubes; the air temperature was 20°C. Some more conditions varied in the experiments: the plants were exposed to 1) continuous light, 2) half the time darkness and light respectively and 3) continuous darkness during the transpiration period. Other series of experiments were carried out in 1) normal Hoagland nutrient solution and 2) demineralized water. In the latter case the plants were already placed in demineralized water during varying periods before the experiments (1–15 days).

Fig. 2 shows the transpiration (measured by the water uptake) and the successive exudation of the plants, placed in Hoagland solution and receiving continuous light during the transpiration period. The figure gives the average of 6 experiments. Transpiration as well as exudation rates show an optimum at a root temperature of 30°C. At higher temperatures there seems to be a slight decrease. The optimum at 30°C indicates that at this root temperature and under the given conditions of light, air temperature etc. the stomata achieve their maximal opening and transpiration is at its height. At root temperatures below 30°C the water conductivity (k in the formula $W = k\,(S_x - S_m)$, where S_x refers to the suction tension of the xylem vessels and S_m to the suction tension of the root medium), will hamper the water transport. Via the suction tension in the plant this will influence the opening of the stomata. So root temperature is a limiting factor up to 30°C; above 30°C the evaporative conditions are. The slight decrease in transpiration rate at these higher root temperatures, especially in prolonged treatment, indicates that the water conductivity in the root tissue decreases somewhat in the course of time, since in very short experiments (6) it increases up to the highest root temperatures of the range examined. In some of the longer experiments the latter is also observed, but in others the water conductivity decreases rather heavily. The condition of the plant material, used in the experiments, may be important here.

Fig. 3 shows the transpiration rates at different times of light exposure during the

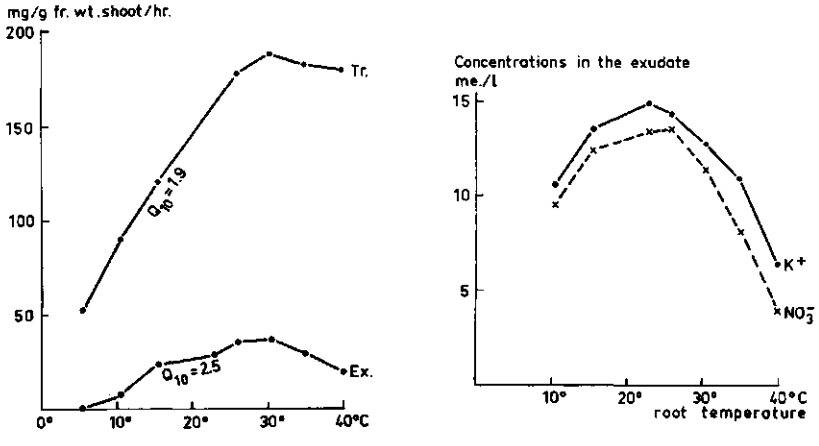

FIG. 2. Rates of transpiration (16 hrs; Tr.) and exudation (4 hrs; Ex.), together with the potassium and nitrate concentrations in the exudate after exposing plants to root temperatures of 5–40°C.

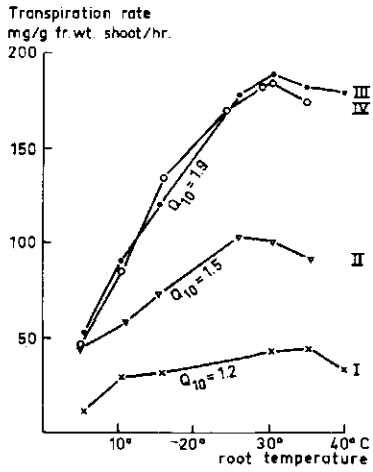

FIG. 3. Rates of transpiration at different root temperatures, after exposing the plants to light for 0 hrs (I), 9.5 hrs (II) and 16 hrs (III and IV) during the transpiration period.

16 hours' transpiration periods. The Q_{10}'s of the curves appear to be different over the whole range of root temperatures from 5 to 30°C.

In considering the exudation rate we see in Fig. 2 an appreciable decrease at root temperatures above 30°C; k as well as O_x in the exudation formula $B = k\ (O_x - O_m)$ may be responsible. Potassium and nitrate concentrations in the bleeding sap are highest at root temperatures below 30°C, with an optimum at 23°C. Since their decrease at higher root temperatures considerably exceeds that in the amount of the bleeding sap itself, it seems likely that the reduced exudation rate is mainly due to diminished salt secretion into the xylem vessels. Exudation rates in these experiments, in which plants were exposed to different root temperatures during 16 hours before bleeding time, show the same trend as in the analogous experiments, described earlier, in which plants were exposed to the different root temperatures during 24 hours before decapitation.

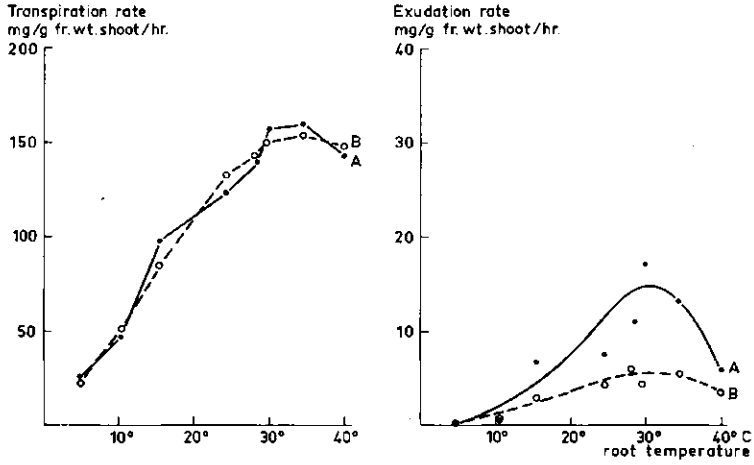

FIG. 4. Rates of transpiration and exudation at different root temperatures of plants, placed in nutrient solution (A) and demineralized water (B) during the experiment and also varying periods before.

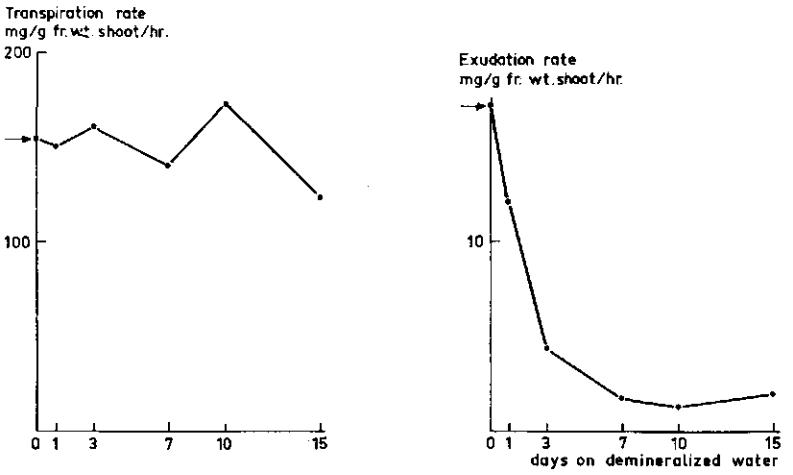

FIG. 5. Rates of transpiration and exudation at a root temperature of 30°C of plants, placed in demineralized water during varying periods before the experiment.

In Fig. 4 (left) the uninterrupted curve shows the transpiration rates in a series of experiments with maize plants, grown in Hoagland nutrient solution before and during the experiments; the interrupted curve reflects the average of experiments with plants placed in demineralized water during the experiments and also 1, 3, 5, 7, 10 and 15 days before. $CaSO_4$ in a concentration of 10^{-4} M was added to the demineralized water, because possible changes may occur in the water permeability of the root tissue in pure demineralized water. Plant ages were 18–32 days and air temperature was 28°C during these experiments. Light sources were TLF tubes. Fig. 5 (left) gives an example of the trend in the transpiration rates (per g fr. wt. shoot/hr.) at a root temperature of 30°C, the individual plants growing in demineralized water during the 6 periods mentioned. Compared to the average transpiration rate of the plants in Hoagland nutrient solution (see arrow), the differences are of minor importance. From Fig. 4 (left) it can be seen that this applies to all root temperatures.

In Figs. 4 and 5 (right) the exudation rates per g fr. wt. shoot/hr. are shown in the same way as the transpiration rates. Here the influence of the pre-treatment in demineralized water and its duration are conspicuous. In conclusion: the transpiration rate of plants in demineralized water did not decrease significantly, but the exudation rate did. If calculated, however, per g fr. wt. *root*/hr., the transpiration rate also shows a decrease, because root growth still continued in demineralized water, whereas growth of the shoots completely stopped.

DISCUSSION

According to BRIEGER (1) the rate of water transport through the living root tissue, as mentioned before, may be expressed by the formula: $W = k\,(S_x - S_m)$ where k is the conductivity of the root tissue (mg hr^{-1} atm^{-1}) and S_x and S_m are the suction tension in the xylem vessels of the root and the suction tension in the root medium (atm.). In the intact plant water movement through the root is not an independent process, but part of a catena along which water moves through the plant tissue (2) from the root medium to the air environment. In this catena the leaf-air interface

(stomata + adjacent air layers) offers by far the most resistance to the flow, and it follows that changes in the rate of water movement may be brought about only by changes in this resistance. It is obvious, however, from these experiments and others (2, 6, 7), that root temperature can induce important changes in the rate of overall water flow. This is comprehensible after the following events. If root temperature is lowered, the resistance to the water flow in the root tissue increases and water absorption is hampered. At the same time transpiration continues and a temporary decrease in the amount of water in the plant body results. This increased water deficit within the plant is accompanied by a loss of turgidity (transference to low root temperatures induces wilting) and enhanced suction tension. The enhanced suction tension gives rise to partial stomatal closure and an increased water uptake ($S_x' > S_x$). This regulation ultimately results in a new equilibrium between water absorption and water loss, the rates of both being lower than the original ones. The values of the rates of water movement, shown in this paper, are achieved therefore in a rather complicated way. They are not only determined by the increased resistance at lower temperatures, but also by the indirectly influenced suction tension. The complexity of the underlying processes can best be proved by the Q_{10}-values. In darkness a Q_{10}-value of about 1.2 is found between 10 and 30 °C, whereas in light this value is 1.9–2.6; it has values in between for intermediate light and dark periods. KUIPER (7) mentioned values as high as about 8 in bean plants with much higher light intensities. The data mentioned here do not allow a separation of the share of each of both quantities: suction tension or conductivity. This is even the case for the water transport in bleeding plants. The Q_{10} of this process is about 2.5.

It has been seen that the concentration of the bleeding sap shows an optimum (Fig. 2) at about 23 °C. Assuming that the conductivity increases regularly with temperature, it is clear that the rate of bleeding will show an optimum too (Fig. 4), if the decrease in salt secretion is greater than the increase in conductivity.

Since the effect of root temperature on water transport in bleeding as well as in transpiring plants results from changes in the driving force and in the water conductivity and since these processes also depend on various other aspects of the environment during treatment and pre-treatment, more detailed studies are necessary before a full explanation can be found.

SUMMARY

The effect of root temperatures on the rates of transpiration and exudation of maize plants was investigated. The plants were grown in nutrient solution or in demineralized water. Exudation of plants, already exposed for 16 or 24 hours to different root temperatures before decapitation, showed a clear optimum at 30 °C (with plants directly decapitated after exposure to different root temperatures the optimum was at higher root temperatures). In the latter group of plants K^+- and NO_3^--concentrations of the bleeding sap decreased at increasing root temperatures, whereas in the first group they showed an optimum at 23 °C.

Transpiration, measured over a 16 hours' period of exposure to different root temperatures, increased up to 30 °C and showed no significant decrease at higher root temperatures.

Transpiration rate of plants, placed in demineralized water, did not decrease significantly, but the exudation rate did.

Enlarging the evaporative conditions resulted in an increased Q_{10}.

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HET VERHINDEREN VAN VIRUSINFECTIE BIJ POOTAARDAPPELEN DOOR GROEIREMMENDE MIDDELEN

with summary

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INLEIDING

Om virusinfectie van de knollen te voorkomen wordt in Nederland het loof van pootaardappelen doodgespoten. Bij het doodspuiten treedt echter vaak hergroei op: de knoppen van de stengels of stengelstompen lopen uit. Deze uitloop is gevaarlijk, omdat het door luizen overgebrachte virus via de jonge, groene bladeren van de spruiten in de knollen kan komen. Door de concentratie van het doodspuitmiddel te verhogen of herhaaldelijk te spuiten kan men wel deze uitloop sterk verminderen, maar vaak niet geheel voorkomen (REESTMAN en SCHEPERS (11)). Door aan het doodspuitmiddel een groeiremmende stof toe te voegen kon deze uitloop geheel onderdrukt worden (BODLAENDER (4)). Voor de onderdrukking van deze uitloop waren echter vrij hoge concentraties van de groeiremmende middelen nodig.

Uit onderzoek van BEEMSTER (1, 2, 3) bleek, dat het virus (bladrol, X- en Y-virus) slechts na voldoende vermeerdering vanuit de bladeren naar de knollen getransporteerd wordt. Een dergelijke vermeerdering treedt in jonge, groeiende bladeren op; in volwassen bladeren vermeerdert het virus zich daarentegen weinig of niet. Deze ouderdomsresistentie van de planten kan echter doorbroken worden door groeiende spruitjes in de bladoksels of nieuwe bladeren aan de toppen der stengels. Zo vond BEEMSTER een volledige besmetting van de knollen met X-virus bij inoculatie van een nieuwe scheut op een oude stengel (2) en infectie van de knollen met bladrolvirus bij inoculatie van jonge spruiten, die zich op afgesneden stengels ontwikkelden (1).

DOEL DER PROEVEN

In een aantal proeven, genomen in de jaren 1960 tot en met 1963, is getracht het optreden van spruitgroei en van jong blad te voorkomen door in juli groeiremmende middelen op het loof te spuiten, om zodoende een ouderdomsresistent gewas te verkrijgen en daardoor toename van de virusinfectie van de knollen te verhinderen. Het volwassen blad zou door deze middelen weinig of niet beschadigd moeten worden, zodat de produktie van droge stof nog enige weken na het spuiten kan voortgaan. Op deze wijze zou een hogere knolopbrengst verkregen kunnen worden dan bij vroeg doodspuiten het geval is.

Proefopzet en resultaten van de in 1963 genomen proef worden hier uitvoerig behandeld. De gegevens der andere proeven zullen slechts kort besproken worden.

VOORAFGAANDE MIDDELENPROEVEN

In enkele kleinere proeven zijn ruim 20 middelen op het loof van pootaardappelen gespoten om hun invloed op de spruitgroei en de mate van bladbeschadiging vast te

stellen. Op grond van de resultaten van deze proeven zijn voor de eigenlijke virusinfectieproeven vier van deze middelen uitgekozen, nl. 2,4,5-T, 2,4-D, 4-CPA en fenac. Andere middelen, zoals MH, Cl-IPC, TCNB, MENA e.d., die de kieming tijdens de bewaring goed remmen (PERLASCA (10), FISCHNICH en PÄTZOLD (6)) bleken óf de spruitgroei in het loof onvoldoende te remmen óf een duidelijke nawerking in de nateelt te vertonen. Een duidelijke remming van de strekkingsgroei van aardappelplanten werd ook door een nieuw type groeiremmende stoffen verkregen (KRUG (7)). In eigen kasproeven bleken deze stoffen, zoals CCC en B 995, wel de lengtegroei, doch niet de vorming van nieuwe, jonge bladeren aan de stengeltop tegen te gaan.

PROEFOPZET DER VIRUSINFECTIEPROEVEN

a. Pootgoed, teeltgegevens

De proef van 1963 werd aangelegd op een perceel humeuze zandgrond bij Maasbree in Midden-Limburg. Gebruikt werd het ras Voran (klasse S). Dit ras vertoont een sterke neiging tot hergroei na doodspuiten.

De voorgekiemde knollen werden gepoot op 25 en 26 april. Het aantal planten was 43 000 per ha en de potermaat 35-45 mm. Als bemesting werd gegeven 120 kg N, 120 kg P_2O_5 en 160 kg K_2O per ha.

b. Beïnvloeding van de besmettingskans

Om een voortijdige besmetting door luizen te verhinderen, werden de planten op 24 mei met het insecticide Systox bespoten. Bij selectie in mei en juni bleken er geen bladrolzieke planten in het proefveld te zijn.

Elk veldje was omgeven door een rij haver om wederzijdse beïnvloeding van de veldjes te voorkomen. De luizen kunnen niet over deze haver heenlopen.

Om ook bij beperkte aantallen luizen een duidelijk effect van de groeiremmende middelen te kunnen aantonen, werden bladrolplanten in de veldjes geplaatst om zodoende een grotere besmettingskans te verkrijgen. Om besmetting in het proefveld vóór de spuitdatum te voorkomen groeiden de bladrolplanten op een ander perceel in potten op en werden pas kort voor het spuiten in het eigenlijke proefveld geplaatst.

c. Bespuitingen

Er waren twee spuittijden, nl. 4 en 19 juli. Het gewas was op die data respectievelijk ca. 50 en 90 cm hoog.

Op deze spuittijden werden 4 groeiremmende middelen (zie tabel 1) op het volle gewas gespoten. Bovendien werden op dezelfde spuittijden de planten van het object ‚doodspuiten na maaien' afgemaaid (om loofklappen na te bootsen) en vervolgens doodgespoten met 40 l DNOC per ha. Bij uitloop werd opnieuw 40 l DNOC gespoten. De bladrolplanten werden niet bespoten met een groeiremmer of DNOC. Voorts was er het object ‚onbehandeld'. Er werd steeds gespoten met een hoeveelheid water overeenkomende met 1000 l per ha.

De ene helft van alle veldjes (ook van de ‚onbehandelde' en van de reeds eerder doodgespotene) werden doodgespoten met 9 l diquat per ha na 2 weken (R_1), de andere helft na 3 weken (R_2).

d. Rooiingen en nateelt

De knollen van bovengenoemde objecten werden bij de eerste spuittijd 2 à 3 weken en bij de tweede spuittijd 1 week na het doodspuiten met diquat gerooid. Bovendien werd op beide spuittijden een veldje groen gerooid om de virusbesmetting van de knollen op het moment van spuiten te bepalen.

TABEL 1. Gegevens betreffende de gespoten middelen; proef 1963.
Data on chemicals sprayed on potato plants; experiment 1963.

Afkorting *Abbreviation*	Actief bestanddeel *Active compound*	Handelsprodukt *Commercial product*	% actieve stof *% active substance*	Gebruikte hoeveelheid in liters per ha *Quantity used in liters per ha*
2,4,5-T	2,4,5-trichloorphenoxyazijnzuur[1]) (ester)	Aanetos-L	52,5	5
2,4-D	2,4-dichloorphenoxyazijnzuur[1]) (ester)	Lyrinox	50	10
4-CPA	4-monochloorphenoxyazijnzuur[1])	Weedone bracken control	50	18
fenac	2,3,6-trichloorphenyl azijnzuur[2]) (Na-zout)		18	16,6 (4/7) 33,3 (19/7)
DNOC	3,5-dinitro-orthocresol (in olie)[3])	AAlomort	20	40
diquat	1,1′-ethyleen-2,2′-dipyridilium (dichloride)	Reglone	20	9

[1]) -chloorphenoxyazijnzuur = *chlorophenoxy acetic acid*
[2]) -chloorphenylazijnzuur (Na-zout) = *chlorophenyl acetic acid (sodium salt)*
[3]) in olie = *in oil*

Bij het rooien werden de knolgewichten per half veldje bepaald. Elk half veldje bestond uit 3 rijen van 17 netto-planten. Alle objecten waren in drievoud aangelegd.

Van elke netto-plant werden 3 knollen verzameld en bewaard. Deze knollen (per object 450) werden in het volgende jaar uitgepoot en de planten die hieruit opgroeiden, werden beoordeeld op opkomst en op ziekteverschijnselen om het bladrolpercentage der verschillende objecten te bepalen.

WAARNEMINGEN EN GEGEVENS

a. Loofwaarnemingen

Reeds enkele uren na het spuiten van de groeiremmende middelen gingen de stengels ombuigen. Dit was vooral bij 2,4,5-T het geval. De stengels waren later ook tamelijk broos. De lengtegroei van de stengels werd door deze middelen geremd. Dit had tevens tot gevolg, dat zich praktisch geen nieuwe bladeren aan de stengelknoppen ontwikkelden. Door de inwerking der remmiddelen werd ook de groei van spruitjes uit de okselknoppen voorkomen of geremd. De okselknoppen werden a.h.w. weggebrand (‚dode' spruitjes) of de reeds aanwezige spruitjes werden omgebogen (‚vervormde' spruitjes) (fig. 1). Het volwassen blad werd daarentegen weinig of niet beschadigd.

In de proef van 1963 zijn slechts schattingen gedaan over de groei van okselspruiten en stengeltoppen. De onderdrukking van de spruitgroei door de verschillende middelen was bij de tweede spuittijd volledíger dan bij de eerste. De spruitgroei trad vooral op in de basale delen van de stengels, waar de okselknoppen en bijbehorende bladeren waarschijnlijk weinig of niet door de middelen geraakt zijn. Er was ook een groot verschil in werking tussen de middelen. De spruitgroei werd het sterkst geremd door 2,4,5-T, zeer sterk ook door fenac bij de tweede spuittijd (hoge concentratie) en het

a

b

FIG. 1. Remming van de spruitgroei; proef 1962.
Inhibition of sprout growth; experiment 1962.
Objecten van links naar rechts/*Treatments from left to right:* onbehandeld/*untreated* – 2,4,5-T – 2,4-D – 4-CPA – fenac.
De volwassen bladeren zijn verwijderd. De foto's zijn genomen op 25 juli 1962. a: 21 dagen na eerste spuittijd; b: 7 dagen na tweede spuittijd.
Mature leaves were taken away. Pictures were taken on 25 July 1962. a: 21 days after first date of spraying; b: 7 days after second date of spraying.

minst door 2,4-D. De remming door 4-CPA was aanvankelijk ook vrij sterk, maar de werking van dit middel hield niet zo lang aan als die van 2,4,5-T of fenac. Drie weken na het spuiten trad hernieuwde spruitgroei op, vooral na de vroege spuittijd. Bij 2,4-D was deze hergroei het sterkst aan de basis van de stengels. Bij 4-CPA groeiden vooral de toppen der stengels weer door.

In de proef van 1962 zijn de aantallen levende, vervormde en dode okselknoppen of spruitjes geteld (tabel 2). Hieruit blijkt duidelijk, dat het aantal okselknoppen bij de onbehandelde planten groter was dan bij de behandelde en dat door de bespuitingen een groot aantal spruitjes vervormd of gedood werd. De resterende levende spruitjes of knoppen waren echter vaak klein en groeiden meestal niet. Ruim drie weken na de eerste spuittijd (27 juli) trad hergroei op in een groot aantal aanvankelijk dode okselknoppen en het aantal levende spruitjes nam weer toe, vooral bij 2,4-D en 4-CPA.

TABEL 2. Aard en aantal spruitjes per 15 stengels; proef 1962.
Nature and number of sprouts per 15 stems; experiment 1962.

Objecten *Treatments*	Datum van waarneming *Date of observation*	Aantal spruitjes per 15 stengels *Number of sprouts per 15 stems*			
		Levend *Alive*	Vervormd *Deformed*	Dood *Dead*	Totaal *Total*
	Spuittijd/*Date of spraying* 4/7				
Onbehandeld	13/7	240	0	0	240
Untreated	20/7	282	0	0	282
	27/7	309	0	0	309
2,4,5-T	13/7	127	63	39	229
	20/7	49	23	171	243
	27/7	57	21	142	210
2,4-D	13/7	129	12	69	210
	20/7	108	24	105	237
	27/7	173	16	66	255
4-CPA	13/7	104	20	124	248
	20/7	70	12	160	242
	27/7	136	23	95	254
fenac	13/7	81	0	139	220
	20/7	93	5	122	220
	27/7	125	7	101	233
	Spuittijd/*Date of spraying* 18/7				
Onbehandeld	27/7	309	0	0	309
Untreated	2/8	325	0	0	325
2,4,5-T	27/7	104	46	119	269
	2/8	42	37	189	268
2,4-D	27/7	180	29	72	281
	2/8	132	72	81	288
4-CPA	27/7	126	22	140	288
	2/8	76	32	172	280
fenac	27/7	88	13	159	260
	2/8	61	35	177	273

De middelen beschadigden in de proeven de volwassen bladeren bij de eerste spuittijd vrijwel niet, maar bij de tweede spuittijd vrij sterk (dode randen of plekjes op de bladeren, afsterving van gehele bladeren). Vooral 2,4,5-T liet een sterke beschadiging zien. De groei van de bovenste bladeren ging nog enkele dagen na het spuiten enigszins door.

b. Knolopbrengsten

In tabel 3 zijn de totale knolopbrengsten der verschillende objecten vermeld. De opbrengsten der met groeiremmende middelen behandelde planten lagen aanmerkelijk boven die van het object ‚doodspuiten na maaien' (betrouwbaarheid 99,9 %): na 2 weken (R_1) ca. 15 en 8 ton/ha, na 3 weken (R_2) ca. 20 en 10 ton/ha (bij de eerste, resp. tweede spuittijd). De knolopbrengsten der behandelde veldjes lagen in het algemeen slechts iets beneden die der onbehandelde veldjes; in deze proef had het object 4-CPA zelfs een hoger knolgewicht dan de onbehandelde planten. Het object 2,4,5-T vertoonde in het algemeen het laagste knolgewicht van de behandelde veldjes. In de andere proeven werden soortgelijke resultaten verkregen.

TABEL 3. Totale knolopbrengsten in kg/are; proef 1963.
Total tuber yields in kg/are; experiment 1963.

Objecten/*Treatments*	Spuittijd/*Date of spraying*			
	4/7		19/7	
	R_1[1])	R_2[2])	R_1[3])	R_2[4])
Onbehandeld/*Untreated*	262	321	339	384
Doodspuiten na maaien *Haulm killing after cutting*	102	111	242	250
2,4,5-T	234	301	321	315
2,4-D	253	301	328	349
4-CPA	265	342	322	369
fenac	241	293	319	348
Rooien op 19/7/*Lifting on 19/7*	–	–	252	–

[1]), [2]) = doodgespoten 19/7, resp. 26/7, gerooid 8/8.
haulms killed 19/7 and 26/7 respectively, lifted 8/8.
[3]), [4]) = doodgespoten 1/8, resp. 9/8, gerooid 8/8, resp. 15/8.
haulms killed 1/8 and 9/8 respectively, lifted 8/8 and 15/8 respectively.

De opbrengsten aan knollen in de maat 28–45 mm (tabel 4) vertonen grote gelijkenis met de totale knolgewichten. De met groeiremmende middelen behandelde veldjes verkregen een aanzienlijke meeropbrengst t.o.v. de gemaaide en daarna doodgespoten veldjes (betrouwbaarheid 99,9 %), vooral bij de eerste spuittijd. De objecten 4-CPA en onbehandeld hadden van alle objecten de hoogste pootgoedopbrengst.

c. Nateelt en virusbesmetting

De in 1963 verzamelde knollen werden na voorkiemen in april 1964 uitgepoot. De opkomst van alle objecten was normaal. Het object fenac vertoonde echter sterke groeiafwijkingen in het loof. Bovendien bleek fenac ook verschillende navruchten (o.m. rogge, stoppelknollen) zeer sterk in de groei te remmen.

Uit de aantallen bladrolzieke knollen (tabel 5) blijkt het volgende: De knollen die

TABEL 4. Knolopbrengsten in de maat 28–45 mm in kg/are; proef 1963.
Tuber yields in the size 28–45 mm in kg/are; experiment 1963.

Objecten/*Treatments*	Spuittijd/*Date of spraying*			
	4/7		19/7	
	R_1[1])	R_2[2])	R_1[3])	R_2[4])
Onbehandeld/*Untreated*	199	219	199	211
Doodspuiten na maaien *Haulm killing after cutting*	83	94	178	184
2,4,5-T	177	197	205	174
2,4-D	188	199	212	218
4-CPA	201	206	194	218
fenac	173	201	204	196
Rooien op 19/7/*Lifting on 19/7*	–	–	221	–

[1]), [2]), [3]), [4]) = zie tabel 3/*see Table 3.*

op de spuitdata gerooid werden (‚rooien op 4/7, res. 19/7') waren vrij van bladrol. Door het inbrengen van bladrolplanten en de aanwezigheid van luizen trad besmetting op. Na 2 weken had het onbehandelde object 9, resp. 29 bladrolzieke knollen (bij de eerste, resp. tweede spuittijd). Het object ‚doodspuiten na maaien' vertoonde na de eerste spuittijd een grotere besmetting dan de onbehandelde planten; de tweede DNOC-bespuiting van de nieuwe uitloop heeft kennelijk de besmetting niet kunnen voorkomen. Na 3 weken liep het bladrolpercentage zelfs tot 8,7 op.

Bij de eerste spuittijd had alleen 2,4,5-T een goed effect, terwijl bij de tweede spuittijd alle groeiremmende middelen een betrouwbaar (99,9 %) lager aantal bladrolzieke planten hadden dan het onbehandelde object.

Drie weken na het spuiten werden voor 2,4,5-T, 2,4-D en 4-CPA weer grotere aantallen viruszieke knollen gevonden dan na twee weken. De werkingsduur van deze

TABEL 5. Aantal bladrolzieke planten per 450 knollen; proef 1963.
Number of leaf roll diseased plants per 450 tubers; experiment 1963.

Objecten/*Treatments*	Spuittijd/*Date of spraying*			
	4/7		19/7	
	R_1[1])	R_2[2])	R_1[3])	R_2[4])
Onbehandeld/*Untreated*	9	10	29	17
Doodspuiten na maaien *Haulm killing after cutting*	20	39	15	18
2,4,5-T	3	5	3	26
2,4-D	14	29	5	21
4-CPA	12	10	2	8
fenac	9	18	3	3
Rooien op 4/7 en 19/7 *Lifting on 4/7 and 19/7*	0		0	

[1]), [2]), [3]), [4]) = zie tabel 3/*see Table 3.*

middelen is kennelijk beperkt. Bij fenac evenwel werd bij de tweede spuittijd na drie weken hetzelfde aantal bladrolzieke knollen gevonden als na twee weken.

In de proef van 1960 bleek 2,4,5-T ook een sterke vermindering van het viruspercentage te geven t.o.v. het onbehandelde object. Andere middelen werden in die proef niet onderzocht. In de proeven van 1961 en 1962 werden slechts zeer weinig bladrolzieke knollen gevonden; er waren in die jaren weinig luizen in juli. De resultaten van 1961 wijzen in dezelfde richting als die van 1963. In 1962 trad vrijwel geen besmetting op.

Discussie en conclusies

In de virusinfectieproeven bleek het mogelijk met bepaalde middelen de groei van de spruitjes in de bladoksels gedurende bepaalde tijd (omstreeks twee weken) te onderdrukken en het doorgroeien van de toppen der stengels te voorkomen, terwijl het oude, reeds volwassen blad betrekkelijk weinig of niet beschadigd werd. Ongeveer 3 weken na het spuiten waren de middelen echter meestal uitgewerkt en trad er hernieuwde groei in de bladoksels en aan de stengeltoppen op. De remming van de spruitgroei was vollediger bij de tweede spuittijd dan bij de eerste. Het gewas was bij die eerste spuittijd nog zeer krachtig en had een sterke neiging tot spruitgroei. De beschadiging van het blad was bij de eerste spuittijd zeer gering, daarentegen bij de tweede veel groter.

De remming van spruit- en topgroei en de bladbeschadiging weerspiegelen zich in de mate van besmetting der knollen en de knolopbrengsten. Alle behandelde veldjes lagen met hun knolopbrengst aanmerkelijk boven die van de doodgespoten veldjes en dichtbij de opbrengst van het onbehandelde object. Van de vier onderzochte middelen vertoonde 2,4,5-T de sterkste bladbeschadiging en ook de laagste knolopbrengst. Bij goede spruitremming was het aantal viruszieke knollen gering (bij 2,4,5-T, bij fenac en 4-CPA bij de tweede spuittijd; twee weken na het spuiten der middelen).

In deze proeven bleek het dus mogelijk door het spuiten van een groeiremmend middel op het loof van pootaardappelen een relatief hoge knolopbrengst met geen of een geringe virusbesmetting te verkrijgen. Het object ‚onbehandeld' had in deze gevallen een aanmerkelijk grotere virusbesmetting der knollen en het object ‚doodspuiten na maaien' een veel lagere knolopbrengst en ook een grotere mate van virusaantasting dan de behandelde objecten. Als men door late knolzetting en/of vroege luizenvluchten het gewas in een relatief jong stadium zou moeten doodspuiten (met een grote kans op een lage knolopbrengst en hergroei), kan men derhalve door een bespuiting met een groeiremmer het doodspuiten nog enige tijd uitstellen. Hierdoor wordt een hogere knolopbrengst verkregen zonder het risico van een sterke virusbesmetting.

De tot nu toe verkregen gegevens zijn nog beperkt en vereisen verder onderzoek. Zo zal onder meer het effect van groeiremmende middelen op de besmetting met andere virussen nagegaan moeten worden. Het is echter wel duidelijk, dat men door een bespuiting met een groeiremmend middel wel een verschuiving van de rooi- of doodspuitdatum, maar niet een absolute bescherming tegen infectie kan verkrijgen. Bij te vroeg spuiten zal men de spruitgroei slechts met zeer hoge concentraties kunnen onderdrukken, terwijl enige tijd na het spuiten het effect van de meeste groeiremmers weer is uitgewerkt. Gebruikt men een middel, dat de spruitgroei zeer sterk en langdurig remt, dan is het gevaar groot, dat het middel ook een nawerking in de nateelt vertoont. Om deze reden bleek bijv. fenac onbruikbaar te zijn. Een zwakker middel als 4-CPA heeft bij vroeg spuiten een geringe invloed en is gauw uitgewerkt. Dit middel vertoont echter geen nawerking, beschadigt het oude blad weinig en geeft een hoge knolopbrengst. 2,4,5-T daarentegen vertoont een sterke groeiremming, maar

ook een sterkere bladbeschadiging en een lagere knolopbrengst. Afhankelijk van de ontwikkeling van het gewas op de spuitdag zal men dus het middel moeten kiezen. Hoe later men spuit, des te zwakker kan het middel zijn.

De concentraties voor de remming van de spruitgroei in het volle gewas liggen aanmerkelijk lager dan bij het tegelijk spuiten van een groeiremmer en een doodspuitmiddel op een vroeg tijdstip (BODLAENDER (4)). De uitloop in de bladoksels wordt namelijk bij dit vroege doodspuiten sterk gestimuleerd. Bovendien kan door eerst alleen een groeiremmer te spuiten en pas twee weken later dood te spuiten een veel hogere knolopbrengst verkregen worden. De met een groeiremmer bespoten planten kunnen op dit latere tijdstip gemakkelijk doodgespoten worden.

De werking der groeiremmende middelen kan wellicht als volgt verklaard worden. Gibberellinezuur bevordert bij aardappelplanten de lengtegroei van stengeltoppen en okselknoppen (KRUG en FISCHNICH (8), BOOTH (5)). OKAZAWA (9) vond aanwijzigen voor de aanwezigheid van gibberellinen vooral in okselknoppen en stengeltoppen, maar hij vond ook remmende stoffen. BOOTH (5) vond deze remmende stoffen in volwassen bladeren. De concentratie was vooral hoog in bladeren die begonnen af te sterven. Deze gegevens doen veronderstellen, dat de verhouding gibberellinen/remmende stoffen tijdens de ontwikkeling ten gunste van de laatste verschoven wordt, waardoor de knoppen op een gegeven ogenblik niet meer kunnen uitlopen. Door toediening van een remstof treedt deze verschuiving eerder op. De plant wordt a.h.w. ouder gemaakt. De verhouding gibberellinen/remmende stoffen wordt ten gunste van de laatste gewijzigd.

Prevention of virus infection in seed potatoes by growth inhibitors

1. In the Netherlands the haulms of potato plants are generally killed in July before lifting to prevent virus infection of seed tubers, by spraying substances as DNOC in oil and sodium arsenite, or by cutting the foliage with a rotorbeater, followed by spraying a haulm killer.
2. A disadvantage of this method is the regrowth occurring from the buds on the stems. It was possible to suppress this regrowth by adding a growth inhibiting substance to the haulm killer; however, quite large quantities of the growth inhibitors were required (BODLAENDER (4)).
3. Potato plants show a mature plant resistance for virus multiplication and infection of the tubers (BEEMSTER (1, 2, 3)). Virus multiplication takes place especially in young leaves. The occurrence of young, growing leaves in the buds and on the tops of the stems in July is therefore dangerous and should be prevented.
4. In field experiments with the late variety Voran growth inhibiting chemicals (Table 1) were sprayed on the foliage on two spraying dates in July; these plants were killed with the haulm-killing agent diquat two and three weeks later respectively (R_1 and R_2). Growth of buds and tops could be prevented by some inhibitors (Fig. 1, Table 2; mature leaves were only damaged to a small degree or not at all. The concentrations needed for this growth inhibition in the foliage are lower than for sprout inhibition after haulm killing.
5. Tuber growth continued after spraying the growth inhibitor; treated plots obtained a much higher tuber yield than those which were killed on the same day with DNOC (Tables 3 and 4).
6. Virus infection could be prevented by spraying a growth inhibitor; treated plots had a lower number of virus-diseased tubers than the untreated ones or those which were killed early with DNOC (Table 5).
7. This, however, was not always the case: inhibition of sprout and top growth was not complete if the growth-inhibiting substance was weak and sprayed early or in

a low concentration. About two weeks after spraying the action of the growth inhibitor diminished and new growth started. Then the plants were killed by the haulm killer diquat.

8. The used growth-inhibiting substances differed in their action: Fenac, a strong growth inhibitor, is useless, because it has a distinct after-effect in the tubers the following year and on the next crop in the same field; 2,4,5-T showed good bud inhibition when sprayed early, but of the treated plots it had the lowest tuber yield; 4-CPA, however, the highest one, but sprout inhibition by this substance disappeared rather soon after the first spraying date.
9. These experiments with growth-inhibiting substances show the possibility of delaying haulm killing for about two weeks, resulting in a much higher tuber yield (also with respect to seed size) without increasing the virus in the tubers.

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NEUE GESICHTSPUNKTE AUF DEM GEBIET DER ROGGENDÜNGUNG MIT STICKSTOFF IN WESTEUROPA[1])

with summary

W. H. VAN DOBBEN

Der Roggen wird meistens angebaut auf ärmeren Böden, sodass hohe Düngergaben notwendig sind, um eine gute Ernte zu erzielen. Stickstoff nimmt in dieser Hinsicht eine besondere Stellung ein, da er nicht im Übermass gegeben werden darf, um Lagerfrucht und Verschwendung von Dünger zu vermeiden. Für Stickstoff gilt mehr als für andere Düngemittel, dass er genau in der richtigen Menge und zur richtigen Zeit angewendet werden muss. Das gilt besonders, wenn man sich bemüht, die Stickstoffgaben in der Praxis zu steigern.

In Holland wird zu Winterroggen oft eine Gabe von etwa 60 kg Reinstickstoff pro ha gegeben im Monat März.

In den letzten Jahren sind viele Feldversuche durchgeführt worden, um herauszufinden wie die Gabe noch gesteigert werden kann ohne Gefahr für Lagerfrucht. Es hat sich dabei herausgestellt, dass es vorteilhaft sein kann, die Stickstoffgabe ganz oder teilweise zu verschieben bis ins spätere Frühjahr. Fig. 1 gibt dafür ein Beispiel.

Eine Verspätung der Stickstoffgabe gibt immer einen niedrigeren Strohertrag. Das ist begreiflich, denn das Stroh fängt frühzeitig an zu wachsen und es braucht die Nahrung also schon unmittelbar nach dem Winter. Beim Korn liegen die Verhältnisse ganz anders. In der Serie von immer späteren Gaben steigert sich der Kornertrag anfangs und erst nach Erreichen eines Höchstwertes sinkt er unter den bei der Frühgabe erzielten Wert. Das gilt besonders für fruchtbare Böden.

Um diese Erscheinung zu erklären ist es notwendig, die pflanzliche Produktion und die Wirkung des Stickstoffs etwas näher zu analysieren.

Die Produktion eines Feldbestandes hängt einerseits davon ab, inwiefern eine geschlossene Pflanzendecke entsteht, die das einfallende Licht so vollständig wie möglich ausnützt zur Fotosynthese, und anderseits von der Länge der Wachstumsperiode, oder besser gesagt von der Lebensdauer der grünen Organe.

Eine frühe Stickstoffgabe führt zur Bildung grosser individueller Pflanzen und damit einer geschlossenen Pflanzendecke. Eine späte Stickstoffgabe – während des Schossens – kann die individuelle Pflanzengrösse kaum mehr beeinflussen (Fig. 2). Wie THORNE und WATSON (Rothamsted) ausgeführt haben für Weizen, kann sie aber das Absterben der Blätter und übrigen grünen Organe der Pflanze hemmen und dadurch die Fotosynthese während der Periode nach dem Schossen sehr stark fördern. Das gilt auch für Roggen (Fig. 3). Wenn die Körner wachsen, wird dem Spross Stickstoff entzogen zu Gunsten der Ähre. Wenn zu dieser Zeit kein Neustickstoff geboten wird, vergilben die Blätter und die grünen Teile des Halmes. Obwohl die Spätgabe die Pflanzlänge und die Halmzahl unter Feldbedingungen nur wenig ändern kann, erhöht sie neben

[1]) Text eines Vortrages, gehalten während der Internationalen Roggenkonferenz im Mai 1965 in Poznan (Polen).

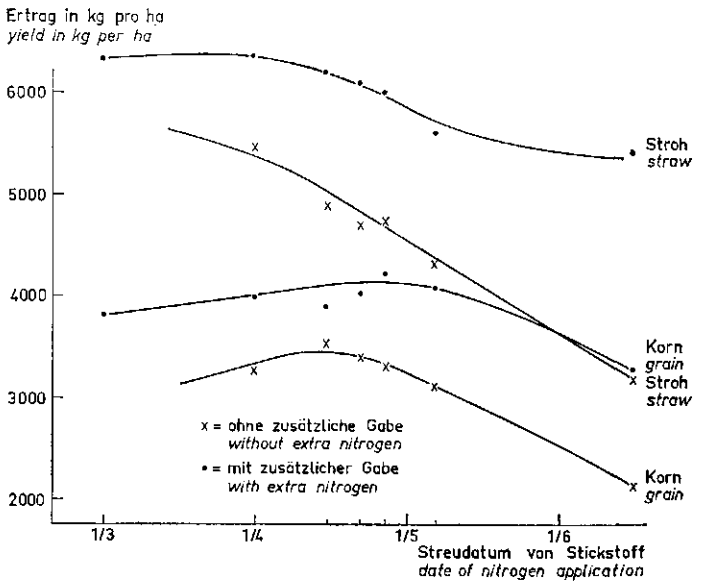

FIG. 1. Korn- und Stroherträge in einem Feldversuch mit verschiedenen Streudaten von Stickstoff (40 kg pro ha) zu Winterroggen.
• =mit einer zusätzlichen, frühen Gabe von 40 kg Reinstickstoff für alle Objekte
× = ohne zusätzliche Gabe
Das Schossen begann etwa am 20. April, das letzte Blatt wurde sichtbar am 6. Mai.
Kernel and straw yield in a field experiment with several dates of application of nitrogen (40 kg per ha) on winter rye.
• = *with an early additional application of 40 kg pure nitrogen for all treatments*
× = *without additional nitrogen*
Shooting began about April 20, the last leaf became visible on May 6.

dem Kornertrag doch auch den Strohertrag durch Steigerung des Halmgewichtes (Tabelle 1). Die Bruchfestigkeit der Halme wird auch erhöht; die kurzen Halme der Spätdüngung sind standfester als die längeren der Frühdüngung.

Während Frühstickstoff Strohertrag und Kornertrag etwa im Verhältnis 2:1 erhöht, liegt dieses Verhältnis bei Spätstickstoff näher bei 3:2 oder sogar 1:1. Die späte Gabe wirkt sich also aus in kurzem, standfestem Stroh und einem hohen Korn/Strohverhältnis. Darin liegt auch die Erklärung der Tatsache, dass Spätstickstoff hinsichtlich des Kornertrages Frühstickstoff oft überlegen ist, besonders wenn es sich um hohe Gaben handelt. Wenn im Frühjahr genügend Stickstoff zur Verfügung steht um eine gut geschlossene Pflanzendecke zu bilden, ist eine noch stärkere Strohentwicklung unerwünscht. Im schlimmsten Fall führt dies zu Lagerfrucht, aber schon das Biegen vom Stroh kann den Kornertrag senken, wahrscheinlich infolge des schlechteren Lichteinfalles in den Bestand.

Dazu kommt für Trockenböden noch ein sehr wichtiger Faktor. Beim Strohwachstum im Frühjahr wird Wasser verbraucht proportional der Höhe der sich bildenden Pflanzendecke. Auf Trockenböden ergibt sich die Gefahr, dass bei starkem Strohwachstum der Wasservorrat im Boden erschöpft ist zur Zeit, wo die Körner entstehen, so dass die Produktion infolge der Anwelkung des assimilierenden Gewebes gehemmt wird. Besonders bei Roggen mit seinem hohen Wuchs ist diese Gefahr wesentlich. Die Sommergerste verdankt ihre relative Trockenresistenz ohne Zweifel teilweise dem kurzen Stroh.

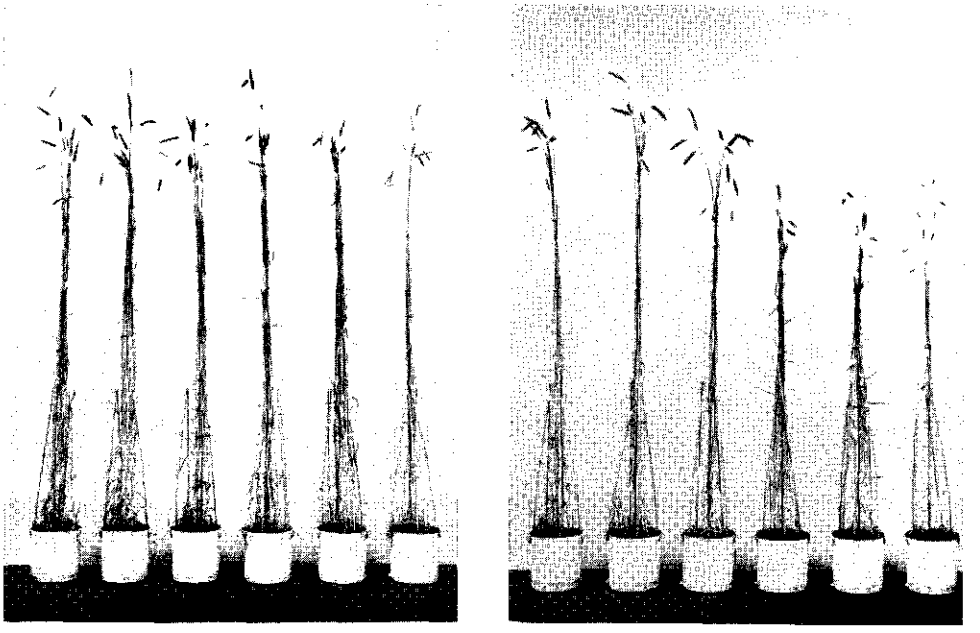

FIG. 2. Gefässversuch mit Winterroggen. Stickstoffgabe (von links nach rechts) am 13. März, 1. April, 20. April (Beginn des Schossens), 29. April, 8. Mai (Ährenschieben) und ohne Stickstoff.
A = mit einer frühen, zusätzlichen Gabe für alle Objekte
B = ohne zusätzliche Gabe
Pot experiment with winter rye. Applications of nitrogen (from left to right) on March 13, April 1, April 20 (beginning of shooting) April 29, May 8 (heading) and without nitrogen.
A = *with an early additional application for all treatments*
B = *without additional nitrogen*

TABELLE 1. Vergleich der Wirkung von Frühstickstoff und Spätstickstoff (gegeben wenn das letzte Halmblatt sichtbar wurde) zu Winterroggen. Armer, trockener Sandboden.
Comparison between the effect of early and late nitrogen (the latter applied at the appearance of the last leaf) on winter rye. Poor, dry sandy soil.

Düngung in kg/ha Reinstickstoff *Fertilization in kg pure N/ha*	Ertrag *Yield in kg/ha* Korn *Kernel*	Stroh *Straw*	Korn/Stroh-Verhältnis *Kernel/straw ratio*	Strohlänge *Straw length* in cm	Halmgewicht *Culm weight* in g	Festigkeit[1]) *Strength*[1])
0 N	1500	3310	0.45	125	14.3	11.7
Früh *Early*						
20 N	1940	4350	0.44	130	16.0	13.2
40 N	2520	5540	0.45	143	17.0	13.5
60 N	2790	5970	0.46	141	17.9	12.8
Spät *Late*						
20 N	2120	4090	0.51	129	16.5	13.8
40 N	2570	4730	0.54	129	17.2	15.0
60 N	2910	5190	0.56	132	17.4	15.8

[1]) Bruchfestigkeit des ersten Halmgliedes
Breaking strength of the first internode

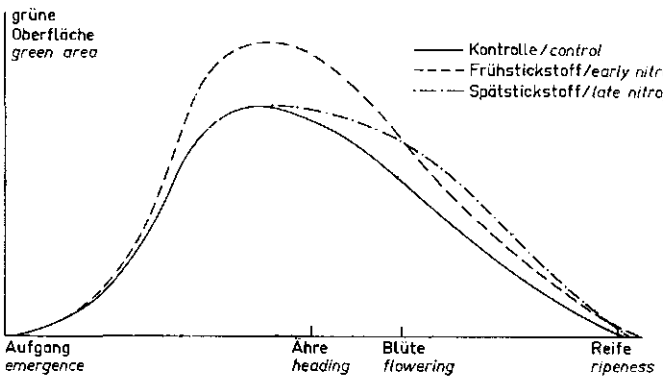

FIG. 3. Schema für die integrierte grüne Oberfläche an einem Roggenhalm (Blätter einbegriffen). *Scheme for the integrated green area of a rye stalk (leaves inclusive).*

Die Spätdüngung von Roggen gibt uns die Möglichkeit, eine Frucht mit bescheidener, eben genügender Strohentwicklung zu bekommen, wobei die Lebensdauer der assimilierende Organe verlängert wird und genügend Wasser im Boden erhalten bleibt, um die produktive Wirksamkeit dieser Organe sicher zu stellen.

Aus dem Gesagten geht hervor, dass es nicht möglich ist, ein einheitliches Rezept für eine 'richtige' Stickstoffdüngung zu Roggen zu geben. Wie hoch die Gabe sein soll und wann die Gabe anzuwenden ist – geteilt oder ungeteilt – das alles hängt von der Fruchtbarkeit des Bodens ab.

Das Erste, was wir verlangen, ist eine geschlossene, aber nicht zu üppige Pflanzendecke. Bei Böden, die nach dem Winter genügend Freistickstoff enthalten, bildet sich eine solche Pflanzendecke auch ohne Frühdüngung. Bei sehr armen Böden soll mindestens 40 kg Reinstickstoff verabreicht werden, sobald das Wachstum wieder beginnt. Später – während des Schossens – kann dazu nochmals dieselbe Menge gegeben werden, oder mehr, wenn die Nachlieferung von Stickstoff durch Mineralisierung oder aus tieferen Schichten des Bodens im späten Frühjahr gering ist.

Eine ähnliche Methode wurde in Frankreich von Y. COIC für Weizen ausgearbeitet.

Der Spätstickstoff soll vor dem Ährenschieben verabreicht werden. Eine spätere Gabe – eine sogenannte Blütendüngung nach W. SELKE (Deutschland) – erhöht den Ertrag nur wenig, aber eben daher den Eiweissgehalt der Frucht sehr stark.

Auf Böden mittlerer Fruchtbarkeit kann man oft mit einer einzigen Gabe auskommen, was Arbeit spart. Dort kann man z.B. genau vor dem Schossen – also bevor die Halme sich bilden – 60 bis 80 kg Reinstickstoff pro ha verabreichen. Diese einmalige Gabe kann bis später verschoben werden, je nachdem der Boden fruchtbarer ist. Fig. 1 gibt dafür ein anschauliches Bild. Der Verlauf der Kurven für den Kornertrag hängt ab von der Menge des im Boden vorhandenen Freistickstoffs.

Die Erklärung liegt auf der Hand. Wenn der Boden selbst der jungen Pflanze im Frühjahr mehr Stickstoff bietet, tritt weniger bald Mangel auf.

Mit einer späten, einmaligen Gabe kann in vielen Fällen eine genügende Jugendentwicklung wie auch eine gute Nahrung zur Zeit des Kornwachstums erzielt werden.

Für eine richtige Abstimmung der Stickstoffdüngung braucht man die Erfahrung des Bauern und die Einsicht des Wissenschaftlers. Der Bauer kennt das betreffende Feld und die Vorfrucht. Er weiss, ob es viel oder wenig Stickstoff braucht, ob es z.B. lagergefährlich ist (das weist hin auf das Vorhandensein von viel Stickstoff im Frühjahr), ob das Korn/Strohverhältnis niedrig ist (das weist hin auf eine dürftige Nachlieferung von Stickstoff im späten Frühjahr) und dergleichen. Der Wissenschaftler soll diese Erfahrungen interpretieren und in jedem konkreten Fall die richtige Dün-

gungsmethode ausarbeiten, wobei auch der Niederschlag im Winter zu berücksichtigen ist, welche die Auswaschung von Stickstoff fördert.

In dieser letzten Hinsicht wird die holländische Landwirtschaft vom Institut für Bodenfruchtbarkeit in Groningen beraten.

Hauptthesen

Frühstickstoff (verabreicht unmittelbar nach dem Winter) verstärkt die Bestockung und vergrössert die Ausmasse der Pflanzen. Er erhöht Korn- und Strohertrag etwa im Verhältnis 1:2 und vermehrt die Lagergefahr.

Spätstickstoff (verabreicht während des Schossens) hat kaum einen Einfluss auf die Ausmasse der Pflanzen. Er verlängert die Lebensdauer der assimilierenden Organe durch Verhütung von Stickstoffentzug zu Gunsten der Körner. Er erhöht Korn- und Strohertrag etwa im Verhältnis 2:3 oder sogar 1:1 und vergrössert die Lagergefahr nicht.

Die Spätdüngung gibt immer weniger Stroh, aber oft – besonders bei hohen Gaben oder fruchtbaren Böden – mehr Korn als die Frühdüngung. Das ist dadurch zu erklären, dass die Strohentwicklung bald ein Optimum überschreitet, worüber hinaus der Kornertrag geschadet wird, im extremen Fall durch Lagerfrucht aber auch schon wegen Biegen vom Gewachs und auf Trockenböden durch vorzeitige Erschöpfung des Wasservorrates.

Bei idealer Versorgung verfügt der Bestand im Stadium der Bestockung nur über die Menge an Stickstoff, die gerade genügt um eine vollständig geschlossene Pflanzendecke zu bilden. Nach dem Schossen soll genügend Stickstoff vorhanden sein um das vorzeitige Absterben von grünem Gewebe zu verhüten.

Ein Boden von idealer Fruchtbarkeit enthält nach dem Winter einen beschränkten Vorrat an Freistickstoff und stellt später, zur Zeit des Schossens, allmählich mehr Stickstoff zur Verfügung durch Mineralisierung von organischer Substanz.

Auf armen Böden kommt man dem idealen Zustand nahe durch eine mässige Gabe im Frühjahr (z.B. Anfang März 40 kg Reinstickstoff pro ha) und eine zweite Gabe während des Schossens (in Holland etwa am 1. Mai).

Auf Böden von mässiger Fruchtbarkeit kann eine einmalige Gabe verabreicht werden, z.B. eben vor dem Schossen (in Holland etwa am 15. April) oder um so später je nachdem der Boden selbst mehr Stickstoff zur Verfügung stellt.

Die richtige Höhe und Anwendungszeit der Gabe kann nur von Fall zu Fall festgestellt werden auf Grund der praktischen Erfahrung mit dem betreffenden Feld.

Summary

Recent views in the field of nitrogen fertilization of rye in western Europe

Early available nitrogen (applied just after winter) increases tillering and the ultimate size of cereal plants. It increases the yield of kernel and straw in a ratio of about 1:2 and increases the risk of lodging.

Late nitrogen (applied during shooting) has hardly any influence on size. It increases the longevity of green assimilating tissue. It raises the yield of kernel and straw in a ratio of about 2:3 or even 1:1 and does not promote lodging.

Late nitrogen applications always give less straw but often more kernel than early applications, especially in the case of high applications or a fertile soil. The explanation is that straw development soon exceeds an optimal level beyond which the kernel yield is unfavourably affected, in the extreme case by lodging and even by bending of the shoots – causing unfavourable light interception – and on dry soils by exhaustion of the water supply.

In the ideal case the plants in the tillering stage have just enough nitrogen at their disposal to form a completely closed crop. After shooting they should have enough nutrients at their disposal to prevent a premature yellowing of green tissue.

The ideal soil therefore contains a limited supply of free nitrogen in early spring and offers later on – during shooting and afterwards – more nitrogen by mineralization of organic matter.

On very poor soils the ideal case may be imitated by a limited application (for instance 40 kg pure nitrogen per ha) in early spring – in Holland about 1 March – and a second one during shooting – in Holland about 1 May.

On moderately fertile soils the nitrogen may be applied in a single dose, for instance just before shooting – in Holland about 15 April.

The rate and application time of nitrogen on cereals can only be assessed from case to case on the basis of practical experience with the field concerned.

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DE FYSIOLOGISCHE OORZAKEN VAN PRODUKTIVITEITSVERSCHILLEN BIJ HAVERRASSEN

with summary

H. D. GMELIG MEYLING en W. H. VAN DOBBEN

INLEIDING

Het is voor de methodiek van de plantenveredeling uitermate belangrijk om te weten, waarom de rassen van één soort verschillen in produktiviteit. Is de omvang van het assimilatorisch oppervlak, de efficiëntie van dit oppervlak, zijn levensduur of de verdeling van de gevormde stof over de organen of meer dan een van eerdergenoemde factoren verantwoordelijk voor de rasverschillen?

THORNE (3, 4, 5) en WATSON, THORNE en FRENCH hebben bij twee gerstrassen (7) en bij oude en nieuwe tarwerassen (8) de fysiologische achtergrond van de verschillen in korrelopbrengst trachten te analyseren. Hoewel zij op grond van hun resultaten niet tot definitieve uitspraken konden komen omtrent de feitelijke oorzaken van de produktieverschillen, bleek toch wel dat de meer produktieve rassen, per eenheid van assimilatorisch oppervlak en tijd, een hogere korrelopbrengst gaven. De moderne produktieve rassen onderscheiden zich bij rijpheid door een hoge korrel/stroverhouding. Bij tarwe werden aanwijzingen verkregen, dat reeds bij het verschijnen van de aar het gewicht hiervan relatief hoog is.

Teneinde hieromtrent enig inzicht te verkrijgen bij haver, zijn enige rassen, die in de praktijk een verschillende produktiviteit aan de dag legden, in onderzoek genomen.

METHODE VAN ONDERZOEK

De keuze van de rassen werd gebaseerd op de verhoudingsgetallen, ontleend aan de Beschrijvende Rassenlijst voor Landbouwgewassen (tabel 1).

In het vroege voorjaar werd gezaaid in potten van 6 liter inhoud, gevuld met zandgrond en voorzien van optimale hoeveelheden P, K en water. De proeven stonden opgesteld in een met gaas en glas afgesloten ruimte onder vrijwel natuurlijke omstandigheden van licht en temperatuur.

In elke pot groeiden 5 planten. Naast een stikstofgift van 70 mg N per plant is ook een hogere gift gebruikt nl. van 105 mg N per plant, verdeeld in een gift van 70 mg even na opkomst van het gewas en een van 35 mg, toegediend op een tijdstip tussen stadium 7 en 8 (stadia volgens FEEKES); dit is een stadium dat ligt tussen het tevoorschijn komen van het voorlaatste en het laatste blad.

De gedeelde N-gift is aangewend om ook over objecten te beschikken zonder ernstig stikstofgebrek tegen het einde van de groei.

Nadat in 1962 en 1963 oriënterende proeven waren genomen met een beperkt aantal rassen, zijn in 1964 alle in tabel 1 weergegeven rassen nogmaals vergeleken. Bij het in pluim komen van een ras (stadium 10.1 volgens FEEKES) werden steeds 2 of 3 potten van elke serie geoogst en bemonsterd, terwijl de laatste bemonstering geschied-

TABEL 1. De verhoudingsgetallen voor korrel- en stro-opbrengst en strolengte van enige haverrassen, ontleend aan de Beschrijvende Rassenlijst voor Landbouwgewassen.
Index figures for grain and straw yields and length of straw of some oat varieties; data taken from the Dutch List of Varieties.

Haverrassen *Oat varieties*	Korrelopbrengst *Grain yield*	Stro-opbrengst *Straw yield*	Strolengte *Length of straw*	Jaar van introductie *Year of introduction*
Gele haver *Yellow oats*				
Gouden Regen II	90	104	8,5	1932
Civena	102	99	6,5	1955
Witte haver *White oats*				
Adelaar	96	105	7,5	1930
C.I.V. 1374	99	102	7,5	1963
Marne	99	98	7	1946
Zonne II	100	104	7,5	1944
Condor	102	96	6	1958

de bij de oogstrijpheid. De pluimen werden in hun geheel, zowel in stadium 10.1 als bij de oogstrijpheid bemonsterd, zodat een indruk kon worden verkregen omtrent de grootte en toename aan droge stof van het gehele generatieve orgaan van de plant.

Naast deze produktiemetingen werden geregeld vanaf het tijdstip van in pluim komen van het gewas tot aan het einde van het groene oppervlak (dus op een tijdstip waarop alle weefsel praktisch geel is) het totale assimilatorische oppervlak van de planten gemeten.

De bladoppervlakken werden verkregen door de bladwaarde (lengte × breedte op het midden) te herleiden tot ware oppervlakken door middel van bestaande verhoudingen tussen bladwaarde en bladoppervlakken. Als oppervlak van de bladscheden en groene stengels werd aangehouden het produkt lengte × gemiddelde omtrek, en het pluimoppervlak werd bepaald door een van te voren vastgesteld standaardgemiddelde van het oppervlak der kroonkafjes van een pakje te vermenigvuldigen met het aantal pakjes op dat moment.

Zodoende kregen wij de beschikking over cijfers betreffende de grootte en levensduur van het assimilatie-apparaat in de postflorale periode, uitgedrukt als de T.O.-waarde in cm^2 dagen (GMELIG MEYLING (2)), het equivalent van WATSON's 'L.A.D.' (leaf area duration (6)).

RESULTATEN

De produktiviteit vóór het in pluim komen

In tabel 2 zijn een aantal gegevens vermeld, waarbij de gele en de witte haverrassen gescheiden zijn gehouden, omdat het wenselijk is de vergelijking tussen meer en minder produktieve rassen te maken binnen groepen van verwanten.

Het blijkt dat in beide groepen de produktieve rassen (Civena, Condor) op dit moment in totaal plantgewicht geheel achteraan komen. Bij Civena kan dit in verband worden gebracht met de korte duur van de periode van opkomst tot in pluim komen, maar bij Condor is deze periode niet opvallend kort.

Wanneer de organen apart worden bekeken, blijkt dat het lagere totale gewicht van Civena en Condor speciaal in de vegetatieve delen (stengel, blad en wortel) tot uitdrukking komt. Het gewicht van de pluim is op het moment van zijn verschijnen bij deze rassen hoger dan bij de oudere rassen Gouden Regen II en Adelaar. Vooral

TABEL 2. Opbrengsten aan droge stof in gram per plant, het groene oppervlak op het moment van in pluim komen en de planthoogte.
Dry matter yields in grams per plant, the green area at the moment of panicle emergence and the plant height.

Ras *Variety*	Stengels, blad *Stems, leaves*	Wortels *Roots*	Pluim *Panicle*	Tot. plant *Total plant*	Pluim/plant-verhouding *Panicle/plant ratio*	Wortel/plant-verhouding *Root/plant ratio*	Groen oppervlak in cm^2 *Green area in cm^2*	Plantgewicht/ Groen opp. *Plant weight/ Green area*	Pluimgewicht/ Groen opp. *Panicle weight/ Green area*	Aantal dagen van opkomst tot in pluim komen *Number of days from emergence to heading*	Planthoogte in cm *Plant height in cm*
	1	2	3	4	5	6	7	8	9	10	11
70 mg N per plant											
Gele haver *Yellow oats*											
Gouden Regen II	4,65	1,34	0,576	6,57	0,09	0,20	545	12,1	1,06	71	92,0
Civena	4,46	1,17	0,720	6,35	0,12	0,18	625	10,2	1,15	68	83,5
Witte haver *White oats*											
Adelaar	5,49	1,74	0,680	7,91	0,09	0,22	555	14,0	1,21	73	98,0
C.I.V. 1374	5,43	1,48	0,968	7,88	0,12	0,19	650	12,1	1,49	72	84,0
Marne	5,36	1,39	0,969	7,72	0,13	0,18	600	12,9	1,61	70	83,0
Zonne II	5,03	1,70	0,811	7,54	0,11	0,23	570	13,2	1,42	74	86,0
Condor	4,16	1,15	0,972	6,28	0,16	0,18	540	11,6	1,80	72	82,0
105 mg N per plant											
Gele haver *Yellow oats*											
Gouden regen II	5,48	1,45	0,663	7,59	0,09	0,20	705	10,7	0,94	71	97,0
Civena	4,30	1,38	0,701	6,38	0,11	0,21	715	8,9	0,97	68	87,5
Witte haver *White oats*											
Adelaar	5,52	1,52	0,923	7,96	0,10	0,19	655	11,9	1,16	73	98,0
C.I.V. 1374	5,46	1,90	1,041	8,40	0,12	0,23	725	10,8	1,34	72	88,0
Marne	5,17	1,60	1,089	7,86	0,14	0,20	720	10,9	1,51	70	90,5
Zonne II	5,36	1,93	0,987	8,28	0,12	0,23	690	12,0	1,43	74	90,0
Condor	4,96	1,46	1,211	7,63	0,16	0,19	700	10,9	1,72	72	87,0

TABEL 3. Eindopbrengsten in gram d.s. per plant; de aanwas in de periode na het in pluim komen en het geïntegreerde groene oppervlak (T.O.-waarde).
Final yields in grams of dry matter per plant; the increment in the period from heading until ripening and the integral of the green area (T.O.-value).

Ras *Variety*	Lengte periode in dagen *Length of period in days*	Stengels en blad *Stems and leaves*			Wortels *Roots*		Pluim *Panicle*			Totale plant *Total plant*				
		Opbrengst *Final yield*	Aanwas *Increment*	T.O.-waarde 10^3 cm^2 dagen *T.O.-value 10^3 cm^2 days*	Opbrengst *Final yield*	Aanwas *Increment*	Opbrengst *Final yield*	Aanwas *Increment*	T.O.-waarde 10^3 cm^2 dagen *T.O.-value 10^3 cm^2 days*	Opbrengst *Final yield*	Aanwas *Increment*	T.O.-waarde 10^3 cm^2 dagen *T.O.-value 10^3 cm^2 days*	Produktiviteit in mg cm^{-2} dg^{-1} *Productivity in mg cm^{-2} day^{-1}*	Aanwas pluim/ Aanwas plant *Increment panicle/ Increment plant*
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
70 mg N per plant														
Gele haver *Yellow oats*														
Gouden regen II	38	5,03	+0,38	9,00	1,19	−0,15	3,78	+3,20	1,30	10,00	+3,43	10,30	*+0,33*	0,94
Civena	41	5,16	+0,70	12,90	0,89	−0,28	5,90	+5,18	2,30	11,95	+5,60	15,20	*+0,37*	0,92
Witte haver *White oats*														
Adelaar	36	5,14	−0,35	9,76	1,11	−0,63	4,00	+3,32	1,14	10,25	+2,43	10,90	*+0,22*	1,42
C.I.V. 1374	37	6,01	+0,58	11,21	1,27	−0,21	5,00	+4,03	1,39	12,28	+4,40	12,60	*+0,35*	0,92
Marne	34	5,30	−0,06	8,27	1,04	−0,35	5,30	+4,33	1,73	11,64	+3,92	10,00	*+0,39*	1,10
Zonne II	35	5,89	+0,86	9,45	1,73	+0,03	4,73	+3,92	1,25	12,34	+4,80	10,70	*+0,45*	0,81
Condor	37	4,70	+0,54	9,64	0,80	−0,35	5,85	+4,88	1,16	11,35	+5,07	10,80	*+0,47*	0,96
105 mg N per plant														
Gele haver *Yellow oats*														
Gouden Regen II	38	5,51	+0,03	11,40	1,20	−0,25	4,64	+3,98	1,70	11,35	+3,76	13,10	*+0,29*	1,06
Civena	41	5,88	+1,58	13,68	1,10	−0,29	7,52	+6,82	2,12	14,49	+8,11	15,80	*+0,51*	0,84
Witte haver *White oats*														
Adelaar	36	6,25	+0,73	12,74	1,11	−0,41	5,79	+4,87	1,96	13,15	+5,19	14,70	*+0,35*	0,93
C.I.V. 1374	37	6,49	+1,03	13,06	1,18	−0,72	6,47	+5,43	1,34	14,14	+5,74	14,40	*+0,40*	0,95
Marne	34	5,85	+0,68	12,20	1,06	−0,54	7,30	+6,21	2,20	14,21	+6,35	13,40	*+0,47*	0,98
Zonne II	36	7,08	+1,72	13,76	1,68	−0,25	7,01	+6,02	2,14	15,78	+7,50	15,90	*+0,47*	0,80
Condor	37	5,43	+0,47	11,02	0,91	−0,56	7,12	+5,91	1,88	13,46	+5,83	12,90	*+0,45*	1,00

bij de witte rassen is dit verschil opvallend. Het aandeel van de pluim op het totaal gewicht is bij de produktieve rassen dus hoog. In het aandeel van de wortels in het totale gewicht zit geen systematisch verschil.

In tabel 2 is ook het geschatte groene oppervlak van de vegetatieve delen van de plant ten tijde van het in pluim komen weergegeven. Bij Civena en Condor werd de indruk verkregen dat zij een wat groter aantal spruiten bezaten, zodat vermoedelijk als gevolg hiervan en door het korter blijven van het gewas de efficiëntie van dit groene oppervlak, gezien het relatief gering totaal plantgewicht, laag is (tabel 2, kolom 8). Dit kan aan een sterkere onderlinge beschaduwing van de spruiten worden toegeschreven. Ten aanzien van de pluim was de efficiëntie echter hoog (tabel 2, kolom 9).

De produktiviteit ná het in pluim komen

In de periode van in pluim komen tot rijping is de totale aanwas van het plantgewicht bij de produktieve rassen opvallend hoog (tabel 3). Deze aanwas komt de

TABEL 4. Korrel- en strogewichten in gram per plant, halmlengte, halmgetal en het aandeel van de wortels bij rijping.
Grain and straw weight in grams per plant, culm length, number of culms and root/plant ratio at ripeness.

Ras *Variety*	Korrelgewicht *Grain weight*	Produktiviteit voor de korrelopbrengst, na het in pluim komen in mg cm^{-2} dag^{-1} *Productivity for grain yield after panicle emergence in mg* cm^{-2} day^{-1}	Stro- + kafgewicht *Straw + chaff weight*	Korrel/stroverhouding *Grain/straw ratio*	Wortel/plant verhouding *Root/plant ratio*	Halmgetal *Number of culms per plant*	Halmlengte in cm *Culm length in cm*
	1	2	3	4	5	6	7
70 mg N. per plant							
Gele haver *Yellow oats*							
Gouden Regen II	3,24	*+0,31*	5,58	0,58	0,12	1,9	131,5
Civena	5,03	*+0,33*	6,03	0,83	0,08	1,7	126,5
Witte haver *White oats*							
Adelaar	3,27	*+0,30*	5,87	0,56	0,11	1,8	126,5
C.I.V. 1374	4,06	*+0,32*	6,95	0,58	0,10	2,0	132,0
Marne	4,38	*+0,44*	6,22	0,70	0,09	2,2	125,0
Zonne II	3,86	*+0,36*	6,75	0,57	0,14	2,1	129,0
Condor	4,84	*+0,45*	5,72	0,84	0,07	2,1	114,0
105 mg N per plant							
Gele haver *Yellow oats*							
Gouden regen II	3,64	*+0,28*	6,52	0,56	0,10	2,1	134,0
Civena	6,42	*+0,41*	6,98	0,92	0,08	2,2	127,5
Witte haver *White oats*							
Adelaar	4,60	*+0,31*	7,44	0,62	0,09	2,1	129,0
C.I.V. 1374	5,32	*+0,37*	7,64	0,70	0,08	2,5	131,0
Marne	6,25	*+0,47*	6,90	0,80	0,07	2,1	121,5
Zonne II	5,88	*+0,38*	8,22	0,71	0,10	2,5	129,0
Condor	6,10	*+0,47*	6,45	0,95	0,07	2,5	113,0

bovengrondse delen ten goede, want het wortelgewicht neemt in deze periode bijna steeds af. Deze afneming toont geen systematisch verschil tussen de meer en minder produktieve rassen. De grote aanwas in de bovengrondse delen leidt er echter toe, dat bij de produktieve rassen uiteindelijk lage waarden worden bereikt voor het aandeel van de wortels in het totale gewicht (tabel 4).

Afneming van gewicht kwam in twee gevallen bij de laagste stikstofgift ook voor bij stengel en blad, de rest der objecten liet een geringe winst zien, het duidelijkst bij de hoogste stikstofgift (tabel 3).

Bij produktieve rassen, speciaal Civena en Zonne II, is deze aanwas hoog. Verreweg het grootste deel van de aanwas in de betreffende periode komt voor rekening van de pluim. Hier komt de bijzondere prestatie van de produktieve rassen duidelijk tot uiting.

Een voor de pluim gunstiger distributie van de totale hoeveelheid gevormde droge stof, zoals in de periode vóór het in pluim komen, kan bij hen echter niet worden aangetoond. De verhouding tussen aanwas van de pluim en totale aanwas is bij de produktieve rassen gemiddeld niet hoger (tabel 3, laatste kolom). Dat de betreffende cijfers niet ver van de waarde 1 liggen, is uiteraard een gevolg van het feit, dat de aanwas voornamelijk in de pluim terecht komt. Dit is bij matige stikstofvoeding regel, evenals het afnemen van het wortelgewicht in de postflorale periode (1).

Hoe moet nu de relatief hoge aanwas van de produktieve rassen worden verklaard?

De T.O.-waarden (dus het geïntegreerde groene oppervlak) voor de totale plant en voor de pluim liggen beide bij het produktieve ras Civena veel hoger dan bij Gouden Regen II. Dit hangt mede samen met de lange postflorale periode van Civena.

Bij de witte haverrassen verschillen de betreffende waarden veel minder duidelijk en bij het produktieve ras Condor liggen de T.O.-waarden niet hoger dan bij Adelaar. De aanmerkelijk hogere aanwas van het plantgewicht kon hier alleen worden toegeschreven aan een grotere efficiëntie van het groene oppervlak (tabel 3, kolom 13).

Bespreking van de resultaten

De verhoudingen tussen de onderzochte rassen, zoals deze in de potproeven zijn vastgesteld, komen in grote lijnen goed overeen met de resultaten van veldproeven, weergegeven in de Rassenlijst (vgl. tabel 1 en 4). De volgens de Rassenlijst produktieve rassen onderscheiden zich ook in de potproeven door een hoog korrelgewicht en een hoge korrel/stroverhouding

De overeenstemming tussen de resultaten van pot- en veldproeven kan worden opgevat als een aanwijzing, dat de produktiviteitsverschillen tussen de rassen weinig afhankelijk zijn van de standruimte. Ze lijken ook niet sterk afhankelijk van de stikstofvoeding, die overigens in de potproeven slechts met gering verschil is gevarieerd.

Kenmerkend voor de produktieve rassen is de distributie van droge stof in de periode van het schieten, die gunstig is voor de pluim. Bij het verschijnen van de pluim is het gewicht van dit orgaan bij de produktieve rassen relatief hoog, het gewicht van de vegetatieve delen relatief laag. De stengel blijft ook korter (tabel 2 en 4).

In de periode na het in pluim komen kan bij de produktieve rassen slechts in zoverre van een gunstig distributiepatroon worden gesproken, dat het aandeel van de wortels in het totaal tenslotte lage waarden bereikt. De verhouding tussen de pluim en de overige delen van de plant wordt in deze periode bij de produktieve rassen niet sterker verschoven ten gunste van de pluim dan bij de minder produktieve rassen. Hun totale produktievermogen ligt nu echter veel hoger.

In de periode van stengelgroei is hun produktie dus relatief laag, in de periode waarin de aanwas vooral aan de pluim ten goede komt relatief hoog. Het resultaat is een hoge korrel/stroverhouding.

Bij de gele haver Civena gaat de hoge produktie tijdens de rijping samen met een veel groter geïntegreerd groen oppervlak zowel van de totale plant als van de pluim afzonderlijk, alsmede met hogere waarden voor de efficiëntie (tabel 3, kolom 13 en tabel 4, kolom 2).

Bij de witte haver daarentegen gaat de hoge produktie van de moderne rassen in het algemeen niet samen met hoge waarden voor het groene oppervlak; hier kan de hoge produktie alleen een gevolg zijn van een grotere efficiëntie (tabel 3, kolom 13 en tabel 4, kolom 2).

Een dergelijke stelling werd door WATSON *et al.* (8) geformuleerd, die voor produktieve tarwerassen eveneens een voorsprong constateerden in het aargewicht bij het doorschieten, maar nagenoeg geen verschil in geïntegreerd groen (blad)oppervlak vonden.

The physiological causes of variation in yield between oat varieties

A comparison was made between oat varieties showing a different productivity in respect to grain yield under field conditions. The investigations were carried out with plants in pots harvested at the moment of panicle emergence and ripeness. Roots, vegetative parts of shoots and panicles were weighed separately. Green area of leaves, stems and panicles was estimated periodically. From these data T.O.-values (Table 3) and index figures for productivity (net weight of dry matter in mg cm^{-2} day^{-1}) were calculated.

Conclusions

In respect of the relations between varieties the results of the pot experiments showed an agreement with those obtained in the field. In the 'productive' varieties relatively high grain yields were obtained with a high grain/straw-ratio.

At the moment of heading the 'productive' varieties had a lower plant weight, but a higher panicle weight than the other ones, so that the distribution of dry matter appeared to be favourable for the generative organ. Root/plant-ratio did not differ significantly between groups of different productivity. Apparent efficiency of leaf area was low in the 'productive' varieties in the first period of development, presumably because of a relatively high number of tillers and more mutual shading.

In the period after panicle emergence increment in weight was particularly high in the 'productive' varieties. Distribution of dry matter in the shoot, however, was not more favourable for the panicle than in the less productive ones. In alle cases this dry matter went almost exclusively to the panicle.

Thus in the 'productive' varieties production was relatively low in the period of stem and leaf formation and relatively high in the period of grain formation. This together with the favourable dry matter distribution in the period of shooting resulted in a high grain/straw-ratio.

In the period after heading the productive group showed a more favourable distribution of dry matter only in this respect that the root/plant-ratio was relatively low. In almost all cases root weight showed a decline in this last stage of development.

The high productivity of Civena in comparison with the other yellow oat variety (Gouden Regen II) may also be related to its much higher integral of total green area over the last growth period for both panicle and rest of the shoot (T.O.-value, comparable to 'leaf area duration' (6)), although apparent efficiency showed higher values (Table 3, column 13 and Table 4, column 2) than for 'Gouden Regen II'.

In the white oat group there was no significant difference in the integral of green area, so that the apparent efficiency, which was very high in the productive varieties

(Table 3, column 13 and Table 4, column 2), can only be responsible for the higher yield.

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NITRATE AND AMINO-ACIDS IN THE BLEEDING SAP OF CUT TOMATO PETIOLES

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INTRODUCTION

Grasses show a negative correlation between nitrate and the soluble sugars in all plant parts (1). In this connection the hypothesis was put forward that increased downward sugar transport raises that part of the nitrate that is assimilated into organic form during its upward transport in the plant. Since grass was not suitable, tomato plants were used to test this hypothesis. By cutting off all petioles at the base and collecting the exudation sap, it is possible to investigate changes in the nitrate and amino-acid content of the sap dependent on the position on the stem.

MATERIAL AND METHODS

Tomato plants of the Ailsa Craig variety were cultivated in a greenhouse at 20 °C in a half-strenth Hoagland nutrient solution. The young seedlings were planted on 1 litre glass jars and the solutions were changed twice a week. After some time they were transferred to 5 litre plastic buckets and later to 10 litre buckets; the solution was then changed daily to ensure a liberal supply of nutrients. The solutions were continuously aerated.

A few days before the experiment the plants were brought into a growth room illuminated with 400 W high-pressure mercury-vapour lamps (Philips HPL) during 17 hours a day at a light intensity of about 5×10^4 ergs cm^{-2} sec^{-1} at plant height.

To collect the bleeding sap the plants were cut with a sharp knife at the correct locations and a special rubber tubing ('python' patent latex) was slid around the stump. Because of the longitudinal grooves in the stem it was usually necessary to tighten the tubing by copper wire to prevent leakage. The other end of the tube was placed in a plastic flask. When the sap was collected the tube was squeezed empty into the flask, the amount was weighed and a sample for analysis was stored at a temperature of —20°C.

To determine the total amino-acid concentration squares of 6×6 cm were drawn with a pencil on a sheet of Whatman I chromatographic paper. A 0.1 ml sample of the bleeding sap was placed in the centre of each square and dried by a commercial hair drier. The spots were coloured, extracted and analysed as described earlier (6). Since about 70 per cent of the amino-acids appeared to consist of glutamine, this was used as a standard. Ammonium salts failed to show any colour with the ninhydrin reagent used, so that they cannot interfere. The total amino-acid concentrations are expressed as μmol glutamine per ml of bleeding sap.

Nitrate in the bleeding sap was determined colorimetrically with phenoldisulphonic acid according to the method of SNELL and SNELL (8) and potassium with a flame photometer.

Results

Experiment 1

Three tomato plants of one month old were brought into growth rooms at a temperature of 10, 20 and 30°C respectively, two days before cutting the petioles at a few cm from the base; only the very young ones were left intact. The bleeding sap was collected for a certain time and analysed for total amino-acids and nitrate.

At 20 and 30°C bleeding stopped after some time and the wound was sealed by callus tissue. At 10°C this callus formation did not occur and bleeding continued, though at a low rate.

After bleeding had stopped in the 20 and 30°C-plants the stems were cut at the top, just below the cluster of very young leaves and the bleeding sap was collected for 1 hour. Thereafter the plants were cut at the stem base and the sap was again collected for a 1-hour's period.

The results are given in Fig. 1. The ordinate represents the length of the plant with the cut petioles roughly in position. The rate of bleeding and the concentrations of total amino-acids and nitrate are given on the abscissae. At the right-hand side of the figure the rate of bleeding and the nitrate concentration are given for the 20 and 30°C-plants cut at the top and at the base.

The rate of bleeding from the petioles was highest at 20°C; at all three temperatures the petioles a little below the middle of the stem height showed the highest rate of bleeding; these petioles carried the larger leaves. Both the nitrate and the amino-acid concentration were approximately independent of the position on the stem, perhaps the very young leaves excluded. At any rate the concentration in the youngest leaf cut at 10°C was much higher than that in the leaves below. Whether this was also the case at higher temperatures could not be determined since the rate of bleeding from the younger leaves was too low. There was no great influence of the temperature on the amino-acid and nitrate concentration.

When the stem was cut at the top the rate of bleeding was much higher than that of the cut petioles. When, thereafter, the plants were cut at the stem base, there was a difference between the rate of bleeding here and at the top at 20°C, where it was somewhat higher at the base; at 30°C there was no difference.

The nitrate concentration at the top and at the base was approximately the same

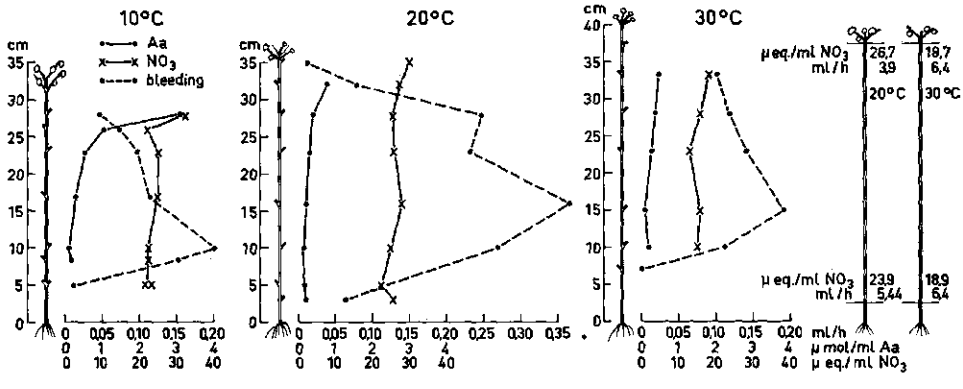

FIG. 1. Rate of bleeding (●-------●) and nitrate (×———×) and amino-acid (●———●) concentration in the sap from cut petioles of 4 weeks' old tomato plants at 10, 20 and 30°C. At the extreme right of the figure the data are presented for the same plants after petiole bleeding has stopped, cut first at the top and then at the base (see text).

as that found in the petioles. Unfortunately, the total amino-acid concentration was not determined.

Experiment 2

One tomato plant, six weeks old, was placed in a growth room at 20 °C. Alongside the main stem there was one side branch in the axil of a leaf and one cluster of flowers. Both these and the leaves were cut as in the preceding experiment and the bleeding sap collected for a certain period. Fig. 2 shows the mean rate of bleeding during this period and the mean concentration of total amino-acids and nitrate. As far as the leaves are concerned the results are comparable to experiment 1. The rate of bleeding was highest with the larger leaves a little below the middle of the stem and there were only minor changes in nitrate concentration along the stem. For total amino-acids the concentration increased somewhat at increasing height to decrease again rather sharply at the youngest leaf.

In this experiment the top of the stem with the very young leaves was also removed, but no bleeding occurred. The branch stem had a much higher rate of bleeding than the leaves, but also a much higher total amino-acid content, whereas the nitrate concentration did not differ from that in the bleeding sap of the cut petioles. The cut stem of the flower cluster had a much lower rate of bleeding but, compared to the petioles, the same concentration of nitrate and amino-acids.

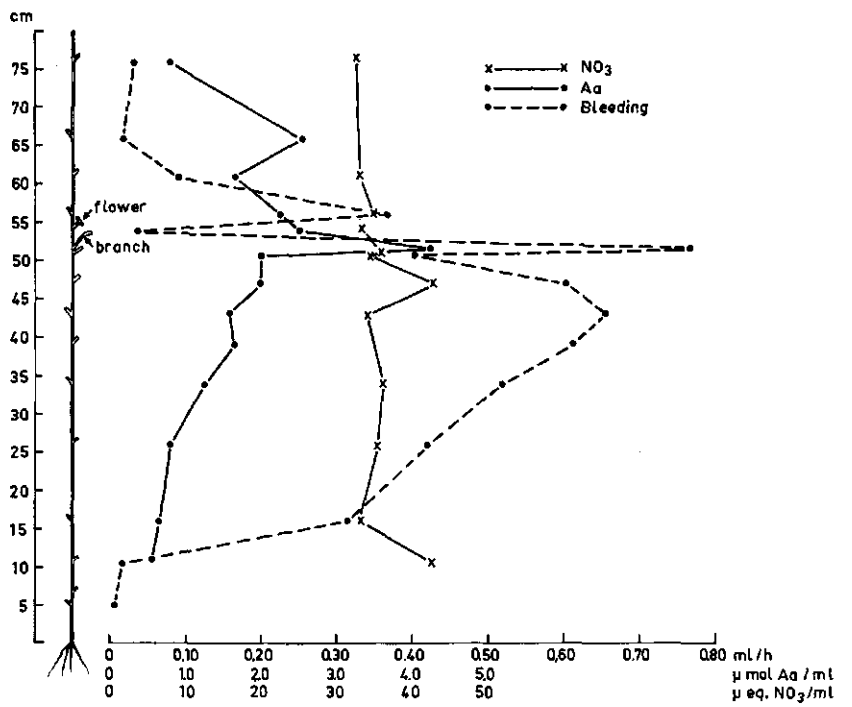

FIG. 2. Rate of bleeding (●-------●) and the nitrate (×———×) and amino-acid (●———●) concentration in the sap from cut petioles, a cut branch and a cut flower cluster of a 6 weeks' old tomato plant.

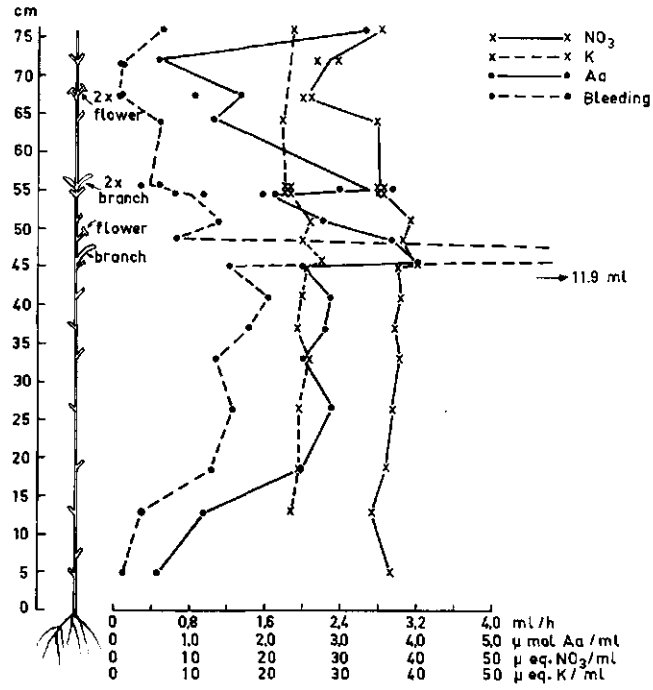

FIG. 3. As Fig. 2 but with a 9 weeks' old tomato plant. The potassium concentration (×------×) is also given.

Experiment 3

Experiment 2 was repeated with a plant of the same batch as in experiment 2, but 9 weeks old at the start of the experiment and with three flower clusters and three branches. These were cut together with the petioles and the main stem just below the very young leaves at the top. Bleeding sap could be collected from all the cut surfaces.

The results shown in Fig. 3 are comparable to those of the preceding experiment. The rate of bleeding from the cut petioles was highest at the middle of the stem, the amino-acid concentration showed a similar trend, whereas the nitrate concentration remained the same all over the stem.

In this experiment potassium was also determined and its concentration in the bleeding sap showed the same trend as the nitrate concentration, but at a somewhat lower level. At the stem tip the rate of bleeding and the amino-acid concentration was much higher than at the petioles just below. The lowest branch had a much higher rate of bleeding than the petioles and also an increased total amino-acid concentration. The lowest flower cluster had also an increased amino-acid concentration, but a rate of bleeding comparable to the neighbouring petioles. The same applied to the two upper branches, but the two upper flower clusters did not show a distinct difference in amino-acid concentration with the nearby leaves.

Experiment 4

Three tomato plants, eleven weeks old, were brought into a growth room at 20°C. After 5 days the main stem was cut 10 cm above the base and the bleeding sap was collected at regular intervals. Two hours after cutting the stem base of one of the plants was chilled with melting ice for two hours. The next day cooling was repeated. The amino-acid concentration was determined in the bleeding sap of all three plants

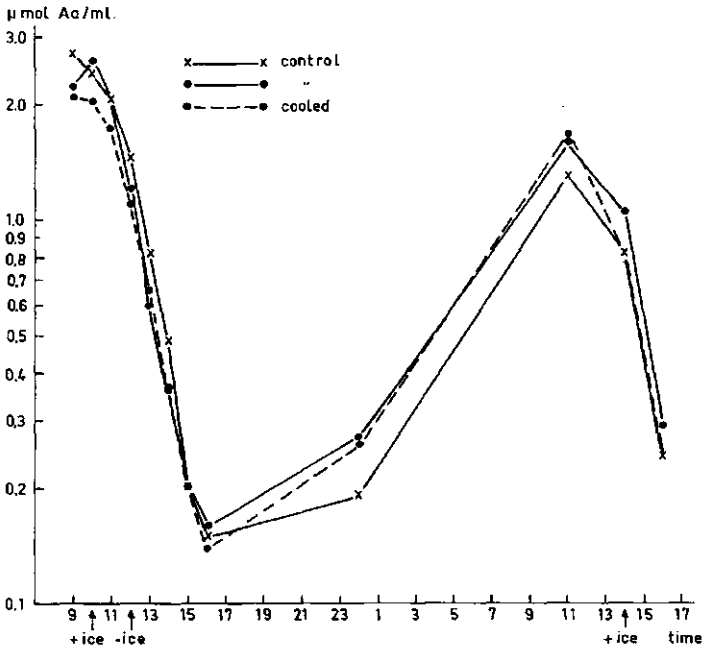

FIG. 4. The amino-acid concentration in the bleeding sap from tomato plants cut at the base. Two control plants (×——×), (●——●) and one plant (●- - - - -●) with the stem cooled in melting ice on 2 successive days during 2 hours. For the sake of convenience data are indicated half-way each sampling period and connected.

and is plotted on a logarithmic scale in Fig. 4. There was a distinct periodicity in this concentration in all three plants, but the cooling did not show any effect.

Experiment 5

To investigate whether the cooling period of two hours was long enough to chill the whole stem, the preceding experiment was repeated with three tomato plants of 14 weeks old. The experiment was carried out in the greenhouse in winter with an additional illumination of 400 Watt mercury vapour lamps. The chilling period was six hours for two successive days. Fig. 5 shows the results. The amino-acid concentration of the chilled plant was below the controls during the first cooling period, but on the following day there was no difference. The periodicity in the concentration was the same as in the preceding experiment; only the concentration at the start was lower and the initial drop less severe.

DISCUSSION

Figs. 4 and 5 show a distinct periodicity in the amino-acid concentration in the bleeding sap. The maxima always occur around noon and are usually at the same level. In Fig. 4 the second peak is slightly lower than the first one, but in Fig. 5 the reverse takes place. The latter trend is also present in Fig. 6, which shows the rate of bleeding together with the amino-acid concentration during 50 hours. The rate of bleeding also shows a definite periodicity, but here each maximum is distinctly lower than the preceding one.

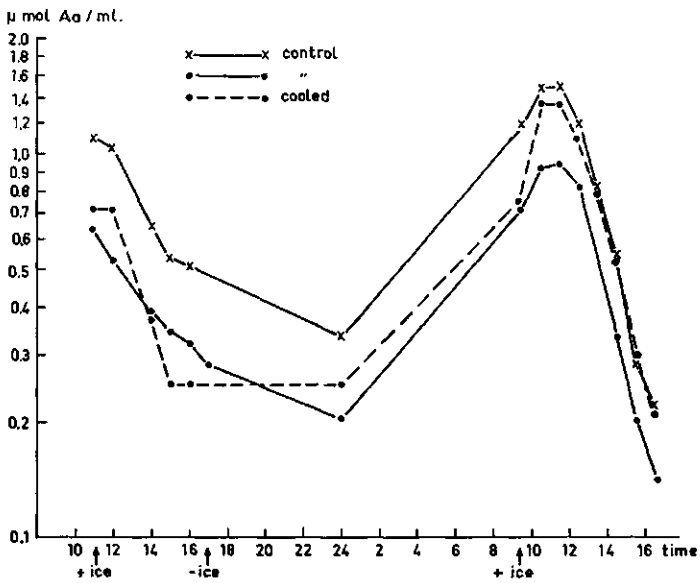

FIG. 5. As Fig. 4, with 6 hours' cooling on 2 successive days.

These data on the rate of bleeding and amino-acid concentration correspond with those of VAN DIE (4) and, for bleeding, also with those of other authors (for a summary see VAN ANDEL (3)).

The rate of bleeding along the stem is highest at the petioles of the largest leaves (Fig. 1). If side branches are present, their rate by far exceeds that of the largest leaf.

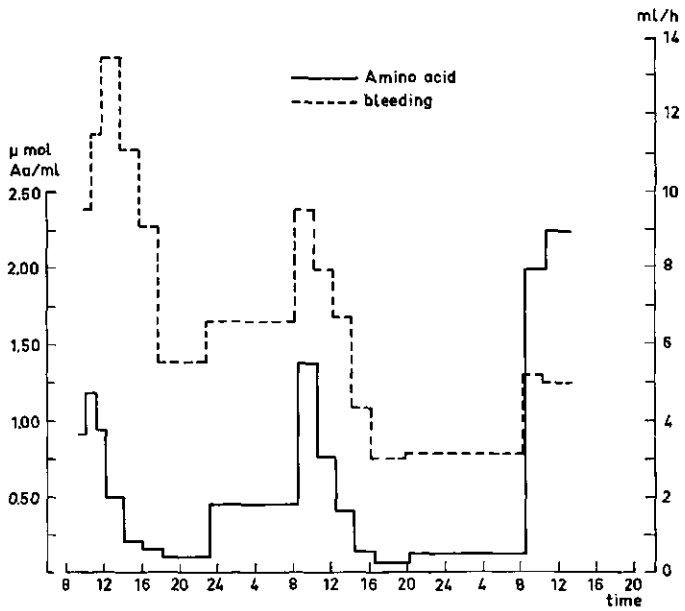

FIG. 6. The rate of bleeding (------) and amino-acid concentration (———) in the bleeding sap from a tomato plant cut at the base. Samples were taken at intervals during 2½ days and expressed as the mean value during each interval.

In flower clusters the rate is usually lower than that of the petioles immediately above or below. These findings agree with the statement of PATE *et al.* (7) that the rate of bleeding is related to the diameter of the cut surface. Perhaps it should even be better to relate the rate of bleeding to the total cross-sectional area of the xylem vessels, but this is difficult to measure.

The nitrate and potassium concentration in the bleeding sap shows only small variations with the position on the stem and with the kind of organ cut (stem, petiole, side branch or flower cluster). There are, however, differences between the experiments.

In Fig. 1 the temperature has a much greater influence on the rate of bleeding than on the nitrate concentration of the exudate. Between 10 and 20 °C the bleeding increased from 0.20 to 0.36 ml/h, whereas the nitrate concentration remained at approximately 25 me./l. At a temperature of 30 °C the rate of bleeding dropped to 0.20 ml/h and the nitrate concentration to 18 me./l.

These data correspond with the earlier findings (2), that the nitrate concentration in the bleeding sap remains constant at 20 to 25 me./l.

It was assumed that the active accumulation mechanism could not establish a concentration difference greater than this value. In Fig. 2 and 3, however, a nitrate concentration of 35 me./l was found. In more recent unpublished experiments it turned out that nitrate concentration was almost invariably between 20 and 25 me./l, so that the value of 35 me./l may be considered an exception, possibly associated with a short preceding period of nitrogen shortage (see 2).

In contrast with the nitrate concentration of the bleeding sap the total amino-acid concentration showed considerable differences. In Fig. 1 there is not much difference along the stem, except maybe at the very young top leaves, but the Figs. 2 and 3 show that the amino-acid concentration in the bleeding sap from a side branch is always considerably higher than in that from the leaf petioles. The flower branches usually showed the same trend as the petioles; only in one case the concentration was higher than that of the petioles directly above or below.

This great difference in amino-acid concentration in the bleeding sap of a petiole and of the side branch in its axil is remarkable. It could be assumed that amino-acids are also transported to rapidly growing regions through the sieve tubes, but since chilling of a part of the stem to 0 °C had no effect on the amino-acid concentration (Figs. 4 and 5) this possibility may be excluded. If these differences were caused by differences in the amino-acid concentration in the cut parenchyma cells, the concentration difference in these cells should have to be at least 50 mol/l which is, of course, impossible. Consequently the differences found are located in the bleeding sap from the xylem. VAN DIE (5) stated that amino-acids are present as a pool in the stem and that only a part of these enters the transpiration stream.

To compare the amino-acid concentration in the bleeding sap with that in the stem, 10 cm sections of the latter were analysed for total amino-acids and nitrate. The sections were cut into small pieces and homogenized in 80 per cent ethanol. After centrifuging the residue was again extracted twice with 80 per cent ethanol. The combined extracts were evaporated to dryness in vacuo and the residue was dissolved in 100 ml of distilled water and analysed. The results are presented in Table 1.

It is seen that the concentration of amino-acids in the stem sections decreased at increasing distance from the base, but that in all sections this concentration was four to five times higher than the highest concentration found in the bleeding sap. It seems likely therefore that also from this pool amino-acids are transported through the xylem vessels to growing regions.

The nitrate concentration is also higher in the stem than in the bleeding sap, which

TABLE 1. Concentration of nitrate and total amino-acids in successive stem sections.

Distance from stem base cm	Amino-acid concentration μmol/g fresh	Nitrate concentration μmol/g fresh
80–90	19.0	54.1
70–80	18.5	55.2
60–70	19.5	42.8
50–60	21.2	41.8
40–50	24.7	55.5
30–40	25.1	64.6
20–30	26.6	75.4
10–20	27.1	72.7
0–10	27.4	54.7

was also found by many others. Apparently, accumulation in the parenchyma cells occurs at a level twice as high as that in the xylem vessels.

Due care should be taken not to generalize te present data too much, especially since PATE *et al.* (7) found completely different ranges of amino-acid and nitrate concentration in different plant species. Therefore, conclusions about any influence of the regional sugar concentration on the reduction and assimilation of nitrate cannot be drawn from the present data.

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THE DETERMINATION OF SULPHIDES IN RUMEN LIQUOR: METHOD AND EXPERIMENTAL RESULTS

MARIA S. M. BOSMAN

INTRODUCTION

Earlier investigations (2) have shown that the copper from fresh grass is converted into less soluble form in the rumen of cattle. It was suggested that this is due to its conversion into copper sulphide.

The presence of hydrogen sulphide in the rumen of ruminants was shown by several other investigators. It was found that greater quantities occur in the rumen gas during the feeding of grass to goats (7). Concerning the origin of hydrogen sulphide, LEWIS (6) and MILLS (8) reported that reduction of sulphate to sulphide occurs in the rumen of sheep. In this relation attention is drawn to the fact that the copper content in the liver of cows is decreased by adding sulphate to the feed (4). Furthermore, hydrogen sulphide may originate from the decomposition of organic sulphur compounds present in the feed.

More quantitative data were collected on sulphide in the rumen liquor under different conditions of nutrition to consider the possible interference of sulphide formation in the utilization of copper from the feed by ruminants.

METHOD

The method described below is based on the decomposition of the sulphides by acidification of the liquor and subsequent transfer of the hydrogen sulphide evolved to a zinc acetate solution. The zinc sulphide is dissolved by acidification and the hydrogen sulphide determined by colorimetry of the methylene blue formed with p-amino-N, N-dimethylaniline.

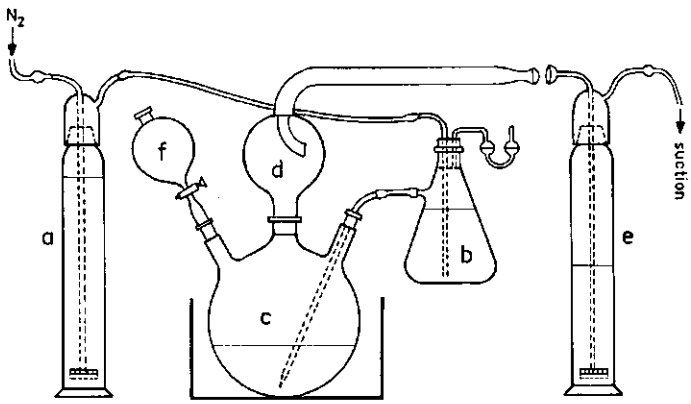

FIG. 1. Apparatus for sulphide determination.

Apparatus

The assembly shown in Fig. 1 consists essentially of a 500 ml three-necked distilling flask (c) provided with a dropping funnel (f), a splash head (d) and a suction flask (b). The air in the sample in (c) and in the apparatus is replaced by nitrogen and the sample acidified. Nitrogen can be passed continuously through the wash bottle (a) filled with pyrogallol-sodium phosphate solution, the suction flask (b) filled with water, and the acidified sample in flask c. The hydrogen sulphide passes with the nitrogen stream through the splash head (d) and the connection with ball joint into the wash bottle (e) where it is absorbed in a solution of zinc acetate. The inlet tubes of the wash bottles have sintered glass outlets.

Reagents

1. Pyrogallol-sodium phosphate solution. Dissolve 10 g of sodium dihydrogen phosphate and 10 g of pyrogallol in 100 ml of distilled water under constant passage of nitrogen through the liquid. Prepare fresh if required.
2. Zinc acetate, 1% solution in distilled water.
3. Sodium hydroxide, 12% solution in distilled water.
4. Hydrochloric acid s.g. 1.18.
5. p-Amino-N, N-dimethylaniline sulphate (The British Drug Houses Ltd.) 0.5 g in 500 ml of 5.5 m hydrochloric acid.
6. Ferric chloride, 0.023 m in 1.2 m hydrochloric acid.

Procedure

Wash bottle (a) is filled with 100 ml of the pyrogallol solution *(1)*, the suction flask (b) and the air valve with water, and wash bottle (e) with 50 ml of the zinc acetate solution *(2)* and 2 ml of the sodium hydroxide solution *(3)*.

The apparatus is assembled, all joints being sealed with vaseline to ensure air-tight connections.

Flask (c) is placed in cold water and the nitrogen flow is adjusted to a steady bubbling. The air in the apparatus is completely replaced by nitrogen in about 30 minutes.

The sample of rumen liquor, usually 50 ml, is introduced into the dropping funnel and carefully transferred to flask (c) by handling the stopcock. Washings with oxygen free water are repeated until the volume of the diluted sample is 100 ml, finally 40 ml of the hydrochloric acid *(4)* is added via the dropping funnel. The additions are made with care to avoid excessive fluctuations of the pressure inside the apparatus.

Heating is applied and the liquid in flask (c) is kept in boiling water for one hour.

Thereafter wash bottle (e) is removed by disconnecting the ball joint. The stopper is removed and 10 ml of the p-amino-dimethylaniline *(5)* are added. The stopper is immediately replaced and the liquid swirled. Slight suction may be applied to dissolve the zinc sulphide adhering to the sintered gas outlet completely (3, 5).

Subsequently, 2 ml of the ferric chloride solution *(6)* are added. The liquid is mixed as before, and set aside for 10 minutes. Thereafter the liquid is transferred to a volumetric flask and the volume made up to 100 ml with distilled water.

After at least 30 minutes and within 20 hours, the optical density is measured at 670 mμ in a 10 mm cuvette.

The extinction of the dilutions of a sodium sulphide solution, previously standardized according to (9), showed a linear relationship with a concentration within a range of 1 to 60 micrograms S per 100 ml of the test solution. The extinction depends on the acidity of the solution obtained. If it is necessary to dilute the coloured solutions prior to measurement in the above range of concentrations, this should be done with

a solution of p-amino dimethylaniline sulphate and ferric chloride of the same concentration as the test solution.

Some applications

Samples from cows on a basal ration and from fistulated cows receiving sulphate and ureum in addition, were obtained from Dr. J. van der Grift (I.V.O., Hoorn).

The progress of fermentation was checked by adding 25 ml of 1 N sodium hydroxide per litre of rumen liquor, which raised the pH of the liquor to about 8 (6). It was shown with cysteine that sulphhydril does not give hydrogen sulphide at this pH. The sodium hydroxide also prevents losses due to volatilization of hydrogen sulphide.

The alkaline liquor was filtered through a nylon gauze, stored at 2 °C and analysed as soon as possible.

The fistulated cows were sampled at short intervals during one day. The other animals could not be sampled at this frequency.

In Fig. 2 the results are shown, curve 1 represents the non-fistulated cow on the basal ration, curve 2 the fistulated cow on the basal ration with sulphate and ureum, and curve 3 a fistulated grazing cow.

In addition the results of a fistulated cow from the Department of Animal Physiology of the Agricultural University, Wageningen, fed on hay, are given in curve 4. The fistulated cows were sampled with a Janet Syringe and the non-fistulated cows with a stomach tube (1).

Table 1 shows that sampling with the stomach tube invariably yields higher values for the sulphide contents of the liquor.

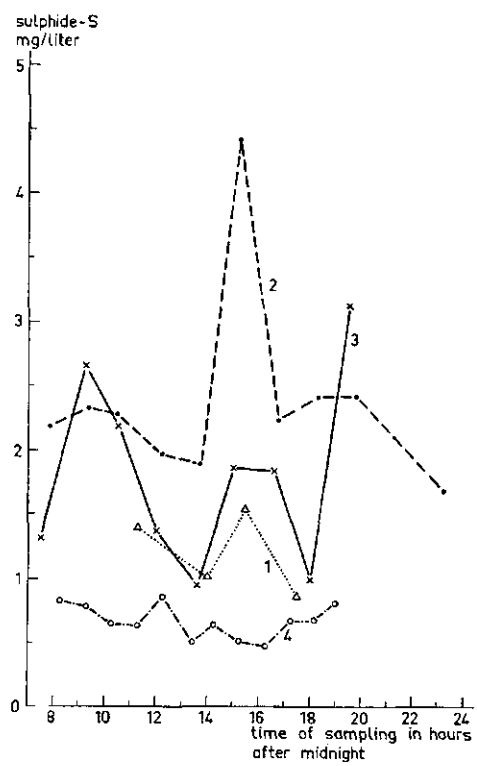

Fig. 2. Total sulphide-S in rumen liquor.
Rations:
1. 10 kg hay, 1½ kg ground linseed and 2 kg sugar-beet pulp (Oct. 12, '64).
2. 10 kg hay, 1½ kg ground linseed, 2 kg sugar-beet pulp and an infuse of 270 g urea + 170 g ammonium sulphate in 4 l of water per 24 hours (Oct. 12, '64).
3. grazing (Oct. 12, '64).
4. 10 kg hay (Feb. 3, '65).

The sampling dates of the rumen liquor are given in parentheses.

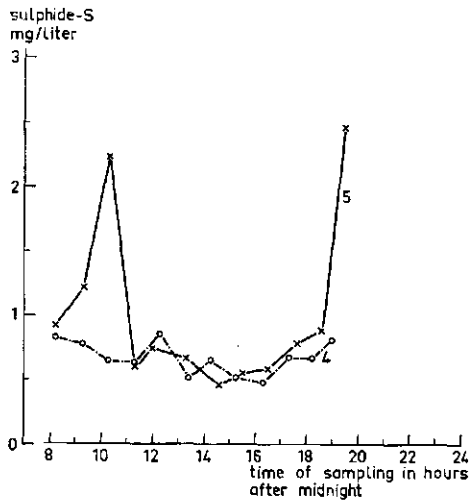

FIG. 3. Total sulphide-S in rumen liquor.
Rations:
4. 10 kg hay (Feb. 3, '65).
5. 2 kg hay and 18 kg wilted silage (Ma. 4, '65).
The sampling dates of the rumen liquor are given in parentheses.

Fig. 3 records the results, curve 4 representing a fistulated cow fed on hay, and curve 5 the same cow fed on wilted silage and some hay.

The feed composition is given in Table 2. The feed consumed by the grazing cow was not sampled.

The rations were fed for at least 2 weeks prior to sampling.

TABLE 1. Total sulphide in mg S per litre of rumen liquor. Average values for samples from three different cows. The rations of the control animals were the same as those mentioned in Fig. 2 (curve 1), and of the experimental cows in Fig. 2 (curve 2) without urea.

Date	Time	Experimental cows		Control animals
		Fistula	Stomach tube	Stomach tube
Dec. 3 '64	9.30	3.31	4.59	1.25
Dec. 3'64	12.15	1.74	3.21	1.32
Dec. 9 '64	10.45	2.65	3.33	1.78
Dec. 9 '64	13.30	1.68	2.83	1.06

TABLE 2. Feed composition.
d.m.: dry matter percentage of the fresh material.
c.p.: crude protein; t.p.: true protein; c.f.: crude fibre; w.s.c.: water-soluble carbohydrates; S: sulphur (all values in percentages of the dry matter); Cu: copper (in mg per kg of dry matter).

Feed	Date	d.m.	c.p.	t.p.	c.f.	ash	w.s.c.	S	Cu	pH
Hay	Oct. 12 '64	96.0	12.5	8.4	35.3	8.5	1.6	0.29	9.0	–
Ground linseed	Oct. 12 '64	97.1	37.8	31.7	10.4	6.4	3.7	0.38	25.6	–
Sugar-beet pulp	Oct. 12 '64	97.2	9.3	8.3	23.2	4.0	3.4	0.35	7.0	–
Hay	Feb. 3 '65	83.1	14.7	10.8	34.8	8.6	3.5	0.31	9.3	–
Wilted silage	Ma. 4 '65	28.4	14.1	6.9	33.5	11.7	1.2	0.38	15.4	5.2

SOME IN VITRO EXPERIMENTS ON HYDROGEN SULPHIDE FORMATION IN RUMEN LIQUOR

Samples of 50 ml of fresh rumen liquor were added to various sulphur compounds in 50 ml bottles. Oxygen was replaced by nitrogen gas and the liquors incubated for

2 hours at 38 °C. Each bottle received a constant amount of 9 mg S, in one of the various compounds. After incubation hydrogen sulphide was determined. The results are given in Table 3. No hydrogen sulphide was formed after adding cysteine to the residue in the distilling flask and continued boiling for another hour.

TABLE 3. Sulphide-S content in rumen liquor, incubated for 2 hours at 38°C with 180 mg S of the various compounds per litre.

Liquor	Substance added	mg S per litre
I	no addition	2.52
I	amm. sulphate	2.58
I	methionine	2.35
I	glutathione	6.13
I	cystine	6.40
I	cysteine	7.86
II	no addition	1.37
II	amm. sulphate	0.85
II	methionine	2.07
II	penicillamine	2.56
II	cystine	2.15
II	cysteine	6.03

DISCUSSION

Fig. 2 shows the effect of the ration on the sulphide content in the rumen liquor. The administration of urea and ammonium sulphate caused high sulphide contents in the liquor.

From Table 1 it may be concluded that there is also an increase in the sulphide in the rumen liquor of the experimental cows fed on the basal ration with ammonium sulphate only. In this relation attention is drawn to the fact that HARTMANS and VAN DER GRIFT (4) found a decrease in liver copper in cows receiving additional sulphate.

The sulphide contents in the rumen liquor of the grazing cow (Fig. 2) were much higher than the low values obtained from cows fed on hay. Fig. 3 shows that wilted silage also causes higher sulphide contents in the rumen liquor. Such results may suggest that fresh grass promotes the formation of copper sulphide in the rumen compared to the conditions in cows fed on a diet of hay.

In studying the daily fluctuations in the sulphide contents fistulated cows should be preferred to enable a sampling at sufficiently short intervals. The inorganic sulphate content in the feed may be important to sulphide formation, but perhaps also the sulphur-containing amino acids, especially the sulphhydryl-compounds. Table 3 shows that several organic sulphur compounds, especially the sulphhydril-compounds, if incubated with rumen liquor for 2 hours at 38 °C, give rise to an increase in the sulphide content.

SUMMARY

A method to determine sulphides in rumen liquor is described.

Some sulphide contents in rumen liquor of cows fed on hay, silage, fresh grass, a basal ration and a basal ration with sulphate or sulphate and ureum are given. The sulphide contents in rumen liquor of cows receiving hay or the basal ration were low compared to the grazing cow, the experimental animals receiving additional sulphate or sulphate with ureum, and the cow fed on silage.

The results suggest that grass feeding gives rise to conditions in the rumen promoting the formation of copper sulphide.

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THE DETERMINATION OF THE HIGHER FATTY ACIDS IN GRASS AND COW-FAECES

H. J. IMMINK, J. H. GEURINK and W. B. DEIJS

INTRODUCTORY

In a previous communication (3) the determination of free fatty acids and soaps in cow-faeces according to (4) was discussed. Analyses for total fatty acids, including free acids, soaps and the acids bound to the fats and set free by saponification, can be carried out after saponification of the samples of fresh grass and faeces. In the present communication the values thus obtained have been compared with those found after saponification of the substance obtained by diethyl ether and petroleum ether extraction of dried grass and faeces samples. In addition, the relationship between crude protein and higher fatty acids in grass is demonstrated and the apparent digestion coefficient of the higher fatty acids is inferred from data on contents in grass and faeces consumed and excreted by cows, submitted to a digestion trial.

METHODICAL

Reagents

1. 96% ethanol, containing 0,4% amyl alcohol
2. KOH in water (33%)
3. HCl in water (25%)
4. Ethanol (96%), neutralized just before use on thymol blue
5. Petroleum ether (b.p. 40–60°C), washed with a dilute solution of KOH and finally washed with water. The distillation residue should be neutral on thymol blue
6. Isobutanolic KOH (0,1 N). 5 litres of isobutyl alcohol are refluxed over 100 g of NaOH during 3 hours. In one litre of the fraction distilling at 105–108°C, 15 g of KOH (50%), diluted with 20 ml of methanol, are dissolved. Before use the isobutanolic KOH is standardized with 0,1 N HCl on thymol blue.
7. 0,1 N hydrochloric acid
8. 2% thymol blue in 50% ethanol
9. Glass tubes (30×4 cm) provided with ground glass stoppers.

Analysis of fresh grass

Samples of deep-frozen fresh grass are ground twice in a meatmincer. 6 grams of the thoroughly mixed wet material are subjected to saponification by boiling under reflux for one hour with 40 ml of ethanol–0.4% amyl alcohol and 10 ml of 33 per cent potassium hydroxyde.

After cooling, the liquid is transferred to a glass-stoppered glass tube, 17 ml of 25 per cent hydrochloric acid and 50 ml of petroleum ether are added and the mixture is shaken vigorously for one minute. Now and then the tube is gently rotated to facilitate separation of the solvent.

After separation, 25 ml of the petroleum ether layer are transferred to an Erlen-

meyer flask containing a 5 × 5 mm piece of filter paper. The solvent is distilled off, the residue dissolved in 25 ml of neutralized ethanol and titrated with standard isobutanolic potassium hydroxyde. The results are expressed as milliequivalents (me.). For corrections required see VAN DE KAMER *et al.* (5).

Analysis of oven-dried grass

1 gram of the powdered, dry sample is suspended in 5 ml of water and submitted to the above procedure.

Analysis of faeces

50 grams of fresh faeces and 75 ml of water are thoroughly mixed during 2 minutes in a mechanical mixer. 10 grams of the suspension, corresponding to 4 grams of faeces, are submitted to the above procedure.

EXPERIMENTAL

The effect of drying the samples

In a few tests the effect of the drying method of the fresh grass on the analytical results was investigated. After drying, the samples were ground to a powder in a hammer mill.

The results are listed in Table 1. The first column indicates the method of drying applied to four grass samples. In addition the me. values are expressed in percentages of the value obtained for the deep-frozen sample.

The vacuum-dried samples and those dried at 70 °C for two hours yielded the same values. However, the iodine values of the fatty acids are likely to be changed by the latter treatment.

If the drying at 70 °C is continued up to 16 or 20 hours, lower values are found.

TABLE 1. The influence of drying the sample on the content of higher fatty acids in grass. The fatty acids (me./kg of d.m.) were determined after total saponification.

Grass-sample	1	2	3	4
Deep-frozen fresh grass	181 (100%)	145 (100%)	195 (100%)	168 (100%)
Fresh grass dried in vacuo				
21 hours at 50°C	180 (99,5%)	145 (100%)		
Fresh grass dried in air-flow				
2 hours at 70°C	180 (99,5%)	150 (103%)		165 (98%)
20 hours at 75°C	169 (93%)			
16 hours at 75°C		135 (93%)		
20 hours at 70°C			164 (84%)	

The gravimetric determination of crude fat

Some samples have been investigated on the crude fat content with procedures outlined in detail in the Norm NEN 3148 of the 'Nederlands Normalisatie-instituut'. The grass samples were dried for two hours at 70 °C and the faeces in vacuo at 50 °C.

First, 10 grams of each sample were Soxhlet-extracted with petroleum ether b.p. 40–60 °C. After 150 minutes the extraction was discontinued and the samples rubbed with 5 grams of sand in a mortar. Thereafter extraction was continued for 2 hours. The sample was again rubbed with sand and the extraction continued for another 2 hours.

After extraction the solvent was evaporated and the residue dried to constant weight at room temperature in vacuo.

In other tests 5 grams of each sample were boiled with 100 ml of 3 N hydrochloric acid. Thereafter, 125 ml of ethanol were added, and the liquid was extracted with 100 ml of carbon tetrachloride. After separation, 50 ml of the carbon tetrachloride layer was removed and evaporated to dryness. The crude fat was dried at room temperature in vacuo to constant weight.

The results are expressed in percentages of the sand-free dry material in the samples, and listed in Table 2.

It is seen that considerably greater quantities of the crude fat are recovered by carbon tetrachloride extraction after digestion of the sample with hydrochloric acid, while much lower quantities are obtained by direct Soxhlet extraction of the solid with petroleum ether.

TABLE 2. Determination of crude fat in samples of dried grass and faeces. The contents are given in % of the sand-free dry matter.

Sample	Crude fat after petroleum ether extraction	Crude fat after boiling with HCl and extraction with CCl_4
Grass 1	4,7	7,1
Grass 8	4,1	6,4
Faeces 5	14,3	22,4
Faeces 6	3,9	6,7
Faeces 7	13,7	21,4

Recovery of the fatty acids by different extraction procedures

A number of samples of grass (dried for 2 hours at 70°C) and faeces (vacuum-dried at 50°C) were tested with the following procedures:

a. direct saponification of the dry samples as outlined above
b. Soxhlet-extraction of the crude fat with petroleum ether, saponification of the residue after evaporation of the solvent and titration of the fatty acids
c. same with diethyl ether extraction of the crude fat
d. extraction of the crude fat with carbon tetrachloride after digestion of the sample with hydrochloric acid, saponification after evaporating the solvent and titration.

The residues of the samples after Soxhlet extraction in the procedures b and c were submitted to saponification and the fatty acids therein titrated to determine the amount which escaped extraction.

TABLE 3. Fatty acids in samples of dried grass and faeces, in extracted crude fat and in the residue after extraction. The contents of fatty acids are given in me./kg of sand-free dry matter.

Method \ Sample	Grass 1	Grass 8	Grass 9	Grass 10	Faeces 5	Faeces 6	Faeces 7*
a.	180	141	147	170	385	111	336
b. In Soxhlet-extract		69	74		133	60	146
In residu		76	73		234	61	194
c. In Soxhlet-extract		85	94				
In residue		56	50				
d.	183	149		166	391	105	385

*) This sample contained 20,4% sand on dry weight.

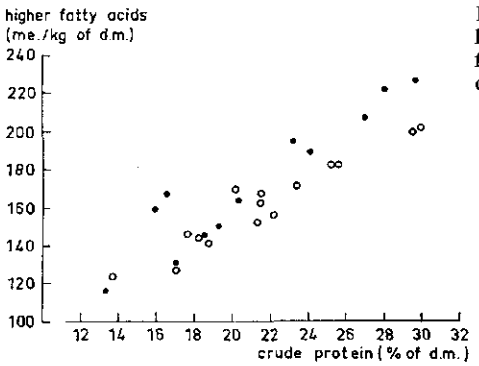

FIG. 1. The relation between crude protein and higher fatty acids in fresh grass.
filled dots: Field 9
open dots: Field 10

The results are listed in Table 3. The data show that Soxhlet extraction of the solid samples with petroleum or diethyl ether (procedure b and c) recovers a small portion only of the total fatty acids from the sample, which agrees with other experience (2). If the quantity recovered by subsequent saponification of the residue of the extraction is added to this amount, the value obtained is very close to that found after direct saponification of the sample (method a). This value also agrees reasonably well with the result of the saponification of the crude fat obtained by carbon tetrachloride extraction after digestion of the sample with hydrochloric acid (method d).

Relation with the crude protein content

Fresh grass was sampled from two permanent-pasture trial fields between April 27 and October 12, 1964. The samples were deep-frozen and analysed for total fatty acids after saponification, and for crude protein. The results, depicted in Fig. 1, reflect the relationship. Such a relationship has earlier been observed by BROUWER (2).

The apparent digestion coefficient

A digestion trial on the utilization of mineral constituents was carried out by KEMP and GEURINK. These workers supplied samples of fresh grass, concentrates and

TABLE 4. Digestion trial with four cows.

Cow No.	1	2	3	4
Intake				
fresh grass (kg of d.m./day)	11.70	12.85	12.32	12.70
concentrates (kg of d.m./day)	1.38	1.39	1.38	1.38
me. of higher fatty acids in 1 kg of d.m.:				
grass	135	135	135	135
concentrates	114	114	114	114
total intake of higher fatty acid (me./day)	1737	1893	1821	1872
Excretion in faeces				
kg of d.m. day	2.954	3.223	3.015	3.080
me. of higher fatty acids in 1 kg of d.m.	135	135	136	180
total excretion (me./day)	399	435	410	554
'Apparent' digestion coefficients				
of dry matter	77	77	78	78
of higher fatty acids (me.)	77	77	77	70

faeces and data on the weights consumed and excreted by the cows under investigation.

From these and from analyses for the total higher fatty acid contents after direct saponification, the daily intake and faecal excretion of the dry material and the fatty acids were calculated.

The amount of fatty acids not excreted in the faeces, expressed in percentages of the intake is called here the apparent digestion coefficient.

The results are listed in Table 4 for each of the four animals of the digestion trial.

It is seen that the apparent digestion coefficients for the total fatty acids and for the dry material are about the same. The average value of 75 is very near to the average of 72 found earlier by BLOM (1) for the me. of different fractions of higher fatty acids.

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DETERMINATION OF THE FIRMNESS OF POTATOES

N. VERTREGT

INTRODUCTION

Potato tubers are more or less injured by modern methods of lifting, transport etc. To a certain extent the injury depends on growing conditions, as is shown by the differing susceptibility of potatoes grown on different fields. To investigate the factors possibly determining the firmness of potatoes an instrument was wanted to estimate potato firmness.

MOHSENIN and GÖHLICH (1) constructed an instrument registrating force-deformation curves of fruit and vegetable tissues. A spectacular result of their experiments is the discovery of a sharp breakpoint in the force-deformation curve, before the point of rupture was reached. This point was called the yield point, because it resembles the yield point as known from the stress-strain curves of metals. This yield point is important, because if the pressure exerted on the tissues is lower than the yield-point pressure, the tissues will not be injured and discolouration will not occur. If pressure exceeds the yield-point pressure, the tissues are injured sufficiently to develop a discolouration. Usually the firmness of potato tissues is defined as the pressure at the point of rupture. It seemed worthwhile to analyse the force-deformation relation of potatoes grown under different conditions of mineral supply according to the method of MOHSENIN and GÖHLICH.

For our purpose a less complicated instrument was constructed. The essential parts, however, are as sensitive as those of the model.

INSTRUMENTAL

Fig. 1 shows the instrument used in the experiments. It consists of a loading device, a load cell to measure the force and deformation, and a galvanometer recorder. The loading device embodies a synchronous motor with gear box. The motor drives an internally threaded hollow shaft which is enclosed in a bearing, at a rotational rate of 6 r.p.m.

The rotating shaft screws a threaded plunger downwards, a wedge preventing the plunger to rotate. The pitch of the screwthread is 0.5 mm, consequently the rate of the plunger is 3 mm per minute. The lower part of the plunger can be exchanged for parts with a diameter of 4, 5, 6 and 7 mm.

The load cell consists of four cantilever beams. In total eight strain gauges are cemented to the upper and lower side of it. The strain gauges are electrically connected in a Wheatstone-bridge arrangement. A platform with vertical guide bearings rests by way of balls on the cantilever beams. By pressing down the platform the cantilever beams bend, effecting a change in the electrical resistance of the strain gauges. The resulting off-balance current of the Wheatstone bridge is registered by the galvanometer recorder.

When a potato is placed on the platform, a part of the displacement of the plunger

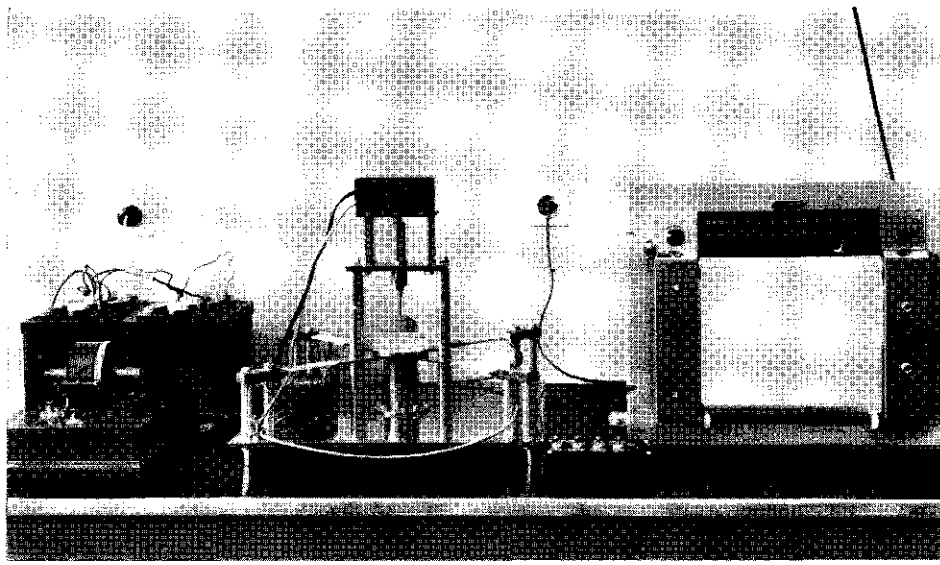

FIG. 1. Instruments used during the experiments.

is absorbed by the potato deformation, and does not result in a displacement of the platform. A calibration line is obtained by loading an undeformable object.

When loading a potato, the deformation of the potato tissue at a certain plunger displacement is reflected in the horizontal distance between the registered curve and the calibration curve. The force exerted on the tissue as well as the tissue firmness may be calculated from the pen deflection.

The deflection of one kilogramforce is determined by placing a kilogram weight on the platform.

Our curves did not show a yield point. However, the clearance of the thread is sufficient to produce a yield point at the moment at which the load on the cantilever beams exceeds the weight of the plunger-bolt assembly. To prevent this the plunger-bolt assembly was tightened with springs to the motor housing. Perhaps MOHSENIN and GÖHLICH's instrument was not protected against simulating a yield point.

RESULTS

Fig. 2 shows the curves resulting from the following experiment.

A cilinder with a diameter of 35 mm was cored from a potato. The cilinder was cut into 11 disks of approximately 10 mm high. Each disk was weighed and immersed in 100 ml of a sucrose solution of various concentration, ranging from 0.25 to 0.50 molar. The weight increase or decrease was determined and the force-deformation relation of the disks was registered.

Because differences in firmness are greatest at small deformations, the firmness of the disks as plotted in Fig. 3 is determined at 0.5 mm plunger displacement. The firmness of the disks is expressed in the pen deflection relative to the pen deflection of the calibration line.

Obviously the firmness measured by this method is proportional to the turgor of

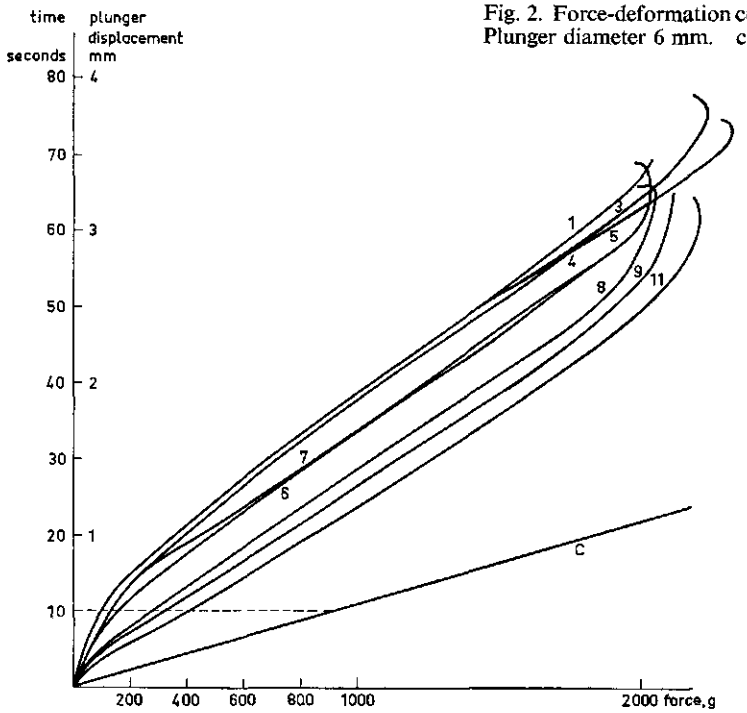

Fig. 2. Force-deformation curves of 9 potato disks. Plunger diameter 6 mm. c = calibration line.

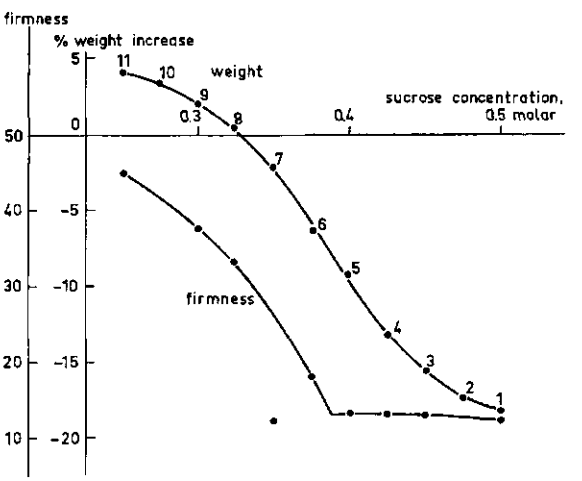

FIG. 3. Weight increase or decrease in potato disks after immersion in sucrose solutions of various concentration.
Firmness calculated from curves of Fig. 2.

the potato tissue. At the point of weight constancy the firmness measured corresponds with the actual turgor of the potato.

The possible relation between turgor and susceptibility of potatoes to bruising may be proved by using the method described.

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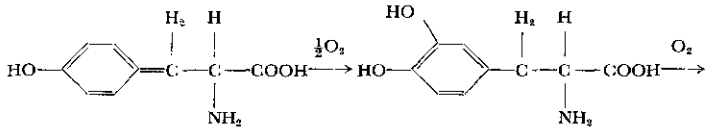
DOPACHROME FORMATION IN POTATOES

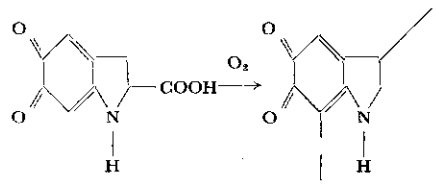
N. VERTREGT and ELISABETH G. PANNEBAKKER

In a previous publication we have described a method to estimate the discolouration of raw potatoes (3). The method is based on determining the dopachrome formation in a defined period from the tyrosine content of potato sap. For a better interpretation of the results obtained by using this method we give some further particulars.

The reaction scheme is:



tyrosine DOPA



dopachrome melanin

The first two stages of the scheme have a relatively high, the last sequence of stages has a low reaction rate. Therefore dopachrome accumulates to a certain extent and the dopachrome concentration can be estimated.

Fig. 1 curve a shows the absorption spectrum of potato sap. In this case the discolouration is prevented by adding sodium diethyldithiocarbamate (dieca). This agent inhibits phenoloxidase activity by sequestering the phenoloxidase-copper. Curve b represents the absorption spectrum of potato sap after 20 minutes' oxidation by air. The absorption spectrum of enzymic oxidized DOPA is given by curve c. The phenoloxidase used was isolated from potatoes according to the method of KERTESZ (1), however without the use of KCN as a copper-sequestering agent. The absorption spectrum of the oxidized DOPA-solution and the subtracted potato sap absorption spectra (b–a) are convincingly similar.

The formation of dopachrome in relation to time is given in Fig. 2. After 30 minutes the melanin formation exceeds the dopachrome formation in the sap of potatoes of the Libertas variety.

At this moment not much melanin is formed in the sap of Bintje and Irene potatoes. At the arbitrarily chosen reaction period of 20 minutes at 25 °C the largest differences of dopachrome concentration between the varieties are found.

To obtain more information about the dopachrome formation from l-tyrosine,

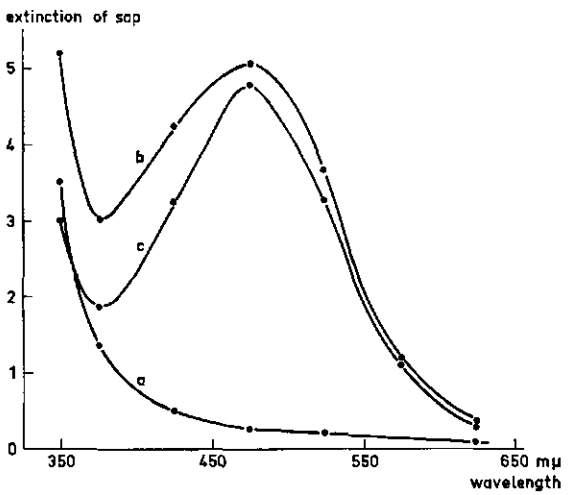


FIG. 1. Extinction of potato sap after 20 minutes stirring and flushing with air at 25°C.
a. phenoloxidase activity in potato sap inhibited with dieca;
b. potato sap without dieca;
c. solution of DOPA and phenoloxidase preparation.

known quantities of tyrosine were added to aliquot portions of potato press-sap of known tyrosine content (2). In these samples the quantity of dopachrome formed in 20 minutes at 25°C was estimated and the tyrosine concentration after the reaction period was determined. The results of these experiments are plotted in Fig. 3.

Differences in the dopachrome formation in the sap at equal tyrosine concentrations are due to different phenoloxidase activities.

From the numbers of Fig. 3 it is possible to compare the dopachrome formation to the tyrosine consumed by the reaction (Fig. 4). In the press-sap of nine varieties the

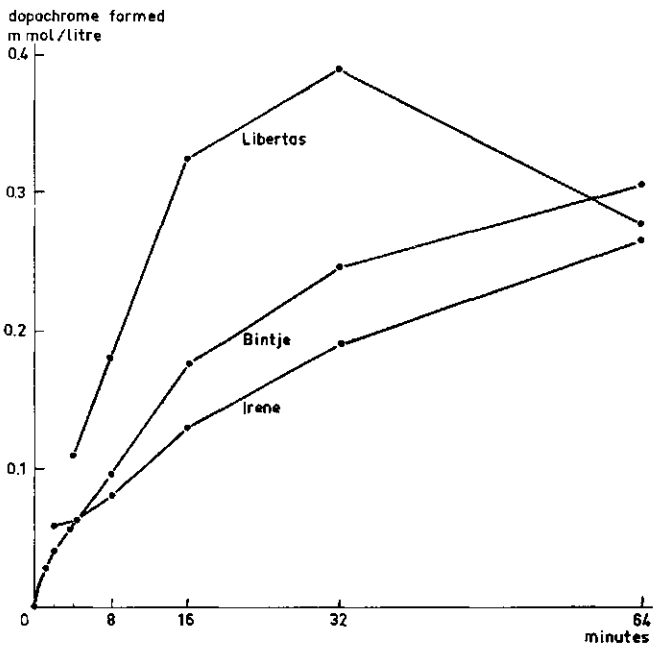


FIG. 2. Dopachrome formation in stirred potato sap at 25°C.

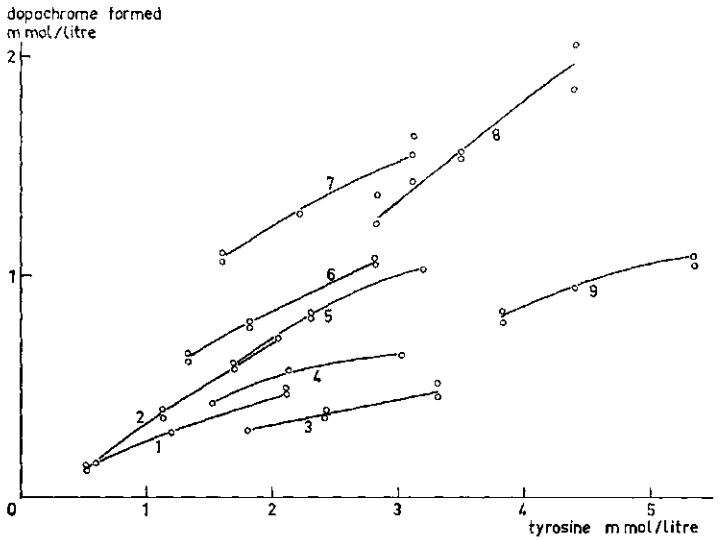


FIG. 3. Dopachrome formation in potato sap after addition of different quantities of tyrosine.

1, 6 Extase	5 Bintje
2 CB 52–51–17	7 Libertas
3 Irene	8 Noordeling
5 MG 54–92	9 Desiree

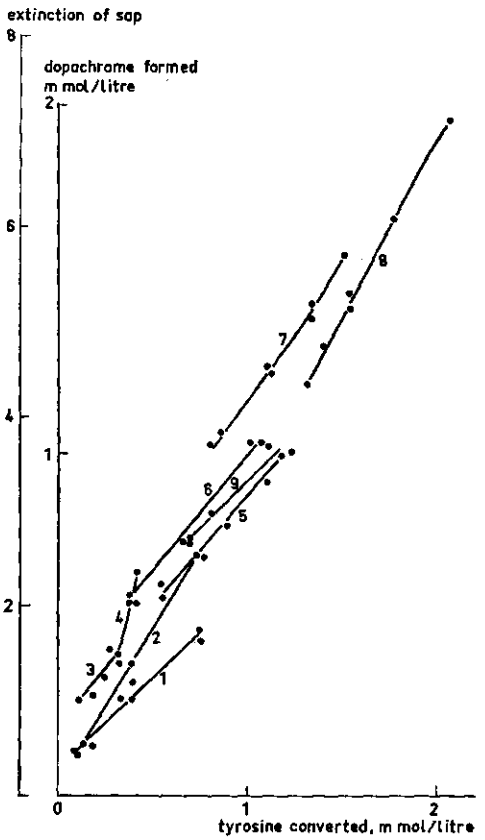


FIG. 4. Formation of dopachrome compared to diminution of tyrosine. Varieties as in Fig. 3.

dopachrome formation is proportional to the disappearing tyrosine. Without isolating the compound the molar extinction-coefficient of dopachrome can be derived from the figure. The value of the molar extinction-coefficient of dopachrome approximated in this manner is: $E = 3600 \pm 700$.

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Mededeling 294 van het I.B.S.

ESTIMATION OF CHLOROGENIC ACID IN POTATOES

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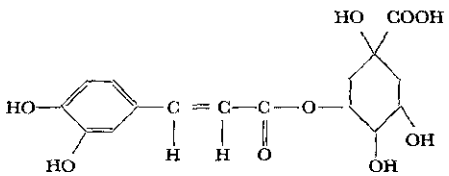
INTRODUCTION

Cooked potatoes, exposed to the air, sometimes develop a bluish-grey discolouration. This phenomenon, 'after-cooking blackening', is caused by the oxidation of a ferrous-chlorogenic acid complex to a ferric complex (2, 3, 4).

Chlorogenic acid is an ester of caffeic acid and quinic acid: 3-caffeoylquinic acid,



To explain the influence of environmental conditions on after-cooking blackening a method was wanted to estimate chlorogenic acid in potatoes. Several more or less specific methods are known:

1. The spectrum of a methanolic solution of chlorogenic acid is subject to a bathochromic shift after adding sodium ethoxide. The effect can be measured at 380 mμ (2). Much time is required to prepare the extract of cooked potatoes. No clear methanolic solutions could be obtained from uncooked potato sap. At 380 mμ the molar extinction of sodium caffeate is twice that of sodium chlorogenate.
2. Determination by quantitative paper chromatography is a good principle (6). Because of the interfering salt and sugar contents deproteinized potato press-sap is not a suitable substrate to be analysed by this method. The method is very time consuming.
3. A convenient method is the modified Arnow procedure published by ZUCKER *et al.* (1, 7). Chlorogenic acid is adsorbed on an aluminum-oxide column. The colour-developing reactions occur on the column, and the coloured compound is eluted and estimated by colorimetry.

EXPERIMENTAL

A chromatographic column is prepared of aluminum oxide not passing a 20 mesh per cm sieve. The column is 4 cm high, with a diameter of 1 cm. The column is washed with water to neutral reaction.

Fourth or eighth parts were longitudinally cut from each potato of a sample of twenty peeled potatoes. The parts were weighed and crushed in a mixer with 3 volumes of 0.1 N sulphuric acid in 90% alcohol. Deterioration of the chlorogenic acid in the supernatant was prevented by centrifuging.

The analysis of the extract must be carried out the same day. 20 ml of the extract are neutralized to pH 6.5–7.5 with 2 N sodium hydroxide and made up with alcohol

to 25 ml. The extract turns turbid and has to be centrifuged. 5 ml of this alcoholic extract are pipetted on the column. The column is washed with 5 ml water. A freshly prepared mixture of 2 ml of 0.5% sodium nitrite and 2 ml of a 5% acetic-acid solution is pipetted on the column. The yellowish-green adsorption zone shifts to orange.

The column is then washed with 5 ml of water. 5 ml of 5 N sodium hydroxide are pipetted on the column. The orange-coloured adsorption zone turns red and elutes. The eluate is collected in a 10 ml measuring flask, water is used as the next eluting solvent to make up to the mark.

The intensity of the red colour is measured at 520 mμ. A calibration curve is obtained in a similar way, using a chlorogenic-acid preparation.

The columns are regenerated by rinsing with 5 ml 5 N sodium hydroxide, followed by water until the eluate is almost neutral. The aluminum oxide of the column is suspended in 5 ml 5 % acetic acid; after settling the column is washed with water until pH 7.

Discussion

The extraction procedure has a recovery of about 85% as not all cells are broken by the mixer. However, as the recovery is fairly constant at 85%, a correction is possible. The efficiency of the extraction procedure was estimated by comparing the

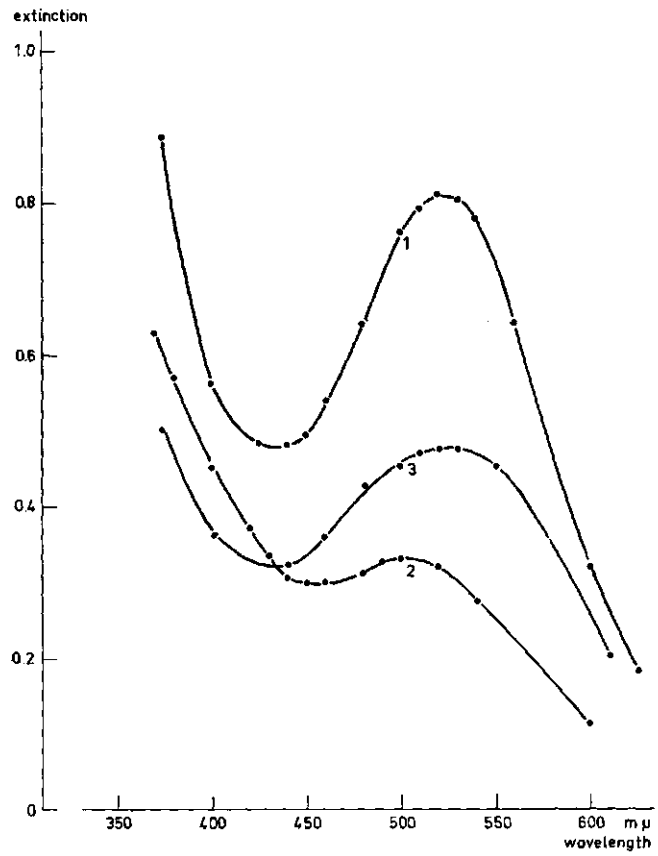


Fig. 1. Absorption spectra of reaction products.
1. chlorogenic acid, 0.1 mmol. litre^{-1}
2. caffeic acid, 0.1 mmol. litre^{-1}
3. potato extract, 100 g potato to 1 litre final solution.

potassium content of a part of the potatoes to the potassium content of the extract of an aliquot part.

The tissue is crushed in acidified alcohol to prevent enzymic oxidation of the chlorogenic acid.

Using chromatographic columns has the advantage that many of the interfering substances are not adsorbed and washed out, while the chlorogenic acid remains adsorbed. The stability of the colour is excellent. No fading of the colour occurs within 30 minutes.

Fig. 1 shows absorption spectra of the coloured compounds obtained from chlorogenic acid, caffeic acid and potato extract using this method. It is shown by paper chromatography that chlorogenic acid is the chief phenolic compound in potatoes (5).

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Mededeling 295 van het I.B.S.

NOTES ON THE ACTIVITY OF EARTHWORMS

IV. General observations on symbiontic bacteria

J. Doeksen

Introduction

The presence of bacteria in the nephridia of earthworms was reported in 1905 by Maziarski (5).

Stephenson (7) in 1930 gave a review of the literature up to that time, part of which (l.c. 202–203) is quoted:

'Maziarski states that the inner surface of the wall of the ampulla is clothed by a layer of rodlets, which by culture show themselves to be bacteria; ...

The subject has been further investigated by Knop ('26); in *Lumbricus terrestris* the individual rodlets are 3–5 μ long, 0.5–0.7 μ thick; they are placed close together and mostly at right angles to the surface of the cells; the poles stain more deeply than the middle, especially at a certain stage of differentiation (hence, perhaps, K. C. Schneider's description of them ('08) as granules).

The eggs and young embryos are always free from bacteria, but just before hatching the nephridial ampullae are full of them. In the adult, at the time of formation of the cocoon, the ampullae become swollen, and the substance which ordinarily causes the bacteria to cling together becomes fluid; the bacteria are thus loosened and pass into the vesicle. Bacteria are found later in the cocoon, less abundantly at first, in larger numbers in older cocoons; there are no morphological differences between those of the nephridial ampullae and those of the cocoons, and the latter are of one kind only – which would not be the case if they had been derived from outside; as a further argument for the identity of the two is the fact that those of the cocoons do not affect the developing embryo prejudically. Hence it is concluded that the bacteria are squeezed out of the vesicle into the cocoon as the worm is extricating itself from the latter. A number of *Lumbricidae* were investigated by Knop with similar results.

The association of worm and bacteria is supposed by Knop to be symbiotic; in support, he brings forward the universality of the occurrence of the bacteria in the *Lumbricidae* of all countries; their strict localization to a definite site; the regularity in the mode and time of their discharge; the invariable infection of the fully-formed embryo; and the absence of injury to the nephridium. It is conjectured that the bacteria are probably beneficial to the worm, but how is not known.'

It is remarkable if not astonishing that these facts, known for nearly 40 years, have been ignored by physiologists, especially those who have been studying excretory processes in earthworms. It is hard to believe that excretes from the nephridia, after passing a layer of bacteria, are comparable with the products really excreted by the earthworms.

EXPERIMENTS

Considering the possible role of the nephridial bacteria of earthworms, two facts seem especially suggestive:

1. The contents of the egg cocoons, which are excreted at the outside of the worms while in the soil – hardly a sterile surrounding – can remain in the cocoons in the soil for many months, without being attacked by micro-organisms. This strongly points to a protection, possibly by a bacteriostatic or bacteriocidal product, harmless to the developing embryo.
2. Uric acid has been reported to be present in 'uric cells' of the peritoneum (WILLEM & MINNE (8); *cf.* also STEPHENSON (7)), whereas in the 'urine' no uric acid or only traces of it are found (*cf.* LAVERACK (4)).

Supposing that the nephridial bacteria have to do with these phenomena, it seems reasonable to try to culture them on uric acid media with inorganic salts only.

In a fluid medium (0.5 g uric acid, 0.1 g K_2HPO_4, 0.05 g $MgSO_4$, 100 ml tap water) bacteria from various origins (*Eisenia foetida, Allolobophora caliginosa)* develop well, even in bottles closed with Kapsenberg caps; others need a good aeration to grow *(Lumbricus terrestris, L. rubellus)*. In all cases shaking of the culture flasks is necessary to keep the uric acid partly suspended. Care has to be taken to sterilize uric acid at 130°C, as this chemical proved to be contaminated with resistant micro-organisms.

Uric acid agar plates were prepared of 0.1 g K_2HPO_4, 0.05 g $MgSO_4$, 0.5 g uric acid, 2.5 g agar-agar, 100 ml tap water. The uric acid is first sterilized for 30 min. at 130°C with a small part of the water. After addition of the other constituents, the whole is sterilized for 30 min. at 110°C. Even under these conditions the agar is much affected by the uric acid. Therefore a good result is obtained with less agar if the sterile uric acid is mixed with the hot sterile water-agar-salts mixture just before pouring the plates.

On these plates, bacteria from all available origins (earthworm species) did very well. The whole plates become clear in a short time. The easiest inoculum proved to be the contents of the egg cocoons of the earthworms. The cocoons were superficially sterilized with 0.2% mercuric chloride. The same results, however, were obtained by using nephridia or well rinsed whole worms as an inoculum. Even the excrements of earthworms, kept for some weeks on daily changed sterile filter paper could be used.

Foreign infections of either plates or bottles seldom occurred, suggesting that an antibiotic might be involved, which would explain the ease of isolating nephridial bacteria and the resistance of the contents of the egg cocoons to microbial attack.

Contrary to the findings of KNOP (3) two types of bacteria were found in each colony. The two could not be separated on uric agar plates; when inoculating sparingly, no growth could be observed at all, suggesting either a symbiosis of the two bacteria or the necessity of the presence of traces of certain chemicals to start new colonies.

Miss F. DE JONG of the I.T.B.O.N. at Arnhem, kindly tried inoculations on different plates and found it possible to separate the bacteria (FIG. 1) on plates according to the formulation of KING, WARD and RANEY (2), *viz.:*

Proteose Peptone nr. 3 (Difco)	2.0%
Bacto Agar (Difco)	1.5%
Glycerol C.P.	1.0%
K_2HPO_4. anh.	0.15%
$MgSO_4$.7 aq.	0.15%
pH 7.2	

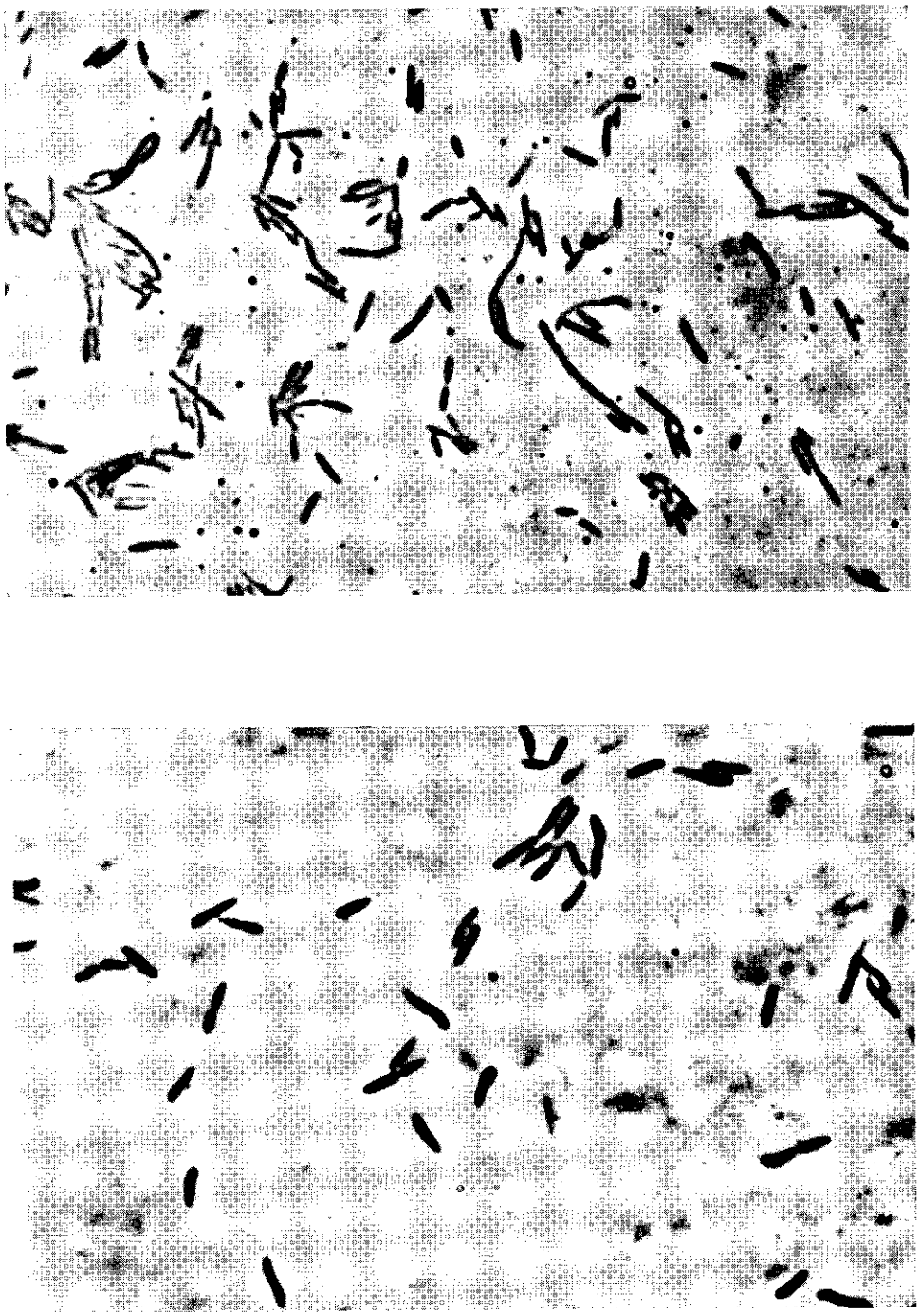

FIG. 1. The two types of bacteria from *Allolobophora caliginosa*. On uric acid agar plates they normally form mixed colonies. Here separated on King's medium.
Separation and photograph: I.T.B.O.N., Arnhem (phase contrast, c. 1800 ×).

Apart from uric acid, a number of nitrogenous compounds have been tried. Allantoine proved to be at least as good as uric acid for bacteria originating from a number of red pigmented earthworm species *(Lumbricus terrestris, L. rubellus, Eisenia foetida, Dendrobaena rubida)*, but less good for those from the non-red pigmented species *Octolasium cyaneum, Allolobophora longa, A. caliginosa* and *A. chlorotica*. The same holds for glycine. Growth on alloxan, parabanic acid and formamide was poor.

Urea can be used as a source of nitrogen in the presence of glucose. As in cultures on uric acid, urea is formed, addition of glucose to the uric acid plates gives a much more profuse growth of the colonies.

So far one of the most interesting observations seems that the nephridial bacteria are very sensitive to physiological drought. At concentrations of over 0.16 osmolar they do not grow at all. It can hardly be coincidental that this for bacteria surprisingly low value equals the osmotic value of the body fluid of earthworms reported by ADOLPH (1).

DISCUSSION

The arguments by which KNOP (3) assumed that the relation between earthworms and bacteria should be a symbiotic one, need not be repeated here. Most of them are still valid, but more can be said as to the character of this supposed symbiosis.

In all cases where uric acid is produced by animals as one of the most important end-products of nitrogen metabolism, the loss of extra energy, compared to the formation of urea, seems to be counterbalanced by the possibility to economize on water. This, however, suggests an adaptation of the excretory organs, which must be able to excrete the uric acid in crystalline form, like in birds, reptiles and insects.

Because the water economy is one of the most vital problems in terrestrial *Oligochaeta*, excretion of insoluble metabolic end-products would be very important.

However, in the earthworms, uric-acid crystals are formed in the coelomic cavity and they cannot, at least do not, pass through the nephridia, but are deposited in special cells in the coelomic wall, mainly around the nephridia (8). The latter are lined with bacteria which are now known to be able to transform uric acid into soluble products even at relatively great distances from their colonies, presumably by enzymatic action. These soluble products are excreted by the nephridia, pass along the bacteria which undoubtedly metabolize them partly at least; the remains, together with the metabolites of the bacteria, can be found in the 'urine' of the earthworms.

This rather complicated process would not be of any use to the water-economy of the earthworm but for the fact that the bacteria stop their activity at a relative humidity, comparable with the osmotic value of the body fluid of the worms.

So long as the worms are able to absorb water through the body wall and to excrete the excess, the bacteria will make soluble products out of the uric acid present. If no excess water is excreted by the nephridia, the uric-acid crystals will remain in the 'uric-acid cells'.

One of the aspects of the symbiosis between earthworms and their nephridial bacteria seems to be that they promote the water economy in the earthworm. The presence of bacteria in the nephridia is therefore considered an adaptation of *Oligochaeta* to terrestrial life.

Another advantage of the symbiosis to the worms seems to be the protection of the food reserve in the egg cocoons.

Much remains to be studied on the relationship between earthworms and bacteria, especially whether the presence of two bacteria species is essential and, if so, the interrelation of these species, the specificity of the bacteria etc.

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