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Aspects of assimilation  
and accumulation of nitrate  
in some cultivated plants

BIBLIOTHEEK  
DER  
LANDBOUWHOGESCHOOL  
WAGENINGEN

A. Darwinkel

IN08201.640

**Aspects of assimilation and accumulation of nitrate in some cultivated plants**

Dit proefschrift met stellingen van Albert Darwinkel, landbouwkundig ingenieur, geboren te Tynaarlo op 25 juni 1942, is goedgekeurd door de promotor, ir. M. L. 't Hart, hoogleraar in de leer van de landbouwplantenteelt en het grasland.

De rector magnificus van de Landbouwhogeschool,  
J. P. H. van der Want

Wageningen, 1 september 1975

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# Aspects of assimilation and accumulation of nitrate in some cultivated plants

Proefschrift

ter verkrijging van de graad van

doctor in de landbouwwetenschappen

op gezag van de rector magnificus,

dr. ir. J. P. H. van der Want, hoogleraar in de virologie,

in het openbaar te verdedigen

op vrijdag 24 oktober 1975 des namiddags te vier uur

in de aula van de Landbouwhogeschool te Wageningen



*Centre for Agricultural Publishing and Documentation*

*Wageningen – 1975*

## Abstract

Darwinkel, A. (1975) Aspects of assimilation and accumulation of nitrate in some cultivated plants. Agric. Res. Rep. (Versl. landbouwk. Onderz.) 843, ISBN 90 220 0586 0, (viii) + 64 p., 21 tables, 27 figs. 98 refs, 2 appendices, Eng. and Dutch summaries.  
Also: Doctoral thesis, Wageningen.

In a study on the accumulation of nitrate nitrogen in plants, turnip, rape, oats, Italian ryegrass, Westerwolth's ryegrass, carrot and spinach were used. During growth the production and distribution of dry matter and the contents of total N and  $\text{NO}_3\text{-N}$  were measured.  $\text{NO}_3$  accumulation occurs when the N uptake exceeds assimilation. The uptake was largely dependent on N supply and plant species, whereas the conversion was closely associated with production and distribution of dry matter. Leaf blades contain high organic N and low  $\text{NO}_3$  concentrations. The activity of the nitrate reductase was mainly located in leaf blades. This activity was highest in a young immature leaf and was lower the older the leaf. Production of leaves affected the  $\text{NO}_3$  conversion considerably. During the growth period a gradual decrease of the conversion took place per unit dry matter produced, because of a smaller demand for proteins. The total N content in the dry matter, above which  $\text{NO}_3$  accumulation takes place, was high in a young plant, but decreased during growth, whereas an increasing part of the extra  $\text{NO}_3$  absorbed accumulated simultaneously. Turnips accumulated most  $\text{NO}_3$ , because of their high N uptake. Other plant species could also reach  $\text{NO}_3$  levels that are toxic for cattle. In rape and Italian ryegrass this is caused by a high N uptake, in oats and carrot by a poor conversion.

**Key words:** plant age, leaf age, plant parts, dry matter yield, nitrogen content, nitrate content, nitrogen uptake, nitrate accumulation, nitrate assimilation, nitrate reductase activity.

This thesis will be published as Agricultural Research Reports 843.

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# Stellingen

1. Het accumulatieproces van nitraat in de plant hangt meer af van de nitraatopname dan van de nitraatverwerking.

Dit proefschrift.

2. Onvoldoende kennis omtrent de hoeveelheid stikstof in drijfmest en de snelheid van het mineralisatieproces van deze stikstof leidt mede tot het frequent voorkomen van een overmatige stikstofvoeding.

3. Als maatstaf voor eiwitgehalte en nitraatophoping in stoppelknollen is de loof/knol-verhouding geen goed criterium.

Dit proefschrift.

4. De door Te Velde aangegeven daling van het nitraatgehalte van stoppelknollen later in de herfst is uitsluitend door afval van afgestorven bladeren tot stand gekomen.

H.A. te Velde, P.A.W. Meded. nr. 139 (1967): 1-43.

5. Verhoging van het aandeel bladschijven van de totale drogestof levert een positieve bijdrage tot de eiwitproductie van groenvoedergewassen.

Dit proefschrift.

6. De indeling waarin Kingsbury plantensoorten op basis van nitraatgehalten aangeeft als zijnde giftig voor vee, is niet algemeen geldend.

J.M. Kingsbury, J.Dairy Sci. 41 (1958): 875-907.

7. Gezien de verscheidenheid aan stikstofverbindingen in voedermiddelen is het de vraag of de toegepaste Kjeldahl-methode voor de waardering van voedermiddelen nog wel aan de huidige eisen voldoet.

8. Voor hogere opbrengsten is het bij de teelt van granen nodig en mogelijk meer rekening te houden met een regelmatige plantverdeling van het gewas over de beschikbare oppervlakte.

9. Uit de door A.D.A.S. verzamelde gegevens mag niet worden afgeleid, dat granen op met cultivator bewerkt land eenzelfde opbrengst bereiken als op geploegd land.

A.D.A.S., Non-ploughing for cereals (1973): 14-15.

10. Om het oorspronkelijk karakter van het stroomdallandschap 'Drentsche A' zo goed mogelijk te handhaven, is het nodig de rivier zo nu en dan buiten haar oevers te laten treden. Technisch zijn daarvoor mogelijkheden aanwezig.

11. Door de bekendheid van "Wageningen" als "landbouwkundig centrum" staan elders in den lande werkende landbouwkundige instellingen bij vele buitenstaanders minder in de aandacht.

Velen hebben tot de totstandkoming van dit proefschrift bijgedragen. Mijn erkentelijkheid hiervoor betreft dan ook een groter aantal personen en instellingen, dan welke ik in onderstaande met name noem.

Professor 't Hart, mijn promotor, ben ik veel dank verschuldigd voor de begeleiding van het onderzoek en het doornemen van het proefschrift. Zijn constructief kritische instelling hierbij heb ik zeer op prijs gesteld. Mijn erkentelijkheid gaat verder in het bijzonder uit naar Bauke Deinum, die het gehele onderzoek heeft begeleid en nauw betrokken is geweest bij het op schrift stellen van het proefschrift.

De aanleg en uitvoering van de proefvelden was in goede handen van de heer Mol en zijn medewerkers; bij de kas-experimenten was de hulp van de heer Möhring en zijn technische dienst onmisbaar. Het verzamelen en verwerken van het plantmateriaal zou zonder de hulp van vele laborantes onmogelijk zijn geweest. Met de medewerking van Evelien Zantman en Tyne van der Ven werd ook het chemische analysewerk tot een goed einde gebracht. De samenwerking met de andere medewerkers van de afdeling was zeer goed en heeft bijgedragen tot een plezierige werksfeer.

Heel hartelijk dank ik Miss A. G. Davies voor de zorgvuldige correctie van de engelse tekst, en de medewerkers van het Pudoc voor de redactie en uitgave van dit proefschrift.

Het onderzoek kon worden uitgevoerd dank zij de financiële steun van de Commissie van Bijstand inzake Stikstofonderzoek. De directie van het Proefstation voor de Akkerbouw dank ik voor de gelegenheid die mij werd geboden om dit proefschrift te voltooien.

Tenslotte dank ik Nellie, Marianne, Hermjan en Jaap voor alle steun en afleiding, die zij mij thuis steeds hebben bezorgd.

*Aan mijn ouders*



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# 1 Introduction

Much research has been done in the last 30 years into the occurrence of  $\text{NO}_3$  in plants and the circumstances under which accumulation takes place. Presence of  $\text{NO}_3$  in plants is important for two main reasons. On the one hand, a large quantity of  $\text{NO}_3$  in plants is undesirable, because it is poisonous to humans and animals, but on the other hand, the presence of  $\text{NO}_3$  in the plant is desirable as an indication of a good supply of nitrogen.

In the Netherlands, high contents of  $\text{NO}_3$  in stubble turnips have caused recurrent signs of poisoning in cattle since 1966 (Te Velde, 1967). In the USA, this symptom was already known, particularly in oats, under the name of oat hay poisoning (Gilbert et al., 1946). Symptoms of poisoning in babies caused by too high contents of  $\text{NO}_3$  in spinach have also been recorded. However, for a maximal production of dry matter an optimal N supply is necessary and this is coupled with a degree of  $\text{NO}_3$  accumulation in the plant. In field conditions, grasses reach a maximum yield of dry matter only if the content of  $\text{NO}_3\text{-N}$  is at least 0.14% in the crop (Van Burg, 1965).

Accumulation of  $\text{NO}_3$  takes place if uptake of  $\text{NO}_3$  is surplus to the requirements necessary for production of dry matter. Because  $\text{NH}_4$  and  $\text{NO}_2$  occur in plants only in negligible quantities, the accumulation of  $\text{NO}_3$  in the plant can be given as the difference between the quantities of total N ( $=\text{N}_t$ ) and organic N ( $=\text{org. N}$ ).

The content of  $\text{NO}_3$  in the dry matter is the difference between uptake and conversion of  $\text{NO}_3$  per unit of dry matter and depends therefore on all those factors which influence these three processes. In many investigations the content of  $\text{NO}_3$  only was measured, which does not provide enough information to solve the problem of the accumulation of  $\text{NO}_3$  in plants and crops. The aim of this research therefore was a closer investigation into the accumulation of  $\text{NO}_3$  in plants using the production of dry matter in particular as the determining variable. By means of these productivity studies, supplementary information was obtained to explain accumulation of  $\text{NO}_3$ . The extent to which special arable crops accumulated  $\text{NO}_3$  and the circumstances in which this accumulation occurred were also investigated.

The experiments carried out related to the influence of plant species and nitrogen fertilization on the nitrate accumulation. Effects of factors such as light, temperature and drought were not studied separately, but some information on the influence of climate was obtained by carrying out the trials in different seasons.

## 2 Review of the literature

The total of N compounds in the plant is often summarized by the term crude proteins. The majority of these are organic compounds, such as proteins, aminoacids and amides. Also included are small quantities of a large number of compounds active in the physiology of the plant. Of the inorganic compounds represented in the crude protein fraction  $\text{NO}_2$  and  $\text{NH}_4$  are only present in small amounts (McKee, 1962; Suzuki & McLeod, 1970). On the contrary,  $\text{NO}_3$  is shown to be found in large quantities in plants.

Nitrogen is an essential component of proteins. The function of proteins is versatile as enzymes which are necessary in the numerous physiological processes for maintenance and further development of the plant. Moreover, proteins can be stored in the plant as a reserve.

Dry matter production in the plant is closely related to nitrogen metabolism. Maximum production of dry matter requires a sufficient supply of nitrogen and sufficient carbohydrates for the efficient conversion of  $\text{NO}_3$  to protein. The content of  $\text{NO}_3$ , being a ratio between the quantity of  $\text{NO}_3$  and the quantity of dry matter, will be dependent on those factors which influence the production of dry matter. This chapter will deal firstly with the production of dry matter and secondly with the uptake and conversion of  $\text{NO}_3$  as determining factors in the accumulation of  $\text{NO}_3$ .

### 2.1 Production of dry matter

The production of dry matter is determined to a high degree by the difference between photosynthesis and respiration. Factors which have different effects on photosynthesis and respiration will influence the production of dry matter. The most important factors are light intensity, temperature, nutrients and water. Photosynthesis is largely determined by light intensity and temperature (Gaastra, 1962), but is also influenced by the availability of nutrients (Bottrill et al., 1970; Nätr, 1972) and water (Baker & Musgrave, 1964; Murata et al., 1965). Respiration is strongly dependent on temperature (Murata & Iyama, 1962). An increase in light intensity leads to an increase in dry matter production, but temperature may increase or decrease dry matter production as each plant species reacts to an optimal range. Under field conditions the production of dry matter mainly depends on water and nutrients. Drought brings the production of dry matter to a halt (Deinum, 1966; Flynn et al., 1957). Shortages of the major nutrients nitrogen, phosphate and potassium severely restricts the production of dry matter. It should also be noted that shortages of other nutrients, both macro and micro elements, can cause considerable yield depressions.

In general, the pattern of production of dry matter during the growing period can be represented by a S-shaped curve (Fig. 1). In the beginning most of the assimilates

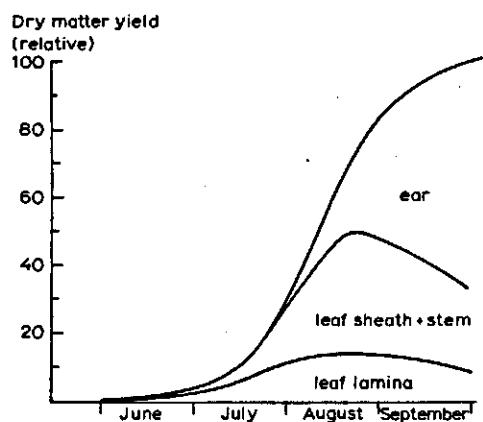


Fig. 1. Diagram of the dry matter production of a maize plant, divided in some plant parts.

produced is used in the production of leaves. After that, a growing share of the dry matter is put into other parts, which brings about a decrease in the share of weight of leaves in the total dry matter production. External factors also influence the distribution of dry matter. According to Brouwer (1962) the distribution of dry matter between shoot and root in perennial ryegrass is based on a functional equilibrium reflected in a constant shoot/root ratio, found for each separate set of external conditions.

## 2.2 Uptake of nitrogen

Sufficient supply of N is necessary for a maximal production of dry matter. In general, it can be stated that for the production of one kg dry matter, 15–30 grammes of  $N_t$  are required. This means a requirement of about 250–400 kg of nitrogen for an annual yield of dry matter of 15 tons/ha.

The uptake of nutrients depends on the activity of the root system. As the plants get older, the roots get a corky covering which restricts the uptake of water and nutrients. A good supply of carbohydrates permits continuous root growth, maintaining a young root system and thus giving a greater nutrient uptake capacity. Cessation of root growth leads to ageing of the root system and to a lower uptake capacity (Brouwer, 1968).

Generally, plants absorb nitrogen quickly. Under favourable growth conditions a large uptake of nitrogen is found especially shortly after nitrogen fertilization in an early stage of development. In wheat, Italian ryegrass and kale (Spratt & Gasser, 1970b), it was found that most of the nitrogen was absorbed in the first stage of development of these crops (Fig. 2). The uptake of N subsequently increased less, because of exhaustion of N supply in the soil, eventually falling behind the production of dry matter. The effect of this was a gradual decrease of the  $N_t$  content.

The uptake of nitrogen by plant roots takes place almost exclusively in the form of  $NO_3$  and  $NH_4$ . Under field conditions uptake of  $NO_3$  will dominate, because  $NH_4$ -based fertilizers undergo nitrification in the soil. Trials with wheat and grasses have showed that the presence of  $NO_3$  in the plants after fertilization with  $(NH_4)_2SO_4$  had to be accredited to nitrification of  $NH_4$  (Nowakowski & Gasser, 1967). The high content of  $NO_3$  after application of ammonium sulphate in experiments of Balks & Plate (1956) can be ex-

Percent of maximum values of dry matter and N uptake

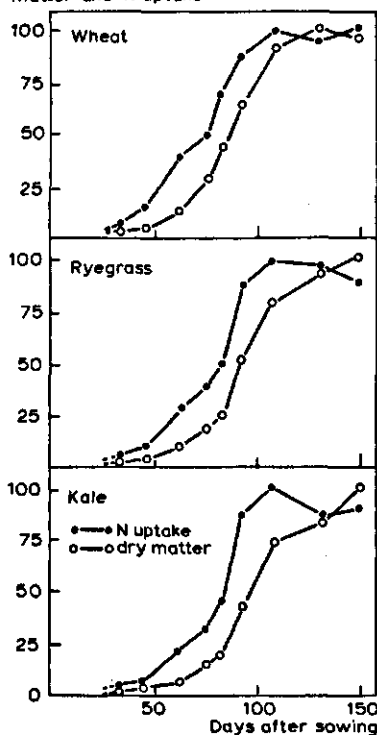


Fig. 2. Dry matter production and N uptake by wheat, ryegrass and kale. According to Spratt & Gasser (1970).

plained in the same way.

In general the uptake of nitrogen, as the level of fertilizer increases, can be represented by a saturation curve (Kreil et al., 1965). Figure 3 shows, that the yield of dry matter as well as the N uptake increases with increasing nitrogen levels up to 400 kg/ha. With further increase of N application, up to 600 kg/ha, only the N uptake increases still further. It can be deduced from the shape of both curves that the more nitrogen is applied, the higher is the  $N_t$  content. Similar results with various plant species have been described by others.

The N uptake is the highest under favourable growth conditions, just as is the production of dry matter. Both are being influenced by almost the same factors such as light intensity, temperature, supply of water and minerals. The production of dry matter in general is influenced more strongly by these factors than the uptake of nitrogen is; application of nitrogen, however, influences the N uptake more than the dry matter production. In better growing conditions with sufficient N supply the production of dry matter increases relatively more than the N uptake, whereas in poorer conditions the production of dry matter is more affected than uptake. The effect of this is a decrease or increase of the  $N_t$  content respectively.

Under circumstances of low light intensities the plant has a high  $NO_3$  content, but a small total N uptake and a small dry matter yield (Alberda, 1965; Bathurst & Mitchell, 1958; Deinum & Dirven, 1967; Nowakowski & Cunningham, 1966). With increasing light

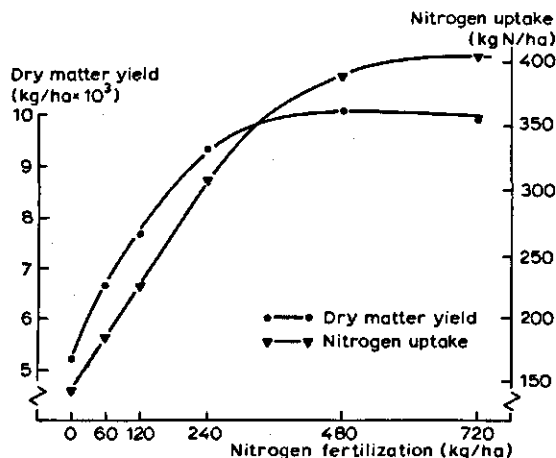


Fig. 3. Production of dry matter and N uptake of permanent pasture under increasing N applications (annual averages 1958–1963). According to Kreil et al. (1965).

intensity the production of dry matter increases relatively more than the N uptake, so that the  $N_t$  content decreases.

The uptake of  $NO_3$  is also strongly influenced by temperature (Lycklama, 1963). In experiments with perennial ryegrass on hydroponics under artificial conditions, Alberda (1965) found a steep increase of the nitrogen uptake with increasing temperature up to 25° C. In this experiment an increase of the  $N_t$  content was found, because the N uptake increased more than the dry matter production. However, in general it appears that raising the temperature to its optimum increases the production of dry matter more than the uptake of N, resulting in a lower  $N_t$  content (Colman & Lazenby, 1970; Deinum, 1966; Younis et al., 1965). A rise in temperature also stimulates mineralization in the soil, so that more nitrogen is made available for the plants (Nielsen & Cunningham, 1965).

Shortages of nutrients (Adams & Sheard, 1966; McLeod & Carson, 1969) and water (Deinum, 1966) restrict uptake of nitrogen. Low yields of dry matter with a high  $N_t$  content are often found together with shortages of minerals, except for nitrogen deficiency. Conversely a more generous supply of nutrients and water leads to a higher N uptake, but a lower  $N_t$  content, due to a stronger increase of the production of dry matter.

### 2.3 Conversion of nitrate nitrogen

For the building of organic N compounds the absorbed  $NO_3$  is firstly reduced to  $NH_3$ . Transformation of  $NO_3$  into  $NH_3$  is accomplished in a series of reduction reactions, which can be represented in simplified form as follows (McKee, 1962; Richter, 1969):

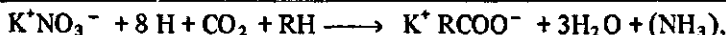
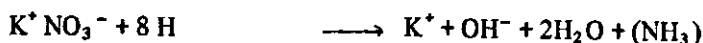


The intermediate products and the necessary enzymes are not yet completely clear. This reduction of  $NO_3$  to  $NH_3$  is also subdivided as follows (Beevers & Hageman, 1969; Hewitt, 1970):

1.  $\text{NO}_3$  reduction:  $\text{NO}_3^- \longrightarrow \text{NO}_2^-$  under influence of enzyme nitrate reductase

2.  $\text{NO}_2$  reduction:  $\text{NO}_2^- \longrightarrow \text{NH}_3$  under influence of enzyme complex nitrite reductase

The reduction of  $\text{NO}_3$  is the rate limiting step and determines the rate of the  $\text{NO}_3$  conversion. In this reduction the negative nitrate ion is changed into non-organic nitrogen. The negative charge is transferred to a newly formed organic anion or carboxylate (Dijkshoorn, 1962). During the conversion the nitrates are replaced by carboxylates as follows:



where  $(\text{NH}_3)$  represents the org. N and RH is a metabolite, which is transformed into carboxylate ( $\text{RCOO}^-$ ) by carboxylation.

For a good nitrogen metabolism in the plant, it is important, that enough  $\text{NO}_3$  is available in those parts of the plant in which intensive  $\text{NO}_3$  reduction can take place. In that case the possibilities are available for optimal  $\text{NO}_3$  conversion, by which the production of organic nitrogen is stimulated and the accumulation of  $\text{NO}_3$  is reduced accordingly.

Transformation of  $\text{NO}_3$  takes place in all parts of the plant (Sanderson & Cocking, 1964a, 1964b; Wallace & Pate, 1965). Under circumstances of sufficient availability of nitrogen, much  $\text{NO}_3$  is reduced in the leaves. This was found by measuring the activity of the enzyme nitrate reductase (NRA). Light activates NRA to a high degree (Hageman et al., 1961). Furthermore, it appears that the activity of the enzyme is genetically defined (Bowerman & Goodman, 1971).

Accumulation of  $\text{NO}_3$  or limited reduction of  $\text{NO}_3$  is often associated with deficiencies in a number of micro-elements which are indispensable in the enzymatic reduction reactions. This is most clearly evident in the cases of molybdenum and manganese (Hewitt et al., 1949; Mulder et al., 1959), but also to a lesser extent of iron, copper and zinc (Kessler, 1964; McKee, 1962).

The distribution of org. N in the various parts of the plant is very different. High contents of org. N are found in the leaves (Dilz, 1964; Houba, 1973). The content is considerably lower in other parts of the plant, such as roots, stems and storage tissues.

In his experiments with perennial ryegrass Alberda (1965, 1968a) determined the maximum content of org. N in herbage, stubbles and roots with high N application. In herbage the upper level of organic nitrogen achieved a value of 3% N in the dry matter. Research with Italian ryegrass (Darwinkel, unpublished data) also indicated maximal org. N contents in herbage, stubbles and roots (Fig. 4). This experiment was carried out with three N levels and two light intensities. The maximum content of org. N in leaves was found to be related to the age of the tiller. In leaf blades of older tillers the content of org. N was significantly lower than in those of the younger tillers.

A clear influence of the age of leaves on the content of org. N was also found in oats (Dilz, 1964) and sugar-beets (Houba, 1973). In the youngest leaves, the content of

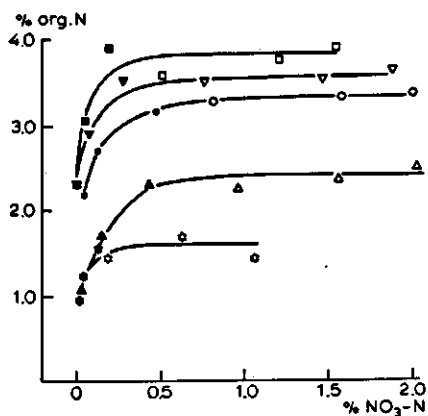


Fig. 4. Relation between contents of organic N and  $\text{NO}_3\text{-N}$  in different parts of Italian ryegrass (Darwinkel, unpublished data). Light intensity 100%, black symbols; 45%, open symbols. Leaves of tillers less than 2 weeks old  $\square$ ; leaves of 2-4 weeks old  $\nabla$ ; leaves of tillers older than 4 weeks  $\circ$ ; stems  $\triangle$  and roots  $*$ .

org. N was significantly higher than in the older leaves.

It can be deduced from this review that the production of org. N is dependent on the total yield of dry matter and on the distribution of the dry matter. Conversion of  $\text{NO}_3$  decreases as the plant develops, and an increasing share of dry matter yield consists of older plant parts with lower org. N contents. From data of Van Burg (1962, 1970) it can be deduced that for the same high content of  $\text{NO}_3$ , the content of org. N is considerably higher in grass cut in a young stage than cut in an older stage.

## 2.4 Accumulation of nitrate nitrogen

Accumulation of  $\text{NO}_3$  in the plant occurs when the uptake of  $\text{NO}_3$  exceeds its conversion. The influence of a factor on the  $\text{NO}_3$  accumulation depends on the extent to which this factor influences uptake and conversion.

Conversion of  $\text{NO}_3$  is linked with the processes of synthesis and transformation of organic matter, and is not or only slightly influenced by the  $\text{NO}_3$  content, as long as this is above a certain minimum level. The accumulation of  $\text{NO}_3$ , being the difference between uptake and conversion, will be determined to a high degree by the amount of the  $\text{NO}_3$  uptake. The quantity of  $\text{NO}_3$  in the plant per unit dry weight is reflected in the  $\text{NO}_3$  content of the dry matter.

From the relationship between contents of  $\text{N}_t$  and  $\text{NO}_3$  in grasses (apGriffith, 1960) as shown in fig. 5 and also from experiments with thousand headed kale and rape (apGriffith & Johnston, 1961), it can be deduced, that below a  $\text{N}_t$  content of 3% only little  $\text{NO}_3$  occurs in the plants. Similar results were obtained by Alberda (1965) with perennial ryegrass. Peterson (1968) found in tobacco leaves, that any increase of  $\text{N}_t$  content above 3% consisted mainly of an increase of the  $\text{NO}_3$  content. From nitrochores, introduced by Van Burg (1962), it can be deduced that the relationship between  $\text{N}_t$  and  $\text{NO}_3$  content depends on the age of the herbage. At a same  $\text{NO}_3$  content, the  $\text{N}_t$  content in herbage, harvested in a young stage of growth is considerably higher than harvested in an older stage.

In review articles, Becker (1967), Nienstedt (1967) and Wright & Davison (1964) described the factors which influence the  $\text{NO}_3$  content. In general their results indicate



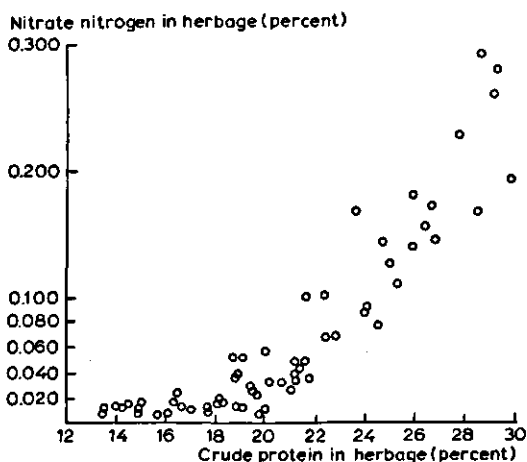


Fig. 5. Relation between percentage crude protein and nitrate nitrogen in herbage. After apGriffith (1960).

that high  $\text{NO}_3$  contents are specially found in circumstances which stimulate the  $\text{NO}_3$  uptake or reduce the production of dry matter.

Accumulation of  $\text{NO}_3$  is closely linked with N supply to the plant from the soil. This supply is strongly connected with the fertility of the soil (Lovelace et al., 1968), and is also influenced by previous crops (Hanway & Englehorn, 1958), a fallow period (Kretschmer, 1958) and mineralisation (Nowakowski & Cunningham, 1966).

High fertilizer nitrogen gifts leads to accumulation of  $\text{NO}_3$  (Alberda, 1965; Balks & Plate, 1956; Blanc, 1959; Van Burg, 1965; Deinum, 1966; apGriffith, 1959; Murphy & Smith, 1967). The  $\text{NO}_3$  content increases shortly after the application of nitrogen, followed by a gradual decrease during the growing period (McCreery et al., 1966; Nösberger & Fessler, 1968; Te Velde, 1967). Although plants fertilized with  $\text{NO}_3$  have significantly higher  $\text{NO}_3$  contents (Balks & Plate, 1956; Van Burg, 1965; Nowakowski, 1962), after fertilization with  $\text{NH}_4$  considerable quantities of  $\text{NO}_3$  can also occur in the plant, because of nitrification (Dijkshoorn, 1960; apGriffith & Johnston, 1961).

Differences in  $\text{NO}_3$  accumulation have been found between plant species (Brown & Smith, 1966; Gilbert et al., 1946; Liebenow, 1971) and also between plant varieties (Barker et al., 1971; Gul & Kolp, 1960). The following plant families are reckoned to belong to the  $\text{NO}_3$  accumulating group: Amaranthaceae, Chenopodiaceae, Compositae, Convulvaceae, Polygonaceae, Solanaceae, Gramineae and Cruciferae by Kingsbury (1958) and Wright & Davison (1964). Distinguished as  $\text{NO}_3$  accumulators among the Gramineae, are: oats, maize, rye, wheat and barley; and as non-accumulators timothy, brome-grass and cocksfoot (Crawford et al., 1961).

Especially high  $\text{NO}_3$  contents are found in grasses in the year of sowing (Thomas & Willemsen, 1971). According to Kretschmer (1958) high  $\text{NO}_3$  contents occur especially in annuals. In pot experiments with the perennial grasses, perennial ryegrass and meadow fescue Balks & Plate (1956) found a high  $\text{NO}_3$  content in the first cut after sowing. The  $\text{NO}_3$  content in the second cut was lower and even more so in the third one.

In research in the United States, occurrence of high  $\text{NO}_3$  contents in field crops is often related to drought (Muhrrer et al., 1955). In investigations on the effect of drought on the  $\text{NO}_3$  content higher  $\text{NO}_3$  contents were observed by Deinum (1966) and Flynn

et al. (1957) but not by Wright & Trautman (1962).

High  $\text{NO}_3$  contents are often recorded in late summer and in autumn (Carlier & Cottyn, 1971; Te Velde, 1967), and may be related to lower light intensities at that time of the year.

Accumulation of  $\text{NO}_3$  is highest under favourable growing conditions, just as the uptake is (Deinum, 1966; Nowakowski & Cunningham, 1966), but the nitrate content is low because of a high dry matter yield. Under unfavourable growing conditions a high  $\text{NO}_3$  content is often found in association with small dry matter production.

High  $\text{NO}_3$  contents are found especially at lower light intensities (Alberda, 1965; Bathurst & Mitchell, 1958; Deinum, 1966; Nowakowski & Cunningham, 1966; Scharrer & Seibel, 1956). The influence of temperature is less clear. Alberda (1965) and Bathurst & Mitchell (1958) found a higher  $\text{NO}_3$  content at increased temperature; Deinum & Dirven (1967) and Younis et al. (1965) did not find a clear connection between temperature and  $\text{NO}_3$  content.

The influence of other nutrients on the  $\text{NO}_3$  accumulation has been investigated repeatedly, but did not always give similar results. In general, higher  $\text{NO}_3$  contents are found under conditions of mineral deficiencies (Hewitt, 1970). This is seen most clearly in cases of deficiencies in molybdenum and manganese (Hewitt et al., 1949; Liebenow, 1972), but also in association with shortages of phosphorus (Scharrer & Seibel, 1956), of potassium (McLeod & Carson, 1965), of sulphur (Adams & Sheard, 1966) and boron (Hulewicz & Mokrzecka, 1971). In wheat seedlings, however, Harper & Paulsen (1969a, 1969b) found higher  $\text{NO}_3$  contents, only in cases of deficiencies of molybdenum, manganese or boron.

Fairly soon after its application  $\text{NO}_3$  is found in almost all organs of the plant. High  $\text{NO}_3$  contents are found in stems, petioles and midribs, whereas leaf blades contain rather low contents (Crawford et al., 1961; apGriffith & Johnston, 1961; Pimpini et al., 1970). According to Pate (1971) transport organs have a storage function for soluble N compounds. From these organs, the  $\text{NO}_3$  is transported to the leaf blades, where the reduction takes place.

Differences in  $\text{NO}_3$  contents also exist between leaves of one and the same plant. The content is considerably higher in older leaves than in the younger ones (Pimpini et al., 1970). Kelley & Ulrich (1966) arranged sugar-beet leaves according to age and found a steep increase of the  $\text{NO}_3$  content with increasing age of the leaves. Most of the  $\text{NO}_3$  was present in the older leaves. Data of Coic et al. (1972) and apGriffith & Johnston (1961) were not in agreement with these results, possibly because applications of nitrogen were relatively low.

In many plants little or no nitrate is found in organs in which assimilates are stored. Hardly any  $\text{NO}_3$  was available in the seed of small grains (Crawford et al., 1961; Dilz, 1964), maize (Walsh, 1971), potatoes, beans (Maynard & Barker, 1972) and in the head of cauliflower, (Pimpini, Vetter & Wünsch, 1970) notwithstanding high  $\text{NO}_3$  contents in other parts of the plant.

In literature, plant families and species are distinguished on the basis of recorded  $\text{NO}_3$  contents in accumulators and non-accumulators (Crawford et al., 1961). A straightforward explanation of these differences in nitrate accumulation found cannot be given, as the production of dry matter and the uptake of nitrogen are often not known. Only by knowing about all these factors a better explanation can be given for the differences in

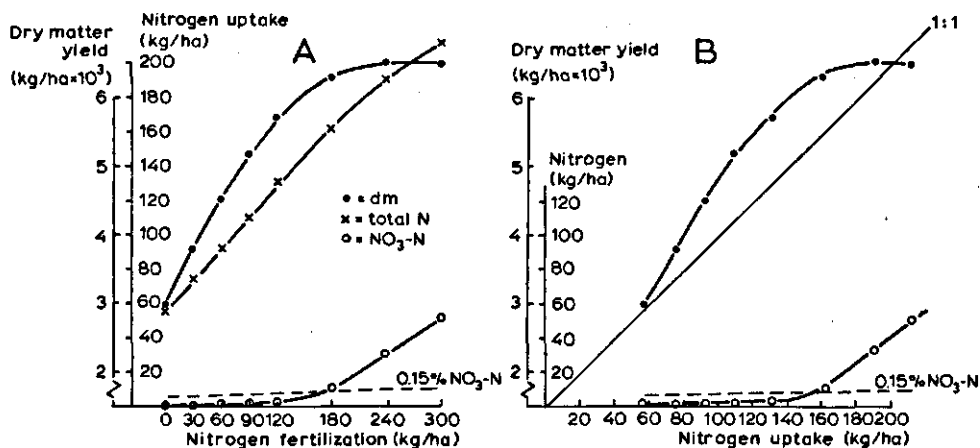


Fig. 6. Relations in grasses, between (A) dry matter yield, N uptake,  $\text{NO}_3$  accumulation versus N application and between (B) dry matter yield,  $\text{NO}_3$  accumulation versus N uptake. According to data of Van Burg (1970).

nitrate contents between plant species.

Accumulation of nitrate nitrogen is illustrated in Fig. 6a with increasing N applications. The patterns of dry matter production and of nitrogen uptake, which are also given in the figure, enable a clear picture of the relationships between these components and nitrate accumulation to be obtained. In grasses it seems that the production of dry matter achieves a maximum; the production does not increase any more beyond a certain N application. N uptake, on the contrary, keeps on increasing with fertilization. Where, in Fig. 6a, the production of dry matter does not increase any further, the surplus N uptake is largely not being converted into organic compounds, but is stored as  $\text{NO}_3$ .

In Figure 6b, N uptake is plotted on the abscissa, using the same data as in fig. 6a. In this graph it is now shown that once the maximum dry matter yield is reached about 75% of the extra N uptake is accumulated as  $\text{NO}_3$  and only 25% is fixed as organic N. This ratio is fairly constant from this point onwards.

By means of this method of graphic representation it is possible to register closely the effects of plant species and external conditions. This way of representation will also be used to discuss the causes of differences in nitrate accumulation in Chapter 4.

### 3 Research

In this research into the accumulation of nitrate nitrogen in crop situations much attention has been paid to influences of plant species, nitrogen fertilization and season on the pattern of dry matter production and the uptake and conversion of nitrate nitrogen. Separately grown plants were used to study in more detail the distribution and reduction of nitrate nitrogen.

The research was carried out in 1969, 1970 and 1971 and included field trials as well as indoor experiments. All trials were carried out on the experimental fields and in the greenhouses of the Department for Field Crops and Grassland Husbandry of the Agricultural University at Wageningen.

In the field trials, the dry matter yield, the nitrogen uptake and quantity of org. N were periodically determined, so that the accumulation of  $\text{NO}_3$  in the plant could be well studied during the growing period. The data of the periodical harvests contributed towards a better interpretation of the results of the final harvest. Conversion and accumulation of  $\text{NO}_3$  were looked at more closely in indoor experiments to obtain a better insight into the nitrogen metabolism of the plant. In these experiments the activity of the enzyme nitrate reductase was also measured.

The material harvested during the growing period was separated into a number of plant parts in which the quantities of dry matter,  $\text{N}_t$ , org. N and  $\text{NO}_3\text{-N}$  were determined. In this way it was possible to study the changes in the distribution of these components during the growing period. With these data a better interpretation can be given for differences in  $\text{NO}_3$  accumulation found at the final harvest.

In this research, the stubble turnip (*Brassica campestris* L. Var. *rapa* (L.) Hartm.) had a central place because of its strong  $\text{NO}_3$  accumulating character. Oats (*Avena sativa* L.) (Bradley et al., 1939) and spinach (*Spinacia oleracea* L.) (Van Burg et al., 1968; Nicolaisen & Zimmermann, 1968) were chosen in connection with high  $\text{NO}_3$  contents found in these species. The following species were added for reasons of comparison: rape (*Brassica napus* L. ssp. *oleifera* (Metzg.) Sinsk.), Italian ryegrass (*Lolium multiflorum* Lam.), Westerwolths ryegrass (*Lolium multiflorum* Lam.) and carrot (*Daucus carota* L.).

#### 3.1 Implementation of the research

The research was started in the spring of 1969 with a field trial in which  $\text{NO}_3$  accumulation was investigated in a number of plant species. After that, several aspects of  $\text{NO}_3$  accumulation were studied in various field and indoor experiments. A review of the experiments which were carried out, is given below:

*Experiment I:* field trial, carried out in early summer – comparative research into the accumulation of  $\text{NO}_3$  in turnip, rape, oats, Italian ryegrass and carrot in relation to different nitrogen applications.

*Experiment II:* field trial, carried out in autumn — effect of nitrogen fertilization on the  $\text{NO}_3$  accumulation in two plant densities of turnip and also in Italian and Westerwolths ryegrass.

*Experiment III:* field trial — effect of the distribution of dry matter on the conversion and accumulation of  $\text{NO}_3$  with three turnip varieties.

*Experiment IV:* field trial — as experiment III with Italian and Westerwolths ryegrass.

*Experiment V:* indoor trial — research into the distribution of organic N and  $\text{NO}_3$  in the turnip.

*Experiment VI:* indoor trial — research into the redistribution of N and the localisation of the  $\text{NO}_3$  reduction during the development of the turnip.

*Experiment VII:* indoor trial — research into the reduction and accumulation of  $\text{NO}_3$  in Italian ryegrass, oats and spinach (comparison with experiments V and VI with the turnip).

In the field trials, all plant species were sown in rows at a distance of 20 cm and N fertilization took place with nitrate of lime ( $\text{Ca}(\text{NO}_3)_2$ ). Also in the indoor experiments, nitrogen was applied in the form of  $\text{NO}_3$ . In all trials, the other nutrients were applied in sufficient quantities.

More extensive information on the implementation of the experiments are given in section 3.3, where the results of the experiments are presented.

### 3.2 Methods of analysis

The plants from the field trials as well as the indoor experiments were always harvested in the mornings between 08.00 and 10.00 hours. This standardization of harvesting time is desirable because the contents of org. N and  $\text{NO}_3$  are subject to diurnal fluctuations (Hageman et al., 1961). The plant material was collected as completely as possible including the roots and dead leaves. After the harvest all samples were put into cold-storage space at a temperature of 2–4° C.

The samples were then processed as soon as possible, the first procedure being to separate the material into a number of fractions. Immediately after separation, the fraction were weighed and put into a drying oven. Plant parts carrying sand, such as roots, were first washed as well as possible. The material was dried at 80° C for at least 20 hours and then weighed. The dried plant material was ground through a 1 mm sieve and stored in airtight plastic bags.

For the determination of  $\text{N}_t$ , inclusively  $\text{NO}_3$ , a modified Kjeldahlmethod was used as described by Dey's (1961). For this analysis 0.3500 to 0.4500 g of dried material were used.

The content of  $\text{NO}_3$  was determined potentiometrically with a specific  $\text{NO}_3$  ion electrode, Orion 92–07 (Paul & Carlson, 1968). For the determination, 0.250 to 0.350 g of dried plant material were weighed and shaken for half an hour with 25 ml demineralized water and then filtered. To 10 ml filtrate, 10 ml 0.2 M  $\text{KH}_2\text{PO}_4$  was added and some Dowex resin (ca. 0.3 g) after which the  $\text{NO}_3$  concentration was measured. Values mentioned in this report for  $\text{NO}_3$  accumulation and  $\text{NO}_3$  contents, must always be read as  $\text{NO}_3\text{-N}$ . The org. N content was calculated as the difference between  $\text{N}_t$  and  $\text{NO}_3\text{-N}$  contents.

For the determination of the nitrate reductase activity a somewhat simplified method was used of Sanderson & Cocking (1964a). At a temperature of 2 to 4° C, fresh plant

material was ground in 25 ml of a solution containing 0.09 M phosphate ( $2 \text{ KH}_2\text{PO}_4/8 \text{ Na}_2\text{HPO}_4$  - pH=7.5), 0.45 M saccharose, 0.009 M glutathione and 0.0009 M EDTA. From the suspension thus prepared 2 ml were added to 10 ml of a solution containing 0.065 M phosphate, 0.01 M  $\text{KNO}_3$  and 2.5 mg NADH. The reaction took place at 30° C and was stopped after 30 minutes by adding 1 ml 2 M Ba-acetate. After centrifuging for 10 minutes at 4000 rpm, the clear liquid thus formed was decanted and coloured with 5 ml 1% sulphonylamide in 2.5 N HCl and 5 ml 0.02% N-1-naphtylethylene-diamine-HCl. The intensity of the colouring was measured colorimetrically 30 minutes later, at 540 nm and the nitrate reductase activity (NRA) was calculated as  $\mu\text{M NO}_2/\text{hour per g fresh weight } (\mu\text{M NO}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1})$ . For the NRA analysis, 2.0 g fresh leaf, 6.0 g stem and 6.0 g root were used.

This method of determination of the NRA is not specifically adjusted to the plant species investigated, which restricts the usefulness of comparisons between them. A comparison between organs of one and the same plant species, however, is quite possible.

After each harvest the plant material was separated in some fractions. The choice of these fractions depended upon the plant species. Leaves of turnip, rape, carrot and spinach were separated into leaf blade, petiole and midrib. The leaf fraction from oats, Italian ryegrass and Westerwolths ryegrass consisted of laminae, including the parallel veins, but the leaf sheaths were included with the stems.

### 3.3 Results of the experiments

The results of all the experiments are presented separately. Just as in the review of paragraph 3.1, the field experiments will be dealt with first and then the indoor experiments. In this chapter the results of the experiments have been compared but only to a very limited extent. In the discussion of the results in Chapter 4, more attention will be paid to this comparison.

#### 3.3.1 Comparative research into the accumulation of $\text{NO}_3$ in some plant species

$\text{NO}_3$  accumulation in some plant species has been studied by means of two field experiments. In 3.3.1.1 a summer experiment (experiment I) is described in which the influence was analysed of N application and of plant species on the  $\text{NO}_3$  accumulation. In other circumstances, a similar experiment (experiment II) was carried out in the autumn on a smaller scale (3.3.1.2).

*Experiment I: Uptake and accumulation of  $\text{NO}_3$  in turnip, rape, oats, Italian ryegrass and carrot.* The experiment was carried out in the summer of 1969 on a light, drought sensitive, sandy soil poor in humus.

Sowing of the crops took place on 15/IV.

N fertilization:

- $\text{N}_1 = 10 \text{ g N/m}^2$  (= 100 kg N/ha)
- $\text{N}_2 = 30 \text{ g N/m}^2$  (= 300 kg N/ha)
- $\text{N}_3 = 10 + 20 \text{ g N/m}^2$  (= 100 + 200 kg N/ha)

The second application of the  $\text{N}_3$  treatment was given with the first harvest of each crop. The crops were sampled on the following dates:

Harvests	I	II	III	IV	V	VI
Plant species						
It. ryegrass + oats	28/V	6/VI	16/VI	24/VI	2/VII	14/VII
turnip + rape	2/VI	11/VI	19/VI	30/VI	9/VII	21/VII
carrot	26/VI	7/VII	16/VII	24/VII	1/VIII	11/VIII

On 24/VII, the  $N_3$  treatment of Italian ryegrass was sampled once again.

The experiment was laid out in a randomized design with four replicates. The net size of the plots was  $1.2 \text{ m}^2$ . To determine weights of roots and stubble of Italian ryegrass and oats,  $0.2 \text{ m}^2$  was sampled. Plots were irrigated on 2/VII, 18/VII, 28/VII and 6/VIII because of drought.

**Results** Carrot showed a very slow growth in the beginning so that the maximum growth rate occurred about four weeks later than in the other plant species. Because of the different weather conditions encountered during growth it is not possible to make comparisons between carrots and the other plant species without complications. Therefore, in the discussion of the results, carrot will be omitted at first.

The total dry matter yield and the uptake, conversion and accumulation of  $\text{NO}_3$  from the final harvest are given in Table 1. At the low level of N application ( $N_1$  treatment) N was deficient and dry matter yields lagged behind in all plant species, except in oats.

The mineralization of N was low in the soil, with the result that in the low N treatment total N uptake hardly exceeded the amount applied. In this  $N_1$  treatment almost all absorbed nitrogen have been converted in organic form. As regards  $\text{NO}_3$  accumu-

Table 1. Total dry matter yield ( $\text{g/m}^2$ ) and uptake, conversion and accumulation of  $\text{NO}_3\text{-N}$  ( $\text{g N/m}^2$ ) and the contents of  $N_t$ , org. N and  $\text{NO}_3\text{-N}$  ( $\text{g/100 g dry matter}$ ) at the final harvest.

	Dry matter	$N_t$	Org. N	$\text{NO}_3\text{-N}$	$N_t$ (%)	Org. N (%)	$\text{NO}_3\text{-N}$ (%)
$N_1$ treatment							
turnip	777	10.2	9.7	0.5	1.30	1.23	0.07
rape	798	10.8	10.4	0.4	1.34	1.29	0.05
oats	1017	10.9	10.6	0.3	1.08	1.05	0.03
It. ryegrass	794	10.1	9.9	0.2	1.27	1.24	0.03
carrot	767	8.9	8.6	0.3	1.15	1.12	0.03
$N_2$ treatment							
turnip	863	25.6	22.2	3.4	2.97	2.57	0.40
rape	939	22.3	20.1	2.2	2.36	2.14	0.22
oats	915	17.4	14.3	3.1	1.91	1.56	0.35
It. ryegrass	899	23.3	20.8	2.5	2.60	2.32	0.28
carrot	913	18.1	15.7	2.4	1.98	1.72	0.26
$N_3$ treatment							
turnip	897	32.3	24.2	8.1	3.60	2.70	0.90
rape	981	29.3	23.3	6.0	2.98	2.38	0.60
oats	924	18.2	14.5	3.7	1.97	1.57	0.40
It. ryegrass	907	27.6	23.2	4.4	3.07	2.56	0.51
carrot	877	19.0	15.8	3.2	2.21	1.82	0.39

lation this treatment is not interesting and will be therefore excluded from further considerations.

With the high N applications, differences in dry matter yield between the plant species and between the N treatments were rather small. Splitting the N application increased the yield somewhat, more with turnip and rape than with Italian ryegrass and oats. In both of the high N treatments, the dry matter yield was highest in rape and lowest in turnip.

Differences in quantities of absorbed nitrogen existed between the N treatments as well as between the plant species. Splitting the N application led to a higher uptake of  $N_1$ ; only oats, which took up significantly less nitrogen, did not show any effect. Turnip took up more nitrogen than rape and Italian ryegrass. The quantity of organic nitrogen was influenced in the same way by nitrogen fertilization and plant species as was the uptake of  $N_1$ . The differences in quantities of org. N however, were smaller than those in the quantities of  $N_1$ . This caused big differences in  $NO_3$  accumulation. Splitting the N application caused a greatly increased  $NO_3$  accumulation; turnip with the highest nitrogen uptake accumulated more  $NO_3$  than rape and Italian ryegrass. In oats org. N production clearly lagged behind N uptake, so that much  $NO_3$  was present in the final harvest.

Big differences in  $N_1$ , org. N and  $NO_3$  contents existed between the plant species, caused by differences in uptake and conversion of  $NO_3$ . The N contents were increased by splitting the high N application. It is remarkable that in the  $N_2$  treatment, the org. N contents were lower than in the  $N_3$  treatment, as there was still much  $NO_3$  available.

The dry matter production curves of the plant species under the  $N_3$  treatment are presented in Fig. 7. The production curves under the  $N_2$  treatment were similar to the  $N_3$  treatment and are therefore not given. All times were measured from the date of the first harvest. The development of the species was very similar, which facilitated a good comparison during the growing period. The maximum time difference in the production curves between turnip, rape, oats and Italian ryegrass was a week, so that an interaction between external circumstances and responses must be fairly small.

The dry matter production curve was largely linear with time. Between the plant species, differences in production rate were rather small. For turnip, rape, oats and Italian ryegrass, these amounted during the growing period to 150, 171, 160 and 158 g  $dm \cdot m^{-2}$ .

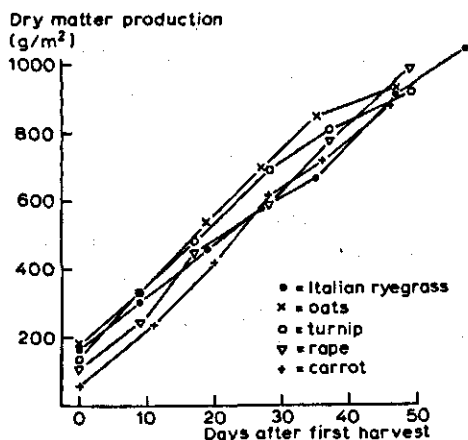


Fig. 7. Trend of the dry matter production of turnip, rape, oats, Italian ryegrass and carrot during the growing period ( $N_3$  treatment).



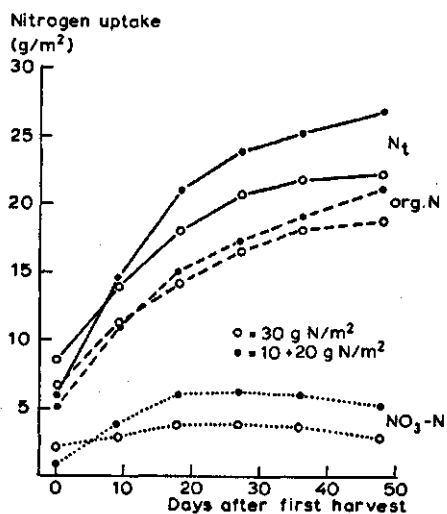


Fig. 8. Effect of splitting the high N application on the trend of the N uptake, org. N production and  $NO_3$  accumulation during the growing period (averages of turnip, rape, oats and Italian ryegrass).

day<sup>-1</sup> respectively. In the beginning turnip and oats appeared to produce somewhat more dry matter than rape and Italian ryegrass; at the end of the growing period this was reversed so that the differences in the final harvest were small.

The total N uptake and the accumulation of  $NO_3$  and org. N is represented in Fig. 8 as an average for the four plant species in the  $N_2$  and  $N_3$  treatments. This shows that at the first harvest more nitrogen had been taken up by the  $N_2$  treatment than was taken up by the  $N_3$  treatment. High N uptake in the  $N_3$  treatment was observed after the second N application, and at the third harvest this treatment contained considerably more nitrogen than the  $N_2$  treatment. After that, uptake of total N started to decrease in both treatments, so that the difference in the quantities of  $N_t$  was maintained almost completely until the final harvest. The pattern obtained was similar to that for the production of org. N. From the third harvest onwards, the quantity of org. N in the  $N_3$  treatment was higher than that of the  $N_2$  treatment. However, the differences between the two treatments in yield of org. N were considerably smaller than the differences in the N uptakes.

The difference between uptake and conversion of  $NO_3$  represents the  $NO_3$  accumulation. At the first harvest most  $NO_3$  appeared in the  $N_2$  treatment. After the second application of the  $N_3$  treatment, uptake of  $NO_3$  was clearly higher than conversion, which was shown by a steep increase in the  $NO_3$  accumulation. At the third harvest of the  $N_3$  treatment, the  $NO_3$  accumulation was already clearly higher than in the  $N_2$  treatment. After that, the accumulated quantity of  $NO_3$  slightly decreased in both treatments.

Although the dry matter yields of the plant species at the final harvest were almost equal, the quantities of  $N_t$  and  $NO_3$  showed clear differences. The effect of the plant species was most clear in the  $N_3$  treatment. N uptake and  $NO_3$  accumulation in turnip, oats and Italian ryegrass are shown for this treatment in Fig. 9; the values for rape were between those of turnip and Italian ryegrass. The graphs show a fairly consistent trend. The irregularity at the fifth harvest in the turnip may be due to leaching of  $NO_3$  from the plants caused by watering, which took place shortly before the harvest.

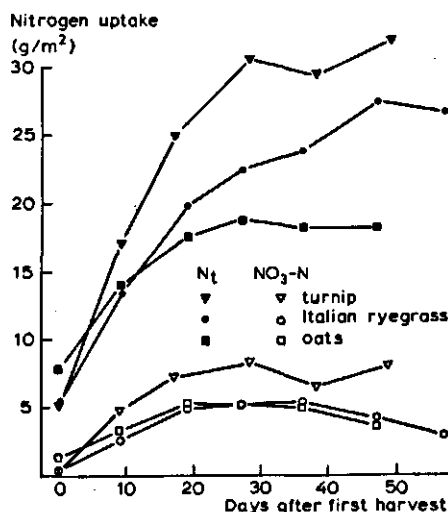


Fig. 9. Pattern of the uptake and conversion of  $NO_3-N$  in turnip, oats and Italian ryegrass during the growing period ( $N_3$  treatment).

Fig. 9 shows that the differences in the final N uptake were caused shortly after the second N application by differences in rate of the N uptake. The N uptake was highest in turnip and lowest in oats; Italian ryegrass took an intermediate position. The rate of N uptake by turnip and, to a lesser extent by Italian ryegrass, decreased because of exhaustion of available nitrogen in the soil after the third harvest. From that time onwards N uptake in Italian ryegrass was slightly higher than the uptake in turnip, so that the difference in the quantities of  $N_t$  at the final harvest slightly decreased. In oats, however, N uptake had almost completely ceased after emergence of the panicle on 24/VI.

Nitrate accumulated until the third harvest and remained almost constant after that in turnip or decreased a little in oats and Italian ryegrass. Conversion of  $NO_3$  until the final harvest took place in turnip and Italian ryegrass. In oats, little  $NO_3$  was converted after emergence of the panicle. Differences in  $NO_3$  accumulation between oats and Italian ryegrass were small during the growing period notwithstanding clear differences between N uptake in those crops (Fig. 9).

The org. N and  $NO_3-N$  contents of the various plant parts are given for the  $N_3$  treatment in Table 2. The contents of the  $N_1$  and  $N_2$  treatments differed only in level (Appendix 1). The distribution of org. N and  $NO_3$  corresponded well in the various plant parts of the investigated plant species. The highest level of org. N was always found in the leaf blades; in all other parts of the plant it was considerably lower.

The distribution of  $NO_3$  in the various organs deviated clearly from the distribution of org. N. The highest  $NO_3$  contents were always found in transport organs and in dead leaves. In roots and leaf blades the  $NO_3$  content was considerably lower and was completely absent from the panicle of oats. The tap root of the turnip had a high  $NO_3$  content and is therefore comparable with stems and petioles.

The behaviour of the carrot plant coincides with that of the other plant species, as can be deduced from Fig. 7 and Tables 1 and 2. The curve of dry matter production showed the same trend during the later part of the growing period. The quantity of  $N_t$  in the plant at the final harvest was smaller owing to a slower rate of N uptake. Org. N was

Table 2. Percentage of org. N and  $\text{NO}_3\text{-N}$  in the various plant parts of turnip, rape, oats, Italian ryegrass and carrot at the final harvest ( $\text{N}_2$  treatment).

	Turnip	Rape	Oats	Italian ryegrass	Carrot
<i>Organic N (g/100 g dry matter)</i>					
herbage				3.18	
leaf-blades + veins	4.64	4.06	3.44		3.11
petioles	2.24	1.83			0.98
stems		2.09	0.93		
panicles			2.10		
dead leaves	1.37	0.98	1.53	1.84	1.92
stubbles				2.87	
roots	2.59	1.71	1.50	1.62	1.43
whole plant	2.70	2.38	1.57	2.56	1.82
<i><math>\text{NO}_3\text{-N}</math> (g/100 g dry matter)</i>					
herbage				0.60	
leaf-blades + veins	0.34	0.19	0.23		0.55
petioles	1.09	0.75			0.81
stems		0.89	0.70		
panicles			0		
dead leaves	1.31	1.09	0.36	0.80	0.65
stubbles				0.34	
roots	0.91	0.60	0.40	0.10	0.08
whole plant	0.90	0.60	0.40	0.51	0.39

produced during the whole growing period and at the final harvest less  $\text{NO}_3$  had accumulated in the carrot than in the other plant species. The distribution of org. N coincided with those of the other plant species. For the distribution of  $\text{NO}_3$  in the root of the carrot, a low content was found which was in contrast with the tap root of the turnip.

In this experiment clear differences occurred between the plant species in N uptake and  $\text{NO}_3$  accumulation. A similar experiment was carried out in the autumn with turnip, Italian ryegrass and Westerwolths ryegrass, to find out whether the influence of species on uptake, conversion and accumulation of  $\text{NO}_3$  depended on the season.

*Experiment II: Uptake and accumulation of  $\text{NO}_3$  in Italian ryegrass, Westerwolths ryegrass and turnips* An experiment in 1968 showed that in late autumn turnips took up considerably more nitrogen than Italian ryegrass at almost the same rate of dry matter production. With the autumn experiment in 1971, uptake and accumulation of  $\text{NO}_3$  were investigated in turnip and Italian ryegrass, for comparison with the results of Experiment I. Westerwolths ryegrass and a second, low plant density of turnip were included to study the possible influence of the morphological composition of the plant on the  $\text{NO}_3$  conversion.

The experiment was done on a sandy peat soil which had received an application of 22 tons of stable manure per ha shortly before sowing. The sowing took place on 18/VIII/1971. After emergence, turnips were spaced to 23 and 69 plants per  $\text{m}^2$ ,

respectively.

The N fertilization (g N/m<sup>2</sup>) and the dates of periodic harvests are mentioned in the schedule below:

N-treatments	Dates of fertilization			Dates of harvests		
	18/VIII	1/X	20/X	1/X	20/X	9/XI
N <sub>1</sub>	10	.	.	x	x	x
N <sub>2</sub>	10	20	.	.	x	x
N <sub>3</sub>	10	.	20	.	.	x

The late top dressings facilitated research into the NO<sub>3</sub> uptake at a late stage of development of the crop.

The net size of the plots at the first two harvests was 1 m<sup>2</sup> and at the final harvest 2 m<sup>2</sup>. For the determination of weights of stubble and roots of both grasses 0.2 m<sup>2</sup> was harvested per plot. The experiment was a randomized block design with 4 replicates.

**Results** The dry matter yields and the quantities of N<sub>t</sub>, org. N and NO<sub>3</sub>-N of the four crops at the final harvest are given in Table 3.

The data show that the yield of turnips was clearly higher than that of both grasses. The differences between the two grasses were small. The yield of turnips at a low plant density lagged clearly behind that at a high plant density. The late N applications did not influence the dry matter yields significantly. A reaction in yield to the extra N applica-

Table 3. Dry matter yields and the quantities of N<sub>t</sub>, org. N and NO<sub>3</sub>-N of Italian ryegrass, Westerwolths ryegrass and turnips (final harvest).

	Dry matter yield (g/m <sup>2</sup> )	N <sub>t</sub> (g/m <sup>2</sup> )	Org. N (g/m <sup>2</sup> )	NO <sub>3</sub> -N (g/m <sup>2</sup> )
<i>Italian ryegrass</i>				
N <sub>1</sub>	346	11.7	10.7	1.0
N <sub>2</sub>	342	14.0	11.3	2.7
N <sub>3</sub>	344	13.7	11.5	2.2
<i>Westerwolths ryegrass</i>				
N <sub>1</sub>	314	11.3	9.8	1.5
N <sub>2</sub>	328	14.4	11.8	2.8
N <sub>3</sub>	337	14.6	11.8	2.8
<i>Turnips (23 plants/m<sup>2</sup>)</i>				
N <sub>1</sub>	431	14.0	11.0	3.0
N <sub>2</sub>	457	17.5	12.5	5.0
N <sub>3</sub>	449	17.0	12.1	4.9
<i>Turnips (69 plants/m<sup>2</sup>)</i>				
N <sub>1</sub>	492	15.4	12.1	3.3
N <sub>2</sub>	519	21.5	14.3	7.2
N <sub>3</sub>	511	20.5	14.4	6.1

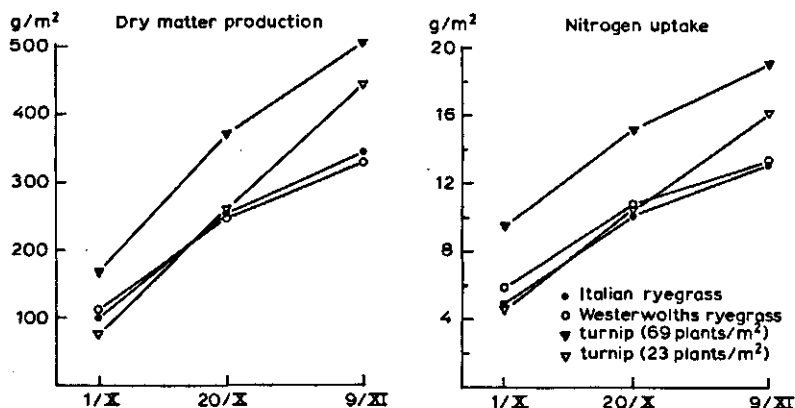


Fig. 10. Production of dry matter and uptake of nitrogen during the growing period in grasses and turnips (averages of all N treatments).

tion should not be expected because of the large quantities of  $\text{NO}_3$  found in the  $\text{N}_1$  treatment.

The turnips have clearly taken up more nitrogen than the two grasses. Differences in quantities of  $\text{N}_t$ , org. N and  $\text{NO}_3\text{-N}$  between the two grasses were very small. The turnips at a low plant density took up less  $\text{N}_t$  than those at a high density, due to the low N uptake in the beginning of the growing period. The second N-application of the  $\text{N}_2$  and  $\text{N}_3$  treatment clearly increased the quantities of  $\text{N}_t$ , org. N and  $\text{NO}_3$  in all crops, but more so in turnips than in the grasses. The date of the late nitrogen dressing hardly influenced these yields.

The yield of org. N was influenced by plant species and N fertilization in the same way as the N yield, but to a lesser extent. Hence a considerable part of the surplus uptake of N was found in the plant as  $\text{NO}_3$ . At the final harvest the turnips had accumulated considerably more  $\text{NO}_3$  than both grasses; there were little differences between the two grasses.  $\text{NO}_3$  accumulation in the dense turnip crop was clearly higher than in the low density crop. The second N application of both treatments increased the  $\text{NO}_3$  accumulation considerably, but this accumulation was clearly not influenced by the date of the second application.

Fig. 10 gives the trend of dry matter production and  $\text{N}_t$  uptake of both grasses and of both plant densities of the turnip. During the growing period the production of dry matter and the N uptake of the grasses lagged more and more behind those of the turnip.

Fig. 11 shows the uptake and conversion of  $\text{NO}_3\text{-N}$  in relation to N application and time. N uptake took place quicker in turnips than in grasses, especially later in the growing season. In turnips the  $\text{N}_t$  yield was considerably higher than the yield of org. N, so that large quantities of  $\text{NO}_3$  were accumulated. In grasses, the difference between uptake and conversion of  $\text{NO}_3$  was considerably lower, so that the measured  $\text{NO}_3$  accumulation was much less.

With an almost equal dry matter yield in all the N treatments, the yield of org. N in the  $\text{N}_1$  treatment clearly lagged behind the org. N yield in both other treatments, notwithstanding the availability of sufficient  $\text{NO}_3$  to reach at least the same amount of org. N. This effect together with the maintenance of a previously accumulated quantity

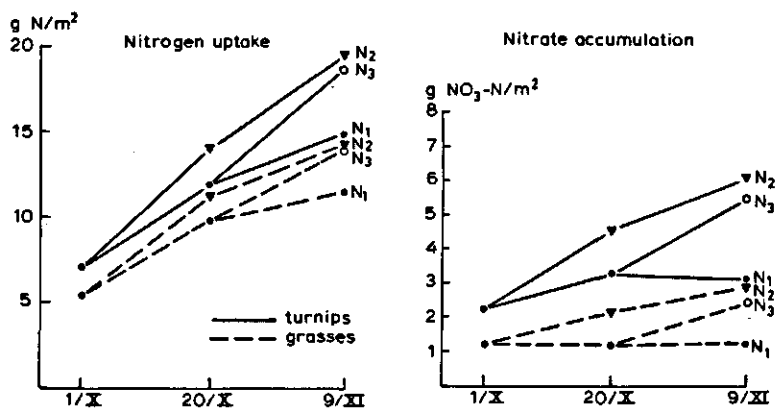


Fig. 11. Uptake and accumulation of NO<sub>3</sub>-N in grasses and turnips in relation to time and level of N application.

of NO<sub>3</sub>, was also found in Experiment I.

Comparison between grasses and turnips of the N metabolism is, however, complicated by big differences in the dry matter yields. Therefore, in Fig. 12, N<sub>t</sub> contents are plotted against the dry matter yield, whereby it is possible to compare the N metabolism of grasses and turnips at the same dry matter yield. As, however, the same dry matter yield was reached on different dates, a possible influence of external conditions and also of physiological age of the plant cannot be excluded (Van Burg, 1962). At a yield of 300 g dm/m<sup>2</sup>, the N<sub>t</sub> contents of grasses and turnips hardly seem to differ. The grasses however, have converted more NO<sub>3</sub> per g of dry matter produced and a higher org. N content and a lower NO<sub>3</sub> content were found.

Changes in the morphological composition are brought about by changes in the distribution of assimilates. Table 4 shows the distribution of dry matter as well as the N content of Italian ryegrass and Westerwolths ryegrass. As both grasses remained in the vegetative stage, only slight differences occurred in the dry matter distribution.

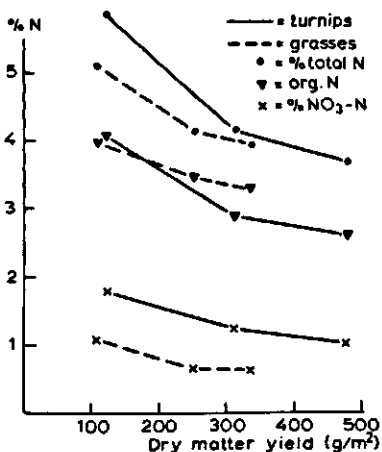


Fig. 12. Relation between the contents of N<sub>t</sub>, org. N and NO<sub>3</sub>-N and the dry matter yields in grasses and turnips (averages of all N treatments).

Table 6. Yield and distribution of the dry matter together with the uptake, conversion and accumulation of  $\text{NO}_3\text{-N}$  in the turnip varieties Siloga and Civasto R (final harvest).

	Yield of dry matter ( $\text{g/m}^2$ )	Distribution of dry matter (%)		Quantities of nitrogen ( $\text{g/m}^2$ )		
		foliage	tap root	$\text{N}_t$	org. N	$\text{NO}_3\text{-N}$
<i>N<sub>1</sub> treatment</i>						
Siloga	720	84.0	16.0	21.0	18.3	2.7
Civasto R	814	58.2	41.8	18.5	17.0	1.5
<i>N<sub>2</sub> treatment</i>						
Siloga	747	90.2	9.8	31.6	21.5	10.1
Civasto R	815	71.7	28.3	30.9	21.3	9.6

**Results** Firstly it can be remarked that the differences in dry matter production, distribution of dry matter and N content between Civasto R and Jobe were minimal, so that results of the morphologically different varieties Siloga and Civasto R only will be given.

The production and distribution of the dry matter at the final harvest, together with the uptake, conversion and accumulation of  $\text{NO}_3\text{-N}$  are shown in Table 6. Civasto R produced more dry matter than Siloga;  $\text{N}_t$  uptake however, was somewhat lower. Both differences were not statistically significant. The dry matter yield was hardly influenced by the second N application.

The distribution of the dry matter between foliage and tap root appeared to be influenced by variety and level of N application. Siloga showed a high production of foliage, so that at the final harvest the foliage yield was higher but the tap root yield considerably lower than in Civasto R. Application of a second N dressing stimulated the production of foliage and suppressed the production of tap roots in both varieties.

The variety effect on the quantities of  $\text{N}_t$ , org. N and  $\text{NO}_3$  was not clear in either N treatment. The nitrogen fertilization, however, strongly influenced the N metabolism. The second N dressing increased the N uptake considerably. The org. N yield however, increased much less, so that a large increase occurred in  $\text{NO}_3$  accumulation. Of the additional nitrogen absorbed,  $11.5 \text{ g N/m}^2$  or 67% was still present at the final harvest as  $\text{NO}_3$ .

Table 7 gives the dry weights and the contents of  $\text{N}_t$ , org. N and  $\text{NO}_3\text{-N}$  of the various plant parts from the  $\text{N}_2$  treatment at the final harvest. Data of the  $\text{N}_1$  treatment are mentioned in appendix 2. Table 7 shows that the higher foliage production of Siloga was achieved by higher yields of the leaf blades as well as petioles + midribs. The tap root production of Siloga was low, roots comprising not more than 10% of the total dry matter produced. With Civasto R the tap root production was almost 30%.

The higher dry matter yield of Civasto R led to a lower  $\text{N}_t$  content, because the  $\text{N}_t$  uptake was not higher. The org. N content in Siloga was small, but significantly higher than in Civasto R, notwithstanding the presence of much  $\text{NO}_3$  in both varieties. Under the same circumstances, Siloga converted more  $\text{NO}_3$  per g dry matter produced but because of the lower dry matter yield and the same N uptake this did not lead to a lower  $\text{NO}_3$  content.

Just as in previous experiments, big differences in contents of org. N and  $\text{NO}_3$  were

Table 7. Dry matter weights, and contents of  $N_t$ , org. N and  $NO_3$ -N in various parts of turnip varieties Siloga and Civasto R from the  $N_2$  treatment (final harvest).

	Leaf blade	Petiole + midrib	Dead leaves	Foliage	Root	Petiole + midrib + deadleaves + root	Whole plant
<i>Dry weights (g/m<sup>2</sup>)</i>							
Siloga	204	371	99	674	73	543	747
Civasto R	163	339	82	584	231	652	815
<i>N<sub>t</sub> contents</i>							
Siloga	5.47	3.99	3.01	4.29	3.71	3.77	4.25
Civasto R	5.42	3.71	2.66	4.04	3.17	3.38	3.79
<i>Org. N contents</i>							
Siloga	5.16	2.31	1.04	2.98	1.85	2.01	2.88
Civasto R	5.13	2.30	0.91	2.90	1.89	1.98	2.61
<i>NO<sub>3</sub>-N contents</i>							
Siloga	0.31	1.68	2.02	1.31	1.86	1.76	1.37
Civasto R	0.29	1.41	1.75	1.14	1.28	1.40	1.18

found between plant parts. Similar organs of Civasto R and Siloga had almost the same org. N contents. The content of org. N was high in the leaf blades but considerably lower in other organs; the reverse was found for the  $NO_3$  content. Between the transport organs, petioles + midribs, and the tap root, differences in contents of org. N and  $NO_3$  were limited. In the tap root, contents of org. N were lower and those of  $NO_3$  higher than in the petioles and midribs, in contrast to both previous experiments.

From this experiment there appeared no clear influence of the morphological composition on the conversion of  $NO_3$ . It is true that a higher content of org. N was found in the leafy variety Siloga, but this did not lead to a lower  $NO_3$  content. In this experiment the total dry matter yield influenced the production of org. N more than the morphological composition. From the results of this experiment it can be expected that with similar dry matter yields and N uptakes, a crop with a large quantity of leaf blades will convert more  $NO_3$  than a crop which is poor in leaf blades.

*Experiment IV: Influence of dry matter distribution on the conversion of  $NO_3$  in Italian and Westerwolths ryegrass* This experiment was to study the influence of the morphological composition of the plant on the  $NO_3$  conversion in closely related grasses Italian ryegrass and Westerwolths ryegrass. During the growing period, the annual Westerwolths ryegrass was in the reproductive phase, so that big differences in morphological composition arose in comparison with the vegetative Italian ryegrass.

The experiment was laid out on heavy river-clay loam. Sowing took place on 11/V/1971. N fertilization: 22.5 g N/m<sup>2</sup> (= 225 kg N/ha). Dates of periodical harvests: 22/VI, 2/VII, 16/VII and 2/VIII. In Westerwolths ryegrass, the first ears appeared on 2/VII. The net plots for the herbage yields were 1 m<sup>2</sup>; for stubbles and roots 0.2 m<sup>2</sup>. All samples consisted of five replicates.



Table 8. Quantities of dry matter,  $N_t$ , org. N and  $NO_3$ -N in herbage, stubble and roots of Italian and Westerwolths ryegrass (final harvest).

	Herbage	Stubble	Roots	Total
<i>Dry weights (g/m<sup>2</sup>)</i>				
Italian ryegrass	731	213	77	1021
Westerwolths ryegrass	771	148	44	963
<i>N<sub>t</sub> yield (g/m<sup>2</sup>)</i>				
Italian ryegrass	24.4	4.0	0.9	29.3
Westerwolths ryegrass	23.3	3.2	0.6	27.0
<i>Org. N yield (g/m<sup>2</sup>)</i>				
Italian ryegrass	22.2	3.6	0.9	26.7
Westerwolths ryegrass	20.5	2.9	0.6	24.0
<i>NO<sub>3</sub>-N accumulation (g/m<sup>2</sup>)</i>				
Italian ryegrass	2.2	0.4	0	2.6
Westerwolths ryegrass	2.7	0.4	0	3.1

**Results** In contrast to experiment II (p. 18), at the final harvest the herbage of Westerwolths ryegrass consisted mainly of reproductive tillers. Italian ryegrass however, did not have any reproductive tiller.

Table 8 gives the quantities of dry matter,  $N_t$ , org. N and  $NO_3$ -N in herbage, stubbles and roots at the final harvest. The differences in the total dry matter yield between both grasses were small and not reliable. The yield of herbage of Westerwolths ryegrass was somewhat higher, the quantity of stubbles and roots however was lower than those of Italian ryegrass, which was in agreement with the results of Experiment II. The uptake of  $N_t$  in Italian ryegrass was not reliably higher than in Westerwolths ryegrass; the same was true in relation to the yield of org. N. Westerwolths ryegrass contained clearly more  $NO_3$ .

At all periodic harvests the herbage was split up in some parts. The dry matter weights of a number of organs of Italian and Westerwolths ryegrass are given in Table 9, together with their contents of  $N_t$ , org. N and  $NO_3$ -N. The distribution of dry matter in the herbage is clearly influenced by the reproductive development. In Westerwolths ryegrass a considerable part of the assimilates was used for the formation of stems and ears, whereas Italian ryegrass had stored more dry matter in the leaf blades. Both grasses had about the same quantity of dead leaves. The differences in weights of stubbles and roots have already been mentioned.

The  $N_t$  content of the total plant did not differ much between the two grasses; Italian ryegrass had a higher  $N_t$  content in the herbage. Just as in previous experiments, the highest org. N content was found in leaf blades. In the other organs of the plant the org. N contents were considerably lower. The differences in org. N content between Italian and Westerwolths ryegrass in herbage (0.38) and in the whole plant (0.14) were limited, but significant. The differences in  $NO_3$  contents between both grasses were also small. In all organs, Westerwolths ryegrass had a higher  $NO_3$  content, which was significant in herbage and in the whole plant. In comparison with Experiment I and II, the contents of org. N and  $NO_3$ -N in herbage, stubble and roots were lower, but the distribution was the same.

In Italian ryegrass, lower  $NO_3$  contents were found in herbage as well as in the whole plant, notwithstanding higher  $N_t$  contents. Westerwolths ryegrass had converted a smaller

Table 9. Dry weights and contents of  $N_t$ , org. N and  $NO_3$ -N (g/100 g dry matter) in various organs of Italian and Westerwolths ryegrass (final harvest).

	Leaf blades	Stems	Ears	Dead leaves	Herbage	Stubble	Roots	Whole plant
<i>Dry weights (g/m<sup>2</sup>)</i>								
It. ryegrass	452	184		95	731	213	77	1021
Ww. ryegrass	248	339	97	86	771	148	44	963
<i>Dry matter distribution (%)</i>								
It. ryegrass	44.3	18.0		9.3	71.6	20.9	7.5	100
Ww. ryegrass	26.0	35.5	10.2	9.0	80.6	15.4	4.6	100
<i>N<sub>t</sub> contents</i>								
It. ryegrass	3.81	2.34		2.14	3.34	1.87	1.15	2.87
Ww. ryegrass	4.11	1.89	2.46	2.52	3.01	2.18	1.34	2.81
<i>Org. N contents</i>								
It. ryegrass	3.50	2.07		1.69	3.04	1.70	1.14	2.62
Ww. ryegrass	3.67	1.52	2.45	1.83	2.66	1.94	1.29	2.48
<i>NO<sub>3</sub>-N contents</i>								
It. ryegrass	0.31	0.27		0.45	0.30	0.17	0.02	0.25
Ww. ryegrass	0.44	0.37	0.01	0.69	0.36	0.24	0.04	0.33

part of the absorbed  $NO_3$  so that at the final harvest, a lower org. N and a higher  $NO_3$  content was found than in Italian ryegrass. The formation of org. N compounds in Westerwolths ryegrass had decreased because of a large production of stems and ears at the growing period. During this period the conversion of  $NO_3$  was less than that of the mainly leaf-producing Italian ryegrass.

From the experiments with turnips (p. 23) and grasses (p. 25), it appeared that the distribution of dry matter between the various plant parts influenced the org. N content of the plant to the extent that a bigger production of leaf blades lead to a bigger quantity of org. N. Changes in the  $NO_3$  conversion during the growing period can be related to a changing distribution of the dry matter. The production of organs poor in org. N, such as tap roots, petioles (p. 23) and stems (p. 25) has restricted the yield of org. N. This resulted in a decreased conversion of  $NO_3$  so that also the decrease of the  $NO_3$  accumulated was small.

Like the distribution of the assimilates between the various plant parts, the conversion of  $NO_3$  into org. N is determined by internal processes of the plant. In order to be better informed about the N metabolism of the plant, more knowledge is necessary about the localisation of the accumulation and conversion of  $NO_3$ , and also about the redistribution of nitrogen from dying plant parts. This research, which was done with plants growing separately under controlled conditions, is described in Section 3.3.3.

### 3.3.3 Closer investigation into the accumulation and conversion of $NO_3$ -N in the plant

In previous experiments, high  $NO_3$  contents were found in transport organs and in dead leaves. The high  $NO_3$  content in dead leaves indicates that much  $NO_3$  is located in

older leaves, and that during the period of senescence the remaining  $\text{NO}_3$  is not withdrawn from these leaves Kelley & Ulrich (1966) found in the leaves of the sugar-beet a regular increase of the  $\text{NO}_3$  content with the age of the leaf. Most of the  $\text{NO}_3$  in the leaf occurred in the petioles.

For a good conversion of  $\text{NO}_3$  in the plant, it is necessary that the physiologically active organs have sufficient  $\text{NO}_3$  available. As for the  $\text{NO}_3$  reduction, the localisation of  $\text{NO}_3$  in the plant is important. For the characterization of the activities of the various organs with regards to the conversion of  $\text{NO}_3$ , nitrate reductase was assayed during the development of the plant. Thus an impression was obtained of the contribution of the various organs to the  $\text{NO}_3$  reduction.

This research was done under controlled conditions with turnip, Italian ryegrass, oats and spinach. During the development of the turnip, the distribution of org. N and  $\text{NO}_3$  in the plant was studied in detail together with the conversion of  $\text{NO}_3$  and the redistribution of nitrogen. The results of this are given on p. 28–35. The distribution of org. N and  $\text{NO}_3$  and the  $\text{NO}_3$  conversion in the various organs of Italian ryegrass, oats and spinach is described on p. 35–40. A comparison between the investigated plant species follows at the end.

*Experiment V: Distribution of org. N and  $\text{NO}_3$ -N in the various parts of the turnip plant* On 2/XI/1970, turnips were sown on sand in 5 litre plastic pots, 20 cm in diameter. After emergence the number of plants per pot was reduced to two.

N fertilization (g N/pot)

Date	27/XI	17/XII
$\text{N}_1$ treatment	0.5	—
$\text{N}_2$ treatment	3.0	—
$\text{N}_3$ treatment	0.5	2.5

Periodical samplings: 23/XII/1970, 4/I/1971 and 15/I/1971. Four pots were available for each harvest. In all treatments, the leaves were separated according to age. The experiment was done in the glass house. In addition to the natural daylight radiation received in the glass house (total radiation  $150 \text{ J.cm}^{-2}.\text{day}^{-1}$ ), extra light, which supplied  $350 \text{ J.cm}^{-2}.\text{day}^{-1}$ , was provided by high pressure mercury lamps. During the experimental period the temperature was kept at  $15/12^\circ \text{C}$  (day/night) and the daylength was 14 hours.

*Results* The quantities of dry matter,  $\text{N}_t$ , org. N and  $\text{NO}_3$ -N of the 3 N treatments are given in Table 10. The dry matter yield of the  $\text{N}_1$  treatment was lower than the dry matter yields of both other treatments, which themselves differed little. In all N treatments, the yield of root was higher than the yield of foliage. The increase of the N dressing stimulated the production of leaves more than tap root production.

The low N dressing reduced the N uptake. Almost all nitrogen in the  $\text{N}_1$  treatment was present in organic form. By increasing the N dressing N uptake, org. N production and  $\text{NO}_3$  accumulation increased clearly. The influence of the splitting of the high N application on the N metabolism was fairly small; the uptake, conversion and accumulation of  $\text{NO}_3$  lagged somewhat behind the single high N application. In both high

Table 10. Quantities of dry matter,  $N_t$ , org. N and  $NO_3$ -N in the turnip plant, grown under artificial conditions, at the final harvest.

	Dry weight (g/pot)	$N_t$ yield (g/pot)	Org. N yield (g/pot)	$NO_3$ -N yield (g/pot)
<i>Whole plant</i>				
$N_1$ treatment	54.4	0.80	0.78	0.02
$N_2$ treatment	74.2	2.03	1.64	0.39
$N_3$ treatment	77.6	1.90	1.56	0.34
<i>Foliage</i>				
$N_1$ treatment	20.0	0.39	0.37	0.02
$N_2$ treatment	30.0	1.15	0.83	0.32
$N_3$ treatment	30.2	1.07	0.80	0.27
<i>Root</i>				
$N_1$ treatment	34.4	0.41	0.40	0.01
$N_2$ treatment	44.2	0.89	0.81	0.08
$N_3$ treatment	47.4	0.83	0.76	0.07

N-treatments, the foliage contained more  $N_t$ , org. N and  $NO_3$  than the tap root. With regards to org. N, the differences were not big;  $NO_3$  was present mainly in the foliage.

The distribution of the nitrogen fractions in the foliage changed with leaf age. In Fig. 13, the contents of org. N and  $NO_3$ -N of the separate leaves of the  $N_2$  treatment are shown; the results of the  $N_3$  treatment hardly deviated from these. The leaves are sub-divided in leaf blades and petioles + midribs. The figure shows that, in agreement with the field experiments, there was a higher org. N content and a lower  $NO_3$  content in leaf blades than in petioles + midribs.

The org. N contents were highest in the young leaves, of which the leaf blades had a considerably higher content than the petioles + midribs. The older the leaves, the lower the content of org. N in the leaf blades. The effect of the age of the leaf on the nitrogen status of the petioles + midribs was small; a high org. N content was, however, found in

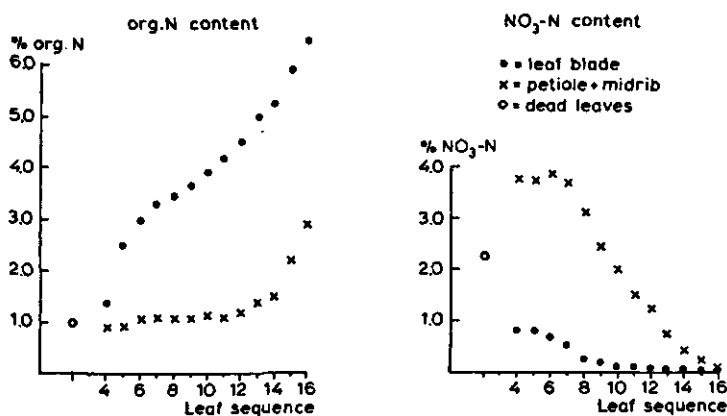


Fig. 13. Contents of org. N and  $NO_3$ -N in leaf blades and petioles + midribs in the turnip plant at the final harvest ( $N_2$  treatment).

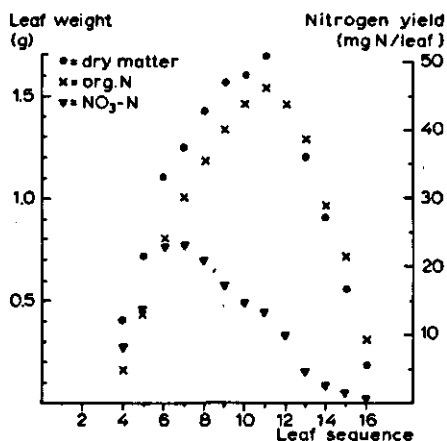


Fig. 14. Quantities of dry matter, org. N and  $\text{NO}_3\text{-N}$  in the various leaves of the turnip plant ( $\text{N}_2$  treatment).

petioles + midribs of immature leaves.

The distribution of the  $\text{NO}_3$  contents between the various leaves was the reverse of that of the org. N contents. The  $\text{NO}_3$  content was highest in the oldest leaves, whereas in the youngest ones hardly any  $\text{NO}_3$  was found. Petioles + midribs had a considerably higher  $\text{NO}_3$  content than the leaf blades. In the petioles + midribs of the four oldest green leaves, more than 3.5%  $\text{NO}_3\text{-N}$  was recorded, whereas the  $\text{NO}_3\text{-N}$  content in the leaf blades of the same leaves amounted to about 0.7%. Almost 80% of the total nitrogen in these oldest petioles + midribs was  $\text{NO}_3$ .

The quantities of org. N and  $\text{NO}_3$  in the various leaves was found by multiplying the leaf weight and the contents of org. N and  $\text{NO}_3$  respectively. In Fig. 14, the quantities of org. N and  $\text{NO}_3$  are plotted on a graph together with the dry matter weights of the leaves, according to age. This graph shows that the oldest and the youngest leaves had a considerably lower dry matter weight than the leaves which were just full-grown. Most of the organic N was in the young mature leaves, and most of the  $\text{NO}_3$  was in the older leaves.

Considerable quantities of  $\text{NO}_3$  appear to occur in the dead leaves. From Table 11 it can be seen, that the  $\text{NO}_3$  content is considerably higher and the org. N content considerably lower in dead leaves than in green leaves. The fairly high  $\text{NO}_3$  content in the dead leaves of the  $\text{N}_1$  treatment is remarkable, because this treatment showed a clear N deficiency at the final harvest.

At a high N supply of the plant, contents of high  $\text{NO}_3$ , but of low org. N, were found in the older as well as in the dead leaves. The occurrence of high  $\text{NO}_3$  levels in the dead

Table 11. Contents of org. N and  $\text{NO}_3\text{-N}$  (g/100 g dry matter) in green and in dead leaves of the turnip plant (final harvest).

	Green leaves		Dead leaves	
	org. N (%)	$\text{NO}_3\text{-N}$ (%)	org. N (%)	$\text{NO}_3\text{-N}$ (%)
$\text{N}_1$ treatment	1.98	0.04	0.94	0.26
$\text{N}_2$ treatment	2.68	0.82	1.16	1.91
$\text{N}_3$ treatment	2.71	1.03	1.01	2.23

leaves might justify the assumption that in the older leaves the synthesis of organic N ceases, translocation of org. N takes place, but no  $\text{NO}_3$  is translocated from these leaves.

The next experiment goes further into the  $\text{NO}_3$  conversion and redistribution of N. In this experiment the  $\text{NO}_3$  reduction and the quantities of  $\text{NO}_3$  and org. N in the various leaves of the turnip plant are measured several times during the development of the plant.

*Experiment VI: Redistribution of N and localization of  $\text{NO}_3$  reduction in the turnip plant* Experiment V showed that distribution of org. N and  $\text{NO}_3$  changed with leaf age in the turnip. Young leaves had high org. N contents and low  $\text{NO}_3$  contents; in older leaves the opposite was found. In this experiment, the redistribution of N and the reduction of  $\text{NO}_3$  is studied more closely.

For this purpose, a pottrial with turnip plants was done in an ordinary glasshouse. The experiment was sown on 4/V/1970 in potting compost, rich in nitrogen. After emergence, the number of plants per pot was reduced to one. Excess nitrogen was applied during the whole growing period to prevent nitrogen deficiency.

#### N fertilization:

Date	11/V	9/VI
g N/pot	1.0	4.5

Harvest dates: 9/VI, 18/VI, 24/VI, 30/VI, 7/VII, 14/VII and 21/VII. At each sampling, 5 plants were harvested of which one was used for enzyme determination in the different leaves of that plant. The plants were always harvested at about 9 h 00. The total quantity of natural radiation received in the glasshouse during the growing period was about  $800 \text{ J.cm}^{-2}.\text{day}^{-1}$ .

**Results** At the sixth harvest (14/VII), one of the five harvested plants was damaged by insects. The results of this harvest are, however, presented without adjustment.

In Fig. 15, the dry matter production per plant is plotted in relation to time. The

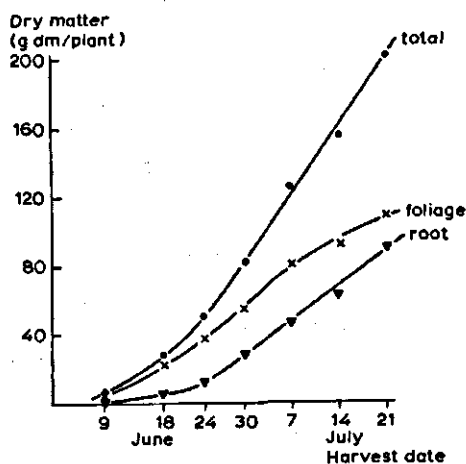


Fig. 15. Production and distribution of the dry matter of the turnip plant during its development.

Table 12. Quantities of  $N_t$ , org. N and  $NO_3$ -N (g/plant) during the development of the turnip plant.

	Harvest date						
	9/VI	18/VI	24/VI	30/VI	7/VII	14/VII	21/VII
<i>N<sub>t</sub> yield</i>							
whole plant	0.42	1.60	2.56	4.05	4.91	5.33	6.09
foliage	0.37	1.36	2.01	3.03	3.35	3.35	3.43
root	0.05	0.24	0.55	1.02	1.56	1.98	2.66
<i>Org. N yield</i>							
whole plant	0.29	0.99	1.59	2.58	3.44	4.11	4.77
foliage	0.26	0.84	1.22	1.85	2.25	2.39	2.49
root	0.03	0.15	0.37	0.73	1.19	1.72	2.28
<i>NO<sub>3</sub>-N accumulation</i>							
whole plant	0.13	0.61	0.97	1.47	1.47	1.22	1.32
foliage	0.11	0.52	0.79	1.18	1.10	0.96	0.94
root	0.02	0.09	0.18	0.29	0.37	0.26	0.38

single plant per pot facilitated a favourable development, so that plant weights were very high at the final harvest. First, the production of dry matter increased exponentially with time, and then more or less linearly. In the beginning, assimilates were mainly used for the production of leaves. Later, a much bigger part of the assimilates was put into the tap root. At the final harvest, 45% of the dry matter was found in the tap root.

The quantities of  $N_t$ , org. N and  $NO_3$ -N in the plant at the various harvest dates are presented in Table 12. Uptake of nitrogen continued during the whole growing period and the quantity of org. N also increased. The generous nitrogen fertilization caused accumulation of large quantities of  $NO_3$  in plant. At the fourth harvest this  $NO_3$  amounted to 36% of the total nitrogen taken up. After that, both the percentage and quantity of  $NO_3$  decreased. At the final harvest, 22% of the absorbed nitrogen was still present in the plant as  $NO_3$ .

Most of the  $N_t$ , org. N and  $NO_3$  was found in the foliage. Until the fourth harvest, the amounts of  $N_t$ , org. N and  $NO_3$  increased rapidly in the foliage. After that, the increase in the quantities of  $N_t$  and org. N became significantly less, and the accumulation of  $NO_3$  in the foliage decreased. A regular increase of  $N_t$  and org. N took place in the tap root until the final harvest; the quantity of  $NO_3$  did not increase after the 4th harvest.

The distribution of org. N and  $NO_3$  amongst the different leaves was the same as in Experiment V, as can be observed in Fig. 16. The org. N content was highest in young leaves; the highest  $NO_3$  content was measured in the older leaves. Just as in Experiment V, the leaf blades had higher org. N, but lower  $NO_3$  contents than the petioles + midribs of the same leaves.

The way in which the quantities of dry matter, org. N and  $NO_3$ -N change during the development of a leaf is shown in Fig. 17. In this figure, the maximum leaf weight of each of the 13 subsequently developing leaves (leaf 5-17) is put at 100. From the trend of the leaf weight of each of these leaves, a general trend can be constructed, which is given in this figure. First, the leaf weight increased and reached a maximum after about 30 days. A gradual decrease started some time after this and the weight of the dead leaves still amounted to 45-60% of the maximum weight.

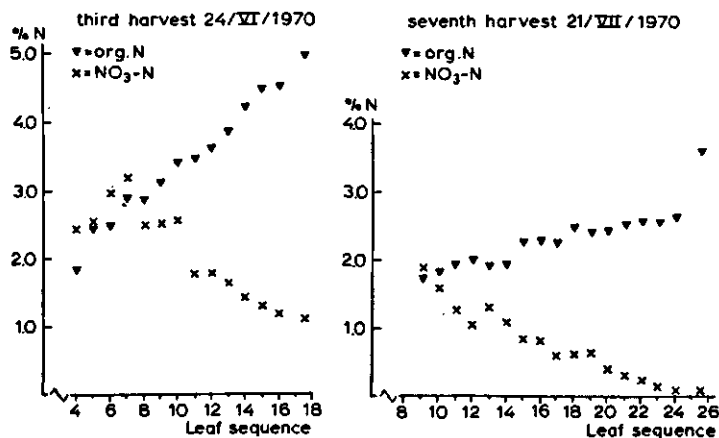


Fig. 16. Contents of org. N and NO<sub>3</sub>-N in the leaves of the turnip plant at the 3rd and 7th periodical harvest.

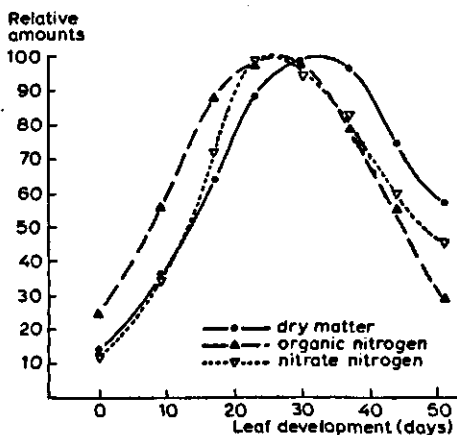


Fig. 17. Changes in the quantities of dry matter, org. N and NO<sub>3</sub>-N during the development of a leaf (relative amounts).

The quantities of org. N and NO<sub>3</sub> are plotted in this figure in a similar way, using the same time scale. The graph showing the org. N in the leaf is situated in front of the dry matter graph, caused by supply of org. N compounds from other organs to the young developing leaf. The quantity of org. N in the leaf reached a maximum shortly before the whole leaf reached its maximum weight. After some time, the quantity of org. N decreased almost constantly. In the dead leaf, still only 20–30% of the maximal amount of org. N was present.

An increase in the quantity of NO<sub>3</sub> in the leaf took place later than the increase of organic N. The quantity of NO<sub>3</sub> reached a maximum quickly and after that, a gradual decrease of the quantity of NO<sub>3</sub> took place. This decrease became smaller during senescence. 40–50% of the maximum quantity of NO<sub>3</sub> observed was still present in the dead leaf. The decrease in quantity of NO<sub>3</sub> is in fact possibly exaggerated because of leaching from the leaves by the weekly watering of the plants.

The conversion of NO<sub>3</sub> in the various organs of the plant was traced by determination



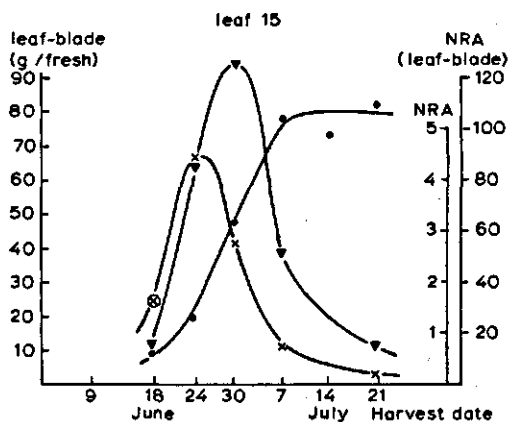


Fig. 18. Changes in the fresh weight (●), nitrate reductase activity per g fresh weight ( $\mu\text{M NO}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ ) (x) and per leaf blade ( $\mu\text{M NO}_2 \cdot \text{h}^{-1}$ ) (▼) of leaf 15 during its development.

of the activity of the enzyme nitrate reductase. Determination of the enzyme activity were carried out at all harvests except the sixth harvest. The determination at the second harvest, however, was not entirely satisfactory.

The contribution of a leaf to the  $\text{NO}_3$  reduction was measured as NRA. This is given in Fig. 18 for leaf 15 during its development. Also given in this figure are the changes in the fresh weight of the leaf blade and the total nitrate reductase activity of the whole leaf blade, calculated as fresh weight multiplied by NRA per gram fresh weight.

It is clear that the NRA in leaf blades of young developing leaves increased quickly to a maximum of  $4.5 \mu\text{M NO}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$  and later decreased to less than  $0.5 \mu\text{M NO}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$  at the final harvest. The maximum NRA was reached on 26/VI, whereas the leaf blade weight did not reach a maximum until 6/VII. Due to this the NRA per leaf blade still increased until the 1/VII, but after that it decreased fairly steeply. At the time that the weight of the leaf blade was maximum, the NRA per leaf blade had decreased already by about 50% and was not more than 10% of its maximum value on 21/VII.

In Fig. 19, the NRA per gram fresh weight of different leaf blades of the turnip plant

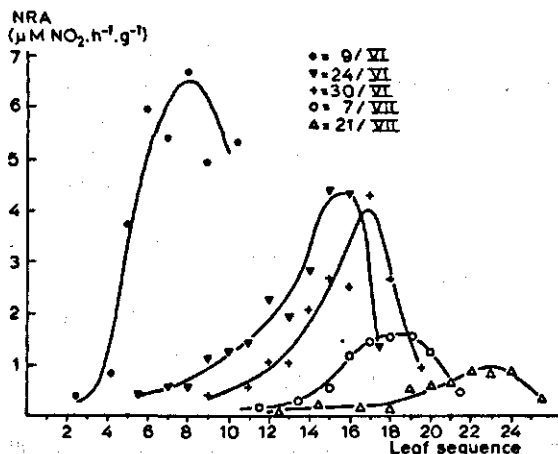


Fig. 19. Nitrate reductase activity per g fresh weight in various leaf blades of the turnip plant during its development.

Table 13. Fresh root weight (g/plant) and nitrate reductase activity per gram root weight and per root during the development of the turnip.

Harvest date	Fresh weight (g/plant)	N R A ( $\mu\text{M NO}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ )	N R A per root ( $\mu\text{M NO}_2$ /root)
9/VI	10	.	.
18/VI	63	0.25	16
24/VI	148	0.30	54
30/VI	338	0.25	85
7/VII	546	0.55	300
14/VII	684	.	.
21/VII	984	0.45	443

is presented at different harvest dates. At all harvests, the highest NRA was measured in the leaf blade of a fairly young leaf. The NRA was considerably lower in leaf blades of older leaves. Very low NRA values were found in the oldest leaves. The level of the NRA was highest in a young plant. In a young growth stage the NRA in leaves was highest and decreased gradually during growth afterwards. At the third harvest, this level was already considerably lower, but dropped to very low values, especially after the fifth harvest. In the same period after the fifth harvest the total quantity of org. N in the foliage increased only a little (see Table 12).

The NRA of the petiole and tap root were considerably lower than that of the leaf blades. During the development of the plant the NRA in petioles of full-grown leaves dropped from  $0.8 \mu\text{M NO}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$  at the 2nd harvest to  $0.2 \mu\text{M NO}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$  at the final harvest. Results from the tap root are presented in Table 13, together with the fresh weights of the tap root and the calculated values of NR. The NRA of the tap root stayed at almost the same level during the whole growing period, in contrast to the NRA level of the leaves. At the end of the growing period a large quantity of  $\text{NO}_3$  could be reduced because of the high weights of the tap root. If the actual reduction of  $\text{NO}_3$  in the plant was in accordance with the measured NRA per gram fresh weight, than calculations show that the nitrate reduction in the tap root was sufficient to explain the increase in org. N in the tap root during the last part of the growing period.

From both indoor experiments with turnips, it appears that the  $\text{NO}_3$  occurs to a large extent in the foliage and particularly in the petioles + midribs of older leaves. The decrease of  $\text{NO}_3$  in older leaves during senescence is limited. Two processes are important for the decrease of  $\text{NO}_3$ , namely the conversion of  $\text{NO}_3$  into org. N and the transport of  $\text{NO}_3$  to other organs. The  $\text{NO}_3$  reduction in dying leaves appears to be very small, so that the decrease of the available  $\text{NO}_3$  by means of reduction into org. N is limited. The transport of  $\text{NO}_3$  from these leaves, however, took place only to limited extent, since the quantity of  $\text{NO}_3$  in a dying leaf decreased by only 50–65%. A big part of this decrease however, took place before senescence, as was shown in Fig. 17. The major part of the decrease of the present  $\text{N}_i$  in the dying leaves, must have taken place by translocation of org. N compounds.

*Experiment VII: Accumulation and reduction of  $\text{NO}_3$  in Italian ryegrass, oats and spinach* In 3 separate experiments, accumulation and reduction of  $\text{NO}_3$  were studied

with Italian ryegrass, oats and spinach, in different parts of the plant. For that purpose, the distribution of org. N and  $\text{NO}_3$  in different organs of these plant species was determined, together with the activity of the enzyme nitrate reductase. In order to compare the results of this experiment with those of Experiments V and VI with the turnip, the plant material of these morphologically very different species was distinguished according to function and age.

The results of the experiments with Italian ryegrass, oats and spinach will be dealt with first, followed by a comparison between these results and the results of the experiments with turnip.

*Italian ryegrass* Seedlings of Italian ryegrass, developed in perlite, were replanted on 16/VI/1970 into a gravel culture. A nutrient solution was circulated through this gravel culture from a 40 l reservoir according the Steiner system (1966). The nutrient solution contained three nitrogen levels (0.667, 2.000 and 6.000 mM/l) and was regularly renewed. At the last renewal on 17/VII, the N levels were changed to  $\text{N}_1 = 1.250$ ,  $\text{N}_2 = 2.750$  and  $\text{N}_3 = 6.000$  mM/l, respectively. This experiment was done in a phytotron. The temperature during the experimental period was  $20/15^\circ \text{C}$  (day/night), the total radiation  $750 \text{ J.cm}^{-2}.\text{day}^{-1}$  and the daylength 14 hours.

The development of the plants was followed by marking the present tillers on 10/VII and 21/VII. At the final harvest on 5/VIII, the tillers were separated according to age into old, medium and young tillers. The laminae of these three tiller groups were analyzed separately, the stems + sheaths were analyzed together. The differences in age between the laminae fractions will thus be smaller than those observed in turnip leaves, because of the continuous formation of new leaves on old and young tillers.

*Results* The effect of the N applications on the dry matter yield and the distribution of dry matter are given in Table 14. The total dry matter yield increased with higher N applications. The differences in root weights between the N treatments were very small; the total weight of tillers increased with increasing N supply. The difference in dry matter yield between the  $\text{N}_2$  and  $\text{N}_3$  treatment is mainly caused by production of young tillers.

Table 15 shows the effect of the N fertilization on the contents of org. N and  $\text{NO}_3\text{-N}$  in the various parts of the plant. In all parts of the plant, increase of N application led to higher contents of org. N and  $\text{NO}_3$ . The distribution of these contents in the plant corresponded to previous experiments; laminae had the highest org. N contents, whereas

Table 14. Effect of N application on the dry matter yield (g/15 plants) and on the distribution of dry matter in Italian ryegrass.

	$\text{N}_1$ treatment	$\text{N}_2$ treatment	$\text{N}_3$ treatment
Total dry matter yield	87.2	120.0	148.1
Laminae of			
young tillers	6.6	15.2	25.3
middle-aged tillers	12.0	23.6	28.8
old tillers	14.7	22.2	22.4
Stems + sheaths	21.9	26.7	37.4
Roots	32.1	32.3	34.2

Table 15. Effect of N application on the contents of org. N and  $\text{NO}_3\text{-N}$  (g/100 g dry matter) in various parts of Italian ryegrass.

	Treatments		
	$\text{N}_1$	$\text{N}_2$	$\text{N}_3$
<i>Organic N contents</i>			
Whole plant	1.48	2.16	2.76
Laminae of			
young tillers	2.31	3.06	3.90
middle-aged tillers	2.32	2.88	3.53
old tillers	2.16	2.69	3.14
Stems + sheaths	1.06	1.72	2.32
Roots	0.96	1.21	1.55
<i><math>\text{NO}_3\text{-N}</math> contents</i>			
Whole plant	0.02	0.10	0.30
Laminae of			
young tillers	0.01	0.06	0.20
middle-aged tillers	0.02	0.08	0.28
old tillers	0.05	0.14	0.47
Stems + sheaths	0.02	0.16	0.43
Roots	0.02	0.04	0.13

the highest  $\text{NO}_3$  contents were found again in transport organs.

Just as in the turnip, the contents of org. N and  $\text{NO}_3$  in the leaves depended on the leaf age. In laminae of young tillers, a high org. N content and a low  $\text{NO}_3$  content were found; in laminae of older tillers, the org. N content was lower and the  $\text{NO}_3$  content higher. This effect of age was shown particularly in the  $\text{N}_2$  and  $\text{N}_3$  treatments.

The reduction of  $\text{NO}_3$  is expressed in Fig. 20 as NRA. An increasing N level led to a higher NRA in all three tiller groups. The highest NRA was measured in laminae of young tillers; laminae of older tillers, which had a higher  $\text{NO}_3$  content had a lower NRA. This corresponds with what was found in the turnip.

Possibly because of a high enzyme activity in the leaf sheath, a fairly high NRA was measured in the fraction stem + sheaths. This NRA increased with increasing N supply from  $0.30 \mu\text{M NO}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$  in the  $\text{N}_1$  treatment to  $0.98 \mu\text{M NO}_2 \cdot \text{h}^{-1} \cdot \text{g}^{-1}$  in the  $\text{N}_3$

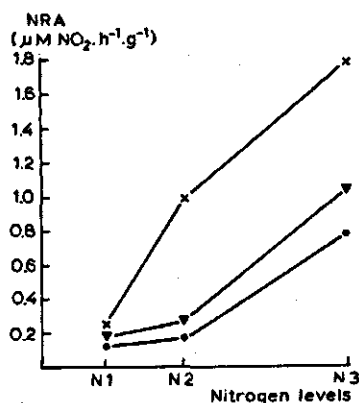


Fig. 20. Relation between NRA of the laminae from tillers of different age and N application in Italian ryegrass at final harvest. Leaf blades of old tillers •; leaf blades of intermediate tillers v and leaf blades of young tillers x.

treatment.

The NRA values of the Italian ryegrass were considerably lower than in the turnip. This result could be caused by the composition of the laminae groups of Italian ryegrass, which consisted of young and old laminae. It is also possible that the method used for determination of NRA was better for the turnip than for Italian ryegrass.

**Oats** The research with oats was done in the glasshouse on a gravel culture with nutrient solution, as described on p. 36. Seven-day-old seedlings of oats were planted on 20/III/1970 and provided with sufficient nitrogen and other nutrients.

The experiment took place at a temperature of 20/15° C (day/night). The natural radiation in the glasshouse was about 600 J.cm<sup>-2</sup>.day<sup>-1</sup> and additional artificial radiation was provided of 300 J.cm<sup>-2</sup>.day<sup>-1</sup> (HPLR). The plants were harvested after 35 days at a fairly young stage of development and divided into different plant parts. Leaves of the main shoot as well as leaves of lateral shoots were separated according to age.

**Results** The dry weights, org. N contents, NO<sub>3</sub> contents and the NRA of the different organs of the plant are shown in Table 16. High contents of org. N and NO<sub>3</sub> were observed in these young oat plants. Big differences in contents of org. N and NO<sub>3</sub> existed again between laminae, stems and roots, as was also found in previous experiments (Fig. 12 and Table 15).

The effect of age on the contents of org. N and NO<sub>3</sub> was less clear than with the turnip. Although the org. N content was higher in young organs, the difference between older and younger organs was small. The NO<sub>3</sub> content was less clearly influenced by age. Young leaves had the lowest NO<sub>3</sub> content, but the highest NO<sub>3</sub> content was found in the middle-aged leaves.

Table 16. Dry weights, contents of org. N and NO<sub>3</sub>-N, and NRA per gram fresh weight in various parts of the oat plant.

	Dry weight (mg/pl)	Org. N (%)	NO <sub>3</sub> -N (%)	N R A (μM NO <sub>2</sub> .h <sup>-1</sup> .g <sup>-1</sup> )
Whole plant	2006	3.52	1.35	
Laminae of the main shoot				
coleoptile	11	4.35	0.65	
lamina nr. 1 (old)	15	4.35	1.33	1.32
lamina nr. 2	40	4.35	1.70	1.96
lamina nr. 3	76	4.42	1.84	4.78
lamina nr. 4	113	4.46	1.44	6.38
lamina nr. 5	135	4.51	0.90	7.19
lamina nr. 6 (young)	36			
total of laminae	426	4.46	1.29	5.56
Laminae of				
lateral shoot nr. 1 (old)	139	4.50	1.41	6.38
lateral shoot nr. 2	135	4.62	1.28	8.09
lateral shoot nr. 3	140	4.66	1.08	7.59
lateral shoot nr. 4 (young)	47	5.58	0.65	5.47
Stems of main shoot	279	3.00	1.93	2.99
Stems of lateral shoots	225	3.43	1.69	2.93
Roots	616	2.27	1.09	

The NRA was strongly dependent on leaf age. This was measured in the separate laminae of the main shoot as well as in the combined laminae of the separate shoots. Earlier formed leaves on the main shoot had a lower NRA. Because the NRA was not measured separately in the youngest leaf (leaf no. 6), it is not possible to say whether this value was somewhat lower than the one in a slightly more developed leaf, as was the case in the turnip (Fig. 18).

A considerable NRA was measured in the sheaths of these fairly young oat plants, just as was the case in Italian ryegrass (p. 37).

*Spinach* For this research, an experiment was done in which 18-day-old spinach seedlings were planted 10/III/1970 on a nutrient solution, in the same manner as on p. 36, and were provided with 4.5 mM N/l and sufficient other nutrients. The experiment was carried out in the glasshouse at a temperature of 20/15° C (day/night) and with additional radiation of 300 J.cm<sup>-2</sup>.day<sup>-1</sup> (HPLR). The total quantity of natural radiation entering the glasshouse amounted to about 500 J.cm<sup>-2</sup>.day<sup>-1</sup>. At the harvest on 7/IV, the leaf blades plus veins were separated according to age.

*Results* The results of this experiment are presented in Table 17. From this table, it appears that the distribution of the org. N content amongst the leaves followed a very regular pattern. In leaf blades of young leaves a high org. N content was found and in older leaves a lower one.

From Table 17 it can be seen that a correspondingly low NO<sub>3</sub> content was present in all leaf blades. Probably the nitrogen supply had been somewhat limited. From data of another experiment, in which spinach was grown with a high N supply, it could however be concluded that the NO<sub>3</sub> content in the older leaf blades was higher than in younger ones. The difference in NO<sub>3</sub> contents between leaf blades and petioles was also considerably higher than the ones mentioned in Table 17. Moreover, the NO<sub>3</sub> content of the root was clearly lower than the NO<sub>3</sub> content of the petioles which is contrary to the situation in Table 17.

The NRA appeared to be dependent to a high degree on the leaf age (Table 17), which is in agreement with the findings in the case of the turnip. Besides the low NO<sub>3</sub> contents,

Table 17. Dry weight, contents of org. N and NO<sub>3</sub>-N, and NRA per gram fresh weight of various organs of spinach.

	Dry weight (g/15 pl)	Org. N (%)	NO <sub>3</sub> -N (%)	N R A (μM NO <sub>2</sub> .h <sup>-1</sup> .g <sup>-1</sup> )
Leaf blade nr. 1 (old)	1.36	2.39	0.03	0.16
Leaf blade nr. 2	1.65	3.05	0.04	1.89
Leaf blade nr. 3	1.35	3.20	0.03	2.66
Leaf blade nr. 4	1.57	3.41	0.03	5.63
Leaf blade nr. 5	1.58	3.88	0.03	6.10
Leaf blade nr. 6	1.55	4.43	0.03	6.84
Leaf blade nr. 7	1.26	5.04	0.03	6.35
Leaf blade nr. 8 (young)	0.96	6.10	0.02	.
Petioles	2.85	2.90	0.13	2.44
Roots	3.37	3.21	0.28	.

a high NRA was measured in the young leaf blades. The NRA was considerably lower in the older leaf blades. The NRA reached also a fairly high value in the petioles.

*Comparison of the results from Italian ryegrass, oats and spinach with the results from turnip* The results of Italian ryegrass, oats and spinach and the results of the turnip can only be compared with each other in a relative way because of the differences in external circumstances between the experiments carried out, such as nitrogen supply, temperature and light intensity, and because of the necessary differences in the way plant parts were divided into morphological and age classes.

Comparing the indoor experiments with turnip, Italian ryegrass, oats and spinach, it appears that the distribution of  $\text{NO}_3$  and org. N in organs with similar functions was largely similar. The org. N content in all plant species was the highest in leaf blades, whereas the transport organs, such as stems and petioles, had the highest  $\text{NO}_3$  content.

The distribution of org. N,  $\text{NO}_3$  and NRA in the leaf blades of Italian ryegrass, oats and spinach was influenced by leaf age in the same way as in the turnip. High org. N contents were found in the young leaf blades whereas leaf blades of the older leaves had lower org. N contents. The opposite was found with regard to the  $\text{NO}_3$  content. Young leaf blades had clearly lower  $\text{NO}_3$  contents than older leaf blades, just as in the turnip. The lowest org. N contents and the highest  $\text{NO}_3$  contents occurred in the oldest leaves; in oats the differences were less clear-cut.

The highest NRA per gram fresh weight was measured in the leaf blades of all plant species. The influence of the leaf age on NRA was high in oats and spinach, corresponding to that found in the turnip. The NRA was highest in the immature young leaves and considerably lower in the older leaves. In Italian ryegrass the shoots were separated according to age so that the groups of laminae thus formed contained young as well as old laminae. The differences in NRA values therefore, were lower but corresponded to those of the other plant species.

The NRA in the other organs of the plant was considerably lower. Nevertheless these organs can possibly contribute to a considerable extent in the total  $\text{NO}_3$  conversion of the plant.

From the results of these indoor experiments it can be deduced that the nitrogen metabolism of Italian ryegrass, oats and spinach was in the main similar to that of the turnip and that the distribution of org. N,  $\text{NO}_3$  and NRA in the whole plant followed the same pattern. The plant species differed only in N level, which may be partly caused by differences in external circumstances of the experiments.

## 4 Discussion

The results of the experiments have so far been presented almost without further consideration. Also hardly any attention was given to a comparison between the results of the experiments. This section will deal more generally with the N uptake, the  $\text{NO}_3$  accumulation and the conversion of  $\text{NO}_3$ .

In the first part, attention will be paid to accumulation of nitrate nitrogen in crops whereby such factors as N uptake, dry matter yield, plant species and season are dealt with. In the second part the physiology of nitrogen metabolism will be considered, including the localization of the accumulation and conversion of nitrate nitrogen together with the translocation of nitrogen from senescent organs of the plant.

### 4.1 Accumulation of nitrate nitrogen

Accumulation of  $\text{NO}_3$  in the plant is caused by differences between uptake and conversion of nitrate nitrogen. According to Ivanko (1971) and Lycklama (1963), uptake of nitrate nitrogen takes place independently of conversion, at least to some extent. Therefore the N uptake in particular plays an important role in  $\text{NO}_3$  accumulation. Accumulation will occur if the uptake of  $\text{NO}_3$  is bigger than that quantity of nitrogen which is necessary for the production of dry matter. Substantial accumulation can only take place if the N supply is more than sufficient for a maximum yield of dry matter (see Fig. 6). An almost total conversion of the absorbed  $\text{NO}_3$  takes place with a small N uptake, but with a high uptake nitrogen can partly accumulate as  $\text{NO}_3$ .

It is often found in literature, that research into  $\text{NO}_3$  accumulation is based on  $\text{NO}_3$  contents. In this manner, a good relationship was found between the  $\text{N}_t$  and the  $\text{NO}_3$  contents (Fig. 5), but after further investigations, it appeared that the level of this relationship was dependent on the dry matter yield (Fig. 23). From the nitrochores of Van Burg (1962) it can be deduced that the  $\text{NO}_3$  content in herbage of an early cut, at a certain  $\text{N}_t$  content, is lower than the  $\text{NO}_3$  content in herbage of an older cut. An interpretation of the results of experiments, based on contents is only possible if the dry matter yield is also taken into consideration.

The discussion in this section is thus based on all factors which determine the contents e.g. dry matter yield, N uptake,  $\text{NO}_3$  accumulation and yield of org. N. It will also be confined to data from treatments in which the N supply was sufficient for a maximum dry matter production. From the relationships between dry matter yield and  $\text{NO}_3$  content, it appeared that presence of at least 0.15%  $\text{NO}_3\text{-N}$  was necessary for a maximum yield of dry matter. This content, which agrees well with values found in grasses (Van Burg, 1965; Hylton et al., 1964), was also considered as lower limit here. Taking this limit as a starting-point the relationship between  $\text{N}_t$  uptake and  $\text{NO}_3$  accumulation during the growing period can be further studied. This relationship is schematically



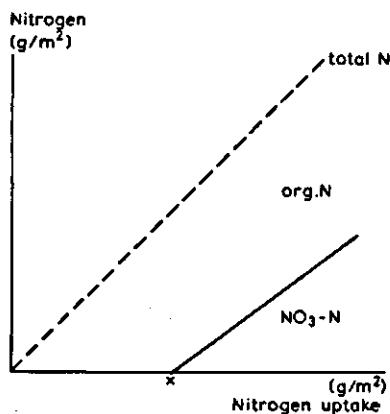


Fig. 21. Schematic presentation of the relationship between uptake and accumulation of nitrate nitrogen in the plant.

presented in Fig. 21, which shows the  $\text{NO}_3$  accumulation increasing in proportion to the total N uptake, from a point indicated X, where the nitrogen supply is sufficient. This relationship can be described by the equation:

$$y_1 = ax - b, \text{ in which } y = \text{NO}_3 \text{ accumulation (g NO}_3\text{-N/m}^2\text{)}$$

$$x = \text{N}_t \text{ uptake (g N/m}^2\text{)}$$

$$a \text{ and } b \text{ are constants}$$

The following equation is presented for the quantity of organic nitrogen ( $y_2$ ):

$$y_2 = (1 - a)x + b$$

The linear relationship between  $\text{N}_t$  uptake and the  $\text{NO}_3$  accumulation in Fig. 21 is drawn as far as the abscissa. This 'critical N-point' (X), can be regarded as the lower limit of the nitrogen quantity which is necessary for a maximum production of dry matter. In reality, this theoretical critical N-point is not reached, because the plant contains some  $\text{NO}_3$  under almost all conditions. At that point however, the quantity of  $\text{NO}_3$  is very small, so that any lower quantity of absorbed nitrogen, is found in organic form. In such situation however, the synthesis of organic N compounds is insufficient for a maximum dry matter production because of the low N uptake.

Above this critical N-point, the dry matter production is not influenced by an increase of the N uptake. Because of this, the synthesis of organic N compounds is limited, so that a large part of the absorbed  $\text{N}_t$  is accumulated in the plant as  $\text{NO}_3$ . The linear relationship indicated between the quantities of org. N and  $\text{NO}_3\text{-N}$  shows that a proportion of the absorbed  $\text{N}_t$  will be accumulated as  $\text{NO}_3$ .

The assumed linear relationship between the  $\text{N}_t$  uptake and  $\text{NO}_3$  accumulation is tested with the data of the turnip, rape and Italian ryegrass of Experiment I. The relationship is given for a number of points of time during the growing period. These points of time are not related to the number of growing days, but indicate a certain dry matter yield. In this way a good comparison can be made with data of other experiments, notwithstanding differences in plant species and season.

Figure 22 shows that the relationship between  $\text{N}_t$  uptake and  $\text{NO}_3$  accumulation is

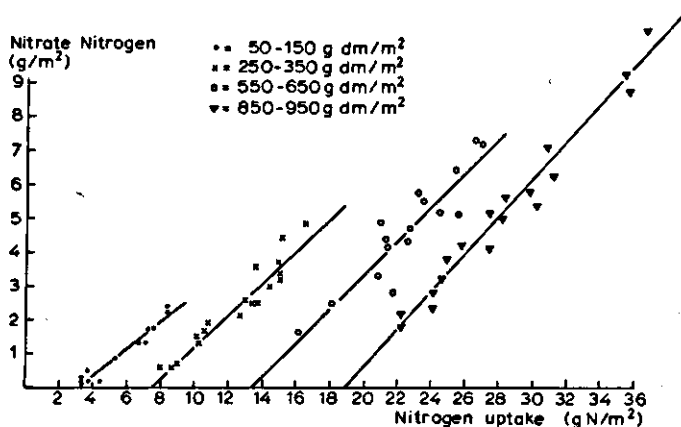


Fig. 22. Relationship between  $N_t$  uptake and  $NO_3$  accumulation at increasing dry matter weights during the growing period (data from Experiment I with turnip, rape and Italian ryegrass).

almost linear. In this figure, relationships are given at 4 levels of dry matter yield, in  $g/m^2$ : 51–150, 251–350, 551–650 and 851–950. A complete review of relationships at increasing dry matter yields is given in Table 18. In connection with this it must be mentioned, that a higher dry matter yield is reached later in the growing period and that a higher dry matter yield as such indicates an older crop.

From the collections of points in Figure 22, together with the high correlation coefficients in Table 18, it can be deduced that the relationship between  $N_t$  uptake and  $NO_3$  accumulation is linear at a certain level of dry matter yield in these plant species. Above this critical N-point, a constant part of the surplus absorbed  $N_t$  is stored in the plant as  $NO_3$ . The other part is converted into organic compounds, so that the quantity of org. N also increases. As the dry matter yield does not alter with N uptake in the

Table 18. Review of the relationships between  $N_t$  uptake and  $NO_3$  accumulation at increasing dry matter weights during the growing period, together with the calculated  $N_t\%$  at the 'critical N-point' with the abscissa.

Dry matter weights ( $g/m^2$ ) class	mean	Equation of regression $y = a x - b$	Coefficient of correlation	% $N_t$ at 'critical N-point'
51–150	114	$y = 0.40 x - 1.24$	0.97	2.72
151–250	182	$y = 0.44 x - 2.04$	0.95	2.55
251–350	306	$y = 0.47 x - 3.55$	0.95	2.47
351–450	403	$y = 0.52 x - 4.79$	0.98	2.29
451–550	489	$y = 0.55 x - 6.50$	0.98	2.42
551–650	612	$y = 0.50 x - 6.68$	0.92	2.18
651–750	700	$y = 0.54 x - 8.23$	0.97	2.18
751–850	805	$y = 0.57 x - 10.25$	0.99	2.23
851–950	907	$y = 0.56 x - 10.53$	0.98	2.07
> 951	1036	$y = 0.62 x - 13.12$	0.86	2.04

circumstances in question, the  $\text{NO}_3$  content will increase steeply with an increase of the  $\text{N}_t$  content and will form an increasing part of it.

In the following sections a closer look will be given into the course of the relationship between uptake of  $\text{N}_t$  and accumulation of  $\text{NO}_3$ . First attention will be paid to changes which occur during the growing period. After that, influence of plant species and season will be discussed.

#### 4.1.1 Aging of the crop

The relationship between the  $\text{N}_t$  uptake and the  $\text{NO}_3$  accumulation depends on the amount of dry matter produced (see Fig. 22). Continuous production of dry matter takes place during the growing period. The associated production of organic N compounds means that accumulation of  $\text{NO}_3$  can only take place at higher  $\text{N}_t$  uptakes. In the figure this is shown in the shifting of the lines to the right. The intersection between the lines and the abscissa shifts at the same time to higher values of  $\text{N}_t$  uptake.

The  $\text{N}_t$  content of the plant, at which maximum production of dry matter can take place without accumulation of  $\text{NO}_3$ , can be calculated as the quotient of the  $\text{N}_t$  uptake at this intersection and the dry matter weight. At a higher  $\text{N}_t$  content, part of the nitrogen in the plant is available as  $\text{NO}_3$ . These calculated  $\text{N}_t$  contents are presented in Table 18 for the different classes of dry matter weight. From this, it appears that as the crop ages, accumulation of  $\text{NO}_3$  starts at a lower  $\text{N}_t$  content. This means that conversion of  $\text{NO}_3$  per g dry matter produced decreases gradually during the growing period. Van Burg (1962, 1970) also found that accumulation of  $\text{NO}_3$  in herbage of permanent grassland started in a young stage of growth at a higher  $\text{N}_t$  content than in a later stage. This is shown very clearly in fig. 23. Changes in morphological composition play an important role in this diminished conversion of nitrate nitrogen, and this subject will be returned to later.

The org. N content decreases gradually under conditions of high  $\text{NO}_3$  contents in the plant during the whole growing period. This is shown in Table 19 for turnip, rape, oats, Italian ryegrass and carrot. In all these plant species the content of org. N decreases clearly, notwithstanding the presence of more than 0.40% of  $\text{NO}_3\text{-N}$  in the dry matter during the whole growing period. Given a sufficient supply of nitrogen during the whole of the growing period, the crop converts more  $\text{NO}_3$  per unit dry weight into organic compounds in a young stage of development than it does in a later stage. This can be seen

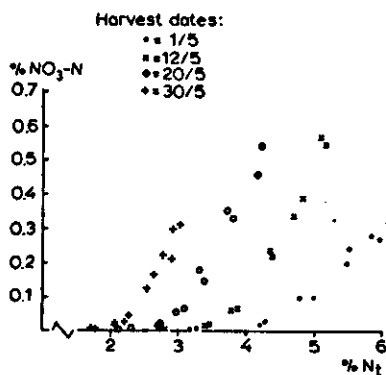


Fig. 23. Relationship between the contents of  $\text{N}_t$  and  $\text{NO}_3$  in herbage during the growing period. Data from Van Burg (1970).

Table 19. Changes in the contents of org. N (g/100 g dry matter) during the growing period with presence of more than 0.40%  $\text{NO}_3^-$ -N in the dry matter (experiment I:  $\text{N}_3$ -treatment).

Periodical harvests	Turnip	Rape	Italian ryegrass	Oats	Carrot
I	4.21	4.39	3.64	4.22	3.55
II	3.66	3.99	3.59	3.24	2.38
III	3.56	3.44	3.27	2.32	2.33
IV	3.25	2.96	3.01	1.97	1.94
V	2.90	2.77	2.78	1.57	1.92
VI	2.72	2.38	2.56	1.55	1.82

also in the data of Van Burg (1970) for herbage, shown in Fig. 23.

It is evident in Fig. 22 and Table 18 that the gradient of the lines relating  $\text{NO}_3^-$  to  $\text{N}_t$  changes during the continued growth of the crops. This means, that as the crop gets older and heavier a smaller part of the surplus absorbed nitrogen is converted into organic form and consequently an increasing part is accumulated as  $\text{NO}_3^-$ . At 100, 300, 600 and 900 g dry matter per  $\text{m}^2$  this amounted to 40, 47, 50 and 56%, respectively.

The gradual decrease of the org. N content in the plant during the growing period occurs in all parts of the plant and is connected with a change in the distribution of the dry matter. Tables 2, 5, 7 and 9 show that org. N content in the leaf blades is considerably higher than in all other organs of the plant. Formation of new leaves which takes place in the beginning of the growth has a positive influence on the  $\text{NO}_3^-$  conversion. During the further development of the crop a change occurs in the distribution of the dry matter. A decreasing part of the assimilates produced is used for formation of new leaves, whereas the quantity which is transported to other organs increases accordingly. The small quantity of organic nitrogen in these organs will probably be connected with the low nitrate reductase activity in these organs. More attention will be paid to this point in Section 4.2. The increase of these plant parts during the further development causes a clear decrease in the org. N content of the plant as a whole.

#### 4.1.2 Plant species

In the relationship between uptake and accumulation of nitrate nitrogen presented in Fig. 22, the results of turnip, rape and Italian ryegrass were reasonably similar. All three plant species grew vegetatively and produced new leaves until the end of the growing period. In comparison with these species, a clearly different relationship between  $\text{N}_t$  uptake and  $\text{NO}_3^-$  accumulation occurred in oats and carrot. This is presented in graphical form in Fig. 24. From this figure, it appears that the differences between all plant species are very small at a fairly low dry matter yield of about 200  $\text{g}/\text{m}^2$ , but at about 600  $\text{g}/\text{m}^2$ , the accumulation of  $\text{NO}_3^-$  in oats and carrot was considerably higher than in the other species at the same  $\text{N}_t$  uptake. The differences between the plant species were still bigger at a dry matter yield of about 900  $\text{g}/\text{m}^2$  at the end of the growing period. This pattern is not entirely unexpected as all plant species had a high production of leaves at an early stage of development. Because the org. N content in leaves is considerably higher than in

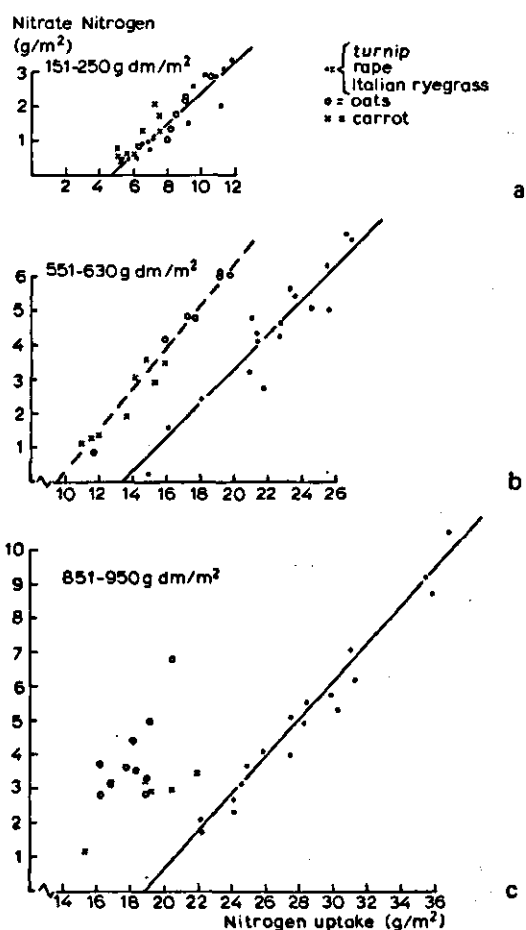


Fig. 24. Relationship between  $N_t$  uptake and  $NO_3$  accumulation at three levels of dry matter yield in turnip, rape, Italian ryegrass, oats and carrot (data from Experiment I).

the other parts of the plant, the production of leaves plays a dominant role in the conversion of  $NO_3$ . Thus the plant species show hardly any differences in the relationship between uptake and conversion of nitrate nitrogen early on in the development, because of the rather limited differences in the production of leaves.

With higher dry matter yields, differences occur in the distribution of the dry matter between oats and carrot on the one hand and turnip, rape and Italian ryegrass on the other. With oats and carrot only a limited part of the assimilates is used for the production of leaves. The assimilates appear largely in organs with considerably lower org. N contents, such as stems and grains in oats and in the root of the carrot. This places a restriction on  $NO_3$  conversion, which explains the different relationship between the  $N_t$  uptake and  $NO_3$  accumulation of these two plant species in Fig. 24 bc.

An effect of differences in morphological composition on the conversion and accumulation of  $NO_3$  also appears in Italian and Westerwolths ryegrass in Experiment IV. The production of leaves of Westerwolths ryegrass ceased at ear emergence, resulting in a considerably reduced conversion of  $NO_3$  in comparison with the vegetatively growing Italian ryegrass (see Table 9).

Fig. 24 c gives the impression that the carrot would take an intermediate position between turnip, rape and Italian ryegrass on the one hand and oats on the other hand. Indeed, at the end of the growing period there existed a difference in  $\text{NO}_3$  conversion between oats and carrot. The increase of the quantity of org. N in the rapidly growing root of the carrot was almost completely achieved by conversion of  $\text{NO}_3$  during the root-growing process. In oats on the contrary, the major part of the org. N compounds in the grains were already present before panicle emergence. This latter aspect will be dealt with in Section 4.2.3.

The different development of the above crops is expressed clearly in the  $\text{N}_t$  content above which accumulation of nitrate nitrogen starts to take place. At a dry matter yield of about  $600 \text{ g/m}^2$ , accumulation of  $\text{NO}_3$  occurred in oats and carrot when the  $\text{N}_t$  content was more than 1.65%, whereas with turnip, rape and Italian ryegrass, this was not the case until a content of 2.18%  $\text{N}_t$  was attained. Furthermore, it can be deduced from the data of Table 9 that the reproductive Westerwolths ryegrass starts to accumulate  $\text{NO}_3$  at a  $\text{N}_t$  content which is about 0.15% lower than that of the vegetatively growing Italian ryegrass.

The different behaviour with regards the uptake and accumulation of nitrate nitrogen can lead to differences in  $\text{NO}_3$  accumulation between plant species. In oats and carrot however, larger quantity of  $\text{NO}_3$  had not accumulated because the N uptake was considerably less than that of turnip, rape and Italian ryegrass at the same nitrogen application and dry matter yield.

#### 4.1.3 Season

It is known from the literature that high contents of  $\text{NO}_3$  in plants can occur especially in late summer and autumn. The quantity of light at that period is small and so the possibilities for synthesis of organic N compounds would be limited. The uptake of  $\text{N}_t$ , however, is generally maintained at a high level because of relatively high soil temperatures and a good supply of water. This means that the  $\text{N}_t$  content of the plant above which  $\text{NO}_3$  accumulation occurs can easily be exceeded because of a limited dry matter production on the one hand and a rather high  $\text{N}_t$  uptake on the other.

Results of Experiment I with turnip, rape and Italian ryegrass were given in Figure 22 and in Table 18. For both autumn experiments II and III, the  $\text{NO}_3$  accumulation is plotted against the  $\text{N}_t$  uptake in Fig. 25. The curves for turnip, rape and Italian ryegrass obtained from the summer experiment are also shown in this figure by a drawn line. With regards to the autumn experiments it should be remarked that the growth rate of the crop in the autumn of 1970 was considerably higher than in 1971 and that this caused big differences in dry matter yields.

In Fig. 25a, the relationship is given for Italian ryegrass at a dry matter yield of  $251\text{--}350 \text{ g/m}^2$  as there were no higher yields available in the autumn experiment II in 1971. At such a yield, there existed no differences in  $\text{NO}_3$  accumulation between summer and autumn, and the morphological composition and org. N levels were similar.

To what extent the relationship between uptake and accumulation of  $\text{NO}_3$  of the grass crop was influenced by the season at higher yields could not be investigated. The data of Van Burg (1970) also show no difference in the relationship between uptake and accumulation of  $\text{NO}_3$  in summer and autumn produced herbage in which the dry matter

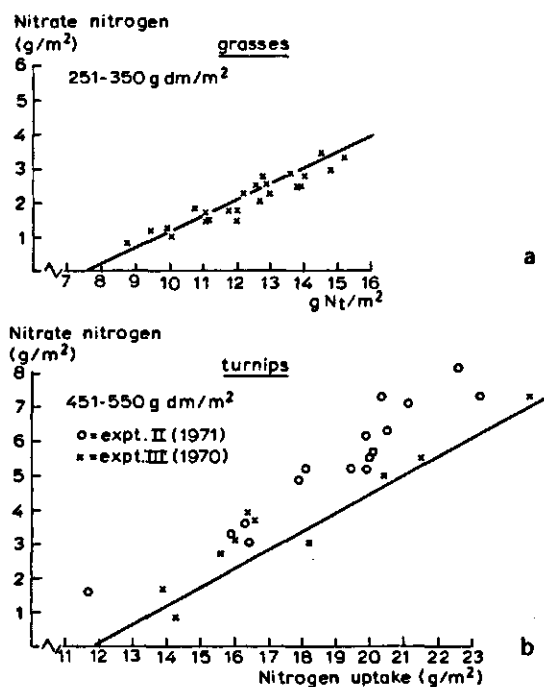


Fig. 25. Relationship between  $\text{N}_t$  uptake and  $\text{NO}_3$  accumulation in Italian ryegrass and turnip of the autumn Experiments II and III, in comparison with that of Experiment I.

yield reached  $400 \text{ g/m}^2$  in autumn; in his experiments carried out in spring, however, it was found that  $\text{NO}_3$  accumulation took place at a higher  $\text{N}_t$  content than in summer and autumn. A conclusive explanation for this could not be given; possibly less rapid death of the leaves in the springtime plays a role. Moreover, in this research neither the stubble nor the roots were investigated.

Unlike the grasses, the turnip did show a seasonal effect. From Figure 25b it appears that more  $\text{NO}_3$  was accumulated in autumn at a dry matter yield of  $451\text{--}550 \text{ g/m}^2$  with the same uptake of  $\text{N}_t$ , particularly in Experiment II. With the higher  $\text{N}_t$  uptake in 1971, the crop accumulated more  $\text{NO}_3$  than in 1970. Differences in the rate of production between both experiments have been previously mentioned. In 1971 a dry matter yield of about  $500 \text{ g/m}^2$  was reached at the end of the growing period in November, whereas the same yield was already found in mid-October in 1970. At the end of the growing period in 1970 almost  $800 \text{ g}$  dry matter had been produced per  $\text{m}^2$ ; at this yield the turnip crop contained about  $2.0 \text{ g NO}_3\text{-N/m}^2$  more than in the summer of 1969.

The higher  $\text{NO}_3$  accumulation in turnips in the autumn must be attributed to a lower  $\text{NO}_3$  conversion per unit dry matter produced. Morphological differences between summer and autumn were not significant. In autumn lower org. N contents were found in the tap root and to a lesser extent in the petioles + midribs. The later in autumn the sampling took place, the larger were the differences in org. N contents.

From the research into the accumulation of  $\text{NO}_3$  in crops, it was found — that clear differences exist in rate and pattern of  $\text{N}_t$  uptake between turnip, rape, oats, Italian ryegrass and carrot; in turnips  $\text{N}_t$  uptake and  $\text{NO}_3$  accumulation were greater than

those of the other plant species

- that accumulation of  $\text{NO}_3$  takes place only if the  $\text{N}_t$  uptake exceeds a certain value at a certain dry matter yield
- that with a sufficient supply of nitrogen the relationship between  $\text{N}_t$  uptake and  $\text{NO}_3$  accumulation is almost linear at the same dry matter yield
- that with higher dry matter yields later in the growing period a decreasing part of the surplus nitrogen absorbed above the critical N-point is converted into organic N compounds
- that differences in  $\text{NO}_3$  accumulation between plant species are connected to a large extent with their morphological composition
- that with high N fertilization, turnips grown in autumn accumulate more  $\text{NO}_3$  than when grown in summer; this was not found in grasses.

## 4.2 Accumulation and conversion of nitrate nitrogen in the plant

### 4.2.1. Localization of nitrate nitrogen and organic nitrogen

Accumulation of  $\text{NO}_3$  in the plant occurs with a high N uptake, which is mostly the case shortly after N application (Fig. 9). The accumulated  $\text{NO}_3$  occurs mainly in stems and petioles and in older and dead leaves of the plant (Tables 2, 7, 9 and 11). Similar results have also been found with other plant species (Houba et al., 1971; Hanway, 1962; Kelley & Ulrich, 1966). In the turnip high  $\text{NO}_3$  contents are present in the tap root, which was also found by Te Velde (1967). This high contents of the root are probably related to the anatomical composition of the plant, in which the so-called tap root is partly a thickened hypocotyl and as such a part of the stem. This is in contrast to the tap root of the carrot, which is a thickened root, and contains considerably lower contents of  $\text{NO}_3$  (Table 2).  $\text{NO}_3$  was not found in the panicle of oats, which is in agreement with data from Dilz (1964).

Further research reveals that the distribution of  $\text{NO}_3$  and also of org. N in leaves depends on leaf age. For example, older turnip leaves appear to contain a higher  $\text{NO}_3$  content (Fig. 13) and therefore most of the accumulated  $\text{NO}_3$  is present in older leaves (Fig. 14). These effects of age were also found in oats (Dilz, 1964), in Italian ryegrass (Hylton et al., 1964), in sugar-beets (Kelley & Ulrich, 1966) and in beans (Martin, 1971). The  $\text{NO}_3$  is situated mainly in the petioles + midribs of these leaves; the  $\text{NO}_3$  content in the associated leaf blades is considerably lower, as can be deduced from fig. 12.

The distribution of the org. N compounds is clearly different from that of  $\text{NO}_3\text{-N}$ . The highest org. N contents are found in the leaf blades, considerably lower org. N contents occur in the other organs of the plant. The org. N content in the leaf also depends on the age of the leaf, as is clearly shown in Fig. 12. The older the leaf, the lower the org. N content of the blades of those leaves; to a lesser degree this is also true for petioles + midribs.

That the age of a leaf is important for the conversion of the absorbed nitrogen can be seen from Fig. 26, which shows the increase in the quantities of nitrogen in each leaf 23 days after a nitrogen application. In all leaves of the turnip the quantity of nitrogen has increased. The distribution of this nitrogen amongst the different leaves corresponds



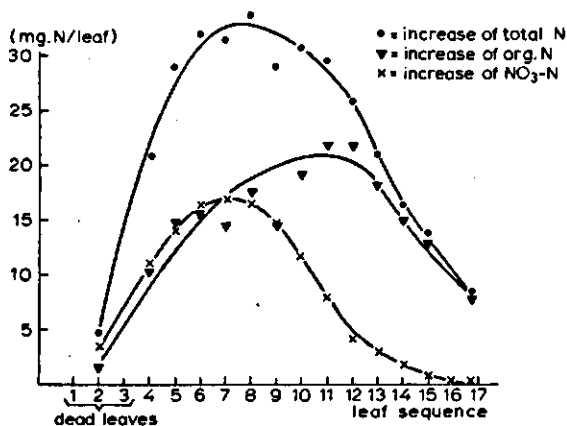


Fig. 26. Increase of the quantities of  $N_t$ ,  $NO_3$ -N and org. N in the different leaves of the turnip at 23 days after a high nitrogen application (Experiment V).

well with the quantity of nitrogen already present in these leaves (Fig. 14). Neither the level nor the time of the N application has any influence on the distribution of the nitrogen amongst the leaves of the plant (Experiment V).

From Fig. 14 it can be deduced, that in the turnip the largest quantity of nitrogen is present in the full-grown leaves. In these leaves, a big increase in the quantity of nitrogen takes place after a N application. Less nitrogen is present in the younger as well as in the older leaves and the increase of nitrogen is smaller than in full-grown leaves. The form in which the nitrogen occurs in the various leaves after the late N application again depends on the age of the leaf. In the older leaves about half of the increase in quantity of  $N_t$  is due to the increase in  $NO_3$ , whereas in the younger leaves, the increase was entirely due to org. N.

#### 4.2.2 Conversion of nitrate and localization of nitrate reduction

The conversion rate of  $NO_3$ -N is indicated by the quantity of org. N compounds produced. The distribution of org. N between the various organs has already been described above as has also the manner in which the production of organs rich in org. N, such as leaf blades, to a large extent determines the  $NO_3$  conversion. In agreement with this the considerable production of org. N compounds is associated with leaf production in the beginning of the growing period. The later decrease of org. N content is caused by a changing distribution of the dry matter (Fig. 8). From Table 12 and Fig. 15 it can be deduced that during the initial growth of turnips 30.1 mg  $NO_3$ -N is converted per g dry matter produced, whereas the conversion during the stage of root formation amounted to only 18.5 mg  $NO_3$ -N.

Differences in the distribution of the dry matter also influenced the conversion of  $NO_3$  in turnips (Table 7) and in grasses (Table 9). With turnips, the greater production of leaf blades in the leafy variety Siloga led to a higher org. N content than in the variety Civasto R, that produces more tap roots as is shown in Table 20. This table also shows that the production of leaves in Westerwolths ryegrass lagged considerably behind the production of shoot and floral tissue. The content of org. N in the dry matter was therefore much less than in the vegetatively growing, leafy Italian ryegrass.

The morphological composition also plays an important role in connection with

Table 20. Distribution of dry matter and contents of org. N (g/100 g dry matter) in (a) turnip varieties Siloga and Civasto R and (b) Italian and Westerwolths ryegrass.

	Distribution of dry matter (%)		Org. N contents	
<i>(a) turnip varieties</i>	Siloga	Civasto R	Siloga	Civasto R
Leaf blade	27.3	20.0	5.16	5.13
Petiole + midrib + dead leaves + tap root	72.7	80.0	2.01	1.98
Whole plant	100	100	2.88	2.61
<i>(b) grasses</i>	It. ryegrass	Ww. ryegrass	It. ryegrass	Ww ryegrass
Lamina	61.8	32.2	3.50	3.67
Ear + stem + dead leaves	38.2	67.8	1.94	1.86
Herbage	100	100	2.90	2.44

differences between plant species in  $\text{NO}_3$  conversion. This matter has already been dealt with in Section 4.1.

Measuring the activity of the enzyme nitrate reductase (NRA) permits a more direct determination of the reduction of  $\text{NO}_3$  in the plant. The quantity of  $\text{NO}_2$  formed by reduction of  $\text{NO}_3$  is measured in vitro, under circumstances which are favourable for the enzyme.

It has been determined, that in turnip, Italian ryegrass, oats and spinach (Section-3.3.3.), the NRA in leaf blades is considerably higher than in other organs of the plant. The  $\text{NO}_3$  reduction occurring in petioles and tap roots in the turnip is considerably less than the reduction in the leaf blades (p. 35); the contribution of the root to the production of org. N is only of significance later in the growing period because of the high root weight (Table 13). In Italian ryegrass (p. 37) and in oats (p. 39) there is less difference between  $\text{NO}_3$  reduction in laminae and stems. It is possible that rapid  $\text{NO}_3$  reduction also takes place in the stem-enveloping leaf sheaths. Differences in NRA between plant parts have also been found in the tomato by Sanderson & Cocking (1964b) and in the cocklebur by Wallace & Pate (1967).

From more detailed analysis it appears that the NRA in the leaves of the turnip (Fig. 18), oats (Table 16) and spinach (Table 17) depends on the age of the leaves. The NRA is highest in the fairly young leaves and the older the analysed leaves, the lower the NRA becomes (Fig. 18). The same effect of leaf age on the NRA was found in the pea (Wallace & Pate, 1965), cocklebur (Wallace & Pate, 1967), soy bean (Harper & Hageman, 1972) and the sugar-beet (Van Egmond & Breteler, 1972). A similar age effect was found between tillers of Italian ryegrass.

The NRA, expressed per unit fresh weight, decreases gradually during the growth of the plant. At the same time, less  $\text{NO}_3$  is converted per unit dry matter produced. This results in a gradual decrease in the org. N content during the growing period. The decrease in the NRA is obviously linked with the aging of the plant. This decrease starts during the stage of leaf production as a result of the gradual aging of the foliage. This causes a decrease in the  $\text{NO}_3$  conversion, and hence a decrease in the org. N content. This is more pronounced after the stage dominated by leaf production, when the production of organs

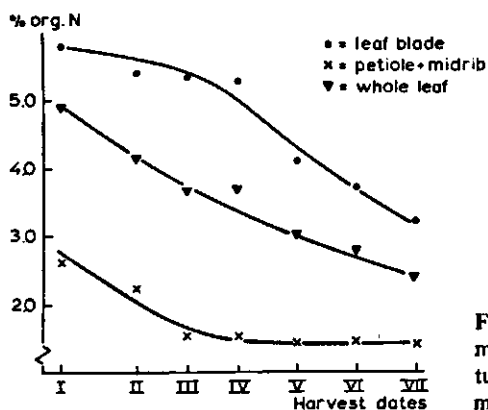


Fig. 27. The org. N content of leaf blade, petiole + midrib and of the whole leaf of a just full-grown turnip leaf during the growing period (Experiment VI).

poor in org. N and rich in carbohydrates, such as stems and storage organs, is greater than leaf production by an ever-increasing amount. To a large extent this change in the distribution of the produced dry matter influences the conversion of  $\text{NO}_3$ .

Besides the above-mentioned changes in the distribution of dry matter during growth, the need for org. N compounds decreases further because of the low org. N content of late-formed leaves, as is shown in Fig. 27. This figure shows that at the same physiological leaf age, a higher org. N content occurs in leaves formed in a younger stage of development of the plant than in leaves formed in a later stage. The org. N content is lower the later the leaf is formed.

#### 4.2.3 Redistribution of organic nitrogen and nitrate nitrogen

A factor at the end of the growing period which contributes to a decreased production of org. N, is the redistribution of organic nitrogen. In Fig. 17, the nitrogen balance is given of a turnip leaf from formation until senescence. The quantity of org. N increased rapidly during the growth of the leaf. Initially the org. N was translocated from elsewhere in the plant, but fairly quickly the leaf was able to provide its own org. N by a rapidly increasing rate of  $\text{NO}_3$  reduction (Fig. 18). On reaching the maximum leaf weight the quantity of org. N in the leaf decreased gradually. During senescence a great part of the org. N was transported away, so that only 20–30% of the maximum quantity of org. N was left in the dead leaf. Similar results were found with pea by Carr & Pate (1967).

A clear case of redistribution of org. N also occurred with oats. The quantity of org. N which was found in the grains at the end of the growing period was to a large extent already present in the plant before the emergence of the panicle (Table 21). The org. N came from the leaves and the haulm. After emergence of the panicle only  $1.88 \text{ g NO}_3\text{-N/m}^2$  was converted whereas the total dry matter yield increased by  $369 \text{ g/m}^2$ .

The data obtained from the experiments described here are less clear about the redistribution of  $\text{NO}_3\text{-N}$ . This redistribution of  $\text{NO}_3$  can be investigated in the turnip, in which the quantities of  $\text{NO}_3$  were measured periodically in the various organs during the growing period (p. 31). A decrease in the quantity of  $\text{NO}_3$  in plant part can take place by conversion to org. N and by translocation.

Table 21. Quantities of org. N ( $\text{g/m}^2$ ) in oats at emergence of the panicle (3rd harvest) and at the final harvest (Expt. D).

	3 <sup>rd</sup> harvest	Final harvest	Difference
Leaves	6.41	3.47	- 2.94
Haulms	5.76	4.21	- 1.55
Roots	0.34	0.33	- 0.01
Panicles		6.38	+ 6.38
production of organic nitrogen after 3 <sup>rd</sup> harvest:			+ 1.88

High  $\text{NO}_3$  contents were found in old and dead leaves. From Fig. 17 it appears that the decrease in the quantity of  $\text{NO}_3$  in a leaf slows down after maturity. This decrease in the leaf will have taken place largely, if not completely, by conversion. So the diminishing decrease of the quantity of  $\text{NO}_3$  can be associated with the gradually decreasing NRA (Fig. 18).

Translocation from mature leaves is not likely. The presence of  $\text{NO}_3$  in phloem sap has never been mentioned. Furthermore there is only a small decrease of the large quantities of  $\text{NO}_3$  in the leaf during senescence. Dijkshoorn (pers. comm.) could not identify  $\text{NO}_3$  in phloem sap from maize plants generously supplied with  $\text{NO}_3$ . Also in sugar-beet redistribution of  $\text{NO}_3$  was not found by Van Egmond (1975). According to Martin (1971), only reduced nitrogen is transportable in phloem. The low mobility of  $\text{NO}_3$  in older leaves of sugar-beets was also shown by Kelley & Ulrich (1966). After an early high  $\text{NO}_3$  application the plant received only a N-free nutrient solution during the whole of its further development. After some time, the young leaves showed a nitrogen deficiency while the oldest green leaves still contained more than 0.3%  $\text{NO}_3\text{-N}$ .

From the closer analysis of the nitrogen metabolism in the plant in Section 4.2, it can be concluded that

- accumulation of  $\text{NO}_3$  in the plant takes places especially in stems and petioles
- $\text{NO}_3$  occurs specially in the older leaves of the plant and org. N specially in the younger ones
- production of leaf blades, which contain a high org. N content, increases the  $\text{NO}_3$  conversion
- $\text{NO}_3$  reduction takes place largely in the younger, just full-grown leaves and that this reduction is very slight in older leaves with high  $\text{NO}_3$  contents
- the quantity of  $\text{NO}_3$  accumulated in a young stage of development will only partly be converted because of its unfavourable localization in the plant.

In 1966 and afterwards in the Netherlands nitrate toxicity occurred in cattle after feeding stubble turnips. This motivated study into the causes of nitrate accumulation in cultivated plants. Field and indoor experiments were carried out with turnip (*Brassica campestris* L. var. *rapa* (L.) Hartm.), rape (*Brassica napus* L. ssp. *oleifera* (Metzg.) Sinsk.), oats (*Avena sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.), Westerwolths ryegrass (*Lolium multiflorum* Lam.), carrot (*Daucus carota* L.) and spinach (*Spinacia oleracea* L.). Besides uptake, accumulation and conversion of  $\text{NO}_3$  the production and distribution of dry matter was also studied. In this way a better knowledge could be gained in the way of  $\text{NO}_3$  accumulation.

During growth  $\text{NO}_3$  accumulates in plants, as uptake exceeds assimilation. The results of the experiments show that the N uptake is dependent on plant species and N supply, whereas the  $\text{NO}_3$  conversion is much influenced by production and distribution of dry matter. In turnip, rape and Italian ryegrass accumulation of  $\text{NO}_3$  is mainly caused by a high uptake, whereas in oats and carrot accumulation was mainly caused by a smaller  $\text{NO}_3$  conversion. With the same dry matter production turnips took up more nitrogen than rape and Italian ryegrass, whereas the  $\text{NO}_3$  conversion was almost equal. The difference in  $\text{NO}_3$  accumulation between these three plant species was mainly attributable to N uptake. In oats and carrots, the N uptake was considerably lower. In carrots, but more so in oats, significantly less  $\text{NO}_3$  was converted per unit dry matter produced. Therefore, much  $\text{NO}_3$  was accumulated in oats; in carrots the accumulation was still considerable, but smaller than in the other plant species investigated.

Accumulation of  $\text{NO}_3$  in the plant occurred when the total N content in the dry matter exceeds a certain value. This value depended on the level of dry matter yield. At the beginning of growth this N content is considerably higher than later, when the dry matter yield is higher. During growth the  $\text{NO}_3$  conversion decreases continuously per unit dry matter produced, which is related to changes in the distribution of dry matter. Firstly a large  $\text{NO}_3$  conversion takes place caused by production of leaf blades, rich in organic N, so that the  $\text{NO}_3$  absorbed is quickly assimilated. Later on when the formation of new leaves is retarded, the conversion of  $\text{NO}_3$  is smaller, resulting in a slower assimilation of the  $\text{NO}_3$  accumulated.

The distribution of  $\text{NO}_3$  and organic N in the various plant parts agreed well between the plant species investigated. The leaf blades differ from other plant parts because of high organic N and low  $\text{NO}_3$  contents. In stems and petioles organic N contents are low and  $\text{NO}_3$  contents high; in roots both contents are low. Much  $\text{NO}_3$  occurs in the tap root of the turnip, whereas less  $\text{NO}_3$  is present in the tap root of the carrot.

Because of differences in organic N contents between plant parts, the distribution of dry matter influences the  $\text{NO}_3$  conversion. Per unit dry matter produced much  $\text{NO}_3$  is converted in crops with a high proportion of total weight present in leaf blades, such as

the turnip variety Siloga and the vegetatively growing Italian ryegrass. Then a relatively high organic N content is found in the dry matter.

In a plant the distribution of organic N and  $\text{NO}_3$  in the various leaves depends on leaf age. The organic N content is high in very young leaves, whereas the  $\text{NO}_3$  content is low. However, in old and dead leaves only small amounts of organic N, but high concentrations of  $\text{NO}_3$  are present.

The nitrate reductase activity was investigated in turnip, Italian ryegrass, oats and spinach. Measurements of this enzyme activity showed that the  $\text{NO}_3$  reduction takes largely place in the leaf blades. Detailed measurements in turnip proved, that per unit fresh weight of the plant the enzyme activity is highest in a young stage of development. The activity decreases gradually with age, which may be associated with an increased production of organs, rich in carbohydrates. Then per unit dry matter produced less protein will be necessary. The nitrate reductase activity per gram fresh weight is highest in a young immature leaf and decreases with age. The activity per whole leaf achieves a maximum value, shortly before leaf weight is at a maximum. In old green leaves the  $\text{NO}_3$  reduction will be limited because of a very low enzyme activity.

Decrease of the present  $\text{NO}_3$  in leaves is small during senescence. Translocation of  $\text{NO}_3$  does not take place from old leaves, whereas the reduction into transportable organic N components is small. Because of this, high  $\text{NO}_3$  contents can be found in dead leaves.

It can be inferred from the results of the experiments that turnip is a  $\text{NO}_3$  accumulator. The accumulation takes place especially with the present-day cultivation methods, where very high N applications are used. But also in crops with a smaller  $\text{NO}_3$  accumulation, such as rape, oats, Italian ryegrass and carrot,  $\text{NO}_3$  concentrations may be still so high that they are a risk to cattle health.

Sinds 1966 trad bij rundvee na het voeren van stoppelknollen nitraatvergiftiging op. Dit was de aanleiding tot het opzetten van een onderzoekprogramma, waarin werd nagegaan op welke wijze en onder welke omstandigheden ophoping van nitraatstikstof in planten plaatsvindt. Hierbij is aandacht besteed aan de invloed van plantensoort, stikstofbemesting en jaargetijde. Middels een gedetailleerde studie van ontwikkeling en productiviteitsverloop van het gewas is tevens getracht een duidelijker beeld van het ontstaan van  $\text{NO}_3$ -ophoping te verkrijgen dan op grond van een uitsluitende bestudering van  $\text{NO}_3$ -gehalten mogelijk is.

In dit onderzoek werd gewerkt met Italiaans raaigras, Westerwolds raaigras, haver, spinazie, voederwortel, bladkool en stoppelknol, waarvan de laatste in praktijk veelvuldig hoge  $\text{NO}_3$ -gehalten heeft getoond. De N-bemesting varieerde in hoeveelheid en wijze van toediening. De toediening geschiedde steeds als  $\text{NO}_3$ , zodat de stikstof opname overwegend in  $\text{NO}_3$ -vorm zal hebben plaatsgevonden.

In gewassituaties is bij genoemde plantensoorten gekeken naar de wijze waarop nitraatstikstof wordt opgenomen en verwerkt, met name onder omstandigheden, waarbij de groei van de gewassen niet beperkt is door het stikstofaanbod. Daarnaast werden in kasexperimenten opname, verwerking en ophoping van  $\text{NO}_3$  in de diverse organen van alleenstaande planten bestudeerd. Alle experimenten werden periodiek bemonsterd, waarbij de vers- en drooggewichten en de gehalten aan totaal stikstof en nitraatstikstof werden bepaald. Op deze wijze was het mogelijk de produktie en verdeling van drogestof en de N-huishouding tijdens de groei te bestuderen.

Tijdens de groei treedt ophoping van  $\text{NO}_3$  in de plant op, wanneer de opname de verwerking van nitraatstikstof overtreft. Uit het onderzoek bleek, dat de N-opname afhankelijk is van plantensoort en N-aanbod, terwijl de  $\text{NO}_3$ -verwerking vooral beïnvloed wordt door produktie en verdeling van droge stof. Bij eenzelfde droge-stofproduktie is de N-opname bij stoppelknol hoger dan bij bladkool en Italiaans raaigras, terwijl de  $\text{NO}_3$ -verwerking vrijwel gelijk is. Verschillen in  $\text{NO}_3$ -accumulatie tussen deze 3 plantensoorten moeten dan ook grotendeels aan de N-opname worden toegeschreven. Bij haver en voederwortel is de N-opname aanzienlijk kleiner. Per eenheid geproduceerde drogestof wordt bij voederwortel, maar met name bij haver, echter duidelijk minder  $\text{NO}_3$  verwerkt. Bij haver vindt zodoende een grote  $\text{NO}_3$ -ophoping plaats; bij voederwortel is de  $\text{NO}_3$ -ophoping nog aanzienlijk, maar is geringer dan bij andere plantensoorten.

Accumulatie van  $\text{NO}_3$  in de plant vindt plaats, wanneer bij een bepaalde droge-stofopbrengst het totale stikstofgehalte een zekere waarde te boven gaat. In het begin van de groeiperiode is dit stikstofgehalte aanzienlijk hoger dan later, wanneer een hogere drogestofopbrengst is bereikt. Tijdens de groei neemt de  $\text{NO}_3$ -verwerking per eenheid geproduceerde droge stof voortdurend af, hetgeen samenhangt met veranderingen in de verdeling van de droge stof. Aanvankelijk vindt door de produktie van organisch N-rijke

bladschijven een grote  $\text{NO}_3$ -verwerking plaats, waardoor een snelle afname van de  $\text{NO}_3$ -ophoping plaats heeft. Door vertraging in de vorming van nieuwe bladeren nadien is de verwerking geringer en daalt het opgehoopte  $\text{NO}_3$  later in de groeiperiode minder snel.

In de verschillende organen van de plant vertoont de verdeling van  $\text{NO}_3$  en van organische N een grote mate van overeenstemming tussen de onderzochte plantensoorten. Hierbij onderscheiden de bladschijven zich van de andere plantdelen door hoge organische N- en lage  $\text{NO}_3$ -gehalten. In stengels en bladstelen en in oudere delen zijn de organische N-gehalten laag en de  $\text{NO}_3$ -gehalten hoog; in wortels zijn beide gehalten laag. In de knol van stoppelknol komt veel  $\text{NO}_3$  voor, terwijl de peen van voederwortel daarentegen weinig  $\text{NO}_3$  bevat. De verdeling van de droge stof over de diverse delen van de plant heeft hierdoor een invloed op de  $\text{NO}_3$ -verwerking. Per eenheid geproduceerde droge stof wordt bij bladschijfrijke gewassen, zoals het stoppelknolras Siloga en het vegetatief blijvend Italiaans raaigras, veel  $\text{NO}_3$  omgezet, hetgeen tot een hoog organisch N-gehalte in de droge stof leidt.

Bij bladeren aan een plant speelt de bladleeftijd een grote rol bij de verdeling van  $\text{NO}_3$  en organische N. Het organische N-gehalte is hoger, naarmate het blad jonger is. Ten aanzien van het  $\text{NO}_3$ -gehalte geldt het omgekeerde. In oudere en reeds afgestorven bladeren komt slechts weinig organische N voor, terwijl in deze bladeren juist het hoogste  $\text{NO}_3$ -gehalte wordt aangetroffen.

Metingen van de nitraatreductase-activiteit hebben bij stoppelknol, Italiaans raaigras, haver en spinazie aangetoond, dat reductie van  $\text{NO}_3$  overwegend in het blad plaats heeft. Uitvoerige metingen bij de stoppelknol hebben aangetoond, dat de enzymactiviteit, uitgedrukt per eenheid vers gewicht, het hoogst is in een jong ontwikkelingsstadium. Nadien vindt een geleidelijke afname plaats van de activiteit van de nitraatreductase, wat kan samenhangen met een toenemende produktie van koolhydraatrijke organen, waardoor steeds minder organische N-verbindingen noodzakelijk zijn.

De nitraatreductase-activiteit, uitgedrukt per eenheid bladgewicht, is het grootst in het jonge, onvolgroeide blad en neemt af met hogere bladleeftijd. De nitraatreductase-activiteit van het gehele blad bereikt een maximum kort voordat het bladgewicht maximaal is. In oude groene bladeren is de reductie van  $\text{NO}_3$  door de geringe enzymactiviteit zeer beperkt.

Vermindering van het aanwezige  $\text{NO}_3$  in bladeren tijdens de fase van afsterving is klein. Translocatie van  $\text{NO}_3$  vanuit deze bladeren vindt niet plaats, terwijl omzetting van  $\text{NO}_3$  in transporteerbare organische N-verbindingen gering is. Dientengevolge kunnen in dode bladeren hoge  $\text{NO}_3$ -gehalten voorkomen.

Uit de resultaten van dit onderzoek kan worden afgeleid, dat de stoppelknol een  $\text{NO}_3$ -accumulator is. Deze accumulatie treedt vooral op bij de huidige teeltwijze, waarbij zeer hoge N-bemestingen worden toegepast. Maar ook in gewassen, die tot een geringere  $\text{NO}_3$ -accumulatie neigen, zoals bladkool, haver, Italiaans raaigras en voederwortel, kunnen na toediening van hoge N-giften toch dermate hoge  $\text{NO}_3$ -gehalten voorkomen, dat gevaar bestaat voor de gezondheid van rundvee.



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Appendix 1A. Contents of org. N (g/100 g dry matter) in various parts of turnip, rape, oats, Italian ryegrass and carrot at final harvest (Experiment 1).

	Herbage	Leaf blades <sup>3</sup>	Petioles	Stems	Panicles	Dead leaves	Stubble	Tap root	Roots	Whole plant
<b>Turnip</b>										
N <sub>1</sub> <sup>1</sup>		2.34	1.23			0.66			0.96	1.19
N <sub>2</sub>		4.20	2.03			1.42			2.08	2.56
N <sub>3</sub>		4.64	2.24			1.37			2.59	2.76
<b>Rape</b>										
N <sub>1</sub>		2.11	1.10	1.15		0.54		1.08		1.31
N <sub>2</sub>		3.57	1.60	1.87		0.91		1.55		2.14
N <sub>3</sub>		4.06	1.83	2.09		0.98		1.71		2.38
<b>Oats</b>										
N <sub>1</sub>		2.37		0.56	1.58	1.08		0.95		1.05
N <sub>2</sub>		3.51		0.90	1.99	1.39		1.70		1.57
N <sub>3</sub>		3.44		0.93	2.10	1.53		1.50		1.57
<b>Italian ryegrass</b>										
N <sub>1</sub>	1.37 <sup>2</sup>					0.69	1.07	0.87		1.25
N <sub>2</sub>	2.51					1.53	2.29	1.41		2.32
N <sub>3</sub>	2.65					1.84	2.56	1.62		2.56
<b>Carrot</b>										
N <sub>1</sub>		2.27	0.65			0.85			0.79	1.13
N <sub>2</sub>		3.04	0.88			1.13			1.30	1.72
N <sub>3</sub>		3.11	0.98			1.92			1.43	1.82

1. N<sub>1</sub> = 100 kg N/ha; N<sub>2</sub> = 300 kg N/ha; N<sub>3</sub> = 100 + 200 kg N/ha.

2. Incl. dead leaves.

3. Incl. midribs.

Appendix 1B. Contents of NO<sub>3</sub>-N (g/100 g dry matter) in various parts of turnip, rape, oats, Italian ryegrass and carrot at final harvest (Experiment I).

	Herb- age	Leaf- blades <sup>3</sup>	Peti- oles	Stems	Pani- cles	Dead leaves	Stub- ble	Tap root	Roots	Whole plant
Turnip										
N <sub>1</sub> <sup>1</sup>		0.06	0.09			0.08		.	0.08	0.07
N <sub>2</sub>		0.11	0.41			0.51		.	0.29	0.33
N <sub>3</sub>		0.34	1.09			1.31		.	0.91	0.90
Rape										
N <sub>1</sub>		0.03	0.07	0.06		0.04		0.03		0.04
N <sub>2</sub>		0.08	0.25	0.36		0.39		0.10		0.25
N <sub>3</sub>		0.19	0.75	0.89		1.09		0.23		0.60
Oats										
N <sub>1</sub>		0.04		0.04	0	0.04		0.03		0.02
N <sub>2</sub>		0.24		0.58	0	0.37		0.33		0.34
N <sub>3</sub>		0.23		0.70	0	0.36		0.27		0.40
Italian ryegrass										
N <sub>1</sub>	0.03 <sup>2</sup>					0.05	0.03	0.02		0.03
N <sub>2</sub>	0.29					0.47	0.22	0.06		0.28
N <sub>3</sub>	0.60					0.80	0.34	0.10		0.51
Carrot										
N <sub>1</sub>		0.05	0.05			0.05		.	0.02	0.03
N <sub>2</sub>		0.39	0.46			0.39		.	0.07	0.26
N <sub>3</sub>		0.55	0.81			0.65		.	0.08	0.59

1. N<sub>1</sub> = 100 kg N/ha; N<sub>2</sub> = 300 kg N/ha; N<sub>3</sub> = 100 + 200 kg N/ha.

2. Incl. dead leaves.

3. Incl. midribs.

Appendix 2. Dry matter yields, dry matter distribution and the contents of total N, organic N and  $\text{NO}_3\text{-N}$  in various parts of the turnip varieties Siloga, Civasto R and Jobe at final harvest (Experiment III).

		Whole plant	Foliage	Tap roots	Leaf blades	Petioles + midribs	Dead leaves	Petioles + midribs + dead leaves + tap roots
<i>Dry matter yield (g/m<sup>2</sup>)</i>								
Siloga	- N <sub>1</sub> <sup>1</sup>	720	605	115	179	339	87	541
	- N <sub>2</sub>	747	674	73	204	371	99	543
Civasto R	- N <sub>1</sub>	813	473	340	127	253	93	686
	- N <sub>2</sub>	815	584	231	163	339	82	652
Jobe	- N <sub>1</sub>	808	495	313	134	274	87	764
	- N <sub>2</sub>	834	602	232	170	338	94	664
<i>Dry matter distribution (%)</i>								
Siloga	- N <sub>1</sub>	100	84.0	16.0	24.9	47.0	12.1	75.1
	- N <sub>2</sub>	100	90.2	9.8	27.3	49.0	13.2	72.7
Civasto R	- N <sub>1</sub>	100	58.2	41.8	15.6	31.2	11.4	84.4
	- N <sub>2</sub>	100	71.7	28.3	20.0	41.6	10.1	80.0
Jobe	- N <sub>1</sub>	100	61.3	38.7	16.6	33.9	10.8	83.4
	- N <sub>2</sub>	100	72.2	27.8	20.4	40.5	11.3	79.6
<i>Contents of total N (g/100 g dry matter)</i>								
Siloga	- N <sub>1</sub>	2.93	3.13	1.89	4.92	2.57	1.60	2.27
	- N <sub>2</sub>	4.25	4.29	3.71	5.47	3.99	3.06	3.77
Civasto R	- N <sub>1</sub>	2.27	2.80	1.54	4.99	2.30	1.14	1.77
	- N <sub>2</sub>	3.79	4.04	3.17	5.42	3.71	2.66	3.38
Jobe	- N <sub>1</sub>	2.45	2.91	1.72	5.10	2.37	1.25	1.93
	- N <sub>2</sub>	3.64	3.91	2.99	5.39	3.50	2.76	3.21
<i>Contents of organic N (g/100 g dry matter)</i>								
Siloga	- N <sub>1</sub>	2.54	2.75	1.42	4.81	2.08	1.13	1.79
	- N <sub>2</sub>	2.88	2.98	1.85	5.16	2.31	1.04	2.01
Civasto R	- N <sub>1</sub>	2.09	2.62	1.35	4.92	2.03	1.03	1.56
	- N <sub>2</sub>	2.61	2.90	1.89	5.13	2.30	0.91	1.98
Jobe	- N <sub>1</sub>	2.22	2.71	1.46	5.02	2.08	1.10	1.67
	- N <sub>2</sub>	2.45	2.71	1.79	5.08	2.00	0.99	1.79
<i>Contents of NO<sub>3</sub>-N (g/100 g dry matter)</i>								
Siloga	- N <sub>1</sub>	0.39	0.38	0.47	0.11	0.49	0.47	0.48
	- N <sub>2</sub>	1.37	1.31	1.86	0.31	1.68	2.02	1.76
Civasto R	- N <sub>1</sub>	0.18	0.18	0.19	0.07	0.27	0.11	0.21
	- N <sub>2</sub>	1.18	1.14	1.28	0.29	1.41	1.75	1.40
Jobe	- N <sub>1</sub>	0.23	0.20	0.26	0.08	0.27	0.11	0.21
	- N <sub>2</sub>	1.20	1.20	1.20	0.31	1.50	1.77	1.42

1. N<sub>1</sub> = 100 kg N/ha; N<sub>2</sub> = 100 + 200 kg N/ha.