

NN8201

no 384

C

ORGANIC SALTS IN PLANTS IN RELATION  
TO NUTRITION AND GROWTH

J. W. VAN TUIL

NN08201.384

WETENSCAPEN  
DER  
LANDBOUWHOGESCHOOL  
WAGENINGEN

H. D. W. van Tuil

# Organic salts in plants in relation to nutrition and growth

## PROEFSCHRIFT

ter verkrijging van de graad van doctor in de landbouwkunde  
op gezag van de Rector Magnificus, Ir. W.F. Eijvoogel,  
hoogleraar in de hydraulica, de bevoeiing, de weg- en waterbouwkunde  
en de bosbouwarchitectuur,  
te verdedigen tegen de bedenkingen van een commissie uit de Senaat  
van de Landbouwhogeschool te Wageningen  
op woensdag 9 juni 1965 te 16 uur.



1965 *Centrum voor landbouwpublikaties en landbouwdocumentatie*  
*Wageningen*

## STELLINGEN

### I

De grootte van het organisch zoutgehalte in populiereblad is mede bepalend voor de mate waarin kaligiften necroseverschijnselen kunnen opheffen.

### II

Dat bemesting van oudere populieren met voldoende kalium resulteert in een geringere ontvankelijkheid voor *Marssonina*, berust op een voldoende afvoer van assimilaten.

### III

De in aanwezigheid van een grotere chlooraccumulatie verhoogde opname van kationen door de plant kan niet als luxeconsumptie worden aangemerkt.

A. BUCHNER, *Z. Pflanzenern., Dung., Bodenk.* 54 (1951) 28

### IV

Aan de uitspraken van BRANTSEG, HOLMES, LAATSCH en WEHRMANN omtrent produktieverhoging van oudere naaldhoutopstanden met behulp van kunstmest wordt in Nederland onvoldoende aandacht besteed.

A. BRANTSEG, *Medd. fra Det Norske Skogforsok* (1962) 243-288, 1962

G. D. HOLMES, Supplement to Forestry, Report of the Third Discussion Meeting Oxford, March 21 to 23, 1963

W. LAATSCH, BVL Verlagsgesellschaft München-Basel-Wien-1963, 1-75

J. WEHRMANN, *For. Cbl.* 78 (1959) 129-149

### V

Wanneer het natrium- en kaliumgehalte van suikerbieten mede worden betrokken in de kwaliteitsbeoordeling door de fabrikant, zal de hieruit voortvloeiende extra zorg voor de teelt zodanig moeten worden gehonoreerd, dat ook de landbouwer profijt trekt van de lagere produktiekosten in de fabriek.

### VI

Een subsidie aan het particuliere bosbezit kan beter worden vervangen door een premieregeling die produktieverhoging stimuleert.

## VII

De kosten verbonden aan de afrastering van jonge opstanden mogen niet vallen onder cultuurkosten doch zullen moeten worden bestreden uit recreatie- en jachtfondsen.

## VIII

De Vereniging tot Behoud van Natuurmonumenten dient de recreatiemogelijkheden voor Amsterdam te verruimen door het Naardermeer tegen een redelijke vergoeding open te stellen voor sportvisserij.

## IX

De voorsprong, welke het nationale park "de Hoge Veluwe" op het gebied van de recreatie bezit, zal verminderen naarmate in de omliggende staatsbossen een grotere wilddichtheid wordt verkregen.

## X

De functie, welke de huisarts in de maatschappij vervult, vraagt niet alleen een vaktechnische opleiding. Aan het dragen van een grote verantwoording moet, mede in het belang van het voortbestaan van dit beroep, tijdens de studie aandacht worden besteed.

## XI

De grondslag voor het Schotse nationalisme werd niet gelegd door MARIA STUART, maar dateert van 27 maart 1306 met de kroning te Scone van ROBERT BRUCE tot KONING ROBERT I.

## XII

Hoewel artikel 63 van het Algemeen Rijksambtenaren Reglement niet in strijd is met artikel 53, lid 1 van dit reglement, kan door toepassing van dit artikel aan de ambtenaar financiële schade worden toegebracht.

## Voorwoord

Vele factoren hebben het resultaat van het onderzoek dat uitmondt in dit geschrift beïnvloed. Naast opvoeding en opleiding zijn de omstandigheden waaronder dit onderzoek heeft kunnen plaatsvinden, van belang geweest. Gaarne wil ik allen die hieraan bijgedragen hebben, dank zeggen.

De hogeschoolgemeenschap heeft, naast haar docerende taak, gelukkig veelal tevens opengestaan voor de ontwikkelingsproblemen van jonge mensen. In deze gemeenschap heeft voor mij het studentenleven op „Ceres” een speciale plaats ingenomen. Al deze factoren hebben ertoe bijgedragen dat ik met veel genoegen aan dit proefschrift heb kunnen werken.

## **Acknowledgements**

The work described was carried out as part of the research programme of the Institute for Biological and Chemical Research of Field Crops and Herbage at Wageningen, the Netherlands.

The author wishes to acknowledge his gratitude to Professor Dr. Ir. G. J. VERVELDE, director of this Institute and to the members of the staff, in particular Dr. W. DIJKSHOORN, for the helpful cooperation.

He also wishes to express his gratitude to the 'Commissie van Bijstand inzake het Stikstofonderzoek' and to the 'Commissie van Bijstand inzake het Kalionderzoek' whose support made his participation in this research programme possible.

The author is much indebted to his teacher, Professor Dr. A. C. SCHUFFELEN of the Agricultural University, for the discussions and constructive criticism during the progress of the work.

# Contents

INTRODUCTORY. . . . .	1
1 COMPOSITION OF THE IONIC BALANCE . . . . .	3
1.1 Earlier evidence on the accumulation of organic salts in dependence on nutrition. . . . .	3
1.2 Uptake from single salts by isolated tissues . . . . .	4
1.3 Uptake, accumulation and utilization of mixed salts by growing plants . . . .	6
1.4 Some examples of the accumulation of organic salts in the leaves of intact plants . . . . .	9
1.5 Internal distribution of absorbed salts. . . . .	13
1.6 Specific properties of potassium . . . . .	17
2 PERENNIAL RYEGRASS. . . . .	21
2.1 The normal organic-salt content. . . . .	21
2.2 Further considerations on the relation between organic-salt content and growth . . . . .	26
2.3 Chromatographic analysis of the organic anions . . . . .	33
2.4 Changes in the individual organic anions . . . . .	38
3 SUGAR BEET . . . . .	41
3.1 Some characteristics of the foliage . . . . .	41
3.2 The normal organic-salt content . . . . .	42
3.3 The organic salts of sugar-beet foliage. . . . .	48
4 POPLAR . . . . .	51
4.1 Growth of crops and leaves . . . . .	51
4.2 The normal organic-salt content . . . . .	54
4.3 Effects of poor fertility . . . . .	56
4.4 Effects of ammonium and nitrate at varying pH of the soil . . . . .	60
4.5 The organic salts of poplar leaves. . . . .	63
5 BIRCH . . . . .	65
5.1 Plants sensitive to nitrate nutrition . . . . .	65
5.2 The normal organic-salt content . . . . .	66
5.3 The effect of ammonium and nitrate fertilization . . . . .	67

CONCLUSIONS AND SUMMARY. . . . .	71
SAMENVATTING . . . . .	77
LITERATURE . . . . .	80



## Introductory

Investigations on the accumulation of salt ions in plants have shown that a part of the inorganic ions absorbed from the soil accumulates in the form of inorganic salts, and the remainder in the form of salts of the organic acids produced by the metabolism.

It has been demonstrated that for optimal growth a so called 'normal organic-salt content' is required. Its value appeared to be characteristic of the plant species (DE WIT *et al.*, 1963).

The purpose of the present investigations is to apply this concept to a few plant species.

Perennial ryegrass (*Lolium perenne* L.) has been reinvestigated on a few characteristics of ion accumulation. A second herbaceous crop, sugar beet (*Beta vulgaris* L., cv. Polykuhn) was chosen as a plant species with a much higher level of organic-salt accumulation in the foliage.

To study accumulation in leaves of trees, poplar (*Populus canadensis* MÖNCH cv. Zeeland) was investigated.

As an example of a plant species of more common occurrence on acid leached soils, use was made of small birch trees (*Betula verrucosa* E.). According to INGESTAD (1962) birch grows better with ammonium instead of nitrate.

All experiments were performed under greenhouse conditions and, if necessary, artificial light was applied from high-pressure mercury vapour lamps (Philips, HPLR) to increase day-length. The technique of pot and nutrient solution experiments are those given by SAID (1959). All the basic data are listed in the corresponding tables.

## 1 Composition of the ionic balance

### 1.1 Earlier evidence on the accumulation of organic salts in dependence on nutrition

The presence of organic anions in plants and their function as a complement to the electrostatic balance of accumulation of inorganic-salt ions absorbed from the soil has been considered since the 19th century (PFEFFER, 1881). The presence of oxalate, malate, citrate, etc. as components of plants was established and the total amount of organic salts was often estimated by the ash-alkalinity.

The ash-alkalinity is the number of equivalents of carbonate found after dry-ashing of the plant material. Since at dry-ashing nitrate is combusted and organic sulphur is partially converted into sulphate, the figure obtained may differ from the organic-salt content of the plant material. If nitrate is low, the ash-alkalinity may very nearly represent the organic-salt content of the sample. Therefore, data on ash-alkalinity can sometimes be applied to estimate the magnitude and variation of the total organic-salt content in plants.

One of the earlier studies of organic-salt formation in relation to the salts supplied is that of WEHMER (1891) on the production of oxalate by *Aspergillus niger*. He showed that oxalate is only produced if the medium is alkaline, or made alkaline by the metabolism when albumoses are added and subsequently converted to ammonia. Oxalate is also formed when nitrate is supplied which during utilization is replaced by oxalate. If ammonium is supplied the medium becomes more acid through utilization of the  $\text{NH}_4^+$  ions which liberates  $\text{H}^+$ , and oxalate is not formed. STOHMANN (1864) showed that the ash of maize contained more carbonate if the plants were supplied with potassium nitrate instead of ammonium nitrate. BENECKE (1903) found that the carbonate content of the ash declines in the following order of nitrogenous salts supplied to the plant: potassium nitrate, ammonium nitrate, ammonium sulphate. He also observed that ammonium salts reduce the formation of calcium oxalate crystals in the tissues, and that these crystals reappear if the medium is rendered more alkaline by the addition of magnesium carbonate.

From this BENECKE concludes that the change in the organic-salt content, resulting from replacement of nitrate by ammonium in the supply, does not originate from the different pathways of their conversion into organic nitrogen, but from the difference in the amounts of unmetabolized salt cations accumulated in the tissues.

This agrees with the older statements of PFEFFER (1881) on the regulation of the

organic-salt content by ion accumulation. He postulates that: 'die Prozesse in denen Basen disponibel werden, sogleich selbstregulierend sind, indem sie Veranlassung zur Entstehung von Säuren geben'.

One of the earlier communications dealing with variations in the sap-soluble organic salts, resulting from variations in the amount and form of the salts supplied, is that of BÖNING and BÖNING-SEUBERT (1932). They showed that the carbonate system is of no importance in tobacco leaves, but that the excess of salt cations over inorganic-salt anions is balanced by organic anions. If ammonium was supplied instead of nitrate the amount of soluble organic salts was considerably lower. If calcium was supplied instead of potassium, the plants developed potassium deficiency and an increased organic-salt content. Older leaves were found to have a higher organic-salt content than younger leaves. These findings were recently confirmed by CHOUTEAU (1960) and VICKERY (1961).

From these and many other data it appears that evidence for the dependence of the organic-salt content on the salt supply has been available since the beginning of this century. However, its direct application to plant nutrition is comparatively recent.

## 1.2 Uptake from single salts by isolated tissues

The results of MEURER (1909) and NATHANSON (1904) on storage tissues have already shown that the two ions of a salt may be absorbed at different rates. According to THOMAS (1958) preferential absorption of salt cations or salt anions involves electrostatic balancing by the passage in or out of the tissue of either  $H^+$  or  $OH^-$ , originating from the dissociation of water.

For the present discussion it is supposed that excess salt-cation uptake is associated with an equivalent uptake of  $OH^-$ , and excess salt-anion uptake with an equivalent uptake of  $H^+$ . This is illustrated by the diagram of fig. 1. From a solution of potassium

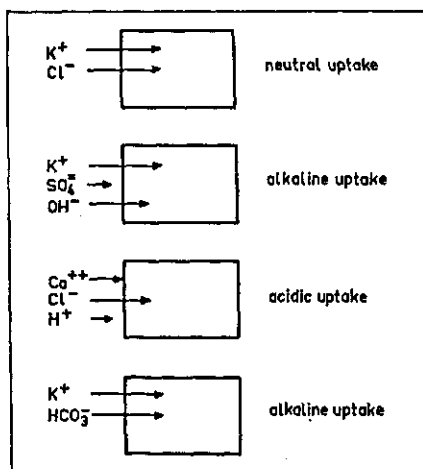


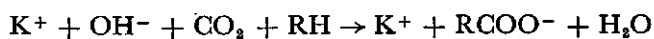
Fig. 1. Diagrammatic representation of differential uptake of salt ions from single salt solutions

chloride  $K^+$  and  $Cl^-$  are considered to be absorbed at equal rates. Consequently, there is no gain or loss of either  $H^+$  or  $OH^-$  in the tissue. Exposed to a solution of potassium sulphate,  $K^+$  enters the tissue in a much greater quantity than  $SO_4^{=}$ .  $OH^-$  enters the tissue in an amount equal to the excess of  $K^+$  absorbed and the uptake is alkaline. If calcium chloride is supplied,  $Cl^-$  is absorbed in much greater quantities than  $Ca^{++}$ . The excess of salt-anion uptake is accompanied by its equivalent of  $H^+$ , and consequently the uptake is acidic.

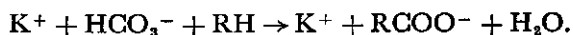
If a tissue is submerged in a solution of potassium bicarbonate, the  $K$ -ions and the  $HCO_3^-$ -ions are considered to be absorbed at the same rate. This is because any excess of  $K^+$  over  $HCO_3^-$  requires its equivalent  $OH^-$ . Since  $OH^-$  is transformed into  $HCO_3^-$  through the presence of respiratory carbon dioxide, there is no possibility to distinguish between these ions. Therefore, introduction of  $OH^-$  as the partner ion in salt-cation uptake is formal. Under common conditions of pH and carbondioxide concentrations the partner anion is more likely to be  $HCO_3^-$ . Any excess uptake of  $HCO_3^-$  from potassium bicarbonate has no significance, because its uptake proceeds with concurrent uptake of  $H^+$ . Thus the uptake of bicarbonate is alkaline to an extent given by the number of equivalents of the salt cation absorbed.

The amounts of  $H^+$  and  $OH^-$  ( $HCO_3^-$ ) involved in the differential uptake of cations and anions from a salt solution are often large compared with the buffer capacity of the plant tissues in the range of pH 5 to pH 7. To maintain tissue pH at its normal value, synthesis or breakdown of organic salts occurs as a direct response to the uptake of excess salt cations or excess of salt anions (BÖNING and BÖNING-SEUBERT, 1932; BURSTRÖM, 1945).

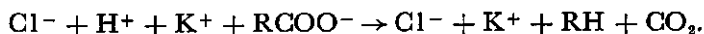
It is often assumed that salt anions and salt cations are absorbed independently of each other (LUNDEGARDH and BURSTRÖM, 1933). The accumulation can be considered in terms of changes in the organic-salt content by decarboxylation and carboxylation (DIJKSHOORN, 1964). A simplified equation for the accumulation of salt cations can be written as:



or



The accumulation of salt anions can be written as:



In this way salt cations accumulate in the tissues as organic salts and salt anions as inorganic salts instead of the organic salts initially present. This concept accounts for the experimental evidence that the differential uptake, salt cations minus salt anions, if expressed as ion equivalents, is numerically equal to the organic-salt content.

As an example of experimental evidence some results of ULRICH (1941) are summarized in fig. 2. In his experiments excised barley roots were kept in single salt solutions for 8 hours and the sap was analysed for inorganic - and organic - salt ions. The organic anions were determined by ether extraction of the acidified sap and subsequent titration.

The differential uptake, salt cations minus salt anions, varied from  $-20$  to  $+55$  me., depending on the salt supplied. Although the buffer capacity of the juice was only 25 me. per liter per unit pH in the range of pH 5 to 6, juice pH remained within the range of pH 5.2 to 5.8. The other graph shows the compensating change in the organic anions, balanced by the differential uptake.

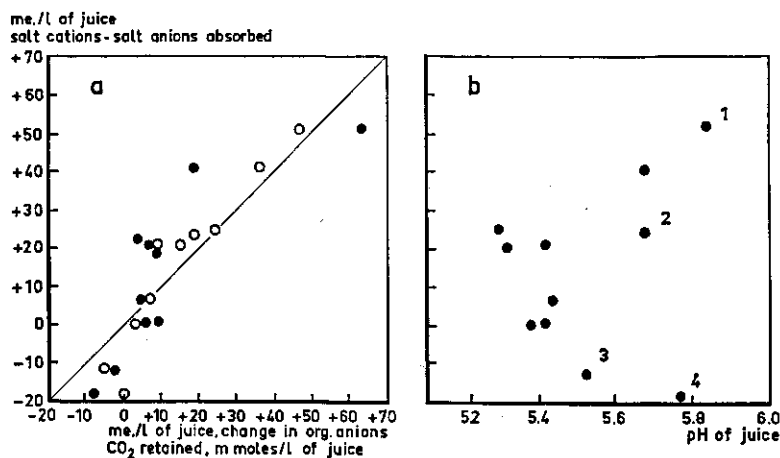


Fig. 2. Differential uptake of salt ions by excised barley roots from solutions of different salts; 1: potassium bicarbonate; 2: potassium nitrate; 3: calcium bromide; 4: calcium nitrate, and other salts not specified here.

a: in relation to the change of the organic-anion content of the juice (open dots) and to the deficit of carbon dioxide evolved with respect to the oxygen consumed (carbon dioxide retention, filled dots).

b: in relation to the pH of the juice.

Data of ULRICH (1941)

Respiration measurements showed that at equal uptake of salt cations and salt anions the carbon dioxide produced equals the oxygen consumed. In the case of differential uptake the deficit of the carbondioxide evolved parallels the excess of salt cations absorbed, indicating carbon dioxide retention for the synthesis of organic salts.

### 1.3 Uptake, accumulation and utilization of mixed salts by growing plants

Differential uptake of salt cations and salt anions from a mixture of nutrient salts by growing plants is of common occurrence. STOKLASA and ERNEST (1908) found that gramineous plants absorb salt anions in excess of salt cations, whereas in potatoes and beets salt-cation exceeds salt-anion uptake. In legumes salt-cation equals salt-anion uptake. These cases refer to ample supply with nitrate and other nutrient salts. The acidic uptake of gramineous plants is responsible for the external alkaline effect of

nitrate nutrition. Other plant species may fail to show this alkaline effect of nitrate nutrition.

For plants grown in soil an inventory of the uptake of cations is obtained by data on the uptake of  $\text{NH}_4$ , K, Na, Mg and Ca. Other salt cations such as Fe, Mn, Zn and Cu are absorbed in very small quantities (fig. 3) compared with the total uptake of 1000 and more mg equivalents of salt cations per kilogram dry material. The equivalent sum of  $\text{NH}_4^+ + \text{K}^+ + \text{Na}^+ + \text{Mg}^{++} + \text{Ca}^{++}$  is further denoted by  $C_u$ .

An inventory of the salt anions absorbed is obtained by analyses for Cl, P, S and total N. Since the pH of the plant tissues is usually about 5 to 6, phosphate enters the plant mainly as  $\text{H}_2\text{PO}_4^-$  independent of soil pH. Any uptake of  $\text{HPO}_4^{=}$  should be considered as the uptake of  $\text{HPO}_4^{=} + \text{H}^+$  and is consequently recorded as  $\text{H}_2\text{PO}_4^-$ . The sum of the equivalents of the anions  $\text{Cl}^- + \text{H}_2\text{PO}_4^- + \text{SO}_4^{=} + \text{NO}_3^-$  represents the total uptake of salt anions and is denoted by  $A_u$ .

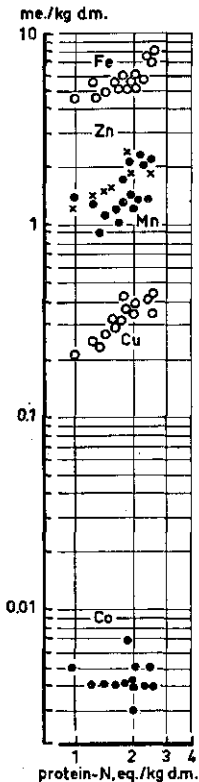


Fig. 3. Some trace elements in perennial ryegrass of varying age and protein content

The difference  $(C_u - A_u)$ , if positive, indicates an alkaline uptake, if negative an acidic uptake, and the alkalinity or acidity is numerically equal to  $(C_u - A_u)$ .

ARNON (1939) analysed whole plants of barley fed with either nitrate or ammonium

as the source of nitrogen in the nutrient solutions. From his data it appears that with nitrate the acidity of the uptake is about 1 equivalent per kilogram dry material. With ammonium the alkalinity of the uptake is about 5 equivalents per kilogram dry material. If the salts would merely accumulate, the considerations of the previous section would predict a much higher organic-salt content in the case of ammonium nutrition. However, it has invariably been found that substitution of ammonium for nitrate in the supply leads to a distinct fall in the organic-salt content of growing plants (section 1.4). It is clear that utilization interferes in the establishment of the final balance of salt accumulation.

By means of ionic equations DIJKSHOORN (1964) demonstrates that the conversion of  $\text{NH}_4^+$ , absorbed together with  $\text{OH}^-$ , proceeds without a change in the total organic-salt content. Also the conversion of nitrate and sulphate together with the  $\text{H}^+$  absorbed, takes place without gain or loss of organic salts. The partial conversion of phosphates into organic phosphates proceeds by esterification and causes no change in the ionic state of phosphate.

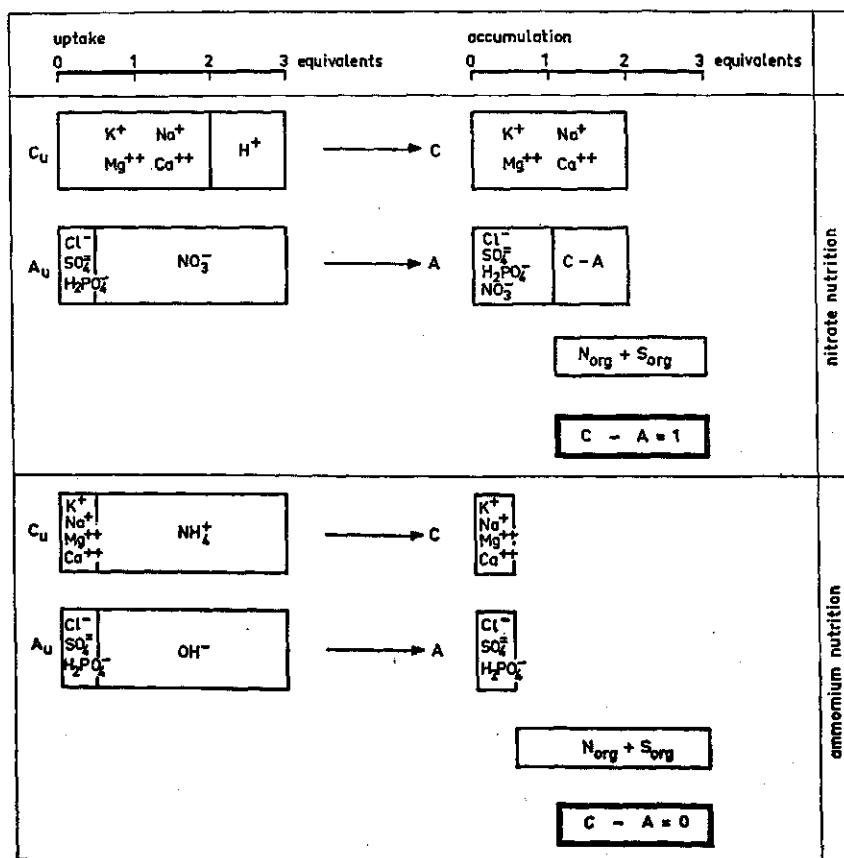


Fig 4. Diagrammatic representation of the uptake, the metabolic utilization and the accumulation of salt ions from a mixture of nutrient salts with nitrate or with ammonium as the source of nitrogen

Since in most plants the ammonium absorbed is completely metabolized without accumulation of organic ammonium salts, ammonium nutrition does not result in a higher organic-salt content. The lower content actually found is caused by a decreased accumulation of the unmetabolized salt cations K, Na, Mg and Ca. The equivalent sum of these cations, the ionic state of which remains unaltered, is denoted by C.

With an adequate supply of nitrate and sulphate the amounts absorbed are only partly converted into organic nitrogen and organic sulphur. The remainder accumulates in the unaltered ionic state as nitrate and sulphate anions. Together with  $\text{Cl}^-$  and  $\text{H}_2\text{PO}_4^-$  the inorganic anions accumulate as inorganic salts. According to section 1.2 these inorganic salts of  $\text{Cl}^-$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{SO}_4^{2-}$  and  $\text{NO}_3^-$  accumulate instead of the organic salts. The equivalent sum of these inorganic salts is further denoted by A.

If the pH of the tissues remains within the range of pH 5 to pH 7, all these ions are present as salts because free acids are practically absent.

Consequently, accumulation in the plant results in a total-salt content C, an inorganic-salt content A and an organic-salt content ( $C - A$ ). By including total P in the inorganic-salt content A, the organic-salt content ( $C - A$ ) refers to the salts of carboxylic acids only.

Usually analytical data include figures for total N, nitrate N and total S. Organic N is obtained by the subtraction of nitrate N from total N. Organic S is calculated from the relation: organic S =  $0.054 \times \text{organic N}$  (DIJKSHOORN *et al.*, 1960). The inorganic-sulphate content is obtained by subtracting organic S from total S.

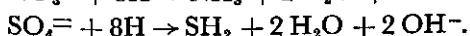
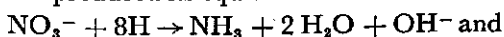
The complete balance of uptake, utilization and accumulation of the inorganic and organic salts is summarized in fig. 4. The numerical values are comparable to those found in perennial ryegrass (DIJKSHOORN, 1963) and in barley (ARNON, 1939). In the case of ammonium nutrition the balance is supposed to operate without accumulation of organic salts. Actually ( $C - A$ ) remains greater than zero.

#### 1.4 Some examples of the accumulation of organic salts in the leaves of intact plants

Results of COIR *et al.* (1961) on leaves of maize plants grown with nitrate or ammonium in the nutrient solutions were used to compose table 1. COIR's records do not include data on nitrate in the leaves. It is assumed that the nitrate content was negligible so that the organic nitrogen equals total nitrogen. Organic sulphur was calculated from the data for organic nitrogen with the proportionality factor mentioned in section 1.3.

When nitrate was supplied the salt anions absorbed ( $A_u$ ) exceeded the salt cations absorbed ( $C_u$ ) by 2346 mc./kg d.m. and the excess equals the acidity of the uptake, ( $C_u - A_u$ ) < 0.

Organic nitrogen plus organic sulphur equals 3436 mc./kg d.m. and their conversion has produced its equivalent of  $\text{OH}^-$  according to the ionic equations:







absorbed is 3596 me./kg d.m. and is listed as an uptake of  $\text{OH}^-$ , representing the alkalinity of the uptake. The conversion of  $\text{NH}_4^+$  into organic nitrogen proceeds to completion in maize. Its utilization is listed under the cations and corresponds to the ionic equation:  $\text{NH}_4^+ \rightarrow \text{NH}_3 + \text{H}^+$ , the  $\text{NH}_3$  being incorporated into the organic-nitrogen compounds and its equivalent of  $\text{H}^+$  released by the metabolism. In addition, 177 me. of  $\text{OH}^-$  are produced by the conversion of sulphate into organic sulphur. The conversion of  $\text{NH}_4^+$  and  $\text{SO}_4^{=}$  liberates  $3270 - 177 = 3093$  me. of  $\text{H}^+$  per kg d.m. The alkalinity of the uptake is 3596 me./kg d.m. Therefore, uptake and conversion results in a final accumulation of 503 me. of  $\text{OH}^-$  per kg dry material. This quantity equals the organic-salt content.

The balance of accumulation shows the presence of 1777 me./kg d.m. of unmetabolized salt cations (C) in the nitrate plants, and of 1362 me./kg d.m. in the ammonium plants. The lower organic-salt content (C—A) of the ammonium fed plants is mainly caused by the reduced accumulation of the unmetabolized salt cations (C).

The last column shows the total amount of organic anions, recovered by extraction and chromatography of their water-soluble acids. In the case of nitrate fed plants the recovery is about 80 per cent of the total organic salts (C—A). The deficit of about 200 me./kg d.m. in the nitrate and ammonium plants probably consists mainly of insoluble polyuronic acids.

COOL (1948) has given detailed records on the constitution of the ionic balance in the leaves of guayule (*Parthenium argentatum*). The plants were grown at three potassium levels with nitrate as the source of nitrogen. Mature and expanding leaves were analysed for the main elements and for organic anions.

Some of his results are listed in table 2.

In the mature leaves the balance of uptake shows an excess of salt cations over salt anions which is balanced by  $\text{OH}^-$ , and consequently the uptake is alkaline. This should be added to the alkalinity produced during the metabolism of nitrate and sulphate. The total alkalinity obtained is transformed into organic salts and equals the organic-salt content (C—A), calculated from the balance of accumulation of the unmetabolized inorganic ions. If the organic-salt content exceeds the sum of organic nitrogen and organic sulphur, the balance of uptake is alkaline.

The expanding leaves have absorbed salt anions in excess over salt cations and the balance of uptake is acidic. To obtain the internal alkalinity transformed into organic salts the acidity of the uptake should be subtracted from the alkalinity produced during metabolism of nitrate and sulphate. Owing to the acidity of the uptake the organic-salt content is lower than the sum of organic nitrogen and organic sulphur.

From analytical data on leaves of different stages of maturity it can often be inferred that older leaves have an alkaline uptake, while younger leaves have a more acidic uptake. For instance, the data obtained by VICKERY (1961) on tobacco indicate that in the bottom leaves the organic-salt content is higher, and in the top leaves lower than the organic-nitrogen plus organic-sulphur content.

It should be borne in mind that the balance of uptake, based on single leaves instead of whole plants, does not mean that all the elements involved have entered the leaf

in the ionic form supplied to the plant. Intake of nitrogen and sulphur from other plant parts may have occurred to a large extent in the form of organic nitrogen and

Table 2. Balance-sheets of mature and of expanding leaves of guayule (*Parthenium argentatum* GRAY), grown at two potassium levels with nitrate as the source of nitrogen. Data of COOIL (1948). All values in me./kg dry matter

Leaf type and treatment	Uptake				Utilization				Accumulation			
	anions		cations		anions		cations		anions		cations	
mature K <sub>1</sub>	N	2940	K	360	N <sub>0</sub>	2500			NO <sub>3</sub>	440		
	Cl	—	Na	20					Cl	—		
	P	164	Mg	960					H <sub>2</sub> PO <sub>4</sub>	164		
	S	1320	Ca	3590	S <sub>0</sub>	135			SO <sub>4</sub>	1185		
	A <sub>u</sub>	4424	C <sub>u</sub>	4930	OH <sup>-</sup>	2635			A	1789	C	4930
	OH <sup>-</sup>	506			OH <sup>-</sup>	506			inorg. salts			1789
						3141			org. salts			3141
mature K <sub>3</sub>	N	2615	K	2560	N <sub>0</sub>	1995			NO <sub>3</sub>	620		
	Cl	—	Na	0					Cl	—		
	P	108	Mg	420					H <sub>2</sub> PO <sub>4</sub>	108		
	S	1760	Ca	1620	S <sub>0</sub>	108			SO <sub>4</sub>	1652		
	A <sub>u</sub>	4483	C <sub>u</sub>	4600	OH <sup>-</sup>	2103			A	2380	C	4600
	OH <sup>-</sup>	117			OH <sup>-</sup>	117			inorg. salts			2380
						2220			org. salts			2220
expanding K <sub>1</sub>	N	3680	K	380	N <sub>0</sub>	3170			NO <sub>3</sub>	510		
	Cl	—	Na	10					Cl	—		
	P	189	Mg	840					H <sub>2</sub> PO <sub>4</sub>	189		
	S	1200	Ca	2890	S <sub>0</sub>	171			SO <sub>4</sub>	1029		
	A <sub>u</sub>	5069	C <sub>u</sub>	4120	OH <sup>-</sup>	3341			A	1728	C	4120
			H <sup>+</sup>	949	H <sup>+</sup>	949			inorg. salts			1728
						2392			org. salts			2392
expanding K <sub>3</sub>	N	3170	K	2300	N <sub>0</sub>	2500			NO <sub>3</sub>	670		
	Cl	—	Na	20					Cl	—		
	P	159	Mg	440					H <sub>2</sub> PO <sub>4</sub>	159		
	S	1430	Ca	1220	S <sub>0</sub>	135			SO <sub>4</sub>	1295		
	A <sub>u</sub>	4759	C <sub>u</sub>	3980	OH <sup>-</sup>	2635			A	2124	C	3980
			H <sup>+</sup>	779	H <sup>+</sup>	779			inorg. salts			2124
						1856			org. salts			1856

organic sulphur. This intake is recorded in the balance as an uptake of  $\text{H}^+ + \text{NO}_3^-$ , and of  $2\text{H}^+ + \text{SO}_4^{2-}$ .

COOL's data on whole plants, harvested in an earlier stage of growth show that in this case the salt cations and salt anions supplied have been absorbed in nearly equal quantities, indicating the absence of alkalinity or acidity of the uptake.

Another difference between older and younger leaves is the higher level of calcium accumulation in the older leaves, and the more even distribution of potassium.

## 1.5 Internal distribution of absorbed salts

In the preceeding section it has been shown that older leaves may differ from younger leaves through higher calcium and organic-salt contents in the dry material. Another example of the distribution of ionic constituents is obtained from data of COIC *et al.* (1962). In table 3 the contents of inorganic and organic ions in the leaves of tomato plants grown with either nitrate or ammonium as the source of nitrogen are given. These results show that when ammonium is supplied instead of nitrate, the total-salt content (C), and the organic-salt content (C—A) are considerably lower. Further, it is obvious that these content are higher in older leaves. Data on the individual cations show that the higher salt content of the older leaves is mainly due to calcium and to some extent also to magnesium. These salt cations show a distribu-

*Table 3. Balance of ion accumulation in the leaves of tomato, grown with nitrate or with ammonium as the source of nitrogen. Data of COIC et al. (1962). Nitrate and sodium were not determined. All values in me./kg dry matter. The 'organic anions found' represent me. of organic acids recovered by extraction after acidification*

Source of nitrogen	Young leaves		Old leaves	
	nitrate	ammonium	nitrate	ammonium
K	770	450	880	620
Na	—	—	—	—
Mg	530	100	930	290
Ca	1380	160	2700	400
C	2680	710	4510	1310
$\text{NO}_3$	—	—	—	—
Cl	190	230	240	220
$\text{H}_2\text{PO}_4$	180	270	140	380
$\text{SO}_4$	180	0	312	236
A	550	500	692	836
(C-A)	2130	210	3818	474
org. anions found	1370	40	2680	40

tion in favour of the older leaves wherein the organic salts accumulate in a greater proportion as organic salts of calcium and magnesium.

A variety of data on the effect on the stage of maturity and leaf position in different plant parts invariably reveals the same pattern of distribution of salt cations and organic salts. This is illustrated by data on the amount of dry material and the number of me. of salt cations in the leaves of citrus and of tobacco, calculated from the results of WALLACE *et al.* (1954) and of VICKERY (1961) (fig. 5).

Higher amounts of dry material and of the salt cations are found in the middle leaves. The points representing the number of me. of the elements scatter along loop

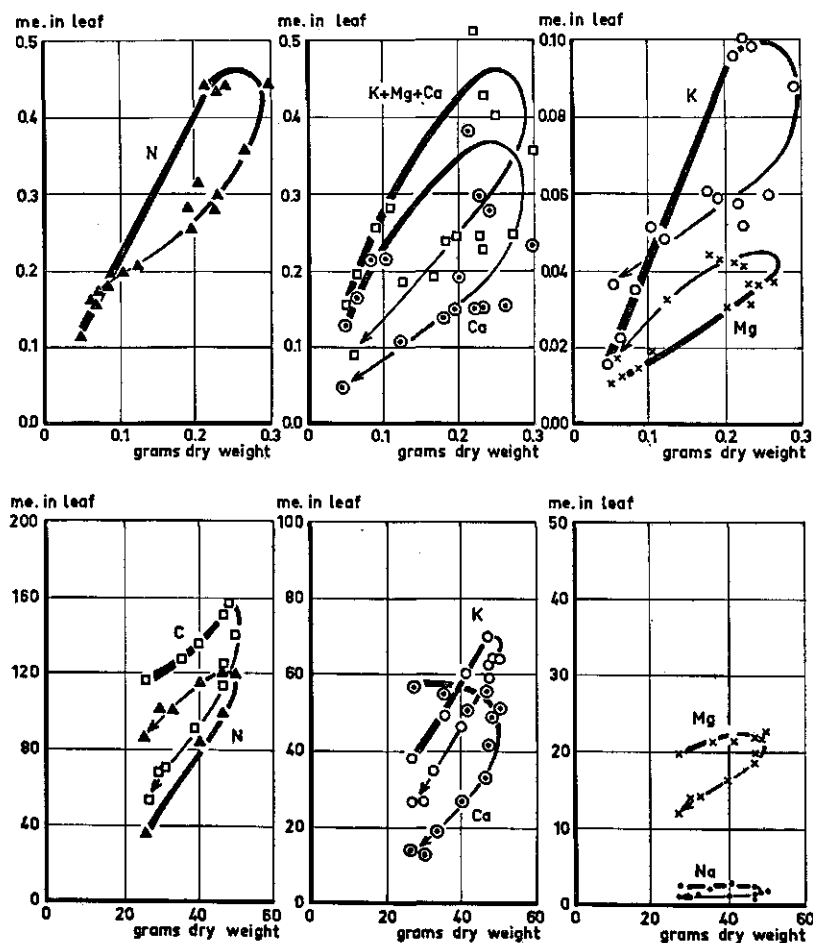


Fig. 5. Absolute amounts of salt cations and nitrogen in leaves in relation to the dry weights and the positions on the stalk.

Upper graphs: Citrus, data of WALLACE *et al.* (1954)

Lower graphs: tobacco, data of VICKERY (1961)

Decrease in thickness of the lines and direction of the arrows indicate transition from bottom to top leaves

shaped curves, which have been drawn with a decrease in thickness when passing from lower to upper leaves. In WALLACE's data sodium was not analysed but certainly low so that the sum of K, Ca and Mg practically equals C. The curves show that the total salt (C) accumulates to higher levels in the bottom leaves, when compared at the same amount of dry material. The contents, expressed in me./g d.m. can be read from the slopes of straight lines through the points and the origin.

It is clear that in citrus and in tobacco the total salt-cation content (C) increases from top to bottom leaves. It is known from other evidence, as e.g. from VICKERY's data on the organic anions, that the organic-salt content of the leaves increases in the same direction. In citrus the curve for total nitrogen indicates that the nitrogen content in the top leaves is about the same as in the bottom leaves. Between lower and upper laves there is a zone of leaves nearer to the top with a lower nitrogen content. In tobacco the top leaves are higher in nitrogen than the bottom leaves. Since both in citrus and in tobacco the bottom leaves have a higher salt-cation content (C) than the top leaves, there seems to be no relation between the distribution of the organic-salt content (C—A) and that of nitrogen. In citrus the distribution of potassium and nitrogen is very similar. In tobacco the distribution of potassium is the reverse of that of nitrogen.

The only possible generalization concerns the relative distribution of the total unmetabolized salt cations (C) and that of potassium and calcium. The distribution of magnesium differs in both species. From the shape of the curves it is clear that potassium tends to become more equally distributed since the width of the loop is small in tobacco and its ends meet in citrus. However, the calcium curves indicate a considerably increased accumulation of calcium in the bottom leaves, relative to the dry weight.

The evidence presented is related to dicotyledonous plants which often absorb calcium as readily as potassium. The selectivity of the gramineous plants exhibited during salt cation uptake is different and shows a rather slow uptake of calcium compared with potassium uptake (DRAKE, *et al*, 1951). However, from data of KEMP and DIJKSHOORN (1956) it can be inferred that the distribution of the salt cations between the upper, more mature part of the herbage and the less mature, expanding tissues follows the same pattern as in the dicotyledones. The ratio of the content in the lower half to that in the upper half for the different salt cations parallels the relative rate of distribution in the herbage. Table 4 represents the values obtained, together with ratio values for manganese, cobalt and copper from other data. The distribution follows the order:  $K = Na > Mg > Ca$ , and for the minor elements:  $Mn > Co > Cu$ .

Efforts were made to interpret the difference in distribution of the salt cations in terms of preferential ion adsorption by or association with immobile organic anions of the plant material. A similarity between the order of relative distribution and that of the affinity to carboxylates of plant origin was obtained by comparing the distribution ratio values with the concentrations of reversal of charge determined by electrophoresis (TEUNISSEN and BUNGENBERG DE JONG, 1938). The lower the concentration of reversal of charge, the higher the affinity to the ionic group of the plant

Table 4. Distribution ratios in perennial ryegrass, calculated from data on contents in the lower and upper half of herbage, given by KEMP and DIJKSHOORN (1956). Concentrations of reversal of charge of some plant colloids, taken from the electrophoretic measurements of TEUNISSEN and BUNGENBERG DE JONG (1938)

	Distribution ratio	log equivalents per liter at reversal of charge			
		soya lecithins		carboxyl colloids	
		I	II	arabinate	pectinate
K	1.22	0.85	0.70	0.50	0.00
Na	1.20	0.40	0.40	0.55	0.30
Mg	1.10		—0.60	0.20	0.15
Ca	0.80		—0.80	0.00	0.05
Mn	1.70	—1.70	—0.90	—0.10	0.00
Co	1.20	—1.20	—0.60	—0.20	—0.25
Zn		—1.30	—0.80	—0.30	—0.75
Cu	0.80	—1.50	—1.05	—0.90	—1.00

colloid. Therefore, the sequence obtained for the different salt cations depends on the type of the charged group carried by the plant colloid. The concentrations of reversal of charge for two lecithins and two carboxylates of plant origin are listed in table 4. It appears that the distribution order agrees with the order of reversal of charge for the carboxylate arabinate.

The relative distribution rate of the cations is found to be smaller if the affinity of the cation to the carboxylate is higher. This suggests that in cation distribution carboxylic groups interfere by ion association or adsorption, and in this way reduce the mobility of the salt cation.

Herbage may contain organic anions of carboxylate origin at about 1000 me. per kg dry material. About 90 per cent of this quantity is represented by anions of the water-soluble plant acids of lower molecular weight (section 2.5). Of the salt cations, calcium is present at 100 to 500 me./kg d.m. This means that if carboxylates are involved in cation distribution, these carboxylates of lower molecular weight should also interfere.

Therefore, the relative rate of distribution of the salt cations reflects the relative rate of distribution of their organic salts. This means that internal distribution of the organic potassium salts proceeds more easily than that of the organic salts of calcium.

The order obtained may depend on the type of carboxylates involved, as evident from the electrophoretic data on arabinate and pectinate, and in this way on the plant species.

The internal distribution should not be connected with solubility of organic salts, because with the occasional exception of calcium oxalate, the organic salts remain in solution in the plant juice. Moreover, plants in which oxalate cannot be detected also show retarded distribution of calcium, as was found for *Plantago lanceolata* (DIJKSHOORN, unpublished).

## 1.6 Specific properties of potassium

In section 1.3 it was pointed out that the salt cations K, Na, Mg, Ca accumulate in the form of organic and inorganic salts in plant tissues. In this respect they act in a similar way and serve the same purpose: a decrease of the acidity of the uptake by the plant during anion uptake and utilization, thus allowing the plant to accumulate organic salts instead of losing them through decarboxylation. Hence, replacement of potassium by, for instance, sodium should not affect the organic-salt content, provided that the plant is able to absorb sodium as readily as potassium.

Perennial ryegrass is able to absorb sodium in considerable amounts when replacement of potassium by sodium leads to potassium shortage. Therefore, substitution of sodium for potassium in the supply does not result in a reduction of the organic-salt content, as it does e.g. in maize, which is unable to absorb sodium in significant amounts even in the absence of potassium.

Actually, if sodium is supplied to potassium deficient perennial ryegrass, organic salts accumulate in the herbage in greater quantities than normal, mainly in the form of sodium salts. The origin of this effect can be demonstrated with data of VOSE (1963) on the effect of replacing potassium by sodium in the nutrient solutions. In fig. 6 the contents in the herbage are plotted against the contents in the roots. The arrows indicate decreasing potassium and increasing sodium in the supply. It appears that sodium increases at the expense of potassium to much higher levels in the herbage than in the roots. The direction of the curves indicates that replacement of potassium by sodium causes sodium to accumulate preferentially in the herbage, while replacement of sodium by potassium indicates preferential potassium accumulation in the roots.

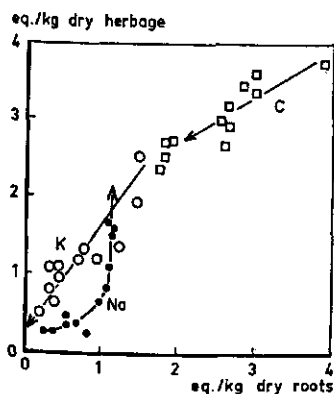


Fig. 6. Potassium, sodium and total salt (C) content of herbage and of roots of perennial ryegrass grown with varying ratios of potassium to sodium in the nutrient solutions. Data of VOSE (1963)

It is generally accepted that the mobility of sodium in the plant is smaller than that of potassium. Therefore, the preferential accumulation of sodium in herbage, and that of potassium in the roots can only be explained by the assumption that in the



absence of potassium sodium moves easily upwards but is hardly able to move downwards to the roots. This means that the accumulation of sodium in the absence of potassium leads to an accumulation of organic salts in the herbage in excess of the normal value found with an adequate potassium supply (DE WIT *et al.*, 1963).

In perennial ryegrass a potassium supply reduces the uptake of sodium by competition during absorption. The accumulation of organic potassium salts does not occur in excessive amounts, because the excess formed in the herbage is continuously removed by back-translocation to the roots. Any excess in the roots is presumably released to the medium as potassium bicarbonate, formed by decarboxylation of the organic potassium salts. This greater internal mobility should be considered as a specific property of potassium, to be connected with the specific potassium requirement. Other salt cations, even if absorbed in similar quantities, are unable to perform the specific functions of potassium, since their rate of internal circulation is insufficient.

It is known that at higher internal potassium levels a partial substitution of other salt cations for potassium does not affect the ionic balance and growth (SAID, 1959) as long as potassium remains present in sufficient amounts to perform its specific function. For further work it is useful to know the internal concentration below which potassium is unable to operate adequately. This can be inferred from data on the relation between potassium uptake and growth. An example referring to potassium uptake and growth of perennial ryegrass on a potassium deficient soil, is shown in fig. 7. The dry weight produced in ten successive cuts is plotted additively against the additive amounts of potassium absorbed by the herbage.

It appears that the low potassium plants produced their dry weight proportionally to the amount of potassium absorbed, which in turn, was determined by the release of available potassium from the soil. Apparently, the growth was controlled by the

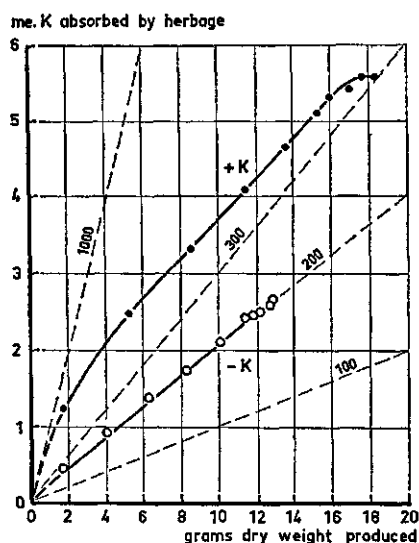


Fig. 7. Cumulative potassium uptake versus cumulative dry weight production by successive cuts of perennial ryegrass, grown on a soil low in potassium, with (+ K) and without (- K) potassium fertilizer. The dashed lines indicate constant potassium contents at four levels in me. per kg dry matter. Data of ARNOLD and CLOSE (1962)

potassium uptake. The slope of the line indicates that the specific potassium requirement is about 200 mc./kg d.m. The higher potassium plants showed higher yields, as evident from the larger abscissae intervals between the successive cuts. They also showed higher potassium contents, which ranged from 1000 to 300 mc./kg d.m.

Generally, all evidence points to the necessity of adequate potassium for maintenance of a normal organic-salt content. The effect of substituting other salt cations for potassium depends on their rate of uptake. If readily absorbed in the absence of potassium, the organic-salt content is increased through poor circulation (sodium in perennial ryegrass). If difficultly absorbed, the organic-salt content decreases through an increased acidic uptake (calcium in perennial ryegrass). Their rate of absorption depends on the plant species. For instance, in tobacco the replacement of potassium by calcium leads to an increased organic-salt content because calcium is readily absorbed.

From the literature on the distribution of salt cations over different plant parts and the relative rate of translocation to growing tissues, it can be inferred that in many species salt cations other than potassium are transported more difficultly to other plant parts. This more difficult transport is considered to proceed mainly in the phloem (MASON and MASKELL, 1931; BUKOVAC and WITTWER, 1957).

## 2 Perennial ryegrass

### 2.1 The normal organic-salt content

Perennial ryegrass has been studied extensively inside the Institute for Biological and Chemical Research on Field Crops and Herbage during previous years.

Calculations of the ionic balance showed that the herbage obtained from pot experiments at low chloride levels, contained about 1000 me. of organic salts per kg dry matter (DIJKSHOORN, 1962). It was found that this value tends to constancy at varying cationic composition, nitrogen content and stage of maturity (DE WIT *et al.*, 1963).

In Dutch pastures the mixed grass herbage occasionally contains 1000 me. of organic salts per kg dry weight, but more often the organic-salt content is lower owing to the presence of more chloride.

Observations on mixed pasture herbage grown in field experiments (VAN BURG, 1963) are summarized in figure 8. The chloride content varies between 200 and 600 me./kg d.m., which is above the chloride content in the herbage of the pot experiments mentioned before. In spite of a large scattering the points indicate that at the decrease of chloride the organic-salt content rises to a value of about 1000 me. Apparently, the presence of more chloride prevents the organic salts to accumulate at the level of 1000 me./kg d.m., which is normal for the foliage of perennial ryegrass.

Depending on the supply, chloride may vary substantially in grass. Its increased accumulation is associated with a considerable increase of the inorganic anions (A) and also of the unmetabolized salt cations (C) (DIJKSHOORN, 1958). However, it appears that the increase of the inorganic anions is not completely balanced by the increase of the salt cations, so that the organic-salt content ( $C - A$ ) drops to lower values. Thus, a large proportion of the chloride accumulates instead of organic salts.

It has been stated that a normal organic-salt content is a condition for optimal growth and it is of interest to decide whether these chloride levels, of common occurrence in pastures, may depress the yield of the herbage.

Another point is the effect of nitrate nitrogen. A higher nitrate content of the herbage is associated with a higher content of the total salts (VAN BURG, 1963). Fresh weights were not recorded in these field experiments, but it is known that the fresh weight of herbage increases relative to the dry weight at the increase of nitrogen.

To demonstrate the relation between the ionic constituents and the fresh weight some other data have been plotted in fig. 9. These data show that the total-salt content (C) in the dry herbage varies with the fresh weight per unit dry weight, and

that the organic salt-content in the dry material is constant (fig. 9). Other evidence, not reported here, also demonstrates that the total salts (C) vary with the fresh weight with constancy of the organic salts per unit dry weight.

Similar results have been obtained in the following experiment (table 5).

Seeds of perennial ryegrass were placed on nylon screens floating on a dilute solution of calcium sulphate. After emergence and some growth the seedlings were transferred to a HOAGLAND solution and allowed to grow during six weeks in a greenhouse. Thereafter the plants were defoliated, placed in a climate room and supplied with

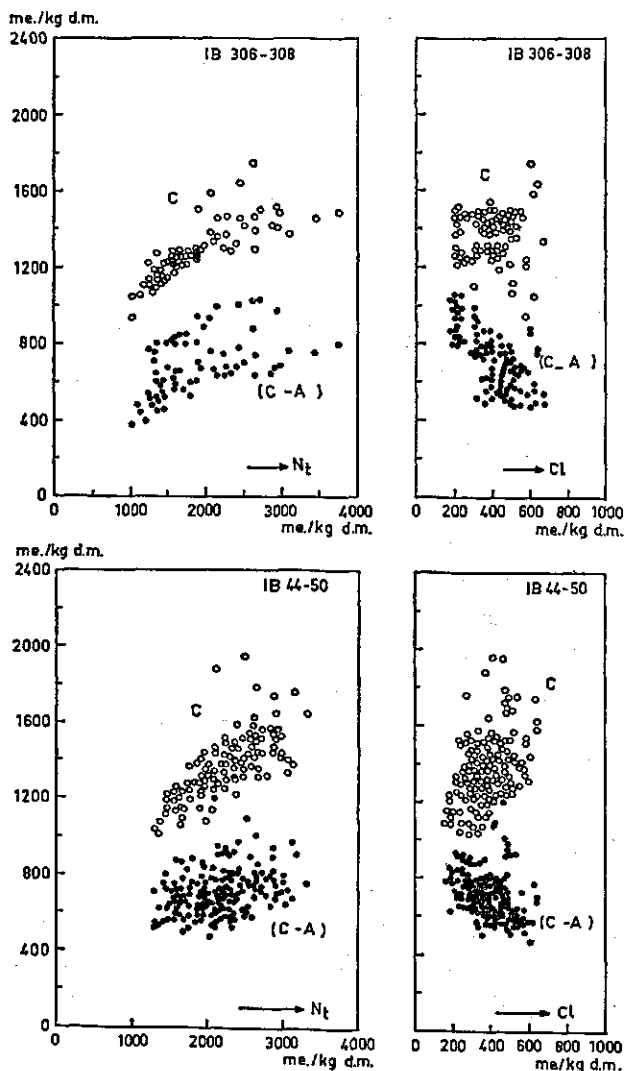


Fig. 8. The organic-salt content ( $C - A$ ) and the total salt content ( $C$ ) of field-grown pasture herbage in relation to the nitrogen and the chloride contents. Data of VAN BURG (1962), partly unpublished

experimental nutrient solutions, containing 3 me. calcium nitrate, 0.4 me. magnesium sulphate,  $x$  me. potassium phosphate and  $(0.5 - x)$  me. sodium phosphate per liter. In addition iron and trace elements were added. The treatments represent a potassium-sodium replacement series, but owing to the potassium content of the tap water used for the daily renewal of the solutions, sufficient potassium was added to prevent deficiency in the zero potassium plants. Three cuts were taken in succession and the herbage analysed for the ionic constituents. From these data the total-salt content ( $C$ ), the inorganic-salt content ( $A$ ) and the organic-salt content ( $C - A$ ) were calculated.

In fig. 10 the ionic constituents, expressed in me. per kg dry weight, have been plotted as ordinates against the fresh weight per unit dry weight. Owing to the increase of the nitrate content from 200 in the first to 1000 me./kg d.m. in the third cut, the inorganic-salt content ( $A$ ) varies over a range from 500 to 1600 me./kg dry material. The total-salt content increases by the same amount as the inorganic salts and the difference ( $C - A$ ) is independent of the large variation of the inorganic-salt content, and remains constant at 900 me./kg dry material. This shows that nitrate accumulates in addition to the organic salts. The data on the total-salt content ( $C$ ) scatter around a straight line through the origin. Though this may suggest constancy

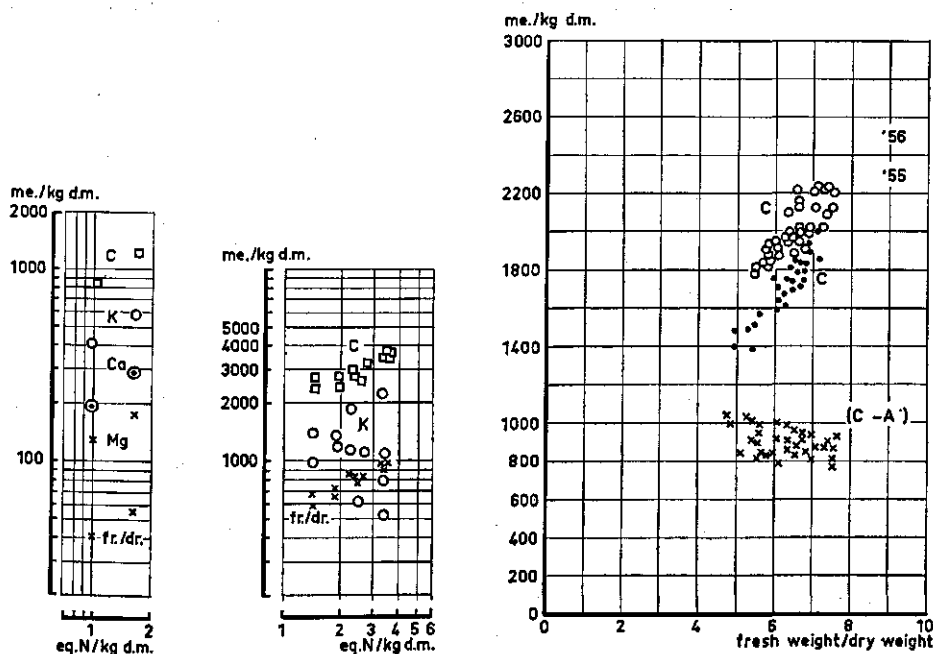


Fig. 9. Total-salt content ( $C$ ), salt cations and organic-salt content ( $C - A$ ) in the herbage of grass in relation to the nitrogen content and the fresh weight per unit dry weight. The ordinates for the ratio of fresh to dry weights should be read at one-tenth (left) or one-hundredth of the indicated values. Left-hand graph: data of MARCUSSEN (1963); middle graph: data of VOSE (1963); right-hand graph: data of DIJKSHOORN and 't HART (1947)

of the total-salt content (C) per unit fresh weight, other data have shown that this is not a rule. However, it has invariably been found that the total-salt content C varies with the fresh weight, while the organic-salt content (C — A) is constant if based on the dry weight.

The normal organic-salt content of 1000 me./kg d.m. is required for optimal growth of the herbage of perennial ryegrass (De Wit *et al.*, 1963). The relations between the organic-salt content and the growth observed by these authors are summarized in fig. 11. At adequate supply with essential elements, and nitrogen in the form of nitrate, growth can proceed at its optimal rate and the organic-salt content is maintained at 1000 me./kg d.m. Suboptimal growth with an organic-salt content below the normal value occurs at potassium deficiency when calcium is supplied instead of potassium. If sodium is supplied to the plants deficient in potassium, the organic-salt content is much higher than the normal value and the growth is also suboptimal. In both cases the addition of potassium salts results in optimal growth with a normal organic-salt content. Suboptimal growth can also occur at the normal organic-salt content if some other factor such as the nitrate supply becomes limiting. With ammonium instead of nitrate the growth is suboptimal at a subnormal organic-salt content. This

*Table 5. Composition of the herbage of three successive cuts of perennial ryegrass, grown in a climate room on nutrient solution with potassium-sodium replacement. N and S refer to total nitrogen and total sulphur. Yield in grams per pot of fresh (f.m.) and dry (d.m.) material*

Cut	Grams per pot		me. per kg dry material									Treatment me. K/liter
	f.m.	d.m.	N	NO <sub>3</sub>	Cl	P	S	K	Na	Mg	Ca	
I	42.4	8.3	2810	230	87	165	228	542	300	114	510	0.0
	57.6	10.5	2741	191	99	168	177	588	249	119	449	-0.1
	72.9	12.9	2615	210	110	148	185	663	200	119	457	0.2
	62.6	11.5	2765	206	85	152	175	788	103	104	442	0.3
	41.5	7.7	3151	307	82	176	266	892	74	114	510	0.4
	67.0	11.8	3073	300	82	180	240	911	90	114	499	0.5
II	99.0	11.3	3155	653	127	176	200	720	484	169	499	0.0
	121	15.6	3052	486	141	179	183	767	429	149	396	0.1
	115	15.4	2846	486	158	169	183	850	310	144	424	0.2
	140	18.1	3212	676	133	182	196	1119	232	149	410	0.3
	108	17.8	2846	319	107	149	175	907	145	114	389	0.4
	164	21.9	3383	752	110	179	219	1264	145	154	400	0.5
III	35.4	3.8	—	1036	296	—	—	1172	481	213	524	0.0
	46.9	5.1	—	974	327	—	217	1383	394	193	528	0.1
	40.4	4.0	—	886	330	—	—	1487	265	184	574	0.2
	62.6	7.0	3543	994	299	231	225	1712	190	164	424	0.3
	71.7	9.1	3429	782	237	228	200	1551	187	129	385	0.4
	82.9	8.8	3703	1132	288	235	223	1897	129	169	474	0.5

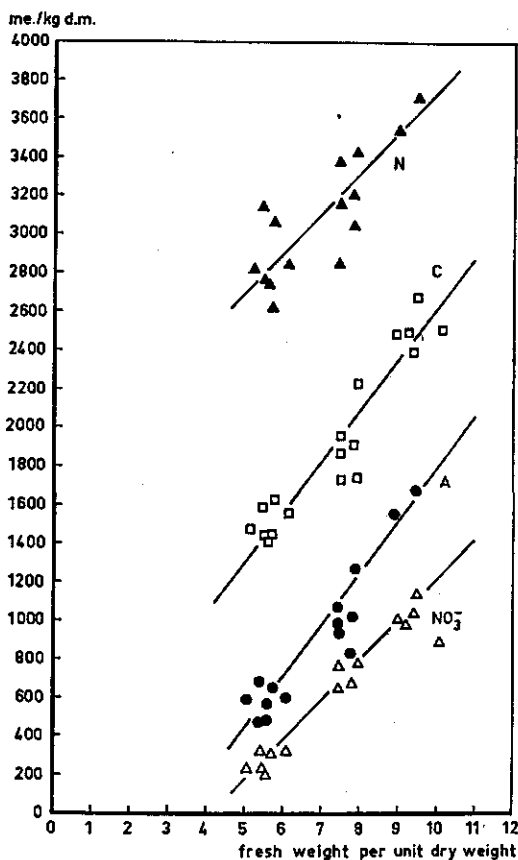


Fig. 10. Nitrogen, total-salt (C), inorganic-salt (A) and nitrate contents in relation to the fresh weight per unit dry weight, in three successive cuts of perennial ryegrass grown on nutrient solutions in a climate room. The constancy of (C — A) in the dry material is reflected in the equidistance of the lines

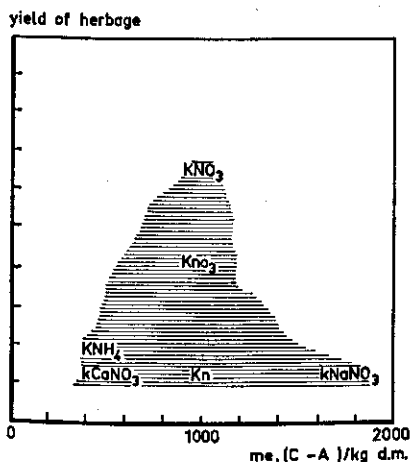


Fig. 11. Diagram, descriptive of the relation between the organic-salt content (C — A) and the yield of perennial ryegrass.  $KNO_3$ : Adequate nutrition with potassium and nitrate in the supply

$Kno_3$ : Suboptimal nitrate nutrition at adequate potassium

$Kn$ : Nitrogen shortage with sufficient potassium

$KNH_4$ : Ammonium as the source of nitrogen at adequate potassium

$kCaNO_3$ : Potassium shortage at ample supply with calcium and nitrate

$kNaNO_3$ : Potassium shortage at ample supply with sodium and nitrate

subnormal organic-salt content may change into the normal content after prolonged exhaustion of the ammonium supplied.

Apparently, at suboptimal growth the organic-salt content may vary continuously from subnormal to supranormal values, depending on the shortage involved and the form of nitrogen supplied. If the nutrient status is made more adequate for growth the deviations from the normal organic-salt content become progressively smaller, and growth can proceed at the optimal rate.

From this experience the authors infer that a normal organic-salt content is essential for optimal growth. Accordingly, it would appear that, per definition, the normal organic-salt content is the value, observed at optimal growth.

## 2.2 Further considerations on the relation between organic-salt content and growth

The relations outlined in the previous section are based upon data on suboptimal growth caused by either potassium shortage or by nitrogen supply with ammonium salts instead of nitrates.

More evidence for the significance of the normal (C — A) content for optimal growth may result from experiments in which the organic-salt content is varied by a change in the accumulation of inorganic salts under otherwise adequate nutrient supply. It has been shown before that chloride may accumulate in considerable amounts instead of the organic salts. Therefore, the introduction of chloride can be used to depress the organic-salt content at adequate supply with other elements.

An example of the effect of chloride on the organic-salt content in perennial ryegrass and the yield is given by DE WIT *et al.* (1963). At a low nitrogen content the application of chloride reduced the organic-salt content from 990 to 580 me./kg d.m. The yield was unchanged, but this may have been caused by nitrogen shortage preventing better growth in the pots without chloride. At a high nitrogen content the plants with and without chloride showed subnormal organic-salt contents and there was no difference in yield between these treatments. Owing to the better nitrogen status they produced more dry matter than the plants of the lower nitrogen series but the growth may still have been suboptimal. Therefore, the data on perennial ryegrass, cited by DE WIT *et al.*, do not permit to decide on the existence of yield depressions associated with an accumulation of chloride instead of the organic salts.

In their experiments on barley these authors claim to have demonstrated the reduction of the organic-salt content due to chloride fertilization as the direct cause of a yield depression. In the discussion of their first experiment on barley at the higher level of salt applications, the possibility of osmotic damage through chloride was recognized. They conclude from their experiment conducted at lower levels of salt application that in the presence of chloride the reduced intake of nitrate has prevented the establishment of the normal organic-salt content. The subnormal organic-salt content was thought to be responsible for the depression of the yield. However, it



seems possible that chloride has reduced the yield through a depression of the utilization of nitrate, as indicated by the lower organic-nitrogen content. Therefore, it remains questionable whether these experiments on barley really demonstrate that chloride may reduce the yield through a subnormal organic-salt content.

Since no other evidence on the effect of chloride on the organic-salt content and the yield was available, some experiments on this feature of ion accumulation were performed with perennial ryegrass.

Pots were filled with 7 kg of a sandy soil of low chloride and potassium content. A basic dressing was added of 100 me. of calcium carbonate, 5 me. of magnesium oxide and 1 me. of calcium phosphate per pot of 7 kg soil. The other salts were added as 1 N stock solutions according to treatment.

*Table 6. Herbage composition of two successive cuts of perennial ryegrass grown in pots with a sandy soil. N and S refer to total nitrogen and total sulphur. Yields in grams of dry (d.m.) material*

Cut	Grams per pot d.m.	me. per kg of dry material									Treatment in me per pot				
		N	NO <sub>3</sub>	Cl	P	S	K	Na	Mg	Ca	K	Ca	NO <sub>3</sub>	Cl	SO <sub>4</sub>
I	11.7	1829	15	85	142	267	1134	32	218	250	40	40	40	0	40
	11.5	1920	32	73	146	217	845	43	253	246	20	40	40	0	20
	11.6	1726	13	429	134	133	773	35	238	253	20	40	40	10	10
	11.9	1783	6	615	149	142	856	35	278	285	20	40	40	20	0
	12.0	1783	8	863	144	125	1126	35	253	275	40	40	40	40	0
II	9.2	2800	269	113	210	292	1121	52	387	339	40	40	40	0	40
	8.3	3189	445	113	207	254	675	107	590	442	20	40	40	0	20
	8.9	2869	231	307	204	217	629	94	561	410	20	40	40	10	10
	9.4	2835	192	561	203	194	576	107	625	467	20	40	40	20	0
	10.3	2652	155	832	191	183	1085	38	437	400	40	40	40	40	0
I	5.1	3349	231	73	163	225	219	177	590	435	0	50	50	0	0
	11.7	2858	147	79	124	167	285	90	407	322	5	45	50	0	0
	13.6	2697	161	59	137	156	465	68	382	314	10	40	50	0	0
	14.4	2560	129	73	138	159	729	45	313	268	20	30	50	0	0
	15.5	2297	90	62	137	179	890	35	278	221	30	20	50	0	0
	16.5	2217	105	59	144	165	1045	29	243	203	40	10	50	0	0
	15.9	2355	90	62	141	185	1126	32	258	152	50	0	50	0	0
II	4.0	3692	521	141	194	256	187	148	655	699	0	50	50	0	0
	10.5	3749	821	108	158	240	930	48	427	482	5	45	50	0	0
	11.5	3075	447	65	184	179	746	55	418	424	10	40	50	0	0
	12.0	3120	495	65	193	177	1217	42	332	317	20	30	50	0	0
	12.8	3098	556	85	208	177	1517	35	298	253	30	20	50	0	0
	13.4	2983	437	62	208	167	1519	29	268	203	40	10	50	0	0
	13.6	3109	555	48	196	175	1585	29	278	196	50	0	50	0	0

Seeds were sown in the untreated 5 cm top layer of the soil on July 2. After four weeks the herbage was harvested, the pots refertilized with 40 me. of calcium nitrate, and a second harvest taken on August 13, 1963.

The results are summarized in table 6 and in figures 12 and 13. Fig. 12 shows the effect of replacing sulphate by chloride at a level of 20 me. and at 40 me. per pot. Herbage composition shows that, except for nitrogen in the first cut, all treatments had adequate supply of the essential elements. Replacement of sulphate by chloride results in a considerable decrease of the organic-salt content. In the first cut of low internal nitrogen level (1800 me./kg d.m.) the organic-salt content decreases from 1200 to 700 me./kg d.m. at the increase of the chloride content. In the second cut of

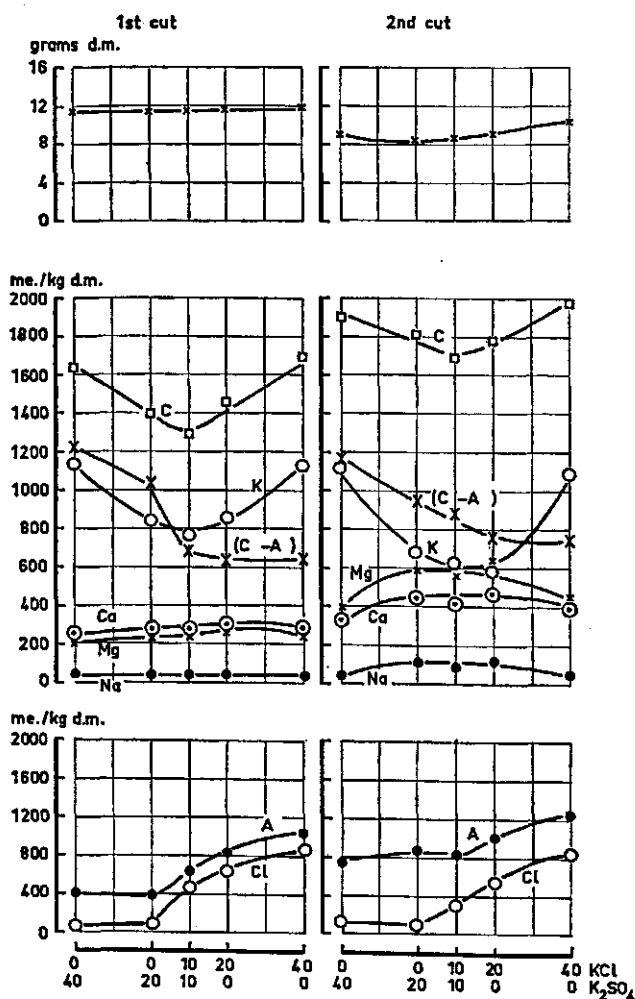


Fig. 12. The effect of replacing sulphate by chloride in the fertilizer salts on the yield and composition of two successive cuts of perennial ryegrass. Potassium was applied at 20 and at 40 me., calcium nitrate at 40 me. per pot

higher nitrogen (2800 me./kg d.m.) the organic-salt content decreases from 1200 to 750 me./kg d.m. In both cuts the yield of herbage was independent of the treatment. In the first cut the growth may have been suboptimal due to shortage of nitrogen. But the second cut with the higher nitrogen level also fails to show any effect on the yield of the chloride applied.

To check on the effect of potassium, an additional series was made with replacement of potassium by calcium. The salts added as 1 N stock solutions were: x me.  $\text{KNO}_3$  + (50-x) me.  $\text{Ca}(\text{NO}_3)_2$ .

The nitrogen content of both cuts was adequate. Fig. 13 shows that in both cuts the zero potassium treatments have low yields. The potassium content of 200 me./kg

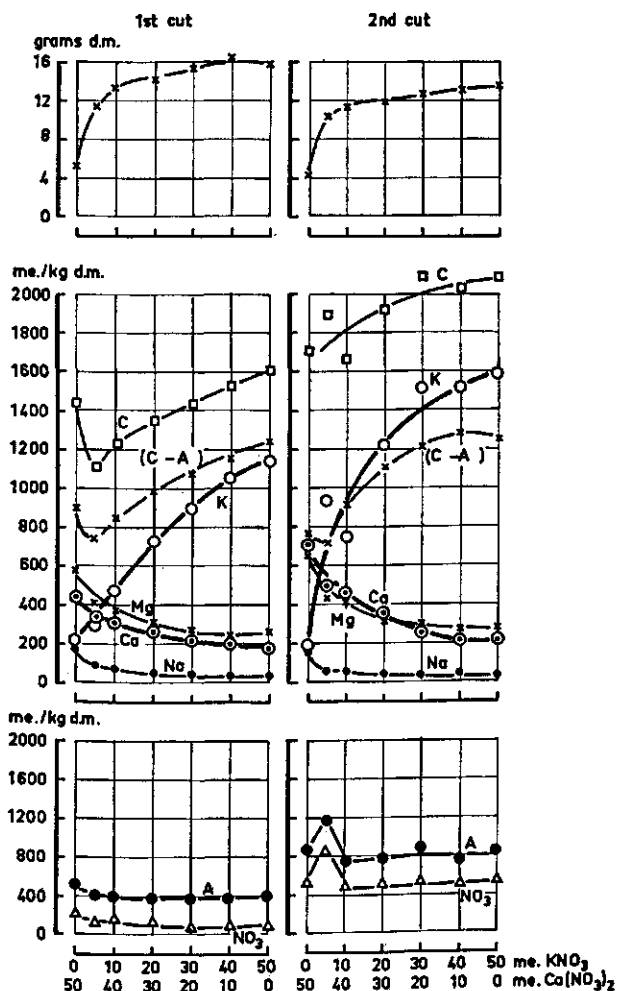


Fig. 13. The effect of replacing calcium by potassium in the fertilizer salts on the yield and composition of two successive cuts of perennial ryegrass

d.m. indicates potassium deficiency (section 1.6). At the increase of the potassium supply the potassium content increases from 200 up to 1400 me. K/kg d.m., the shortage is relieved and the yield of herbage increases considerably. This is associated with a continuous increase of the organic-salt content from a subnormal level of about 800 up to about 1200 me./kg d.m. The latter value is higher than 1000, because the plants were young.

A review of the graphs shows that the greater part of the increase in yield occurs in the range of zero to 10 me. K per pot. In this range the potassium content of the herbage increases from 200 to about 700 me. K/kg d.m. At the further increase of potassium the yield continues to increase slightly. In this range the organic-salt

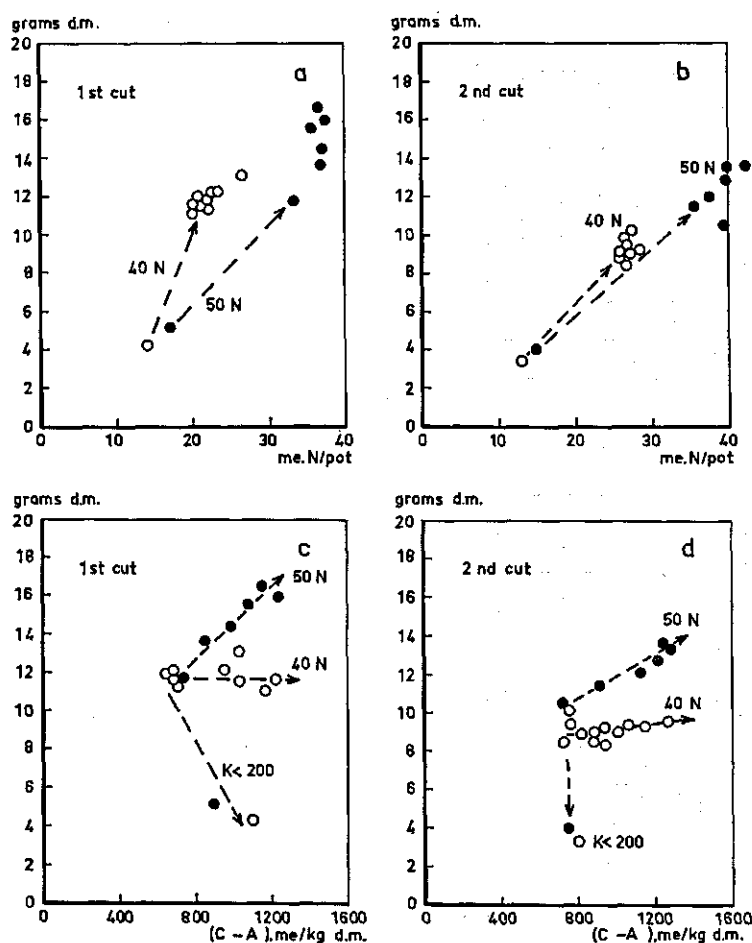


Fig. 14. Relation between the dry-matter yield in grams per pot and the nitrogen uptake in me. per pot (graphs a and b). Relation between the dry-matter yield and the organic-salt content (C - A) in me. per kg dry matter produced (graphs c and d). Two successive cuts of perennial ryegrass with varying rates of potassium applications and two levels of nitrate, 40 and 50 me. per pot, in the fertilizer salts

content increases from about 900 to 1200 me./kg d.m. In the range of higher potassium contents from 700 upto 1600 me. K/kg d.m. the effect of potassium is obviously not related to specific potassium requirements. The increase of the yield is more probably related to the increase of the organic-salt content.

The relation between the organic-salt content and the yield is shown in fig. 14. In this figure the data on the potassium-calcium and on the chloride-sulphate replacement series are summarized. The low yields refer to zero potassium supply.

In figures 14 c and 14 d it is shown that in the chloride-sulphate replacement series, at a 40 me. nitrogen supply, there is no response of the yield when the organic-salt content (C — A) increases from 700 to 1200 me. per kg dry material. In addition, data of another series conducted at a level of 40 me. of nitrate supplied are also included in fig. 14.

In the K-Ca series with 50 me. of nitrate in the supply, the increase of the organic-salt content is associated with a distinct increase of the yield.

Since all series were run side by side in the same experiment, the higher yield reached at 50 me. nitrate in the supply must be connected with the improved nitrogen status, compared with the 40 me. nitrate series.

In figures 14a and 14b the nitrogen balance of the series is reviewed. In the 50 me. nitrate series the nitrogen uptake relative to the dry herbage produced is greater than in the 40 me. nitrate series. In the second cut this difference is reduced owing to the refertilization with nitrate.

This review of the data shows that the replacement of potassium by calcium, at 50 me. nitrate in the supply goes together with a reduction of the yield, a decline of the organic-salt content, and of the potassium content in a range well above the deficiency level of 200 me./kg d.m.

Because at 40 me. nitrate growth was still suboptimal through an insufficient nitrogen supply, the data fail to demonstrate that accumulation of chloride instead of the organic salts can lead to a reduction of the yield.

*Table 7. Herbage composition of perennial ryegrass after two weeks of regrowth on solutions with calcium-potassium replacement in the presence or absence of chloride. N and S refer to total nitrogen and total sulphur. Yields in grams of fresh (f.m.) and dry (d.m.) material*

Grams per vessel		me. per kg of dry material									Treatment in me. per vessel of 6 liters of solution					
f.m.	d.m.	N	NO <sub>3</sub>	Cl	P	S	K	Na	Mg	Ca	NO <sub>3</sub>	Mg	K	Ca	Cl	SO <sub>4</sub>
109	13	2732	400	133	121	268	814	196	321	409	12	10	0	12	0	10
116	14	2572	382	150	114	297	933	191	304	341	12	10	5	7	0	10
121	15	2720	486	121	106	302	1206	155	313	209	12	10	10	2	0	10
116	14	2572	413	395	111	189	809	213	214	534	12	10	0	12	8	2
05	13	2286	223	485	118	220	952	226	193	449	12	10	5	7	8	2
122	15	2332	187	466	118	225	1031	183	185	392	12	10	10	2	8	2

Therefore, another attempt was made to study the effect of chloride at sufficient nitrogen supply (table 7).

Nutrient solutions were prepared in which calcium was replaced by potassium in the absence of chloride. A similar series was made, in which a constant proportion of the sulphate was replaced by chloride. Outside grown plants were cleaned from adhering soil and defoliated. They were transferred to the experimental solutions on October 5 and the herbage produced by regrowth was harvested on October 18, 1963. The results are shown in fig. 15.

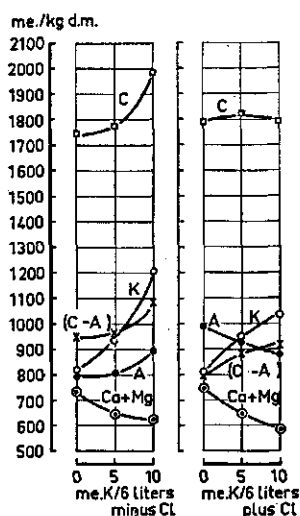


Fig. 15. The effect of replacing calcium by potassium and of sulphate by chloride in the nutrient solutions on the composition of perennial ryegrass

Owing to the potassium carried by the clipped plants, the zero potassium treatments still show 800 me. K/kg d.m., which is well above the internal level of specific potassium shortage. According to the supply, potassium increases from 800 to 1200 me./kg d.m. At the increase of potassium the organic-salt content increases by a 100 me./kg d.m. only and the yield of herbage remains unchanged.

To study the effect of chloride it is useful first to compare the zero potassium treatments. Here, the introduction of chloride has increased the inorganic-salt content (A) in excess to the small increase in total salts (C). Consequently, the common picture of accumulation of chloride instead of the organic salts prevails. The same comparison made at the highest potassium level shows an unchanged inorganic-salt content (A) because chloride accumulates instead of nitrate. In this case the reduction of the organic-salt content is caused by a substitution of chloride for nitrate in the inorganic salts (A), accumulated in the herbage.

This demonstrates again that while the nitrates tend to accumulate in addition to the organic salts, the chlorides accumulate invariably instead of the organic salts.

These findings at the highest potassium supply recalls those encountered in the

experiment of DE WIT *et al.* (1963) on the effect of chloride on oats, showing substitution of chloride for nitrate in the foliage at an unchanged inorganic-salt content (A) and at a reduced organic-salt content (C — A).

## 2.3 Chromatographic analysis of the organic anions

DIJKSHOORN (1962) found that the excess of inorganic cations over inorganic anions (C — A) in the herbage of perennial ryegrass is balanced for about 90 % by the anions of the water-soluble, non-volatile acids obtained by water extraction with decationization, separation on an anion exchange column and subsequent partition chromatography on silica gel. Apart from experimental errors the small portion of (C — A) not balanced by this group of organic anions may be balanced by the anions of volatile organic acids, those of the insoluble organic acids (polyuronic acids, etc.) or by the anions of acids which have otherwise escaped from complete recovery.

To investigate the role of the different organic anions in the building-up of the total balance, a complete analysis of the herbage of perennial ryegrass was made to test the recovery of the constituents of the ionic balance.

Perennial ryegrass was grown in a greenhouse and, after a few previous cuts and adequate dressings with nitrate the herbage was sampled. The greater part of the material was oven-dried and ground for analysis on inorganic and total elements and a portion of the fresh material was analysed for organic constituents in the following way.

*Extraction.* 15 grams of the fresh material were transferred to a beaker suspended in a boiling water bath. Steam was passed through the material for rapid inactivation during 10 minutes and thereafter 100 ml. of water was added and the material was homogenized to a slurry with the 'Ultraturrax' homogenizer. The slurry was cooled by suspending the beaker in ice and stirred during 30 minutes. The liquid was decanted and the fibrous residue gently pressed to remove excess liquid. Extraction of the residue with cold water was repeated 6 times with 100 ml. water. To the combined filtrates, containing suspended tissue fragments, were added 3 grams H-sulphonic acid resin (Merck I) and the liquid was stirred for 1 hour at 0° C. The acidified yellow extract showed a flocculent turbidity which was completely removed by filtration under suction through a dense filter made of cotton wool and powdered cellulose. The extract was kept at 0° C. The resin was collected by careful decantation of the crude extract during its transfer to the filter. The resin was washed with small portions of cold water and these washings were also passed through the filter. The washed resin contained the greater part of the cations and amino acids in the crude extract and the remainder, together with the anions and neutral constituents of the extract was present in the clear yellow filtrate.

To complete decationization and to collect the amino acids a column with 2 grams of H-sulphonic acid resin was prepared and the three grams of resin, separated from

the crude extract by decantation, were transferred on top of the layer, yielding a column of 16 x 0.8 cm. The resin column was kept in an ice jacket and the clear, yellow filtrate was passed through it.

The effluent was a clear solution of the anionic and neutral substances. It was kept in the cold for a few days until required for analysis.

*Isolation of the amino acids.* Elution of the cation exchange column was performed at 0° C. To remove colouring matter 100 ml. of 80 % ethanol was passed through the column. The yellow effluent was evaporated to dryness and nitrogen was determined with a micro KJELDAHL method. The amount of nitrogen found was 7 me./kg dry matter. The elution of the amino acids was performed in two steps:

- a. 20 ml of 1 N  $\text{NH}_4\text{OH}$  were slowly passed through the column, followed by 40 ml water. The combined effluents were evaporated to dryness in vacuo. The residue was dissolved in 8 ml of water. 4 ml of the solution were transferred to a small beaker and 36 ml of 95 % ethanol were added, together with 2 ml 0.25 % thymolphthalein. Titration with 0.01 N NaOH in 85 % ethanol was made until a distinct virage to blue (assay of carboxyl). Thereafter, 7 drops of 0.2 % methyl red in ethanol were added and the solution titrated with 0.01 N HCl until faintly red (assay of amino). Subsequently, the solution was taken to dryness and KJELDAHL nitrogen was determined.
- b. A second elution of the exchange column was made by subsequently passing 100 ml 1 N  $\text{NH}_4\text{OH}$  through the column, followed by 40 ml water. The effluent was taken to dryness in vacuo. The residue was dissolved in 4 ml water, 36 ml of 95 %

Table 8. Ionic balance of herbage of perennial ryegrass, in me. per kg of dry material. Nitrogen, carboxyl and amino me. per kg dry material present as free amino acids

Uptake		Utilization		Accumulation		Organic anions found
anions	cations	anions	cations	anions	cations	
N 2575	K 1013	N <sub>o</sub> 2556		No <sub>3</sub> 19		
Cl 164	Na 126			Cl 164		
P 169	Mg 169			H <sub>2</sub> PO <sub>4</sub> 169		
S 385	Ca 321	S <sub>o</sub> 138		SO <sub>4</sub> 247		
A <sub>u</sub> 3293	C <sub>u</sub> 1629	OH <sup>-</sup> 2694		A 599	C 1629	
	H <sup>+</sup> 1664	→ H <sup>+</sup> 1664		inorg. salts	599	
					1030	
		1030		→ org. salts		930
				free amino acids		
				COO <sup>-</sup> NH <sub>3</sub> <sup>+</sup> N		
		fraction a		89 80 105		
		fraction b		17 18 15		
		sum		106 98 120		



ethanol was added and titration of carboxyl and amino functions performed as described before. The results of the amino acid assays are recorded in table 8.

For paper chromatography Whatman I paper strips (38 x 450 mm) were first treated as follows. A series of 2 liter glass jars with glass covers, filled with, respectively a. 0.1N NaOH, b. deionized water, c. deionized water, d. 0.2 N NaOH, e. deionized water, f. deionized water, g. 0.01 molar  $\text{NaH}_2\text{PO}_4$  (prepared from a normal solution, previously freed from heavy metal impurities by shaking with 25 ml of a saturated solution of dithizone in carbon tetrachloride per liter of solution) was prepared.

The strips were passed through the solutions in the order given, keeping them submerged in each liquid for at least 15 minutes and allowing the excess to drip off prior to each transference. A stock of submerged strips was kept available in the phosphate solution. For use, strips were taken from this solution and dried at room temperature.

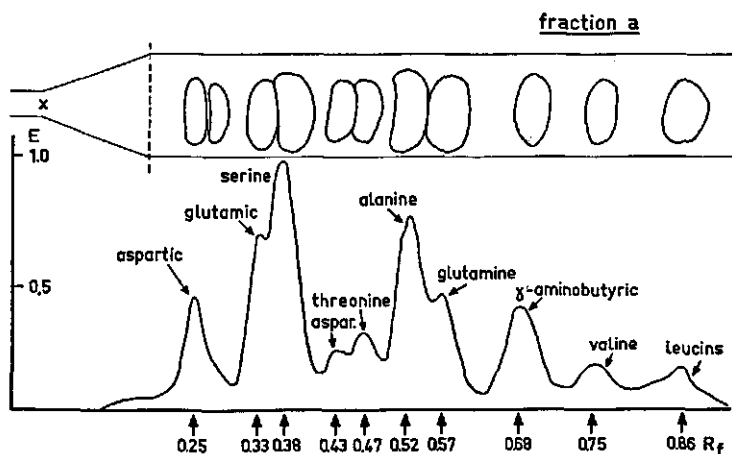


Fig. 16. Paper chromatogram of the free amino acids from herbage of perennial ryegrass

The buffered paper strips were provided with a wedgelike incision leaving a bridge of 1 cm width at the origin where the spot is applied to the paper (fig. 16).

The solution of the residues of the effluents obtained according to (a) and (b) (see above) was sampled prior to the titration procedure, and 20 micro liters (about 1 micro equivalent nitrogen) of the solution (a) were applied to the narrow bridge on the strips and chromatography was performed in the descending fashion with water saturated phenol during 26 hours.

Thereafter, the strips were dried at room temperature. For colour development the strips were passed through a solution of 500 mg ninhydrin in 93 ml. water saturated butanol and 7 ml glacial acetic acid, placed in a shallow trough. The excess reagent solution was evaporated at room temperature for about 10 minutes and subsequently development was completed at 68-70° C. The spots were recorded with a recorder (fig. 16).

*Isolation of the organic anions.* To isolate the organic anions the decationized extract was passed over an exchange column (6 grams of Merck II, a strongly basic anion resin, 120 x 8 mm, exchange capacity 18 me.), previously brought into the OH-form.

The effluent was discarded and the organic anions, other than oxalate, were eluted by passing 100 ml 5N formic acid through the column. The eluate was evaporated to dryness in vacuo, incorporated into silica gel and submitted to partition chromatography.

Oxalic acid was eluted from the anion exchange column by a second elution with 100 ml 1 N sulphuric acid and determined by titration of the effluent with permanganate.

The entire procedure has been described in detail by DIJKSHOORN and LAMPE (1962 a, 1962 b).

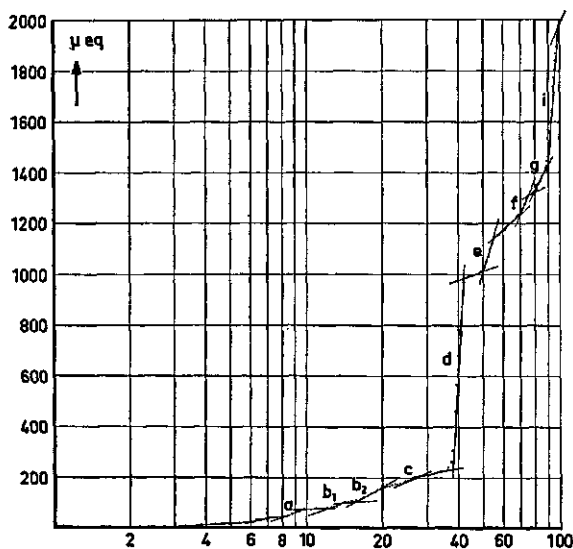


Fig. 17. Partition chromatography on silica gel of the acids of the organic salts from 15 grams of fresh herbage of perennial ryegrass. Ordinates: additive number of microequivalents; abscissae: fraction number.  $b_1$ : succinic;  $b_2$ : malonic; d: malic; e: citric; f: glyceric; g: shikimic; i: quinic + phosphoric acids

In the chromatogram shown in fig. 17, wave i represents a.o. phosphate. Its quantity was determined by pooling the percent fractions 90-100, extraction of the solvent with NaOH, evaporating the water layer to dryness and determining phosphate with a colorimetric procedure, according to the instructions of DIJKSHOORN and LAMPE (1961). The phosphate content, 140 me. is expressed as the divalent form because titration of the fractions was made to pH 8 to 9.

The entire acidity developed off the column was 2000 me., the organic acidity

obtained after subtraction of phosphate was  $2000 - 140 = 1860$  me.

Oxalate was found at 126 me. and consequently the total organic acidity was 1986 me.

Since the herbage sample of 15 grams of fresh material contained 2.14 grams of dry material, the organic anions recovered by the procedure described amount to 930 me./kg dry matter. This value is comparable to the results of DE MAN and HEUS (1947) on the total organic-anion content in herbage of perennial ryegrass, found by a titrimetric procedure.

Analyses on the total elements and nitrate contents were performed in the oven-dried sample. The ionic balance, calculated as outlined in section 1.2, is shown in table 8. The balance shows that the levels of the individual elements are all well above the values critical for specific shortage. The low concentration of nitrate in the herbage indicates nitrogen exhaustion in the soil. However, growth had not yet proceeded to more severe nitrogen depletion, because the total-nitrogen content is sufficiently high in the herbage.

Analysis of the free amino acids shows that this fraction contains only about 120 me. N/kg dry matter. The titration values show that the number of equivalents associated with the amino groups differs only little from the carboxyl equivalents, fraction (a) showing an excess of only 9 me./kg d.m. This confirms insignificance of equivalents associated with the free amino acids to the total ionic balance.

A considerable increase of the free amino acids occurs at sulphur deficiency (DIJKSHOORN *et al.*, 1960). According to findings of COLE *et al.* (1962) the increased content of free amino acids in sulphur deficient barley mainly concerns the neutral amino acids and asparagine and glutamine, so that even in this case there is no significant excess of carboxyl over amino groups. The analysis of the organic anions shows that the anions of the water-soluble, non-volatile organic acids represent about 90 % of the value found for the total organic salts (C — A).

It was shown by DIJKSHOORN and LAMPE (1962) that wave d in fig. 17 represents malate and wave e citrate. According to DAVIES and HUGHES (1954) these anions together constitute about 50 % of the total organic anions in grass. These authors noticed the absence of tartrate and iso-citrate. Their failure to detect oxalic acid results from the fact that the 0.1 N hydrochloric acid used for elution of the organic anions from the exchange column fails to elute oxalic acid (DIJKSHOORN and LAMPE, 1962, ROUX and LESAINT, 1959). HULME and RICHARDSON (1954) investigating a mixed herbage of mainly meadow foxtail (*Alopecurus pratensis*) and meadow fescue (*Festuca pratensis*), dried immediately after cutting in a commercial grass drier, confirmed the presence of malate, citrate, succinate (wave b<sub>1</sub>) and malonate (wave b<sub>2</sub>) and, in addition, demonstrated the presence of quinic and chlorogenic acids.

To identify the organic anions, the fractions beyond wave e (citrate, compare fig. 17) were pooled in two groups. One group contained the acids represented by wave i and the other group contained the acids represented by wave f and g. The solvent was extracted with water, made alkaline to thymolblue with a few drops of sodium hydroxide. The water layers containing the sodium salts were removed and passed

through a cation exchange column. The decationized extracts were evaporated to dryness in vacuo. The free acids were dissolved in 1 ml of water and 0.1 ml of the solutions were applied to Whatman I paper. Chromatography was performed in the ascending fashion, in one direction (I) with ethanol-ammonia, in the other direction (II) with butanol-formic acid-water. For further details the reader is referred to DIJKSHOORN and LAMPE (1962 a).

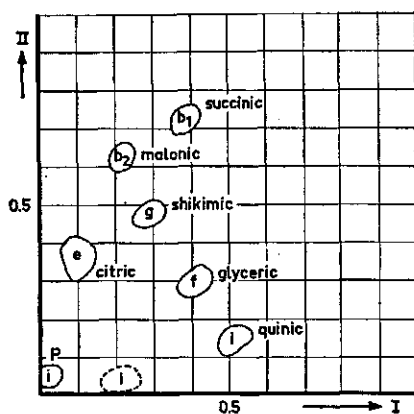


Fig. 18. Paper chromatogram of fractions developed off the silica gel column. Solvent I: ethanol-ammonia-water; solvent II: n butanol-formic acid-water

The results shown in fig. 18 indicate that wave i yielded a spot corresponding to quinic acid and in addition faint spots for phosphate and sulphate were detected. The fraction of waves f and g yields two spots, one corresponding to glyceric and the other, faint spot, to shikimic acid. The order of emergence: e = citric, f = glyceric, g = shikimic and i = quinic, agrees with the sequence found by other workers on partition chromatography on silica gel of the plant acids, as e.g. COIG and LESAINTE (1960).

## 2.4 Changes in the individual organic anions

Chromatographic analysis is a useful means to investigate the contribution of the individual organic anions as malate, citrate, etc. to changes in the total organic-salt content caused by a difference in nutrition.

In this section some observations on the effect of replacing chloride by nitrate in the herbage are discussed. As appears from section 2.2 the organic-salt content, subnormal through the accumulation of chloride, is raised if nitrate is made to accumulate at the expense of chloride. This substitution is a convenient way to vary the organic-salt content at adequate supply with the other elements. An experiment was conducted with a fertile sandy soil of appreciable chloride content. Pots were filled with 7 kg of the soil, fertilized with 20 me. magnesium sulphate and 0 or 20 or 40 me. of either

potassium nitrate or calcium nitrate. The pots were planted, the plants were defoliated and after 18 days of regrowth the herbage was harvested. The results are given in table 9.

Table 9. Herbage composition of perennial ryegrass after three weeks of regrowth in a pot experiment with a fertile sandy soil. *N* and *S* refer to total nitrogen and total sulphur. Yields in grams of fresh (f.m.) and dry (d.m.) material

Grams per pot		me. per kg of dry material									Treatments in me. per pot		
f.m.	d.m.	N	NO <sub>3</sub>	Cl	P	S	K	Na	Mg	Ca	K	Ca	NO <sub>3</sub>
107	15.8	1554	13	536	122	258	1022	74	124	214	0	0	0
124	17.0	1977	36	485	107	292	1243	68	129	235	20	0	20
150	19.0	2526	192	398	96	296	1283	65	144	235	40	0	40
86	11.7	1875	16	550	131	329	1100	87	144	246	0	0	0
107	13.8	2240	39	550	114	279	1032	116	169	292	0	20	20
107	14.1	2549	129	482	100	212	924	142	208	357	0	40	40

Organic anions found in me. per kg of dry material				Treatments in me. per pot		
total	malate	citrate	oxalate	K	Ca	NO <sub>3</sub>
588	125	68	78	0	0	0
860	270	102	73	20	0	20
871	307	102	91	40	0	40
651	110	59	70	0	0	0
746	271	78	81	0	20	20
839	280	100	77	0	40	40

The data show that the nutrition was adequate in all treatments with the exception of nitrogen. But at zero nitrate supply the organic-salt content is markedly below the normal value owing to the high level of chloride accumulation. It appears that at the increase of nitrate, the chloride content is lowered and the organic-salt content is increased.

The herbage of all treatments was analysed for organic anions by extraction and chromatography (section 2.3). In fig. 19a the chromatograms of the 0 and 40 me. potassium nitrate treatments are recorded. The increase of the organic anions due to nitrate supply is mainly caused by malate (wave d).

The results of all treatments are summarized in fig. 19b. The graph shows that about 90 per cent of the total organic-salt content (C — A) is recovered by the chromato-

graphic procedure, and that malate contributes mainly to the change in the organic salts. Similar results were obtained by chromatographic analysis of some of the herbage samples of the experiment on chloride-sulphate replacement of section 2.2. That chloride accumulates instead of the organic salts and mainly at the expense of malate in the herbage is again displayed clearly in these experiments.

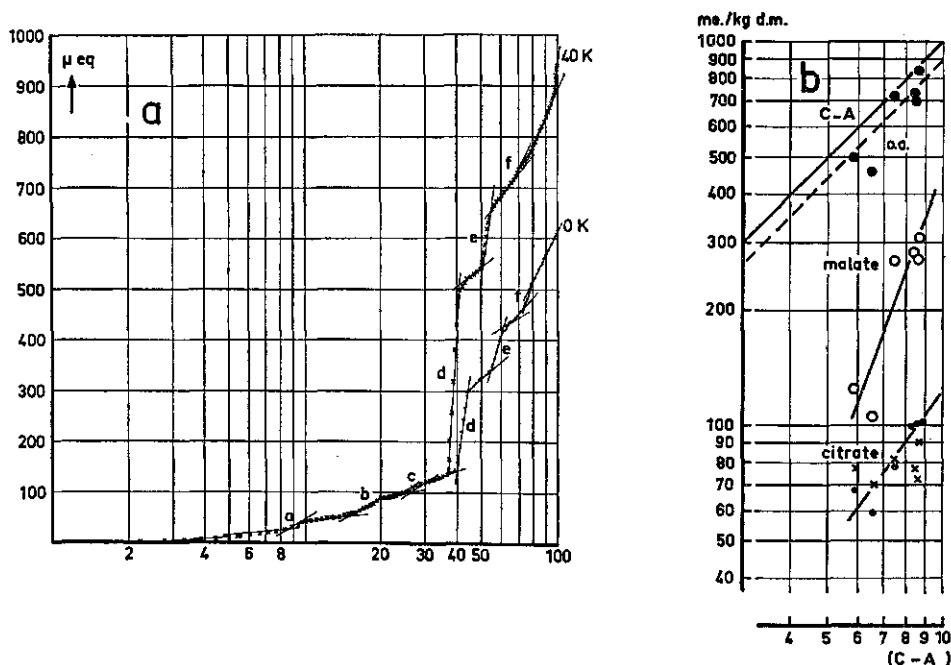


Fig. 19a. Partition chromatography on silica gel of the organic acids from perennial ryegrass, grown at two levels of potassium nitrate application. For indices see fig. 17

Fig. 19b. Water-soluble acids of the organic salts (o.a.), malate, citrate and oxalate (crosses) in perennial ryegrass with varying ( $C - A$ ) contents due to nitrate accumulation in the presence of much chloride

### 3. Sugar beet

#### 3.1 Some characteristics of the foliage

To disclose other aspects of the ionic balance it is instructive to continue the study with sugar beets. This species exhibits an alkaline uptake from nutrient salts with nitrate as the source of nitrogen (STOKLASA and ERNEST, 1908). Furthermore salts accumulate to a considerably higher internal level compared with grass, the selective uptake of salt cations differs from that of grass, and the yield response of potassium deficient plants to sodium is pronounced (WYBENGA and LEHR, 1958).

Changes in the accumulation of salt cations associated with the transition from gramineous plants to sugar beet foliage are evident from the data of LARSSON and PIERRE (1953), which are represented in fig. 20.

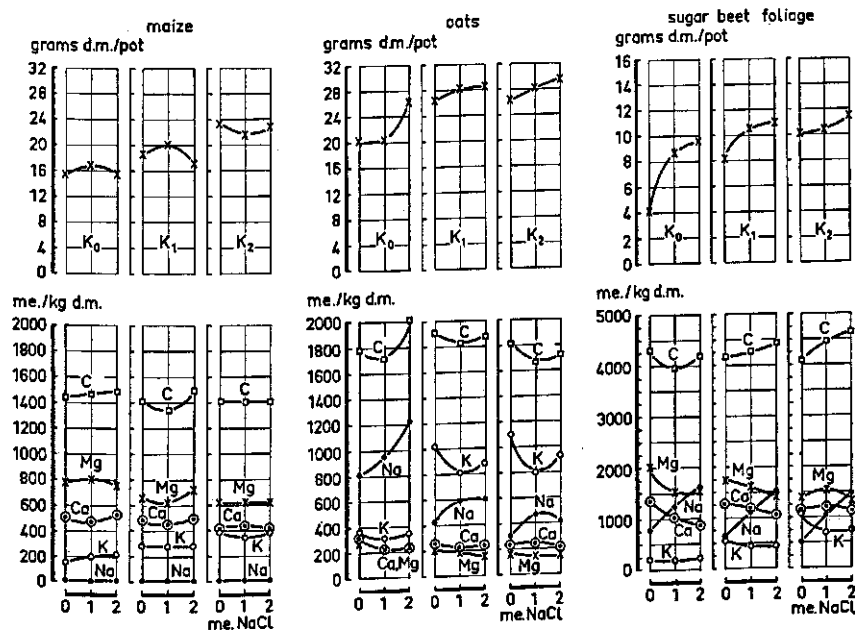


Fig. 20. Dry-matter yields and cationic composition of maize, oats and sugar beet foliage grown in pot experiments at three potassium levels ( $K_0$ ,  $K_1$  and  $K_2$ ) and at increasing amounts of sodium chloride.

Data of LARSSON and PIERRE (1953)

Maize, oats and beets were grown on two soils low in potassium ( $K_0$ ), and at two levels of potassium application ( $K_1$  and  $K_2$ ). At each potassium level sodium chloride was applied at three levels, 0, 1 and 2 me. per pot.

The results show that maize is unable to accumulate sodium. With potassium at about 200 me./kg d.m. the yield is depressed, as shown by the treatments  $K_0$ . At the increase of the potassium supply in the order of  $K_0$ ,  $K_1$ ,  $K_2$ , the potassium content of the foliage increases from 200 to 300 and 400 me./kg d.m., respectively. The first increase to 300 me.K/kg d.m. is associated with an increase of the yield, and there is a further increase of the yield when passing from 300 to 400 me.K/kg d.m. The total salt content is not affected by an increase of the potassium supply.

In contrast to maize, oats are able to accumulate sodium in considerable amounts and more in particular so at low potassium levels. The results of treatment  $K_0$  indicate improvement of the growth at the increase of the sodium chloride supply. At the higher potassium levels this effect of sodium is practically absent. Apparently, in the absence of potassium, sodium can be beneficial to the growth of oats.

The data on sugar beet foliage show that the total salt content (C) is in the higher range of 4000 to 5000 me. /kg d.m. In the treatment  $K_0$  there is potassium shortage at a level of 200 me.K/kg d.m. as apparent from the depressed yield. Here, the yield is considerably improved by the application of sodium, thus exhibiting the well-known effect of sodium on beets deficient in potassium. The other treatments  $K_1$  and  $K_2$ , with potassium contents of 500 to 1000 me.K/kg d.m., have still higher yields which proves that potassium remains superior in its effect on the yield of the foliage.

### 3.2 The normal organic-salt content

The evidence of the previous section illustrates the response of beets to some fertilizer salts. In addition, it was thought useful to study the complete balance in sugar-beet foliage in experiments with varying proportions of the different cations and anions supplied in the fertilizer salts.

For these experiments use was made of a poor sandy soil. Each of the replicates was prepared by mechanically mixing 210 kg of the soil with 2.9 equivalents of calcium carbonate, 2.9 equivalents of magnesium oxide, and 0.50 equivalents of calcium bi-phosphate. Each pot was filled with 7 kg of the soil containing this basic dressing (pH 5.5 in a 1:5 suspension in water). Measured amounts of normal salt solutions were added to obtain the following experimental treatments, listed in table 10.

Seeds were sown on June 14, 1963. The foliage of the plants was harvested on July 17, 1963. On the untreated soil with the basic dressing only the yield is very low (treatment A, table 10, fig. 21). Application of calcium nitrate does not improve growth. Although the nitrogen content of the foliage increases from 2700 to 4100 me./kg dry matter, potassium shortage at the level of 200 me./kg d.m. prevents any increase of the yield. The organic-salt content increases from 2500 to 3400 me./kg d.m., mainly through an increased accumulation of the organic salts of magnesium.



Magnesium is constant in the supply but is absorbed in greater amounts when calcium nitrate is added.

If, instead of calcium nitrate, its equivalent of an equimolar mixture of the nitrates of potassium and sodium (treatment A and E) is added to the soil the yield is considerably increased, because the fertilizer permits potassium to increase above the deficiency level of 200 me./kg per kg of the dry foliage. The organic-salt content increases from 2500 to 3500 me./kg d.m.

The treatments B, C and D (table 10, fig. 21) demonstrate, the effect of replacing 80 me. of calcium in the supply by its equivalent of an equimolar mixture of potassium and sodium, at a constant supply of 40 me. nitrate and 40 me. of either carbonate, sulphate or chloride. The different anions in the fertilizer salts were introduced to compare their effect on growth and composition in addition to the effects of salt cation replacements.

Table 10. Treatments in the pot experiment on sugar beets

Treatments	Basic dressing: 95 me. $\text{CaCO}_3$ ; 95 me. $\text{MgO}$ ; 15 me. $\text{Ca}(\text{H}_2\text{PO}_4)_2$ per pot, and in addition me. per pot:						
	K	Na	Ca	$\text{NO}_3$	$\text{CO}_3$	$\text{SO}_4$	Cl
A <sub>1</sub>	0	0	0	0	0	0	0
A <sub>2</sub>	0	0	20	20	0	0	0
A <sub>3</sub>	10	10	0	20	0	0	0
B <sub>1</sub>	0	0	80	40	40	0	0
B <sub>2</sub>	20	20	40	40	40	0	0
B <sub>3</sub>	40	40	0	40	40	0	0
C <sub>1</sub>	0	0	80	40	0	40	0
C <sub>2</sub>	20	20	40	40	0	40	0
C <sub>3</sub>	40	40	0	40	0	40	0
D <sub>1</sub>	0	0	80	40	0	0	40
D <sub>2</sub>	20	20	40	40	0	0	40
D <sub>3</sub>	40	40	0	40	0	0	40
E <sub>1</sub>	0	0	40	40	0	0	0
E <sub>2</sub>	5	5	30	40	0	0	0
E <sub>3</sub>	10	10	20	40	0	0	0
E <sub>4</sub>	15	15	10	40	0	0	0
E <sub>5</sub>	20	20	0	40	0	0	0
F <sub>1</sub>	0	40	0	40	0	0	0
F <sub>2</sub>	10	30	0	40	0	0	0
F <sub>3</sub>	30	10	0	40	0	0	0
F <sub>4</sub>	40	0	0	40	0	0	0

All three series show that at zero potassium supply plants are deficient with a potassium content of 200 me.K/kg d.m. The poor growth results in 2 grams of dry material per pot. Replacement of calcium by potassium and sodium relieves potassium shortage and increases the yield to about 8 grams of dry matter per pot. The potassium content increases to above the deficiency level and there is a concurrent increase in the sodium content. This shows the ability of beets to absorb sodium as readily as potassium even in the presence of adequate potassium.

The graphs also show that replacement of potassium and sodium by calcium makes calcium to substitute for potassium and sodium in the foliage only to a small extent. Here, magnesium, of which the supply is constant, substitutes for potassium and

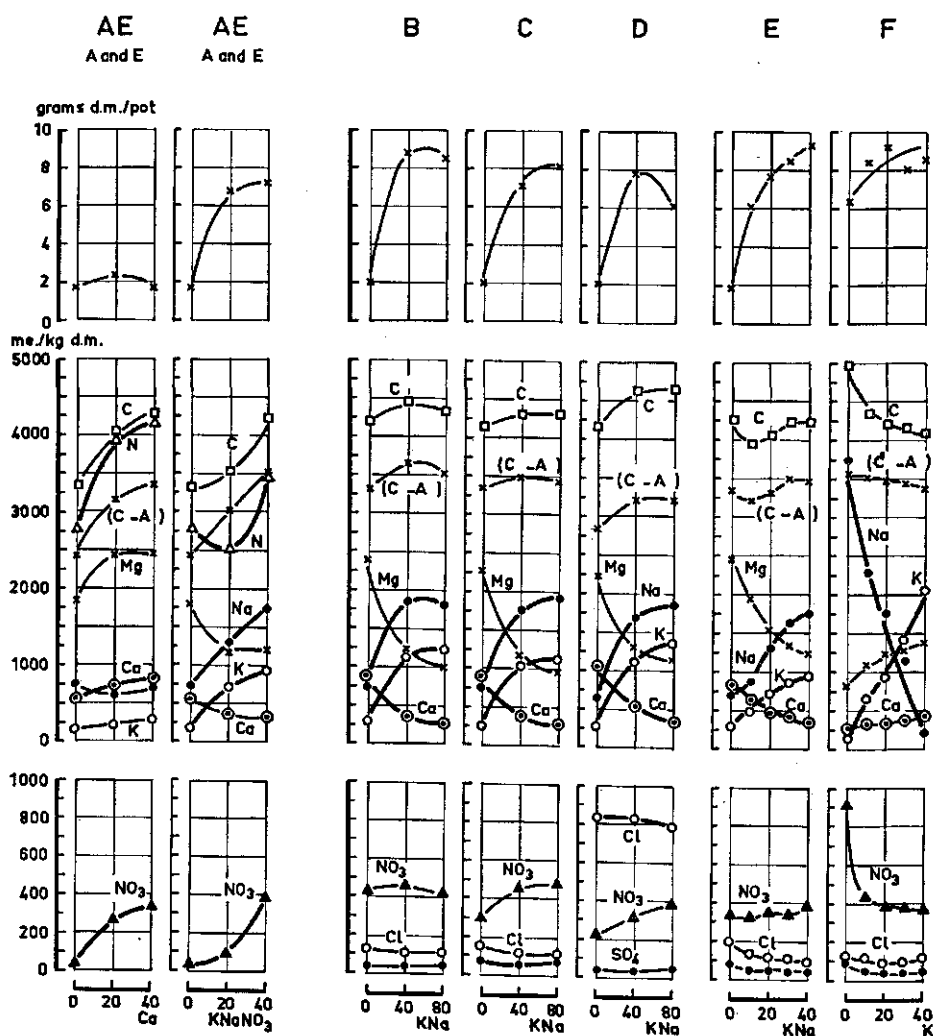


Fig. 21. Yield and composition of sugar-beet foliage grown in a pot experiment. For treatments see table 10

sodium in the foliage to a greater extent. This recalls the behaviour of sodium in potassium deficient perennial ryegrass. However, it is seen in fig. 21 that magnesium is effectively depressed when sodium is supplied to potassium deficient beets.

In all treatments of the three series the nitrogen contents are high. Table 11 indicates that plants which received no potassium contain the highest amounts of total nitrogen, because dilution of nitrogen by growth is diminished.

In the absence of chloride in the fertilizer the organic-salt contents are about 3500 me./kg d.m. They are only slightly lower in the potassium deficient foliage. The increased accumulation of magnesium when potassium and sodium are omitted from the treatments prevents a larger reduction of the organic-salt content.

Compared with the sulphate series (treatment C), the carbonate series (B) shows

Table 11. Composition of the foliage of sugar beet, grown in the pot experiment on cation replacement in the salts applied. For the treatments compare table 10. N and S refer to the total nitrogen and total sulphur contents. Yields in grams of fresh (f.m.) and dry (d.m.) material per pot

Grams per pot		me. per kg of dry material									Treatments me. per pot	
f.m.	d.m.	N	NO <sub>3</sub>	Cl	P	S	K	Na	Mg	Ca		
17	1.7	2777	32	341	346	306	186	745	1830	556	A1	0 K
26	2.3	3943	272	189	273	279	216	626	2445	713	A2	0
85	6.8	2503	82	112	243	229	703	1281	1225	338	A3	10
23	2.0	4114	406	129	252	268	263	732	2381	816	B1	0 K
127	8.9	3543	450	110	223	220	1104	1884	1145	349	B2	20
122	8.5	3543	425	101	218	216	1238	1820	1002	253	B3	40
22	2.0	3989	288	155	253	266	254	735	2272	856	C1	0 K
112	7.1	3680	445	110	219	224	1025	1781	1141	374	C2	20
118	8.2	3691	470	101	211	247	1112	1929	967	278	C3	40
24	2.0	3851	220	829	202	237	276	635	2207	1030	D1	0 K
120	7.8	3486	316	826	202	210	1119	1700	1274	527	D2	20
103	6.1	3566	385	787	221	202	1344	1829	1141	328	D3	40
19	1.8	4137	333	197	270	291	282	716	2430	820	E1	0 K
77	6.1	3623	316	129	226	227	484	897	1935	656	E2	5
102	7.6	3520	346	110	216	220	715	1303	1527	492	E3	10
119	8.4	3486	325	110	221	222	860	1632	1314	420	E4	15
129	9.3	3463	382	95	211	212	943	1752	1205	321	E5	20
116	6.4	4103	903	124	260	264	135	3775	818	238	F1	0 K
127	8.4	3600	427	110	237	222	647	2297	1081	328	F2	10
114	8.1	3486	377	93	214	208	1412	1145	1235	367	F3	30
114	8.6	3417	367	110	212	208	2075	225	1379	410	F4	40

slightly higher, and the chloride series (D) significantly lower organic-salt contents. With an adequate supply of potassium the dry-matter production is higher in the carbonate series and lower in the chloride series. The spreading of the observations does not permit to conclude that the fall of the yield with chloride in the fertilizer salts is of statistical significance. However, the reduction of the organic-salt content observed when passing from the carbonate via the sulphate to the chloride series seems of significance, and indicates that chloride accumulates partly instead of the organic salts. As shown by the other data, the remainder accumulates at the expense of nitrate. The series E (fig. 21), with replacement of calcium by potassium and sodium at the 40 me. level in the supply, gives results very similar to those of series B, C and D at the 80 me. level.

Series F (fig. 21) shows the effect of replacing sodium by potassium at a 40 me. level of supply. If sodium is supplied to potassium deficient plants, sodium accumulates to a very high level in the foliage. In contrast to the behaviour of sodium in perennial ryegrass, sodium accumulates to a large extent as sodium nitrate in the foliage of sugar beets and the organic-salt content is only slightly increased. The graph also shows that at substitution of sodium for potassium in the foliage the magnesium content is diminished.

If the potassium deficient plants are supplied with sodium instead of calcium, the growth is considerably improved. However, the further increase of the yield at the replacement of sodium by potassium indicates that potassium is superior in its effect on the yield of the foliage. This confirms the observations of LARSON and PIERRE (1953) on the effect of sodium and potassium on the yield of the foliage of sugar beets.

To test the effects of the form of nitrogen supplied the following experiment was made. Pots were prepared with the soil and the basic dressing of the previous experiment. Normal salt solutions of potassium carbonate, calcium nitrate, ammonium nitrate and ammonium sulphate were added to obtain the treatments given in table 12.

*Table 12. Treatments of the pot experiment on the effect of the form of nitrogen on the composition of sugar-beet foliage*

Treatment	me. per pot					
	K	Ca	NO <sub>3</sub>	NH <sub>4</sub>	SO <sub>4</sub>	CO <sub>3</sub>
1	40	40	40	0	0	40
2	40	0	20	20	0	40
3	40	0	0	40	40	40

There were 10 replicates in the experiment of which 5 received 70 mg of 'N-serve' per 7 kg of soil per pot, to inhibit nitrification of the ammonium added to the soil (VAN TUIL and LAMPE, 1964). The pH of the soil used in this experiment was 5.5. Seeds were sown on April 17, 1964 and the foliage of the plants was harvested on May 15, 1964.

Table 13. Composition of the foliage of sugar beets, grown in the pot experiment on the effect of replacing nitrate by ammonium in the fertilizer salts. For the treatments compare table 12. A basic dressing of 95 me. of calcium carbonate, 95 me. of magnesium oxide and 15 me. of calcium biphosphate was applied per pot. One half of the replicates received 10 ppm of N-serve (+N.s.). N and S refer to the total-nitrogen and total-sulphur contents. Yields in grams fresh (f.m.) and dry (d.m.) material per pot

Grams per pot		me. per kg of dry material									Treatment
f.m.	d.m.	N	NO <sub>3</sub>	Cl	P	S	K	Na	Mg	Ca	
105	7.6	3360	505	102	168	275	2255	170	1160	390	1: NO <sub>3</sub> + N.s.
90	5.9	4240	1003	130	174	243	2264	191	1044	250	2: NH <sub>4</sub> NO <sub>3</sub> + N.s.
48	3.6	3566	381	195	210	418	2095	244	822	235	3: NH <sub>4</sub> + N.s.
91	6.1	3646	668	118	158	262	2474	191	1201	389	1: NO <sub>3</sub>
82	5.2	4263	1021	133	193	281	2474	204	1040	235	2: NH <sub>4</sub> NO <sub>3</sub>
35	3.1	3143	39	276	387	568	2040	270	789	150	3: NH <sub>4</sub>

The results listed in table 13 show that the treatments with and without N-serve do not result in different foliage composition. From the yields and the nitrogen contents it can be calculated that nitrogen in the foliage amounts to about 25 me. per pot at the most. Since 40 me. of nitrogen was supplied nitrogen has been adequate in all treatments, which is also evident from the nitrate content of the foliage. The data for the other elements also indicate the absence of shortages.

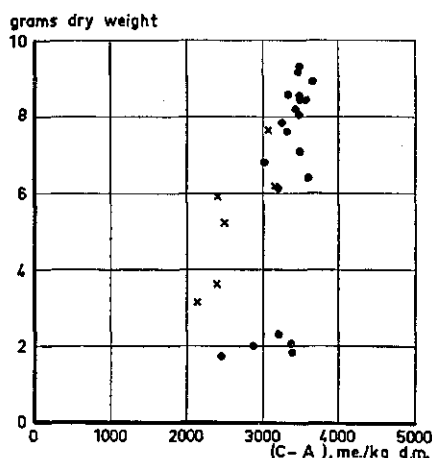
The organic-salt content of the plants which received nitrate is about equal to the sum of the organic nitrogen and organic sulphur content because the salt cations and the salt anions have been absorbed in nearly equal quantities. Consequently, there is no external alkaline or acidic effect of the uptake.

Those plants which received nitrate as the sole source of nitrogen have higher yields than the plants which received ammonium. If nitrate in the medium is replaced by ammonium, the organic-salt content and the yield decrease considerably. The reduction of the organic-salt content is mainly due to a reduced accumulation of the salt cations(C).

In the case of ammonium nitrate nutrition the organic-salt content is also lowered, mainly through an increased accumulation of nitrate in the foliage. The salt-cation content(C) decreases slightly when nitrate is supplied without ammonium. Thus it appears that ammonium nitrate increases the inorganic-salt content (A) at the expense of the organic salts, whereas ammonium in the absence of nitrate lowers the total-salt content (C) at the expense of the organic salts. Although in both cases the organic-salt content is lowered to about 2300 me./kg d.m., ammonium nitrate gives only a slight depression in yield and ammonium in the absence of nitrate a strong depression, compared with the plants receiving nitrate only.

The experiments discussed have shown that the foliage of sugar beets has an organic-salt content of about 3500 me./kg d.m. if nitrate is supplied in sufficient amounts to

obtain a nitrogen content of 3500 me./kg d.m. in the foliage. If potassium deficiency is induced by the replacement of potassium by calcium in the supply, the organic-salt content is only slightly depressed at enhanced accumulation of magnesium and at low yields. If sodium is supplied to potassium deficient beets the organic-salt content is only slightly increased and in this case the foliage shows a high sodium content and a depressed magnesium content while growth is still substantial. If nitrate is replaced by ammonium there is a considerable reduction of both the organic-salt content and the yield of the foliage.



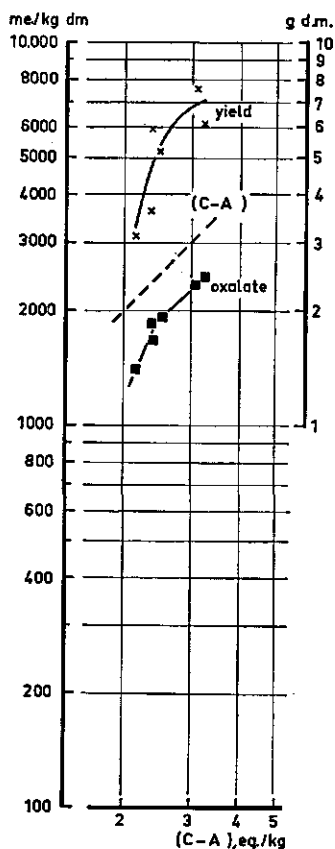


Fig. 23. Yields in grams dry matter per pot (right-hand ordinates), organic-salt (C — A) and oxalate contents of sugar-beet foliage grown with ammonium, with ammonium nitrate or with nitrate as the nitrogenous fertilizer

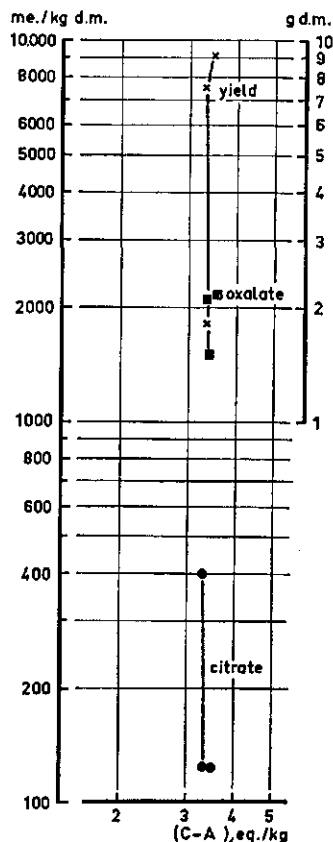


Fig. 24. Yields in grams dry matter per pot (right-hand ordinates), organic-salt (C — A), oxalate and citrate contents of sugar-beet foliage grown with nitrate and varying amounts of potassium in the fertilizer salts

cedure described in section 2.3. The results are recorded in figures 23 to 25.

Fig. 23 shows the depression of the yield and the organic-salt content caused by replacement of nitrate by ammonium in the supply. It is seen that the reduction of the organic-salt content is almost entirely due to a decrease of oxalate.

Fig. 24 refers to depression of the yield caused by potassium shortage following replacement of potassium and sodium by calcium in the supply. The depression of the yield is pronounced, but the organic-salt is only slightly lowered. It should be noted that potassium deficiency has caused a fall in the oxalate content from 2200 to 1500 me./kg d.m., but that the organic-salt content remains unchanged through increased accumulation of citrate, of malate, and of salts of the water-insoluble acids

not recovered by the extraction procedure. The yield depression brought about by either ammonium nutrition or potassium shortage is in both cases associated with the same fall of the oxalate content, from about 2200 to 1500 me./kg d.m. In the plants supplied with ammonium this decrease of the oxalate content is not balanced by an increase of the other organic salts. However, in the potassium deficient plants the other organic salts substitute for oxalate and the total organic-salt content remains unaltered.

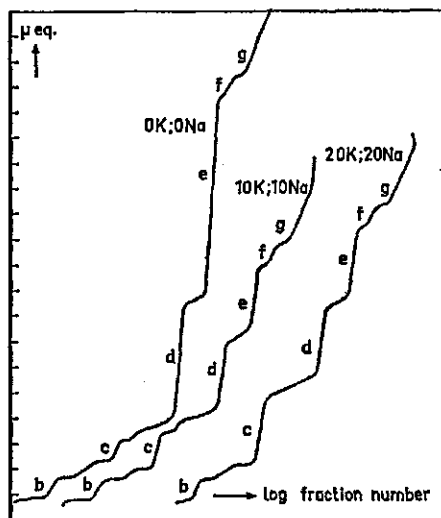


Fig. 25. Partition chromatography of the acids of the organic salts in sugar-beet foliage, grown with nitrate and varying amounts of potassium and sodium in the fertilizer salts. (treatments E of table 10). Acids from 2 grams of dry material; one subdivision = 100 microequivalents. b: succinic; c: unknown; d: malic; e: citric; f: glyceric; g: quinic acids

Chromatograms obtained from the foliage of the plants grown at the replacements of potassium-sodium by calcium are shown in fig. 25. The very high citrate content of the potassium deficient foliage and the higher malate content, compared with adequate potassium, are evident from the size of the corresponding waves e and d. It is also evident that the organic salt of wave c is substantially raised as a result of the increase of the potassium supply. The acid c occupies a position similar to that of oxalic acid, which has been removed previously by anion exchange. By means of paper chromatography it was confirmed that wave c is some other acid and that its presence is not due to incomplete removal of oxalic acid. By its low mobility in the two solvents applied to the paper (ethanol-ammonia and butanol-formic acid-water) it differs from glycolic, pyrrolidone carboxylic and aconitic acids, which also separate in this region when applied to partition chromatography on silica gel.



## 4 Poplar

### 4.1 Growth of crops and leaves

Investigations into the nutritional status of trees are mostly confined to the leaves. Since leaf composition is known to depend on age and position on the branch (section 1.5), selection of certain leaves is often required to standardize the procedure.

However, for the study of more obvious peculiarities of tree species it is convenient to study small tree cuttings and to sample by complete defoliation. In this way the difficulty of defining different classes of leaves is avoided.

The potassium requirements in young apple trees were studied in this way by EDGERTON (1948). His results are summarized in fig. 26 in terms of the total amount of potassium in the leaves and the total-leaf weight. The contents in me. of potassium per kg dry matter are given by the slopes of straight lines through the points and the origin. Depending on the potassium supplied the potassium contents vary in a range from 100 to 900 me./kg d.m.

Two aspects of these results are of interest: the total-leaf weight varies in a relatively small range and the potassium contents of the leaves fall to levels appreciably below 200 me./kg d.m. if the potassium supply is lowered. At these low potassium levels the bottom leaves show severe symptoms of deficiency. Apparently, the total leaf weight

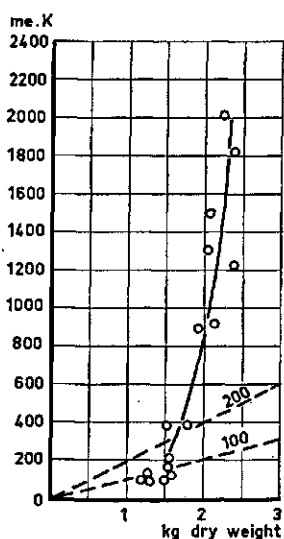


Fig. 26. Relation between the absolute amounts of potassium and the dry material in the leaves of apple trees grown at varying supply with potassium. The dashed lines indicate constant contents at 200 and at 100 me. potassium per kg dry matter. Data of EDGERTON (1948)

depends to a smaller extent on potassium and the minimum potassium content of the total leaf mass is lower than that of grass.

In grass and in earlier stages of growth in other herbaceous crops, abundant tillering occurs and the potassium content does not decline to below 200 mc./kg d.m. However, in shoots of trees with terminal growth the potassium content of the total leaf material is often found to diminish to below 200 mc./kg d.m., because there is a greater proportion of older leaves which have ceased to grow, and are exhausted by the expanding leaves. The possible difference in nutrient requirements which may originate from this difference in growth calls for special note.

First consider planar growth such as, for instance, of *Lemna* fronds floating on the surface of water (WHITE, 1940). Assuming that the surface exposed to the incident light increases proportionally to the plant weight, and that the net assimilation rate remains unchanged, the time ( $t$ ) required for an increase of plant weight ( $W$ ) to twice its initial value is independent of weight. Growth is qualified as exponential and can be represented by:

$$(\log W_2 - \log W_1) (t_2 - t_1)^{-1} = \text{constant (fig. 27a).}$$

Now consider similar fronds which are piled up during growth and have complete absorption of the incident light. Record the time interval required for the first frond to produce a second one of the same weight and dimensions. The second frond becomes located on top of the first one and the latter ceases to assimilate through complete light interception, thus contributing to plant weight by its presence only and not by its activity. The second frond takes over the assimilation and in a same time interval a third frond is produced which again takes over light interception and photosynthesis, and so on. In this case the assimilatory surface remains unchanged and the plant weights recorded over a same interval of time differ by the same value, independent of plant weight. Growth is said to be linear and is given by:

$$(W_2 - W_1) (t_2 - t_1)^{-1} = \text{constant (fig. 27b).}$$

Herbaceous crops have many expanding leaves and growing zones during early growth. A large proportion of the material contributes to expansion of the assimilatory surface. Growth can proceed in an exponential way if plant distance prevents a mutual shading. During more advanced growth the geometric conditions may gradually change into those of a closed crop with complete light interception by the upper zones of the leaves. From there on the expanding upper leaves do not add to total photosynthesis, but take over photosynthesis from the older bottom leaves. Consequently, further growth will proceed in a linear fashion according to fig. 27b.

In single plants with unbranched vertical growth, terminal leaf expansion results in a piling up of the leaves. If illuminated with a light beam along the vertical the conditions are those of fig. 27b, and growth will proceed linearly. Growth will proceed in a more exponential manner if illuminated from all directions.

The growth of unbranched poplar cuttings in the greenhouse with diffuse light was analysed. The total leaf surface of many cuttings, submitted to the experimental treatments has been measured at frequent intervals during the growth. This was

done by first studying the leaf geometrics and establishing the relation between leaf length and leaf surface of a number of leaves. The length of the leaves was measured, and by subdivision into classes of different lengths the leaf surface was calculated by means of factors related to differences in the leaf shape. From many measurements, tested with a computer, it was found that the total leaf area of each cutting increased linearly with time. Associated with the first leaf expansion after defoliation, there was only a short period of exponential growth, which was negligible compared with the main period of linear growth.

If it is assumed that the net assimilation rate of the illuminated leaves remains unchanged, linear growth of the leaf blades must have resulted from the export of substance from the leaves to the stems and roots. When each individual leaf has reached its final size, export becomes complete and the younger leaves take over leaf expansion. If the darkened areas represent leaves of final size with complete export of the substance produced, this case resembles that of fig. 27b.

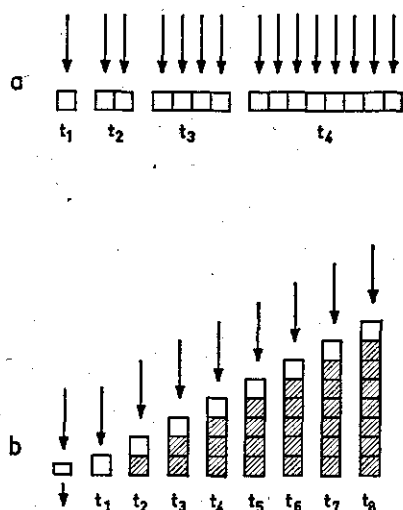


Fig. 27. Diagram illustrating exponential (a) and linear growth (b). The arrows indicate incident light. In fig. b light interception becomes complete at  $t_1$ . The time intervals are those required to double the weight by photosynthesis and are considered constant

Now assume that leaf expansion and growth requires the presence of 200 me. potassium per kg dry weight produced. In exponential growth (fig. 27a) all plant parts continue to expand and this can proceed undisturbed only if the potassium content is kept at this level in the whole material. If it is assumed that with linear growth this critical potassium content is required only in the terminal, expanding leaves, and that potassium moves from older to terminal leaves to be used repeatedly during leaf expansion, the potassium requirements for linear growth in the example given in fig. 27 would be only 1/8 of the requirement for exponential growth with the same total leaf weight production.

Potassium exhaustion of the older leaves does not proceed to completion. However,

it is often reflected in a higher potassium content of the terminal expanding leaves in potassium deficient plants (NIGHTINGALE *et al*, 1930) and in earlier appearance of necrotic symptoms in the older leaves. Owing to the predominance of linear growth it can be expected that the effect of the application of potassium on the leaf weight of poplar cuttings will be small compared with the response of the foliage of herbaceous crops, and that the potassium content may fall to below 200 me./kg d.m. in the total-leaf mass.

## 4.2 The normal organic-salt content

Growth and composition of the leaves of poplar cuttings have been studied by EVERS (1963, 1964). Working with sand cultures irrigated with solutions of nutrient salts, EVERS found that in the range pH 3 to pH 8 nitrate as the source of nitrogen was more adequate than ammonium for dry weight production of leaves. Chloride was not supplied, and in leaf analysis chloride and nitrate were not determined. Since chloride and nitrate contents are always low in the leaves of poplar, the data of EVERS can be used to estimate the organic-salt content ( $C - A$ ). It appears that with nitrate supply the organic-salt content was about 1200 me./kg d.m. at pH 6 and 8, and about 900 me./kg d.m. at a soil pH of 3.3. With ammonium the organic-salt content varied from 1100 at pH 8 to 300 at pH 3.3. At pH 8 both treatments had a yield of about 33 grams, but at the lower pH levels the nitrate treated plants produced 40, and the ammonium plants only 4 grams of dry leaf material.

Although it appears that the poorer growth with ammonium can be improved by raising the soil pH, it is clear from the results that poplar grows better with nitrate as the source of nitrogen. The more vigorous growth obtained with nitrate also indicates that the normal organic-salt content is about 1100 me./kg d.m.

A first exploration of the ionic balance of poplar was made by collecting leaf blades from a seven year old, healthy tree in the garden of the Institute on September 12, 1962 at noon. An organic-salt content of 1346 me./kg d.m. was found. This value is slightly higher than that for cuttings, which may have resulted from a greater proportion of older, more mature leaves in the sample. A higher organic-salt content is often found in leaves of more advanced maturity (compare e.g. COIC *et al*, 1962).

Organic-salt contents similar to those calculated from the data of EVERS were found to occur in the cuttings submitted to the following greenhouse experiment.

Pots were filled with 7 kg of a humic sandy soil of good fertility with pH 5.5. and defoliated cuttings were planted on October 1, 1962. The treatments were applied as measured amounts of 1 N stock solutions on October 15.

The treatments were 0, 20, 40 and 60 me. of the nitrates of either potassium, sodium, magnesium or calcium in each of the series. The leaf blades produced were harvested on November 11, 1962. The results listed in table 14 show that there is some increase in the content of the cation supplied with the nitrate, except for sodium, which apparently fails to accumulate in the leaves when applied to the soil. Further, there is

Table 14. Composition of the leaf blades of poplar cuttings grown in a pot experiment with a sandy soil of good fertility, to which increasing amounts of the nitrates were added. N and S refer to total nitrogen and total sulphur. Yields of the leaf blades in grams fresh (f.m.) and dry (d.m.) material per plant per pot

Grams per pot		me. per kg of dry material									Treatments me. per pot	
f.m.	d.m.	N	NO <sub>3</sub>	Cl	P	S	K	Na	Mg	Ca		
66	21	1600	8	56	63	308	669	13	129	646	0	KNO <sub>3</sub>
71	26	1806	11	62	69	233	525	13	99	649	20	
89	26	2172	6	65	69	323	763	10	114	492	40	
81	24	2206	13	62	76	471	824	13	129	774	60	
62	20	1349	11	65	61	292	582	13	124	531	0	NaNO <sub>3</sub>
72	21	1943	11	59	72	302	729	13	129	663	20	
87	25	1875	10	62	65	354	586	26	134	578	40	
64	17	2595	6	76	83	410	637	19	124	721	60	
66	22	1532	8	39	62	327	586	6	124	635	0	Mg(NO <sub>3</sub> ) <sub>2</sub>
71	25	1532	6	56	55	237	499	6	159	539	20	
72	21	2263	6	65	66	410	582	13	208	760	40	
89	25	2389	13	62	87	342	641	6	198	521	60	
65	18	1394	10	62	59	331	686	6	129	514	0	Ca(NO <sub>3</sub> ) <sub>2</sub>
69	25	2046	13	65	68	287	486	10	149	667	20	
80	22	2320	15	65	77	321	652	6	154	696	40	
77	23	2149	11	65	65	408	567	6	154	835	60	

no indication of any shortage of the elements. With increasing nitrate supply the nitrogen content of the leaves increases from about 1500 to 2400 me./kg d.m., but there is no effect on the total leaf weight. This means that for this short time experiment 1500 me. of nitrogen per kg of dry material is adequate.

The total amount of anions absorbed ( $A_u$ ) varies considerably owing to the difference in nitrate uptake. In fig. 28 the relation between the utilization of organic nitrogen and organic sulphur, the accumulation of the salt cations (C), the apparent intake of hydrogen ions ( $H^+$ ), and the total amount of anionic elements ( $A_u$ ) in the leaves is given. The increase of the total anionic elements is balanced by an apparent intake of hydrogen ions, the intake of the salt cations (C) remaining practically unaltered. Thus there is no indication of any constancy in the ratio of salt cations to salt anions absorbed.

The graph shows that the production of organic nitrogen and organic sulphur proceeds in an excess of about 1100 me./kg d.m. over the apparent hydrogen uptake. This excess accumulates as organic salts. The organic-salt content indicated by crosses, is about 1100 me./kg d.m. and independent of the total amount of anions absorbed.

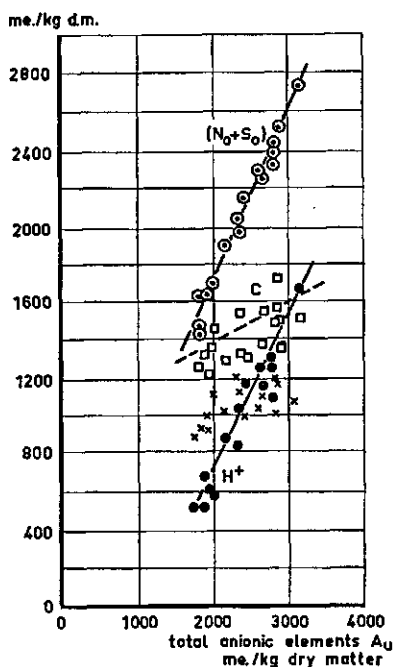


Fig. 28. Organic-nitrogen plus organic-sulphur ( $N_o + S_o$ ), total-salt ( $C$ ) (squares), and organic-salt ( $C - A$ ) (crosses) contents in relation to the total absorption of anionic elements ( $A_u$ ) in me. per kg of dry material in the leaves of poplar cuttings grown at varying rates of nitrate application. Data of table 14. The filled dots represent the calculated uptake of hydrogen ions ( $H^+$ )

The acidity of the uptake, which varies from 600 to 1600 me.  $H^+$ /kg d.m. recalls the conditions in perennial ryegrass. The greater acidity of xylem tissues relative to phloem and other tissues (NIGHTINGALE *et al.*, 1930) and the relation between nitrate reduction and the early stages of photosynthesis (BURSTRÖM, 1946) may support the view that nitrate utilization by the metabolic process mainly occurs in the leaves and that nitrate is transported to the leaves as such (LUNDEGARDH, 1945). In this case the uptake by the leaves is really acidic.

However, if nitrate is converted into organic nitrogen during its transport to the leaves, the intake by the leaves is not actual acidic. This organic nitrogen appears in the balance as an intake of nitrate and its equivalent of hydrogen ions.

Since the soil used was of good fertility and foliar analysis showed the absence of shortages it can be assumed that the organic-salt content of 1100 me./kg d.m. represents the normal value in poplar leaves.

### 4.3 Effects of poor fertility

As found by EVERS (1963) a higher acidity of the substrate lowers the organic-salt content of poplar leaves even in the presence of a sufficient supply with salt cations and nitrate. In the present investigation in a greenhouse this was achieved by making use of an acid soil with a low potassium content. At the subnormal organic-salt contents thus obtained the effect of potassium was studied in detail. After a first defoliation the

fertility of the soil was improved by a second application of the nutrient salts and the effect of potassium was studied again at the higher organic-salt contents obtained.

In this experiment use was made of a poor sandy soil of pH 4.0 and with a cation exchange capacity of 140 me./kg d.m. The pots were filled with 7 kg of soil to which was added 40 me. of calcium biphosphate as a basic dressing. Leafless winter cuttings of 40 cm height were planted on January 15, 1963 (one cutting per pot), and on January 19 salt solutions were supplied according to the following schedule, expressed as me. per pot:

$x \text{ K} + (40 - x) \text{ Na} + 40 \text{ Mg} + 40 \text{ Ca} + 40 \text{ NO}_3 + 80 \text{ SO}_4$

$x \text{ K} + (40 - x) \text{ Mg} + 40 \text{ Na} + 40 \text{ Ca} + 40 \text{ NO}_3 + 80 \text{ SO}_4$

$x \text{ K} + (40 - x) \text{ Ca} + 40 \text{ Na} + 40 \text{ Mg} + 40 \text{ NO}_3 + 80 \text{ SO}_4$ .

Leaf growth was allowed to continue, on March 5, 1963, the leaf blades were harvested for analysis. After this first defoliation, the pots received a second supply with the same amounts of the nutrient salts, and after a second period of leaf growth the cuttings were defoliated on April 8, 1963.

The data on total-leaf weight and composition are listed in table 15. In fig. 29 leaf composition is plotted against the milliequivalents of potassium supplied per pot. Because the change of the other constituents, such as nitrogen, inorganic salts (A), magnesium, calcium, etc. is either small (nitrogen) or as expected (moderate changes in the magnesium and calcium contents according to their supply), the values for potassium, total salts (C) and organic salts (C — A) are plotted only.

In the leaves of harvest I (after 7 weeks of growth) the potassium content is below the specific potassium requirement of 200 me./kg d.m. at zero potassium supply. The organic-salt content of 500 me./kg d.m. is much below the normal value of 1100 me./kg d.m. At the successive increments of the potassium supply the potassium content of the leaves increases up to 400 me./kg d.m. and the organic-salt content to about 650 me./kg d.m. Although the potassium content increased from below the value, associated with specific shortage, to well above this specific requirement, all treatments resulted in plants with necrotic spots on the leaves. These spots are considered to be indicative of potassium shortage. Of course, this could be connected with a higher specific requirement in poplar, but such an assumption would contradict the evidence inferred from many literature data on a variety of plant species and from other experience with poplar. Furthermore the linear fashion of growth does not account for a higher specific potassium requirement in poplar compared with plants of more exponential growth (section 1.4).

Therefore, the persistence of necrosis at the higher potassium contents is not conditioned by potassium alone, as apparent if the data on the second defoliation are considered more in detail. In harvest II (after 5 weeks of growth) plants with a potassium content of 200 me./kg d.m., showed necrotic leaf symptoms of potassium deficiency, whilst at the increase of potassium the symptoms already vanished at the first addition of potassium, raising the content to 300 me./kg d.m., and remained absent at the further increase of potassium.

The main difference is the much higher organic-salt content of harvest II, compared with harvest I.

Apparently, the ability of the plant to recover from necrosis through potassium

*Table 15. Composition of the leaf blades of poplar cuttings. First (I) and second (II) defoliation. Treatments: 40 me. of nitrate per pot and replacement of other salt cations by potassium. N and S refer to total nitrogen and total sulphur. Yields of the leaf blades in grams fresh (f.m.) and dry (d.m.) material per plant per pot*

Grams per pot		me. per kg of dry material									Treatments me. per pot		Defoli- ation
f.m.	d.m.	N	NO <sub>3</sub>	Cl	P	S	K	Na	Mg	Ca			
72	18	1772	15	45	84	167	121	6	228	389	0 K	40 Na	I
104	23	1909	11	48	87	194	225	6	228	364	10	30	
105	25	1657	21	65	77	179	221	6	208	400	20	20	
100	23	1680	11	71	70	190	263	6	218	428	30	10	
143	30	1829	13	62	90	206	406	6	203	328	40	0	
80	20	1715	10	87	80	181	121	6	243	396	0 K	40 Mg	
87	19	1932	11	73	96	177	215	6	233	424	10	30	
127	28	1840	11	76	90	187	340	6	198	375	20	20	
129	30	1806	11	85	89	185	427	6	164	307	30	10	
136	30	1749	15	71	76	177	321	6	174	375	40	0	
—	26	1909	15	164	84	185	138	6	263	428	0 K	40 Ca	II
137	33	1612	16	73	90	115	189	6	233	439	10	30	
127	27	1852	15	59	100	200	285	6	218	442	20	20	
106	23	1875	15	51	103	194	325	6	203	350	30	10	
114	27	1806	18	152	87	192	376	6	193	317	40	0	
18	5	2617	—	59	184	204	195	6	218	649	0 K	40 Na	
33	9	2629	—	76	144	215	319	10	263	528	10	30	
28	8	2423	—	76	137	196	376	6	198	474	20	20	
31	8	2491	—	59	141	223	476	10	203	482	30	10	
48	12	2926	—	59	153	250	567	6	337	531	40	0	
19	5	2503	—	76	187	202	200	16	248	621	0 K	40 Mg	II
28	8	2537	—	62	154	202	316	6	213	585	10	30	
38	10	2606	—	73	149	212	380	6	253	535	20	20	
37	9	2755	—	54	182	219	542	6	193	467	30	10	
42	11	2595	—	56	128	187	552	13	119	507	40	0	
19	5	2617	—	59	186	260	227	13	278	746	0 K	40 Ca	
41	11	2595	—	79	138	246	302	12	283	678	10	30	
40	10	2675	—	54	152	223	395	10	248	546	20	20	
36	9	2995	—	73	163	246	584	10	258	489	30	10	
33	8	2617	—	68	131	225	467	10	258	432	40	0	



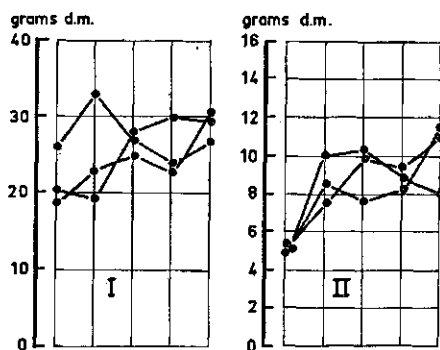
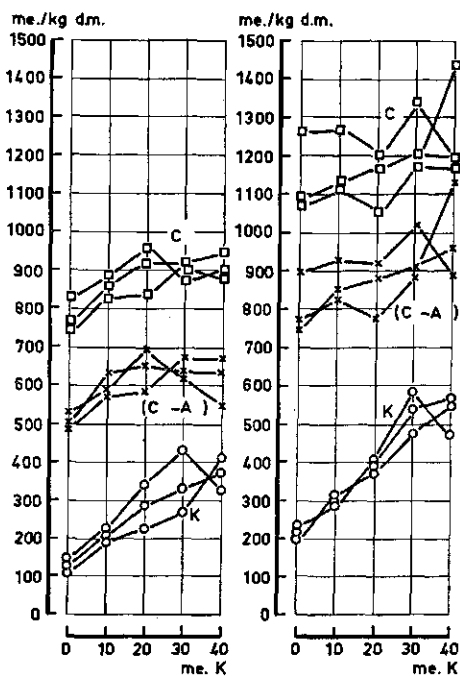


Fig. 29. Composition of the leaf blades of poplar cuttings grown on a poor, acid soil with varying amounts of potassium in the fertilizer salts applied. First (I) and second (II) defoliation. The pots were refertilized after the first defoliation. Data of table 15



uptake depends on the organic-salt content. At organic-salt contents below about 700 me./kg d.m., potassium alone is not able to remove the symptoms. To cure the leaves by the supply of adequate amounts of potassium a higher organic-salt contents of about 900 me./kg d.m. is required.

Another difference between both harvests is the higher nitrogen content of the leaves of harvest II, originating from the improved fertility after refertilization. However, the lower nitrogen content of about 1700 me./kg d.m. in the experiment of section 4.2 was found to be associated with healthy leaves and an organic-salt content of 1000 me./kg d.m.

#### 4.4 Effects of ammonium and nitrate at varying pH of the soil

EVERS (1963) studied the growth and composition of the leaves of poplar cuttings. Compared with nitrate fertilization ammonium reduced both the yield and the organic-salt content considerably. At pH 3.3 nitrate resulted in a good yield with an organic-salt content of about 900 me./kg d.m., while ammonium treated plants showed very poor growth with an organic-salt content of about 300 me./kg d.m. Apparently, at this acidity ammonium is inferior to nitrate, but it has frequently been found that when the acidity is reduced, growth with ammonium is much improved (WANDER and SITES, 1956).

As can be calculated from the data of EVERS, this improvement is associated with a higher organic-salt content. His experiments with irrigated sand cultures were undoubtedly conducted in the absence of nitrification.

For the next experiment a soil was used in which nitrification of ammonium occurs at pH > 5 (VAN TUIL and LAMPE, 1964). To create a range of soil pH from 3.4 to 7.4,

*Table 16. Composition of the leaf blades of poplar cuttings grown in a pot experiment on the effect of soil pH with ammonium or nitrate as the nitrogenous fertilizer. N and S refer to total nitrogen and total sulphur. Yields of the leaf blades in grams fresh (f.m.) and dry (d.m.) material per plant per pot.*

*Treatments: 0 and 50 me. sulphuric acid or 0; 100; 200; 400; 1000; and 2000 me. of calcium carbonate per pot of 7 kg of soil. Exchange capacity of the soil: 140 me./kg. Added nutrient salts, in me. per pot: (30 calcium nitrate; 10 magnesium nitrate; 15 potassium sulphate and 15 potassium biphosphate) or (40 ammonium sulphate; 10 magnesium sulphate; 15 potassium sulphate and 15 potassium biphosphate)*

Grams per pot		me. per kg of dry material									Soil pH	
f.m.	d.m.	N	NO <sub>3</sub>	Cl	P	S	K	Na	Mg	Ca	init.	fin.
died											3.4	—
2.7	0.9	2252	—	—	—	—	599	32	233	992	4.0	3.9
24	7	2229	13	99	155	937	1109	30	203	553	4.5	4.2
41	11	2240	8	87	87	769	1058	26	109	546	5.0	4.3
45	13	2446	10	85	94	614	879	19	109	674	5.9	4.2
36	10	2480	11	87	113	667	826	16	119	842	7.2	5.6
31	8	2549	11	85	90	539	826	13	119	735	7.4	6.9
6.6	1.8	2389	—	—	—	—	847	23	382	927	3.4	4.0
23	6	2389	24	93	177	321	1013	19	184	578	3.7	4.2
41	11	2400	8	85	114	465	990	19	114	549	4.2	4.8
43	11	2377	6	85	132	519	932	19	119	667	4.7	5.0
37	10	2475	11	87	117	512	867	23	119	738	5.6	5.6
32	9	2275	11	85	87	435	811	23	114	842	6.9	6.9
42	11	2400	11	79	89	477	835	19	94	753	7.1	7.3

NH<sub>4</sub>  
series

NO<sub>3</sub>  
series

pots were filled with 7 kg of soil, previously mixed with measured amounts of either 1 N sulphuric acid or solid calcium carbonate. One half of the pots received 40 me. of ammonium sulphate and the other half 40 me. of calcium nitrate. In addition all pots received a constant amount of potassium sulphate, magnesium sulphate and potassium biphosphate. The salts were added to the soil on July 17, 1963, the cuttings

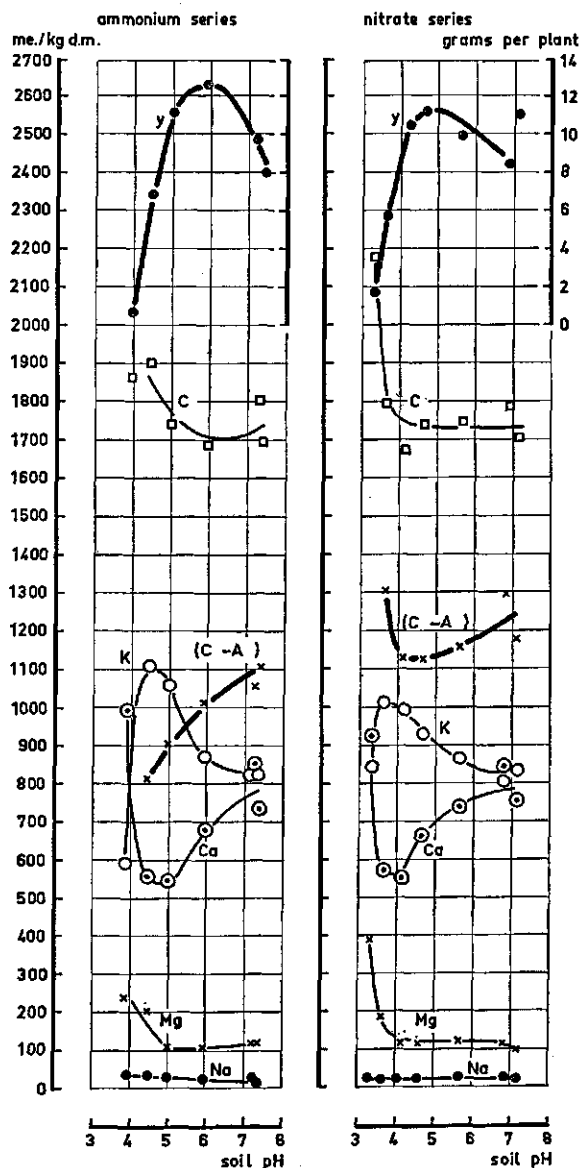


Fig. 30. Leaf composition and yield (grams dry matter per plant per pot) of poplar cuttings, grown at various soil pH with nitrate or with ammonium as the nitrogenous fertilizer. Data of table 16

planted and the initial soil pH measured in a water suspension (1 : 5). The leaves were harvested and the final soil pH was determined on December 13, 1963. During the period of growth the nutrient salt applications were repeated on October 28, 1963.

The results are listed in table 16 and figure 30. The graphs show that the yield falls at the increase of the soil acidity. In the ammonium series the yield depression begins at pH 5 and in the nitrate series at pH 4.

It has been shown that with nitrate the uptake of the salt ions is acidic to an extent comparable to that of perennial ryegrass (section 4.2) and consequently, there is an external alkaline effect of the uptake of nutrient salts with nitrate as the source of nitrogen. The results of the pH determinations show that in this case the soil pH increases e.g. from 4.2 at the beginning to 4.8 at the end of the experiment. In the ammonium series the pH falls e.g. from 5.0 initially to 4.3 at the end of the experiment. Compared with the final soil pH the initial values at the beginning of the experiment are lower in the nitrate series and higher in the ammonium series.

The shift of the soil pH of the pots which received nitrate fertilization is about 0.5 units at pH 4. From the analytical data it can be inferred that the acidity of the uptake by the leaves is about 1.4 me. per gram dry matter at a dry-weight production of 11 grams, which corresponds to 15 me. of hydrogen ions per pot. From the relation between soil pH and the amount of calcium carbonate used for the preparation of the different levels of soil pH it could be inferred that about 100 me. of alkali per pot are required to raise the pH by 0.5 units at this level of pH. Of course, the calculated acidity of the uptake by the leaves cannot account for the total acidity absorbed by the whole plant.

The acidification of the ammonium fertilized pots during the experiment may originate from the alkaline uptake by the plant, and in addition, from the process of nitrification which was found to occur in this soil only after liming to above pH 5 (VAN TUIL and LAMPE, 1964). Assuming that the nitrogen has been completely absorbed in the form of ammonium, the analytical data show that the alkalinity of the uptake is on the average 3 me. per gram dry matter. At pH 5 the yield is 11 grams of dry material per pot which means that the alkalinity of the uptake by the leaves is 33 me. per pot. The shift of the pH during the experiment is from pH 5.0 to pH 4.3 and since this is associated with an acidity of about 100 me. per pot, the alkalinity of the uptake by the leaves accounts only for one third of the shift.

Of course, such calculations should be made with data on whole plant analysis. The figures given before are used only to demonstrate the order of magnitude of the external alkaline and acidic effects associated with nitrate and ammonium as the source of nitrogen. In the case of ammonium nutrition, the process of nitrification at soil pH > 5 also contributes to the acidification of the soil.

The data on yield and nitrogen content of the leaves show that at pH 5 about 25 me. of ammonium has been absorbed at a total supply of 80 me. of the ammonium salt per pot. Uptake by the whole plant is unknown. At the higher pH levels with nitrification and low yields, ammonium has most certainly been supplied in excess of the uptake by the whole plant.

The analytical data show that the substitution of ammonium for nitrate has lowered the organic-salt content to a larger extent in the more acid range of the soil pH. Owing to the onset of nitrification the organic-salt content increases at the increase of pH in the range above pH 5, and approaches the value obtained with nitrate application.

#### 4.5 The organic salts of poplar

Samples from different treatments have been examined on the organic salts by extraction and chromatography with the methods described in section 2.3.

It was found that the procedure recovered 60 per cent of the calculated organic-salt content as the salts of the water-soluble, non-volatile plant acids.

The chromatogram shows the presence of malate, citrate, malonate, succinate and in addition, ketoglutarate. The final wave i of the chromatogram obtained from the column is found to include shikimate and quinate (fig. 31).

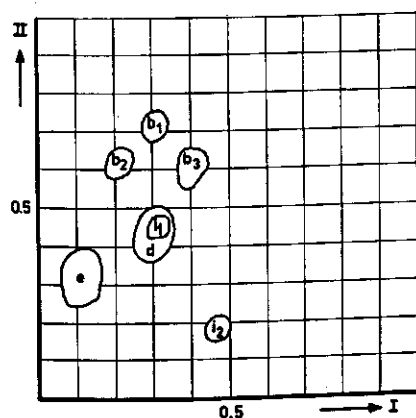


Fig. 31. Paper chromatogram of organic acids from poplar leaves, after previous separation on silica gel.  
*b<sub>1</sub>: succinic; b<sub>2</sub>: malonic; b<sub>3</sub>: ketoglutaric; d: malic; e: citric; i<sub>1</sub>: glyceric; i<sub>2</sub>: quinic acids*

Some samples have also been investigated on polyuronic acids. The samples were first extracted with acidified water and with ether in succession, and thereafter the polyuronates were determined by titrating the carbon dioxide evolved by boiling the residue with hydrochloric acid. It was found that the total recovery increased to about 80 per cent of the organic-salt content if the polyuronates were included.

The residue of the material obtained after the extraction of the water-soluble acids for chromatography, has been re-extracted with boiling hydrochloric acid, and oxalic acid was determined by precipitation of calcium oxalate and permanganate titration. The residue appeared to contain about 300 me. of oxalate per kilogram of the initial dry material. Since the organic-salt content (C — A) was 1500 me. per kg dry material, this represents 20 per cent of the total organic-salt content. Apparently,

the difference between the calculated organic-salt content and the sum of the water-soluble and polyuronic acids represents more difficultly extractable oxalic acid.

Some samples of grass and sugar-beet foliage were also investigated on difficultly extractable oxalic acid by re-extraction of the residue with hydrochloric acid. It appeared that in these species only about 5 per cent of the oxalate was present as more difficult extractable oxalic acid. This is due to the much lower ratio of calcium to oxalate in sugar-beet foliage and to the low calcium and oxalate contents of grass.

## 5 Birch

### 5.1 Plants sensitive to nitrate nutrition

It has been found that a great variety of agriculturally important plant species achieves lower yields when fed with ammonium instead of nitrate. The lower organic-salt content of such plants does not originate from a difference in the utilization of both forms of nitrogen, but is caused by competition of ammonium with the salt cations (C) and of nitrate with the salt anions (A) during the uptake (ARNON, 1939).

Data on a large number of plant species have indicated that ammonium nutrition invariably results in a lower organic-salt content than nitrate nutrition. However, certain plant species exhibit better growth with ammonium. If nitrate is supplied to these plant species, the response may vary from complete inhibition of growth (*Vaccinium oxycoccus*, MARTHALER, 1939) to reduced growth, as for instance in *Erica tetralix*, *Calluna vulgaris*, *Vaccinium spec.* (CAIN, 1952) and *Cornus canadensis* (TAYLOR, 1935).

CAIN (1954) found that the pH of the leaf homogenate of *Vaccinium corymbosum*, grown with ammonium is 3. If ammonium was replaced by nitrate, the growth was reduced and the pH of the leaf homogenate was increased to around 5. This change was associated with chlorosis as a visual symptom of the disorder caused by nitrate fertilization. COLGROVE and ROBERTS (1956) found that the leaf pH in *Rhododendron* increased from pH 4.0 to pH 5.3 and the growth was considerably reduced if ammonium in the supply was replaced by nitrate.

Apparently, these ericaceous plants show better growth with ammonium. Nitrate results in reduced growth, higher tissue pH and chlorosis. As expected, the data of CAIN (1954) show that the total-salt content (C) of the leaves increases if nitrate was supplied instead of ammonium.

INGESTAD (1962) grew small birch seedlings on nutrient solutions. Better growth was obtained when cultivated with ammonium instead of nitrate, although at nitrate supply the plants survived without chlorosis.

In the present investigation birch was chosen as representative for the group of plant species with improved growth when cultivated with ammonium. The purpose was to investigate whether ammonium gives a higher yield, associated with a lower organic-salt content, and to apply the concept of the normal organic-salt content to this particular behaviour.

## 5.2 The normal organic-salt content

Pots were filled with 7 kg each of a poor sandy soil of pH 3.9. Salts were added with a constant supply of 60 me. of potassium, and 60 me. of nitrate, or 30 me. of nitrate plus 30 me. of chloride, or 30 me. of nitrate plus 30 me. of carbonate, or 60 me. of carbonate (compare table 17). Budding birch trees of 40 cm height were previously grown from seed of *Betula verrucosa*. On April 5, 1963 they were selected for equal size and transplanted in the treated pots. On May 24 the addition of nutrient salts was repeated, and on July 1 the leaves were harvested.

The analytical data are listed in table 17. The total amount of nitrogen and of organic salts in the leaves per plant were calculated, and plotted as ordinates against the dry weights of the leaves per plant in fig. 32. At zero nitrate supply (a) the yield and the nitrogen content are low with an organic-salt content of 400 me./kg d.m. The supply with 30 me. of nitrate and 30 me. of chloride (b) improves the growth considerably, the organic-salt content is 530 me./kg d.m. Replacing chloride by 30 me. of carbonate (c) gives a further improvement of growth, with a reduction of the nitrogen content and an increase of the organic-salt content to 580 me./kg d.m. At 60 me. of nitrate supplied (d) the yield is slightly lowered at a higher nitrogen content and the organic-salt content increases to 700 me./kg d.m. If the data are plotted in the man-

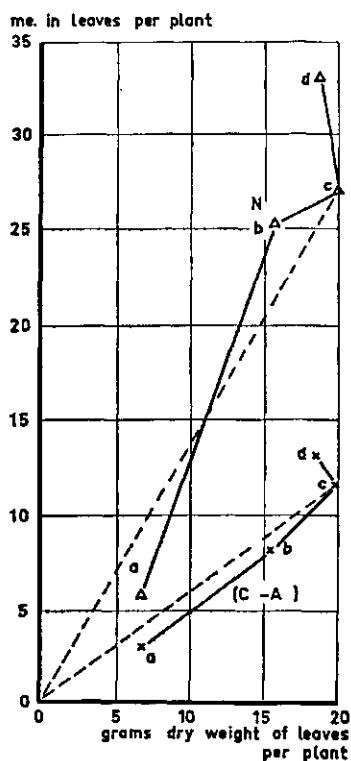


Fig. 32. Absolute amounts in me. of nitrogen (N) and of organic salt (C — A) in relation to the dry weight in the leaves of birch trees grown at various levels of nitrate application to the soil. Data of table 17. The lower dashed line is drawn at 580 me. per kg dry material



Table 17. Composition of the leaf blades of small birch trees grown in a pot experiment on a poor sandy soil (pH 3.9). The treatments have been applied a second time during the growth period. N and S represent total nitrogen and total sulphur. Yields of the leaf blades in grams of fresh (f.m.) and of dry (d.m.) material per plant per pot

Grams per pot		mc. per kg of dry material									Treatments, mc. per pot: 60 K and		
f.m.	d.m.	N	NO <sub>3</sub>	Cl	P	S	K	Na	Mg	Ca	NO <sub>3</sub>	Cl	CO <sub>2</sub>
15	6.9	845	13	56	43	79	306	6	55	189	0	0	60
44	15	1645	15	133	66	115	571	6	35	157	30	30	0
52	20	1370	7	37	49	92	503	13	35	139	30	0	30
55	18	1805	8	28	63	112	616	6	35	157	60	0	0

ner of fig. 32, the contents in mc./g d.m. are given by the slope of a straight line through the point and the origin. It is seen that the highest yield obtained coincides with an organic-salt content of 580 mc./kg d.m., and that lower yields are associated with either higher or lower values of the organic-salt content.

This indicates that for birch the normal organic-salt content is per definition about 580 mc./kg d.m. This value can be obtained with nitrate, if the soil is sufficiently low in pH (compare section 5.3). At the highest yield obtained the supply was 2 x 30 mc. per plant. The uptake by the leaves was 27 mc. Although the uptake by the whole plant is greater nitrate must have been applied in excess.

### 5.3 The effect of ammonium and nitrate fertilization

The natural habitat of birch is a leached acid soil but its occurrence is not restricted to these soils. On more alkaline soils birch trees often occur with a healthy appearance. However, INGESTAD's findings on improved growth with ammonium instead of nitrate seem indicative of better growing conditions on the more acidic soils with retarded nitrification.

Obviously, this reaction to replacement of nitrate by ammonium differentiates the birch from agriculturally important crops. The effect of soil acidity in the presence of ammonium and nitrate has been investigated in a pot experiment with a poor, acid soil (pH 3.9) which was varied in acidity. In order to obtain series of pots with varying pH, weighed portions of 7 kg of the soil were mixed with measured amounts of either solid calcium carbonate, ranging from 0 to 2000 mc. per pot, or of a N sulphuric acid solution ranging from 0 to 100 mc. per pot. In addition, constant amounts of various salts were added: 10 mc. of magnesium sulphate, 10 mc. of potassium biphosphate and 30 mc. of potassium sulphate per pot. One half of the pots of each pH level received 2 x 50 mc. of ammonium sulphate (ammonium series), the other half 2 x 50 mc. of calcium nitrate (nitrate series).

Directly after the preparation of the pots, small soil samples were taken from each pot and their pH was measured in a (1:5) water suspension. The results have been related to these initial values of soil pH. Their change during the experimental period (April 5 to July 1, 1963) has been checked upon by a second sampling at the conclusion of the experiment for measuring final soil pH values. The results are listed in table 18 and summarized in fig. 33.

The graphs show that in the range studied the yield has a distinct optimum in the range of soil pH 4 to 5. In the ammonium series the optimum is located at the initial soil pH of 5, and in the nitrate series at the initial value of 4.

The initial and final values of soil pH show that in the ammonium series pH decreased by 1 to 2 units in the range from pH 4 to 5. In the nitrate series the change is less than 0.5 units. The fall of the pH during the experiment in the ammonium series also occurs in the region below pH 5. From this it is clear that nitrification is not the only cause of acidification. From the data on leaf analysis it can be calculated that the alkalinity of the uptake in the ammonium series is on the average 2000 me./kg d.m. Taking into account the titration curve of the soil, it can be calculated that

*Table 18. Composition of the leaf blades of birch grown in a pot experiment on the effect of soil pH with ammonium or nitrate as the nitrogenous fertilizer. N and S refer to total nitrogen and total sulphur. Yields of the leaf blades in grams fresh (f.m.) and of dry (d.m.) material per plant per pot. Treatments: 0, 50 and 100 me. sulphuric acid or 0, 100, 200, 400 and 2000 me. calcium carbonate per pot of 7 kg of soil. Exchange capacity of the soil: 140 me./kg. Added nutrient salts in me. per pot: (50 calcium nitrate, 10 magnesium sulphate, 10 potassium biphosphate and 30 potassium sulphate) or (50 ammonium sulphate, 10 magnesium sulphate, 10 potassium biphosphate and 30 potassium phosphate). The application has been repeated during the growth*

Grams per pot		me. per kg of dry material									Soil pH	
f.m.	d.m.	N	NO <sub>3</sub>	Cl	P	S	K	Na	Mg	Ca	init.	fin.
9	3.5	—	—	—	—	—	—	—	—	—	3.3	3.2
32	12	1990	6	17	72	248	297	10	124	339	3.5	3.2
65	27	1565	6	23	59	162	272	6	84	253	3.8	3.1
89	34	1430	6	20	59	144	306	6	104	275	5.1	3.2
70	24	1830	6	25	76	171	365	6	124	339	5.8	3.7
31	12	1965	8	37	69	173	346	6	124	400	6.3	4.5
4.4	2	—	—	—	—	—	—	—	—	—	7.3	6.9
died											3.0	—
38	12	2220	6	28	80	204	418	6	129	382	3.3	3.1
75	26	2130	6	28	80	183	412	6	129	378	3.6	3.7
74	29	1590	8	11	58	110	389	6	99	257	4.0	4.6
64	24	1625	8	17	63	127	357	6	114	385	4.9	5.3
59	21	1715	6	25	63	121	331	6	134	382	5.4	5.8
24	9	2115	6	17	63	156	355	6	104	425	6.6	6.0
27	13	1545	5	28	51	131	319	6	84	453	7.4	7.3

NH<sub>4</sub>  
series

NO<sub>3</sub>  
series

uptake by the leaves may have caused a decrease of pH of about 0.5. However, the uptake by the whole plant must have been higher.

The change of soil pH in the ammonium series means that the yield curve of fig. 33 is shifted to the more alkaline side compared with the average conditions during the experiment. Therefore, it seems useful to restrict the discussion to the relation between yield and leaf composition only.

In the ammonium series the optimum of the yield coincides with an organic-salt content of about 550 me./kg d.m., which is near to the normal value (section 5.2).

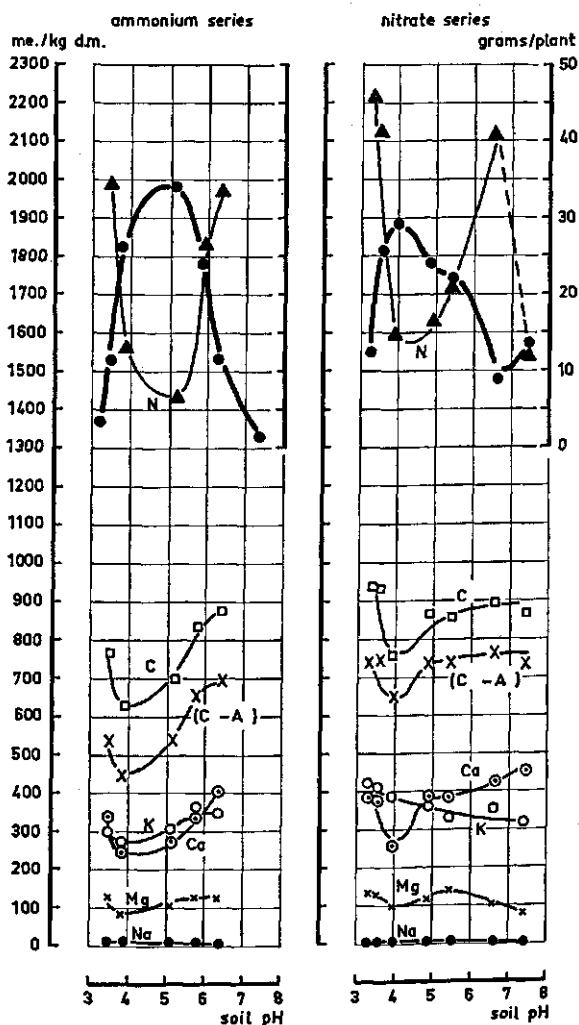


Fig. 33. Yields in grams of dry material per plant per pot (filled circles, heavy lines, ordinates at the upper right-hand side of the graphs) and composition of the leaf blades of birch trees grown at different soil pH and with ammonium or with nitrate as the nitrogenous fertilizer salt

When passing from the optimum to the more acid direction the yield declines and the organic-salt content falls to below the normal value. To the more alkaline side the yield declines with an increase of the organic-salt content to above its normal value.

In the nitrate series the optimal leaf production coincides with an organic-salt content of 650 me./kg d.m., which is above the normal content. All other treatments of the nitrate series show still higher organic-salt contents at about 750 me./kg d.m.

A comparison of both series reveals that the highest production in the nitrate series at 650 me. organic salts/kg d.m. is lower than the highest production at 550 me. organic salts/kg d.m. of the ammonium series. In addition, the nitrogen content, which shows a minimum at the highest yields owing to dilution by growth, is higher in the nitrate series than in the ammonium series.

Apart from further details the results show that the highest yield has been obtained with ammonium as the source of nitrogen at an organic-salt content near to the normal value.

The increase of the organic-salt content at the decrease of the soil acidity in the ammonium series is due to the onset of nitrification above pH 5, which makes leaf composition to approach that of the plants which received nitrate.

The increased organic-salt content at the initial pH 3.5 and 3.3 is associated with very poor growth caused by the extreme soil acidity.

## Conclusions and summary

If excised roots are suspended in solutions of single salts the uptake of the salt cation ( $C_u$ ) may occur in amounts which, depending on the type of salt added, differ from the number of equivalents of the salt anion absorbed ( $A_u$ ). In the ionic balance a positive difference ( $C_u - A_u$ ) represents an uptake of  $OH^-$ , the uptake is alkaline. If ( $C_u - A_u$ ) is negative, the difference represents an uptake of  $H^+$ , the uptake is acidic. The hydroxyl anions, balancing the excess of the salt cation absorbed, are replaced by organic anions formed by metabolic processes. The hydrogen ions, balancing the excess of the salt anion absorbed, are neutralized by the alkalinity produced by the metabolic breakdown of organic salts in the tissue. The pH of the tissue remains unchanged and, if the tissue is not too acid or too alkaline, the ions therein are present as inorganic salts (A) and organic salts ( $C - A$ ) of the salt cations (C) in all states of ion accumulation. A direct experimental verification of this has been carried out by ULRICH (1941).

If growing, intact plants are cultivated on a mixture of nutrient salts with ammonium as the sole source of nitrogen, the uptake of salt cations  $C_u$ , expressed as the sum of the me. of  $NH_4$ , K, Na, Mg, Ca, is much higher than the uptake of the salt anions  $A_u$  expressed as the sum of the me. of  $NO_3$ , Cl,  $H_2PO_4$ ,  $SO_4$ . In this case the alkalinity of the uptake ( $C_u - A_u$ ) is large relative to plant weight and total uptake.

If nitrogen is added in the form of nitrate to the nutrient salts the uptake of salt cations  $C_u$  sometimes equals the uptake of salt anions  $A_u$ . More often, as for instance in the gramineous plants, the salt anions are absorbed in excess and there is a marked acidity of the uptake, as evident from values of ( $C_u - A_u$ ) smaller than zero which are numerically significant relative to plant weight and total uptake. The experimental evidence for this has been supplied by STOKLASA and ERNEST (1908), ARNON (1939), and others.

In plants cultivated with ammonium as the source of nitrogen, uptake and utilization of the ions proceeds in such proportions that the alkalinity absorbed, with a small increment due to the conversion of sulphate anions into organic sulphur, exceeds the acidity liberated during the conversion of ammonium to organic nitrogen. The excess is transformed into organic salts.

In plants cultivated with nitrate, the alkalinity liberated by the conversion of nitrate and sulphate anions into organic nitrogen and sulphur exceeds the acidity of the uptake. The excess is transformed into organic salts.

As a result of the uptake and the partial conversion of the inorganic ions of the nutrient salts there results a final accumulation of the inorganic cations:  $K^+$ ,  $Na^+$ ,  $Mg^{++}$  and  $Ca^{++}$ , and the inorganic anions:  $NO_3^-$ ,  $Cl^-$ ,  $H_2PO_4^-$  and  $SO_4^{--}$ . It is not necessary to account of the very low quantities of other inorganic ions if plants are considered with more common conditions of supply. The sum of these cations and anions in me. per kg dry material is given by C and A respectively. C equals the total salt content, A the inorganic-salt content and  $(C - A)$  the organic-salt content of the plant material.

It is customary to designate total phosphorus as inorganic phosphate. This eliminates the organic phosphates from the organic salts.

This treatment will be limited throughout by the understanding that the pH of the tissues is to remain within the range of 5 to 6, and that the other elements can be eliminated from consideration. Such balance-sheets of accumulation have been worked out in detail by COOIL (1948) and DIJKSHOORN (1962).

If the nitrates of a nutrient solution are replaced by ammonium salts the organic-salt content of plants cultivated thereon is diminished. If the ammonium salts are replaced by nitrates the organic-salt content increases. So it is in general for all plants. The effect of ammonium is due to inhibition of the uptake of the other salt cations. Many workers have confirmed these early findings of BENECKE (1903).

The organic-salt content of plants may vary according to the species. Data on the sap-soluble organic salts in the leaves of twelve plant species have been reported by PIERCE and APPLEMAN (1943). Complete records on the ionic constituents of plants have been supplied occasionally for certain species, but more often incomplete records or data on ash-alkalinity are available for a first approximation of the organic-salt content. It appears that the organic-salt content of the leaves is characteristic of the plant species.

In plants cultivated with insufficient potassium in the supply and with reduced growth the organic-salt content is often diminished to subnormal or increased to excessive values, depending on the other salt cations supplied, and the plant species involved. If potassium is added the growth becomes optimal and the organic-salt content regains its normal value.

Experimental evidence on plant yields and composition has shown that at suboptimal growth the organic-salt content may vary depending on nutrition and other factors, but that optimal growth is invariably associated with the normal organic-salt content. This and other evidence made DE WIT, DIJKSHOORN and NOGGLE (1963) to postulate that the normal organic-salt content is one of the conditions for optimal growth.

The purpose of the present investigations is to collect additional evidence for the importance of the organic-salt content for plant growth.

The experiments with *perennial ryegrass* show that the normal organic-salt content

(C — A) is about 1000 me. per kg dry herbage. The constancy of (C — A) per unit dry weight is maintained at large fluctuations of the inorganic-salt content brought about through varying levels of nitrate accumulation in the herbage. This change of the inorganic-salt content, if due to variations of the internal level of unmetabolized nitrate in the herbage, makes the total-salt content of the dry weight to vary with the fresh weight, but the organic-salt content per unit dry weight remains constant. This invariance of (C — A) relative to the dry weight of the herbage represents a typical feature of nitrate accumulation. The nitrates accumulate independently of the organic salts.

The accumulation of chloride takes a different course. Perennial ryegrass is capable of accumulating chloride in considerable amounts. Depending on the salt supply this is associated with an increase of the inorganic-salt content or, at higher initial nitrate and cation contents, with a partial substitution of chloride for nitrate at an unchanged inorganic-salt content of the herbage. In both cases the organic-salt content is diminished. Thus it appears that chloride partly accumulates instead of the organic salts.

Evidence on this point has also been obtained from data on pasture herbage. If nitrate in the herbage is increased by the application of nitrogenous fertilizers, this results in a slight increase of the (C — A) content to a higher, but still subnormal, value through partial replacement of excessive chloride by nitrate in the foliage. At the increase of chloride in the excessive range of 200 to 600 me. per kg dry matter the (C — A) content falls to 500 me./kg dry material. Extrapolation to zero chloride suggests that in the absence of chloride the organic-salt content of the pasture herbage would have been near to the normal value of 1000 me./kg dry matter. The suggestion has arisen that the subnormal organic-salt content through excess chloride is common in pasture herbage and may play a part in the effect of nitrogen dressings on the yield. This is because the substitution of nitrate for chloride results in a rise of the (C — A) content, eventually to the normal value which is necessary for optimal growth. If that were so one would expect the yield of pasture to be more often sub-optimal through excess chloride.

It was recently noted by UDOVENKO and IVANOV (1964) that plants with a previously improved nutrient status exhibit a reduced accumulation of chloride from sodium chloride solutions. From this it seems that nitrate does not inhibit the uptake of chloride per se during the passage in the root system, but that its presence in the tissues may occupy a certain capacity of accumulation, otherwise available for chloride.

If it were possible to demonstrate data on a decrease of the (C — A) content to subnormal values and a concurrent decrease of the yield through the action of chloride only, important evidence for the view that a normal (C — A) content is necessary to optimal growth would have been available. Unfortunately, the experiments designed to show the effect of chloride failed to provide conclusive evidence on this matter owing to interference of nitrogen with the growth.

Chromatographic investigations have shown that 90 per cent of the (C — A) content

consists of the salts of the water-soluble, low-molecular plant acids. Malate forms the main constituent and varies to a much greater extent with (C — A) than the other organic salts.

The experiments with young *sugar-beet* plants show that the normal organic-salt content (C—A) is about 3500 me. per kg dry matter. In the potassium-deficient foliage magnesium appears to substitute for potassium and the (C — A) content is only slightly diminished. Chromatographic analysis shows that citrate and some other organic salts substitute for oxalate if magnesium replaces potassium at potassium shortage in the foliage.

If ammonium is supplied in place of nitrate, the (C — A) content of the foliage is much lower than normal and chromatographic analysis shows that this is due to a fall of the oxalate content.

The normal organic-salt content is associated with optimal and suboptimal yields of foliage in dependence of the salts supplied, but at subnormal organic-salt contents the yield of the foliage is invariably suboptimal.

Leaf composition of the *poplar* cuttings grown at different salt treatments indicates very low potassium contents in the total-leaf mass if potassium is short in the supply. Compared with the herbaceous crops, the effect of a supply with potassium on the total dry weight of the leaves is small. The lower potassium requirement is connected with terminal growth, internal transport of potassium to and reutilization by the newly expanding leaves.

The normal organic-salt content is about 1100 me. per kg dry leaf material. If ammonium is supplied in place of nitrate the leaf growth diminishes and the organic-salt content falls to a subnormal value.

If grown on a poor, acid soil the organic-salt content of the leaves is very low and it is not possible to cure leaf necrosis of potassium-deficient plants by merely raising the potassium content to above the value critical for otherwise normal plants. It is precisely this circumstance which suggests the importance of (C — A) in relation to the potassium requirements. If (C — A) is too low the leaves suffer from necrosis with potassium contents otherwise associated with healthy leaves.

Certain plant species adapted to growth on leached, acid soils may achieve higher yields if supplied with ammonium in place of nitrate as the source of nitrogen. This behaviour is peculiarly interesting from the point of view of the organic-salt accumulation in its relation to growth.

Birch seedlings, when cultivated on nutrient solutions, have greater plant weights with ammonium instead of nitrate in the solutions (INGESTAD, 1962), though nitrate is not injurious, as it is in some ericaceous plants (CAIN, 1954).

The experiments with small *birch* trees show that ammonium is more favourable to the growth of the leaves than nitrate. Optimum leaf weight is achieved at a soil pH about 5 with ammonium added to the soil. In this case the (C — A) content of the



leaves is about 550 me. per kg dry material and this is the normal value associated with optimum yields. According as the soil is on the acid or alkaline side of the optimum the (C — A) content of the leaves is lower or higher than the normal value and the dry weight of the leaves is diminished. On the alkaline side of the optimum the (C — A) content of the leaves rises to the higher values characteristic of the nitrate fertilized plants because the ammonium added has undergone nitrification.

In the plants fertilized with nitrate the (C — A) content of the leaves remains higher than normal throughout the range of soil pH, but it falls at the optimum yield to a value differing by a 100 me. only from the normal value.

At the extreme soil acidity of pH 3.5 the (C — A) content of the leaves of the ammonium fertilized plants is normal but the leaf growth is much reduced because this soil acidity is injurious.

It is important to remember that a number of economic plants is often capable of more luxuriant growth if lime and nitrate are applied or ammonium is converted to nitrate in the soil. There is every probability that this behaviour reflects a greater sensitivity to soil conditions associated with lower organic-salt contents in the plant. Other plant species, such as birch are ill adapted to grow on more alkaline, fertile soils because, if grown thereon, the plant suffers too quickly from an excessive accumulation of organic salts above the normal content necessary to optimal growth. In this case ammonium is the best source of nitrogen and the plants are capable of greater expansion or development on acid soils. The ericaceous plants form a class having this characteristic more clearly defined as chlorosis and an increased pH of the cell sap if ammonium in the supply is replaced by nitrate. Perhaps, this phenomena can provide some direction to further speculations on the nature of lime induced chlorosis and on the ecology of different groups of plants.

## Samenvatting

Proeven van ULRICH (1941) hebben aangetoond dat afgesneden wortels van planten uit een zoutoplossing kationen en anionen in verschillende hoeveelheden kunnen opnemen afhankelijk van de in de oplossing aanwezige zouten.

Is de kationenopname ( $C_u$ ) groter dan de anionenopname ( $A_u$ ) dan kan van een alkalische opname worden gesproken. Is daarentegen de opname van anionen groter dan die van kationen en is derhalve ( $C_u - A_u$ ) kleiner dan nul, dan is er sprake van een zure opname (sectie 1.2).

Bij een overmaat van kationen in de opname accumuleren deze als organische zouten in de plant, terwijl bij een overmaat van anionen een afbraak van aanwezige organische zouten plaats vindt (sectie 1.2).

Wanneer intacte, groeiende planten worden beschouwd, zal behalve met de opname ook rekening moeten worden gehouden met de verwerking en de accumulatie van de opgenomen zouten. De vorm waarin stikstof aan de plant wordt gegeven is van groot belang (figuur 4). Als aan een plant stikstof in de vorm van ammonium wordt toegediend, overtreft de kationenopname die van de anionen, de opname is alkalisch. Wordt ammonium als stikstofbron vervangen door nitraat dan kan de opname zuur worden door een grotere anionenopname (sectie 1.4).

De opname van ammonium leidt in de plant tot een lager organisch zoutgehalte dan met een nitraat voeding wordt verkregen. De oorzaak hiervan moet niet worden gezocht in de verwerking van de stikstof in de plant die volgens DIJKSHOORN (1964) verloopt zonder dat daarbij organische zouten worden afgebroken of gevormd, maar in de ionenconcurrentie van ammonium met andere kationen.

Onderzoek aan *Engels raaigras*, verricht door DE WIT, DIJKSHOORN en NOGGLE (1963), heeft enkele facetten opgeleverd welke in figuur 11 schematisch zijn weergegeven. Hieruit blijkt dat een sub-optimale produktie van droge stof meer oorzaken kan hebben, zoals kaligebrek, stikstofgebrek en ammonium in plaats van nitraat als stikstofbron. Tegelijkertijd blijkt dat bij een hoog opbrengstniveau het organisch zoutgehalte veel minder afwijkt van het gehalte dat als normaal wordt beschouwd voor optimale groei, dan bij lage produktie niveaus. Hieruit is de conclusie getrokken dat een bepaald organisch zoutgehalte, *het normale gehalte*, één van de essentiële factoren voor optimale produktie is.

Uit eerder onderzoek is reeds gebleken dat het normale organische zoutgehalte, vereist voor optimale produktie van *Engels raaigras*, 1000 mg equivalenten per kilogram droge stof bedraagt.

Uit de experimentele resultaten is gebleken dat deze waarde, uitgedrukt per eenheid drooggewicht constant is ondanks grote variaties welke in het droge stof-gehalte kunnen optreden (sectie 2.1).

Uit proeven in de kas zowel als uit veldproeven blijkt dat bij een hogere chloride-gift aan gras het organisch zoutgehalte ( $C - A$ ) op twee manieren verlaagd kan worden:

- a. door accumulatie van chloorionen, waardoor het anorganisch anionengehalte (A) sterker toeneemt dan het kationengehalte (C);
- b. door een verlaagde nitraataccumulatie bij toenemend chloride in de voedingsoplossing, waarbij het totale anorganische zoutgehalte (A) gelijk blijft doch waarbij de kationenaccumulatie (C) wordt verminderd (sectie 2.2).

Chromatografische analyse van gras leert dat 90 % van het berekende organische zoutgehalte ( $C - A$ ) voorkomt als zouten van in water oplosbare zuren. Appelzuur blijkt quantitatief de belangrijkste component te zijn en tevens het meest te variëren onder invloed van verschillen in bemesting (figuur 19).

Bladeren van de jonge planten van de *suikerbiet* hebben een normaal organisch zoutgehalte van ongeveer 3500 mg equivalenten per kilogram droge stof. Uit de proeven blijkt dat dit gehalte slechts weinig varieert bij kaliumgebrek doch dat ammonium in plaats van nitraat als stikstofbron een sterke verlaging van het organische zoutgehalte geeft (figuur 21).

Bij kaliumgebrek daalt het oxalaatgehalte sterk maar door toename van andere organische zouten, in het bijzonder door de toename van magnesium en citraat, daalt het totale organische zoutgehalte slechts weinig. Wordt daarentegen nitraat in de voeding vervangen door ammonium als stikstofbron dan daalt het oxalaatgehalte zonder dat er enige compensatie tegenover staat (figuur 23).

Blad van de *populier* heeft bij optimale groei een organisch zoutgehalte van ongeveer 1100 mg equivalenten per kilogram droge stof (sectie 4.2). Ammoniumvoeding resulteert, evenals bij suikerbieteloof, in een verlaagd organisch zoutgehalte en in een verminderde opbrengst. Vergeleken met gras en loof van suikerbieten is het effect van kalium op de groei van populiereblad gering, hetgeen toegeschreven is aan een meer lineaire groei van de populier en een meer exponentiële groei in grassen (figuur 27).

Necrose-verschijnselen aan blad dat een zeer laag organisch zoutgehalte heeft als gevolg van kaliumtekorten, kunnen slechts dan door toediening van kalium worden opgeheven, wanneer het organisch zoutgehalte op een redelijk niveau is gebracht (sectie 4.3.)

Van het berekend organisch zoutgehalte ( $C - A$ ) is in blad van de populier slechts 50 tot 60 % teruggevonden in de vorm van zouten van in water oplosbare zuren. Een belangrijk deel van de in deze bladeren aanwezige zouten komt voor in de vorm van onoplosbare zouten van organische zuren (sectie 4.5).

In tegenstelling tot de eerdergenoemde planten en een reeks van planten welke

landbouwkundig van belang zijn, blijken er planten voor te komen welke met een ammoniumbemesting een hogere opbrengst hebben dan met nitraatbemesting (CAIN, 1954; INGESTADT, 1962). Tot deze bijzondere groep van planten behoort de *berk*, die bij optimale groei een laag organisch zoutgehalte van het blad heeft. Hoewel de *berk* nog een tolerantie voor nitraat als stikstofbron bezit, leiden hogere nitraatgiften in vergelijking tot ammoniumvoeding tot een lagere produktie en een organisch zoutgehalte dat boven de normale waarde van 550 mg equivalenten per kilogram droge stof komt te liggen.

## Literature

- ARNOLD, P. W. and B. N. CLOSE 1962 Potassium-releasing power of soils from the Agdell rotation experiment assessed by glasshouse cropping. *Potash Rev.*, October, Int. Potash Inst., Berne.
- ARNON, D. I. 1939 Effect of ammonium and nitrate nitrogen on the mineral composition and sap characteristics of barley. *Soil Sci.* 48, 295-307.
- BENECKE, W. 1903 Über Oxalsäurebildung in grünen Pflanzen. *Bot. Z.* 61, 79-110.
- BÖNING, K. and E. BÖNING-SEUBERT 1932 Wasserstoffionenkonzentration und Pufferung im Presssaft von Tabackblättern in ihrer Abhängigkeit von der Ernährung und Entwicklung der Pflanze. *Bioch. Z.* 247, 35-67.
- BUKOVAC, M. J. and S. H. WITTWER 1957 Absorption and mobility of foliar applied nutrients. *Plant Physiol.* 32, 428-435.
- BURSTRÖM, H. 1945 Studies on the buffer systems of cells. *Ark. Bot.* 32A, 7, 1-18.
- BURSTRÖM, H. 1946 The nitrate nutrition of plants, a general survey of the occurrence and assimilation of nitrate. *Ann. Roy. Agric. Coll. Sweden* 13, 1.
- CAIN, J. C. 1952 A comparison of ammonium and nitrate nitrogen for blueberries. *Proc. Amer. Soc. Hortic. Sci.* 59, 161-166.
- CAIN, J. C. 1954 Blueberry chlorosis in relation to leaf pH and mineral composition. *Proc. Amer. Soc. Hortic. Sci.* 64, 61-70.
- CHOUTEAU, J. 1960 Les équilibres acides-bases dans le tabac Paraguay. Thesis Bordeaux.
- CHOUTEAU, J. 1960 Balance acide-base de la composition chimique des plantes de tabac alimentées en azote nitrique ou en azote ammoniacale. *Ann. Physiol. Vég.* 4, 237-247.
- COIC, Y. and C. LESAINT 1960 Les acides organiques de la féverole (*Vicia faba* L., minor): comparaison entre feuilles, racines, nodosités, influence de la déficience en potassium. *Ann. Physiol. Vég.* 2, 73-83.
- COIC, Y., C. LESAINT and F. LE ROUX 1961 Comparaison de l'influence de la nutrition nitrique et ammoniacale combinée ou non avec une déficience en acide phosphorique, sur l'absorption et le métabolisme des anions-cations et plus particulièrement des acides organiques chez le maïs. Comparaison du maïs et de la tomate quant à l'effet de la nature de l'alimentation azotée. *Ann. Physiol. Vég.* 3, 141-163.
- COIC, Y., C. LESAINT and F. LE ROUX 1962 Effet de la nature ammoniacale ou nitrique de l'alimentation azotée et du changement de la nature de cette alimentation sur le métabolisme des anions et des cations chez la tomate. *Ann. Physiol. Vég.* 4, 117-125.
- COIC, Y., et al. 1962 Influence de la déficience en soufre sur l'absorption des substances minérales et le métabolisme de l'azote et des acides organiques chez l'orge. *Ann. Physiol. Vég.* 4, 295-306.

- COLGROVE, M. S. and A. N. ROBERTS 1956 Growth of the Azalea as influenced by ammonium and nitrate nitrogen. *Proc. Amer. Soc. Hortic. Sci.* 68, 522-536.
- COOIL, J. B. 1948 Effects of potassium deficiency and excess in guayule II. Cation-anion balance in the leaves. *Plant Physiol.* 23, 403-424.
- DAVIES, C. W. and R. B. HUGHES 1954 The organic acids of grass extracts. *J. Sci. Food Agric.* 5, 200-205.
- DE MAN, T. J. and J. HEUS 1946 Organische zuren, fructose en fructosaan in *Lolium perenne*. Med. Inst. Moderne Veevoeding „De Schothorst”, Amersfoort 1946; *Chem. Abstr.* 41, 7458 (1947).
- DE WIT, C. T., W. DIJKSHOORN and J. C. NOGGLE 1963 Ionic balance and growth of plants. Versl. Landbouwk. Onderz. 69, 15.
- DRAKE, M., J. VENGRIS and W. G. COLBY 1951 Cation exchange capacity of plant roots. *Soil Sci.* 72, 139-149.
- DIJKSHOORN, W. 1958 Nitrogen, chlorine and potassium in perennial ryegrass and their relation to the mineral balance. *Neth. J. Agric. Sci.* 6, 131-138.
- DIJKSHOORN, W. 1960 Het effect van nitrificatie van een ammoniumbemesting op de nitraataccumulatie en de kation-anionbalans in Engels raaigras. Jaarboek I.B.S. 1960, 123-134.
- DIJKSHOORN, W. 1962 Metabolic regulation of the alkaline effect of nitrate utilization in plants. *Nature* 194, 165-167.
- DIJKSHOORN, W. 1963 The balance of uptake, utilization and accumulation of the major elements in grass. Proc. 1st Regional Conference, Intern. Potash Inst. Wexford (Ireland), 43-62.
- DIJKSHOORN, W. 1964 Le bilan ionique dans le diagnostic foliaire. Jaarboek I.B.S. 1964, 133-144.
- DIJKSHOORN, W. and J. E. M. LAMPE 1961 Phosphorus fractions in perennial ryegrass. Jaarboek I.B.S. 1961, 101-106.
- DIJKSHOORN, W. and J. E. M. LAMPE 1962 The separation of organic acids from *Lolium perenne*. Jaarboek I.B.S. 1962, 135-139.
- DIJKSHOORN, W. and J. E. M. LAMPE 1962 Partition chromatography of the organic acids of *Lolium perenne* L. on silica gel columns. Jaarboek I.B.S. 1962, 141-148.
- DIJKSHOORN, W., J. E. M. LAMPE 1960 A method of diagnosing the sulphur nutrition status of herbage. *Plant and Soil* 13, 227-241.
- and P. F. J. VAN BURG
- EDGERTON, L. J. 1948 The effects of varying the amounts of potassium on the growth and potassium accumulation of young apple trees. *Plant Physiol.* 23, 112-122.
- EVERS, F. H. 1963 Die Wirkung von Ammonium- und Nitratstickstoff auf Wachstum und Mineralstoffhaushalt von *Picea* und *Populus*, I-III. *Z. Bot* 51, 61-111.
- EVERS, F. H. 1964 Die Bedeutung der Stickstoffform für Wachstum und Ernährung der Pflanzen, insbesondere der Waldbäume. *Mitt. Vereins forstl. Standortsk. Forstpflanzenzüchtung* 14, 19-37.
- HULME, A. C. and A. RICHARDSON 1954 The non-volatile acids of grass. *J. Sci. Food Agric.* 5, 221-225.
- INGESTAD, T. 1962 Macroelement nutrition of pine, spruce and birch seedlings in nutrient solutions. *Meddel. Statens Skogforsk. Inst.* 51, nr. 7
- KEMP, A. and W. DIJKSHOORN 1956 Het verschil in de minerale samenstelling van het bovenste en van het onderste deel van een snede weidegras. Verslag C.I.L.O. 1955, 100-104.

- LARSON, W. E. and  
W. H. PIERRE  
LUNDEGÅRDH, H.
- LUNDEGÅRDH, H. and  
H. BURSTRÖM  
MARCUSSEN, N. R.
- MARTHALER, H.
- MASON, T. G. and  
E. J. MASKELL
- MEURER, R.
- NATHANSON, A.
- NIGHTINGALE, G. T.,  
L. G. SCHERMERHORN  
and W. H. ROBINS  
PFEFFER, W.  
PIERCE, E. C. and  
C. O. APPLEMAN  
ROUX, L. and C. LESAIN
- SAID, I. M.
- STOHMAN
- STOKLASA, J. and A. ERNEST
- TAYLOR, R. F.
- TEUNISSEN, P. H. and  
H. G. BUNGENBERG DE JONG  
THOMAS, M.  
UDORENKO, G. V. and  
N. P. IVANOV
- ULRICH, A.
- VAN BURGH, P. F. J.
- VAN TUIL, H. D. W. and  
J. E. M. LAMPE  
VICKERY, H. B.
- 1953 Interaction of sodium and potassium on yield and cation composition of selected crops. *Soil Sci.* 76, 51-64.
- 1945 Absorption, transport and exudation of inorganic ions by the roots. *Ark. Bot.* 32 A, nr. 12, 1-139.
- 1933 Untersuchungen über die Salzaufnahme der Pflanzen III. *Bioch. Z.* 261, 235-251.
- 1963 Einfluss der Kalidüngung und der Witterung auf Ertrag, Rohprotein- und Mineralstoffgehalt des Grasses auf Marschböden. *Wirtschaftseigene Futter* 9, 280-288.
- 1939 Die Stickstoffernährung der Hochmoorpflanzen. *Jb. Wiss. Bot.* 88, 723-758.
- 1931 Further studies on transport in the cotton plant I. Preliminary observations on the transport of phosphorus, potassium and calcium. *Ann. Bot.* 45, 125-173.
- 1909 Über die regulatorische Aufnahme anorganischer Stoffe durch die Wurzeln von *Beta vulgaris* und *Daucus carota*. *Jb. Wiss. Bot.* 46, 503-567.
- 1904 Über die Regulation der Aufnahme anorganischer Salze durch die Knollen von *Dahlia*. *Jb. Wiss. Bot.* 39, 607-644.
- 1930 Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. N. Jersey Agric. Exp. Sta. Bull. 499, 1-36.
- 1881 Pflanzenphysiologie. Leipzig.
- 1943 Role of ether-soluble organic acids in the cation-anion balance of plants. *Plant Physiol.* 18, 224-238.
- 1959 Mise au point d'une technique de détermination des acides organiques chez les végétaux. *Ann. Physiol. Vég.* 1, 83-91.
- 1959 Cation selectivity and cation-anion balance as factors governing the mineral composition of pasture herbage. *Versl. Landbouwk. Onderz.* 65, 16.
- 1864 Cited after Benecke (1903).
- 1908 Beiträge zur Lösung der Frage der chemischen Natur des Wurzelsekretes. *Jb. Wiss. Bot.* 46, 55-102.
- 1935 Available nitrogen as a factor influencing the occurrence of sitka spruce and western hemlock seedlings in the forest of south-eastern Alaska. *Ecology* 16, 580-602.
- 1938 Negative, nichtamphotere Biokolloide als hochmolekulare Elektrolyte II. *Kolloid Beih.* 48, 33-92.
- 1958 Plant Physiology, 5th Ed., London
- 1964 Effect of total level of mineral nutrition on intensity of entry of chlorine into the plant. *Trans. Dokl. Biol. Sections*, 152, 1358-1360.
- 1941 Metabolism of non-volatile organic acids in excised barley roots as related to cation-anion balance during salt accumulation. *Amer. J. Bot.* 28, 526-537.
- 1962 Interne stikstofbalans, produktie van droge stof en veroudering bij gras. *Versl. Landbouwk. Onderz.* 68, 12.
- 1964 Control of nitrification by 2-chloro-6-(tri-chloromethyl) pyridine. *Jaarboek I.B.S.* 1964, 161-163.
- 1961 Chemical investigations of the tobacco plant XI. Composition of the green leaf in relation to position on the stalk. *Conn. Agric. Exp. Sta. Res. Bull.* 640.

- Vose, P. B. 1963 The cation content of perennial ryegrass, *Lolium perenne* L., in relation to intraspecific variability and nitrogen-potassium interaction. *Plant and Soil* 19, 49-64.
- WALLACE, A., *et al.* 1954 Translocation of nitrogen in citrus trees. *Proc. Amer. Soc. Hortic. Sci.* 64, 87-104.
- WANDER, I. W. and J. W. SITES 1956 The effect of ammonium and nitrate nitrogen with and without pH control on the growth of lemon seedlings *Proc. Amer. Soc. Hortic. Sci.*, 68, 211-226.
- WEHMER 1891 Cited after Benecke (1903).
- WHITE, H. L. 1940 The interaction of factors in the growth of *Lemna*. XV. On a rhythmic growth cycle of *Lemna* colonies associated with transference to a potassium-free solution. *Ann. Bot. N.S.* 4, 495-504.
- WYBENGA, J. M. and J. J. LEHR 1958 Exploratory pot experiments on sensitiveness of different crops to sodium: e. red table beet. *Plant and Soil*, 9, 385-394.