Nitrogen nutrition effects on development, growth and nitrogen accumulation of vegetables

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Stellingen

1. Bij groeiremming van een gewas door stikstofgebrek kan de achterstand in produktie niet meer ingehaald worden.
   Dit proefschrift.

2. Verschillen in totale bladoppervlakte per plant als gevolg van stikstofbehandelingen ontstaan voor het grootste deel door verschillen in oppervlakte van individuele bladeren en niet door verschillen in aantallen bladeren. Verschillen in oppervlakte van individuele bladeren ontstaan hoofdzakelijk door verschillen in groeisnelheid en niet door verschillen in groeiduur.
   Dit proefschrift.

3. Het gewas spinazie heeft eerder te lijden van stikstofgebrek dan prei en spruitkool, omdat bij stikstofgebrek het plantstikstofgehalte eerder een waarde bereikt, waarbij ernstige groeiremming optreedt.
   Dit proefschrift.

4. Herfstteelt van spinazie moest verboden worden.

5. Bladkleur bij prei is een irrelevant kwaliteitskenmerk.

6. De Nederlandse term "onderzoekschool" staat op gespannen voet met de Engelse term "graduate school".

7. Het is verstandig om een omscholingscursus op te nemen in het opleidings- en begeleidingsplan van AIO's en OIO's.

8. Een carrière is een loopbaan, niet een racebaan.

9. Een goede kamergenoot is het halve werk.

Henk Biemond

"Nitrogen nutrition effects on development, growth and nitrogen accumulation of vegetables"

Wageningen, 3 november 1995

ABSTRACT

Biemond, H., 1995. Nitrogen nutrition effects on development, growth and nitrogen accumulation of vegetables. Doctoral thesis, Department of Agronomy, Wageningen Agricultural University, Wageningen, the Netherlands, 171 pp., English and Dutch summaries.

In order to be able to match nitrogen supply and nitrogen requirement of vegetable crops, insight is necessary in the responses to nitrogen of important processes of growth and development. This study focused on effects of amount of nitrogen applied and fractionation of nitrogen supply on leaf attributes, accumulation and partitioning of dry matter and nitrogen in potato (*Solanum tuberosum* L.), Brussels sprouts (*Brassica oleracea* L. var *gemmifera* DC), leek (*Allium porrum* L.) and spinach (*Spinacia oleracea* L.). Effects of amount of nitrogen applied were always much more important than effects of fractionation of nitrogen supply.

Rate of leaf appearance varied among crops from 0.15-0.60 leaves d^{-1}; it increased with more nitrogen in Brussels sprouts and spinach. Rates of leaf senescence were enhanced by nitrogen in Brussels sprouts. Life span of leaves was about 70 d for all crops. Rates of leaf expansion and maximum sizes of leaves increased with leaf number until a certain leaf number after which they gradually decreased. Both characteristics increased with more nitrogen. Duration of leaf expansion varied among crops from 18-40 d and decreased in Brussels sprouts with more nitrogen. Maximum size of a leaf was mainly determined by rate of leaf expansion. Except in potato, more nitrogen increased specific leaf area. Differences among nitrogen treatments in total green leaf area reflected the effects of nitrogen on rates of leaf expansion.

Total dry matter production was strongly related to leaf area duration. Although more nitrogen applied resulted in more nitrogen taken up and more total dry matter produced, considerable variation was observed in the relation between total nitrogen uptake and total dry matter production. Harvest indices for dry matter varied among crops and treatments from about 0.10-0.87; more nitrogen increased it for Brussels sprouts, but decreased it for leek. Harvest indices for nitrogen varied from about 0.22-0.86; more nitrogen increased it for Brussels sprouts. In general, organic nitrogen concentration increased with increasing node number for leaf blades, petioles and leaf sheaths but not for sprouts. The gradient with node number resulted from a decreasing nitrogen concentration during the leaf's life. High nitrate concentrations in the marketable produce were only observed in spinach. Nitrate nitrogen concentrations of leaf blades, petioles and leaf sheaths decreased with increasing leaf number at any time of observation, but were not related to leaf age. However, in stems of Brussels sprouts and stems and tubers of potato, total nitrogen and nitrate nitrogen concentration were closely related.

The present findings elucidate the reactions of the crops to nitrogen fertilisation. This is helpful for the fine-tuning of nitrogen fertilisation and to develop modules on plant development in crop simulation models.

Keywords: potato, *Solanum tuberosum* L., Brussels sprouts, *Brassica oleracea* L. var *gemmifera* DC, leek, *Allium porrum* L., spinach, *Spinacia oleracea* L., leaf appearance, leaf expansion, leaf senescence, leaf size, dry matter production, dry matter partitioning, nitrogen uptake, nitrogen partitioning, nitrogen concentration, nitrogen nutrition, plant structure.

Reference to Chapters 2 and 3 should be made by citing the original publications.

WOORD VOORAF

In dit proefschrift wordt verslag gedaan van het onderzoek, dat is uitgevoerd in het kader van het AIO-project "Analyse van de groei, de ontwikkeling en de stikstofopname van vollegrondgroentegewassen in relatie tot het bemestingsregime". Dit project liep van 1 januari 1991 tot en met 31 december 1994 en maakte deel uit van het onderzoeksthema "Experimenteel en modelmatig onderzoek naar de beheersing van nutriëntenstromen bij de vollegrondsgroenteteelt in relatie tot milieu, opbrengstvorming en kwaliteit", dat gedeeltelijk gefinancierd werd door het Ministerie van Landbouw, Natuurbeheer en Visserij in het kader van het nationale onderzoeksprogramma "Geïntegreerde Plantaardige Produktie". Bij dit onderzoeksthema waren naast de vakgroep Agronomie het instituut voor Agrobiologisch en Bodemvruchtbaarheidsonderzoek (AB-DLO) en het Proefstation voor de Akkerbouw en de Groenteteelt in de Vollegrond (PAGV) betrokken. Ik ben het Ministerie van Landbouw, Natuurbeheer en Visserij zeer erkentelijk voor het financieel mogelijk maken van dit AIO-onderzoek. Tevens zijn in dit proefschrift twee manuscripten opgenomen over effecten van stikstofbemesting op groei, ontwikkeling en stikstofopname van aardappel, gebaseerd op eerder uitgevoerd onderzoek en op een nadere analyse van het cijfermateriaal volgens de onderzoeksbenadering van het AIO-project.

Dit proefschrift zou nooit in deze vorm tot stand gekomen zijn, als niet enkele tientallen personen hieraan hadden meegewerkt. Graag wil ik iedereen, die zijn of haar steentje heeft bijgedragen, hiervoor hartelijk bedanken.

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Henk Biemond

NOTES

(1) Section 2.1 has been published in *Annals of Botany* 70: 27-35;

(2) Section 2.2 has been published in *Netherlands Journal of Agricultural Science* 43: 217-232;

(3) Section 2.3 has been published in *Netherlands Journal of Agricultural Science* 43: 233-246;

(4) Section 2.4 has been published in *Netherlands Journal of Agricultural Science* 43: 247-260;

(5) Section 3.1 has been published in *Annals of Botany* 70: 37-45;

(6) Sections 3.2, 3.3 and 3.4 are submitted to *Netherlands Journal of Agricultural Science*;

(7) The papers as presented in this thesis differ from the original papers in the following ways:

   (a) The 'keywords' of the individual papers have been combined into one list at the end of the 'Abstract';

   (b) The acknowledgements are moved to the 'Woord vooraf';

   (c) Minor changes have been made to standardize presentation.

I thank the Editorial Boards of *Annals of Botany* and *Netherlands Journal of Agricultural Science* for their kind permission to include the papers in this thesis.

CONTENTS

CHAPTER 1

General introduction

General introduction

In the Netherlands each year about 65000 ha of arable land is cropped with vegetables, including onions and green harvested peas. More than 35 different crops are grown on this area. Because the number of crops is large and the area per crop small, research on the nitrogen fertilisation and utilisation in each crop is limited, so that accurate, research-based recommendations are lacking. In practice, for many of these crops large amounts of fertiliser nitrogen are used, to ensure a good yield and quality of the marketable produce. This is enhanced by the fact that fertiliser costs are low, compared to the price of the product (Everaarts, 1994).

Nitrogen as a pollutant

Although they usually sharply respond to nitrogen, most vegetable crops are not very efficient in nitrogen uptake (Greenwood *et al.*, 1989). The nitrogen uptake characteristics of individual crops, together with the large amounts of fertiliser nitrogen used, have given rise to large nitrogen losses. Nitrogen losses from the rootable soil layers occur in two ways. In the first place losses to the air can take place by volatilisation of ammonia and by denitrification. Denitrification occurs under wet conditions of the soil. In the second place nitrate can leach to the ground water or through a drainage system to surface water. Nitrate leaching can take place when there is a precipitation surplus. The nitrate can originate from mineralised organic matter, from fertiliser nitrogen or from atmospheric nitrogen deposition (Addiscott *et al.*, 1991). Versteeg (1988) and Schrage (1990) observed residues of mineral nitrogen of 100-300 kg per ha in the soil layer 0-30 cm after the final vegetable crop in a growing season. According to Versteeg about 50% of this nitrogen is lost after 100 mm precipitation and about 80% after 175 mm precipitation. These losses are higher at a sandy soil than at clay soil. In the Netherlands the average precipitation from mid-September until the beginning of March is 360 mm: this results in a complete loss of the nitrogen residues. Ammonia is much less liable to leaching than nitrate, because it is a cation and adsorbed to the negatively charged soil elements, but in the soil ammonia is readily converted into nitrate (Vos, 1994a).

Vos (1994b) mentions three ways by which it is possible to reduce nitrate losses, see also Addiscott *et al.* (1991) for a more general treatise. The first way is to apply such an amount of fertiliser nitrogen on such dates to the crop, as to achieve the closest match possible between availability of nitrogen and demand of the crop. The second way is to use the nitrogen in crop residues effectively. The third way is to take measures which reduce the emission of nitrate during the winter. This includes for example the growth of catch crops, removal of crop residues and abstaining from growing a vegetable crop,

which leaves large nitrogen residues in the soil, as final crop in a growing season before winter.

Nitrogen as a nutrient

The importance of nitrogen as a nutrient is clear: it has large effects on development and growth of a crop (when other nutrients are available in non-limiting amounts, which is usually the case in Western Europe (Greenwood, 1982)). But what is the optimal nitrogen concentration in a plant? In general the overall nitrogen concentration in cultivated plants decreases with increasing plant mass (Justes *et al.*, 1994; Greenwood *et al.*, 1990). It is possible to define a critical nitrogen concentration curve which represents the minimal nitrogen concentration that produces the maximum amount of dry matter at a given time and field situation. Justes *et al.* (1994) calculated such a curve for winter wheat crops. The curve could be described by the equation

$$N_c = 5.35DM^{-0.442}$$

in which

N_c is the critical nitrogen concentration (% of the dry weight) and

DM the amount of dry matter, accumulated in the shoot (t ha^{-1}).

The equation was applicable when DM ranged between 1.55 and 12 t ha^{-1}. Usually when there is nitrogen shortage not only the rate of dry matter production decreases, but also the plant nitrogen concentration drops below the critical concentration. When there is a surplus of nitrogen, the rate of dry matter production remains the same, but the plant nitrogen concentration is above the critical concentration. Both insufficient and excessive nitrogen availability can affect the quality of the marketable produce. Nitrogen shortage (which by definition results in a yield reduction) in Brussels sprouts for instance can result in sprouts which are too small or have outer leaves which are yellow. Excessive nitrogen in spinach for instance can result in a nitrate concentration exceeding the maximum acceptable level.

Research programme and thesis outline

This study focuses on matching the availability of nitrogen to the demand of the crop, to achieve a reduction of nitrate losses in vegetable production in the field. The final goal of this research is to contribute to minimisation of nitrogen losses without reducing yield and quality. This goal can be achieved by quantifying the effects of amount of nitrogen applied and date of application on 1. leaf attributes (e.g. leaf appearance, rate of leaf expansion, specific leaf area) and 2. accumulation and partitioning of dry matter and nitrogen. Much attention has to be paid to leaf attributes, because it is known that effects of nitrogen nutrition are mainly caused by its effects on leaf area duration. For potato this is illustrated by Gunasena & Harris (1968), Dyson & Watson (1971) and Clutterbuck & Simpson (1978).

The treatments in our experiments consisted of different amounts and different splits of nitrogen. Splitting doses in varying ways was included as treatment, because nitrogen uptake takes place during a large part of the growing season, especially in crops which are still actively growing at the harvest. The common cultivation techniques do not take this into account, because fertiliser nitrogen is applied once, twice or maximally three times during the growing season. The application of large amounts of nitrogen has larger risks of nitrogen losses during the growing period than the application of small amounts, because a short period of precipitation surplus can occur any time during the growing period. This leads to the question whether splitting the nitrogen fertilisation would be a feasible measure in reducing nitrogen losses. Splitting the nitrogen fertilisation has also the advantage that there are more and later moments at which decisions can be made about the total amount of fertiliser nitrogen which has to be applied to the crop. Increased apparent nitrogen recovery could be another beneficial effect of split applications. However, splitting can have the disadvantage that it increases the risk of nitrogen shortage. This leads to the following two questions: 1. How does nitrogen shortage affect the crop; 2. Can a reduction in growth as a result of a temporary nitrogen shortage be nullified by applying extra nitrogen in a later stage of growth? These questions were investigated in several experiments by including a treatment, in which the plants experienced nitrogen shortage at first and had sufficient nitrogen for unlimited growth later on.

The research was carried out with a selection of crops including potato (*Solanum tuberosum* L.), Brussels sprouts (*Brassica oleracea* L. var *gemmifera* DC), leek (*Allium porrum* L.) and spinach (*Spinacia oleracea* L.). Experiments were carried out in the field, but also in glasshouses, to be able to carry out intensive observations. Plants in a glasshouse experiment were also looked upon as a 'crop'. Chapter 2 presents in four different crop-specific sections the effects of the nitrogen treatments on the leaf development and leaf growth. Chapter 3 analyses effects on accumulation and partitioning of dry matter and nitrogen for each of the four crops. Finally, Chapter 4 compares the leaf development and leaf growth, and the dry matter and nitrogen accumulation and partitioning of potato, Brussels sprouts, leek and spinach.

The results from this research can be used in simulation models, which take into account the nitrogen fertilisation.

References

Addiscott, T.M., A.P. Whitmore & D.S. Powlson, 1991. Farming, Fertilizers and the Nitrate Problem. CAB International, Wallingford, UK, 170 pp.

Clutterbuck, B.J. & K. Simpson, 1978. The interactions of water and fertilizer nitrogen in effects on growth pattern and yield of potatoes. *Journal of Agricultural Science* (Cambridge) 91: 161-172.

Dyson, P.W. & D.J. Watson, 1971. An analysis of the effects of nutrient supply on the growth of potato crops. *Annals of Applied Biology* 69: 47-63.

Everaarts, A.P., 1994. Doelstellingen van het onderzoeksprogramma. In: A.J. Haverkort, K.B. Zwart, P.C. Struik & P.H.M. Dekker (Eds.). Themadag Stikstofstromen in de Vollegrondsgroenteteelt. Themaboekje nr. 18. Proefstation voor de Akkerbouw en de Groenteteelt in de Vollegrond, Lelystad, pp. 7-8.

Greenwood, D.J., 1982. Nitrogen supply and crop yield: the global scene. *Plant and Soil* 67: 45-59.

Greenwood, D.J., G. Lemaire, G. Gosse, P. Cruz, A. Draycott & J.J. Neeteson, 1990. Decline in percentage N of C_3 and C_4 crops with increasing plant mass. *Annals of Botany* 66: 425-436.

Greenwood, D.J., D.A. Stone, I.G. Burns & H.R. Rowse, 1989. Improving the efficiency of N-fertilizer use for vegetable crops. *Journal of the Science of Food and Agriculture* 48: 125-127.

Gunasena, H.P.M. & P.M. Harris, 1968. The effect of the time of application of nitrogen and potassium on the growth of the second early potato, variety Craig's Royal. *Journal of Agricultural Science* (Cambridge) 71: 283-296.

Justes, E., B. Mary, J.M. Meynard, J.M. Machet & L. Thelier-Huche, 1994. Determination of a critical nitrogen dilution curve for winter wheat crops. *Annals of Botany* 74: 397-407.

Schrage, R., 1990. Methoden zur Bestimmung des Stickstoff-Düngerbedarfs von Gemüsekulturen mit geringem analytischen Aufwand. Ph.D. Thesis, Fachbereich Gartenbau, Universität Hannover, Hannover, 155 pp.

Versteeg, E.M., 1988. Stikstofbemesting in de intensieve vollegrondsgroenteteelt. *Ad Fundum* 6: 22-35.

Vos, J., 1994a. Effects of dicyandiamide on potato performance. *Journal of Agronomy and Crop Science* 173: 93-99.

Vos, J., 1994b. Hoe verder met de stikstofbemesting van vollegrondsgroenten? In: A.J. Haverkort, K.B. Zwart, P.C. Struik & P.H.M. Dekker (Eds.). Themadag Stikstofstromen in de Vollegrondsgroenteteelt. Themaboekje nr. 18. Proefstation voor de Akkerbouw en de Groenteteelt in de Vollegrond, Lelystad, pp. 103-108.

CHAPTER 2

Effects of nitrogen on development and growth of the leaves of some vegetable crops

SECTION 2.1

Effects of nitrogen on the development and growth of the potato plant. 1. Leaf appearance, expansion growth, life spans of leaves and stem branching

J. Vos and H. Biemond

Abstract

Potatoes (*Solanum tuberosum* L.) were planted in pots in a temperature controlled glasshouse to collect data on the rate of leaf appearance, leaf expansion, apical lateral branching and active life spans of leaves. The treatments consisted of three rates of nitrogen supply, viz. the N1 treatment with 2.5 g N per pot and the N2 and N3 treatments with 8 and 16 g N per pot, respectively.

The rate of leaf appearance was 0.53 leaves per day (one leaf per 28 °C d) and was negligibly affected by nitrogen supply. The rate of leaf expansion was related to leaf number and nitrogen supply. The areas of mature leaves increased with leaf number on the main stem to reach a maximum for leaf number 12-14, and declined for higher leaf numbers. Leaves on apical lateral branches declined in mature area with increase in leaf number. The expansion rate of leaves was the dominant factor that determined the mature leaf area, irrespective of leaf number and nitrogen treatment. The smallest leaves were observed at the lowest rate of nitrogen supply. Nitrogen promoted apical branching and so the total number of leaves that appeared on a plant. The proportion of total leaf area contributed by leaves on apical branches increased with time and nitrogen supply.

Active life span, i.e. the period of time between leaf appearance and yellowing of the leaf, showed a similar relation to leaf number as mature leaf area, at least in qualitative terms. Leaves of the N3 treatment showed systematically longer life spans than leaves of the N1 and N2 treatment in the order of 3 weeks. The number of main stem leaves was not affected by nitrogen supply.

Introduction

Nitrogen nutrition affects tuber yield of potatoes mainly by its effect on leaf area duration

(Gunasena & Harris, 1968; Dyson & Watson, 1971; Clutterbuck & Simpson, 1978). However, there is little quantitative information available on how nitrogen affects leaf growth of potato. An appropriate approach for analysis of leaf growth, which is also directly relevant to modelling crop growth (Ng & Loomis, 1984), is to distinguish the rates and durations of leaf appearance, and of expansion growth (cf Gallagher, 1979; Ong & Baker, 1985). In this paper we report on a pot experiment which was designed to quantify such aspects of leaf growth in relation to nitrogen supply. We deal also with death rates of leaves and life spans of leaves, since information on these aspects is required for a more fundamental understanding of the changes with time in total green leaf area. It is important to bear in mind that potato is in a sense an 'indeterminate' plant: branches can emerge from any axil of leaves on the stem that emerges from the sprout on the mother tuber. Such branches can produce a next order of branches. This process of branching is influenced by the availability of N (cf Millard & McKerron, 1986) and was therefore also examined in the present study.

Materials and methods

Plant culture

Seed potatoes (cv. Bintje) were planted on March 28, 1988 in 20 litre pots, containing sand, free from organic matter. The pots were placed in a glasshouse, kept at 18 °C during the day (12 h) and at 12 °C during the night (12 h). Natural light was supplemented with 400 Watt Philips SON-T fluorescent lamps, installed at a density of 0.7 lamps m^{-2}. The day when 50% of the plants had emerged is considered the date of emergence (April 10). The initial density of the pots was 5 pots m^{-2}. As more pots were removed at intermediate harvests, the remaining pots were spaced wider, down to 3.6 pots m^{-2} from 58 DAE (days after emergence) onwards. Water was administered from the top of the pot until the plants were well established. Afterwards a 5 cm high saucer underneath the pots was filled daily with water to its brim. The pots were covered with polyethylene granules to reduce evaporation and thus the accumulation of salts in the top layer of the soil. Once every two weeks the plants were allowed to absorb all the water from the saucers. Then water was administered from the top once. Subsequently the standard way of supplying water via the bottom saucer was resumed (Datema *et al.*, 1986). The number of stems per plant was reduced to three.

Treatments

The treatments consisted of three different amounts of nitrogen. The other nutrients were supplied in equal amounts to all treatments. Nutrient solutions were supplied on seven occasions at two weeks intervals, starting at one week after emergence. In total 2.5 g N per

pot was supplied to the low N treatment (N1), 8.0 g N per pot to the intermediate N treatment (N2), and 16.0 g N per pot to the high N treatment (N3). One pot of each treatment was regarded as a block.

Morphological definitions

It is important to define the terminology of stems and branches that we adopt in this paper (cf. Vos, 1995). Main stems are those that emerged from the soil surface. Several main stems can emerge from one mother tuber. The main stem terminates in an inflorescence (or its rudiments). Upon completion of the main stem, apical lateral branches (ALB) can develop. Usually two of such branches develop from the axils of the second and third leaf below the inflorescence. Calling the leaf subtending the inflorescence the 'n th leaf', ALBs thus emerge from the 'n-1' and the 'n-2' leaf positions. This pattern is not completely fixed. Occasionally an ALB emerged from the fourth leaf below the inflorescence. We call the two ALBs that emerge from the main stem the first order ALBs. ALBs also terminate in an inflorescence. Each of the two first order ALBs can give rise to two second order ALBs, and so on. In this paper we shall identify each ALB by the leaf position it emerges from. For instance the notation '(n-1)(n-2) 2nd order ALB' refers to the second order ALB that emerges from the axil of the 'n-2 leaf position' on the first order ALB, which in turn emerges from the 'n-1 leaf position' on the main stem.

Very often the potato plant also produces branches from the axils of leaves on the lower half of the main stem. We shall call these the basal lateral branches (BLBs).

The determination of leaf parameters

The changes in leaf area of individual leaves in time were derived from frequent measurements of leaf length. From the time that a new expanding leaf could be distinguished at the apex, i.e. when it reached a length of about 1 cm, its length (from insertion on the stem to its tip) was recorded, usually at intervals of 2-3 days, until further extension was negligible. It is important to bear in mind that leaf appearance was set equal to the first recording of leaf length in this study. The data were collected throughout growth on 15 stems per treatment. The measurements were confined to the leaves on the main stem and the first, second and third order ALBs, emerging from the 'n-1' leaf positions.

Destructive sampling was applied (see *Plant analyses*) at regular intervals throughout growth to obtain paired data on leaf length, and leaf area (determined with a Li-Cor model 3100 electronic area meter; Li-Cor, Lincoln, Nebraska). These data were fitted to a quadratic regression (Table 2.1.1). These regressions were used to calculate leaf areas from routinely measured leaf lengths.

Table 2.1.1. Results of regression analysis, relating leaf area (Y; cm^2) to leaf length (X; mm), using the model $Y = a X^2 + b X$. Standard error of a and b between brackets.

Parameter	Nitrogen treatment					
	N1		N2		N3	
a	0.00208	(0.00012)	0.00268	(0.00013)	0.00239	(0.00013)
b	0.0906	(0.0285)	0.1071	(0.0406)	0.1539	(0.0358)
r^2	0.94		0.92		0.91	
n	210		305		331	

Figure 2.1.1 presents a typical example of the time course of leaf expansion. The shape of the curves is sigmoidal, but the greatest part of the leaf area is formed during a short period of time with practically linear expansion. Therefore, we fitted linear regressions to such data and characterized the expansion rate by the slope of the regression.

The dates of senescence of leaves were not recorded directly, but were reconstructed from data on green leaf area per leaf number at intermediate harvests. The absence of a value for green area meant that that particular leaf had died at the time of measurement. In combination with the rates of leaf appearance (Figure 2.1.2) we calculated the life spans of leaves at each position.

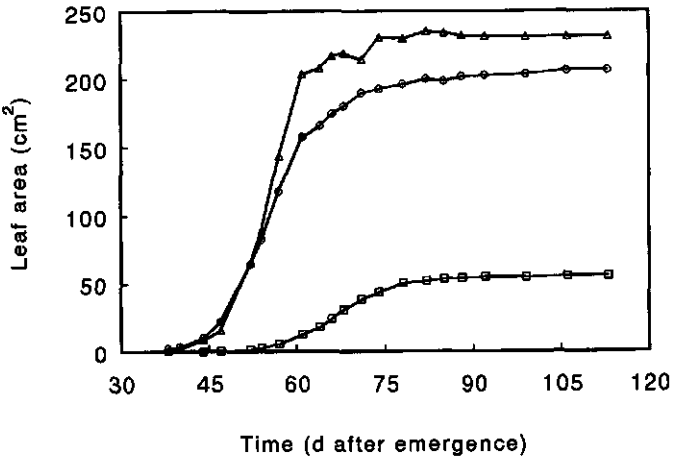

Figure 2.1.1. The change with time in leaf area of leaf 3 on the (n-1) first order apical lateral branch as affected by nitrogen supply. This leaf appeared on 44 DAE. (□) N1; (△) N2; (○) N3. (cf. Figure 2.1.4 for effects of nitrogen on leaf sizes.)

Plant analyses

Destructive analyses of plants were made on six occasions between 30 and 120 DAE. At each sampling date five randomly selected blocks of plants were used. The measurements included leaf area and fresh weight and dry weights of leaves, branches, stolons and tubers. Dried samples were ground and subsequently analysed for nitrate and nitrogen (Biemond & Vos, 1992). The 15 blocks of plants on which non-destructive measurements of leaf growth were made, were harvested after plant senescence (at 127 DAE for the N1 and N2 treatment and at 148 DAE for the N3 treatment).

Results

The rate of leaf appearance and the number of leaves

Figure 2.1.2 shows the number of leaves as a function of time for the main stem and for the selected apical lateral branches. There were no significant differences in the number of leaves at any time between N2 and N3 plants for the main stem and the (n-1) 1st order ALB, hence the rates of leaf appearance were similar. The rate of leaf appearance of the low N treatment was slightly slower. The last leaves of each stem section appeared at a slower rate than the previous leaves; this applied to all N treatments. The last leaves that appeared on the plant in an absolute sense also appeared at a slower rate; that is: using the current definition of

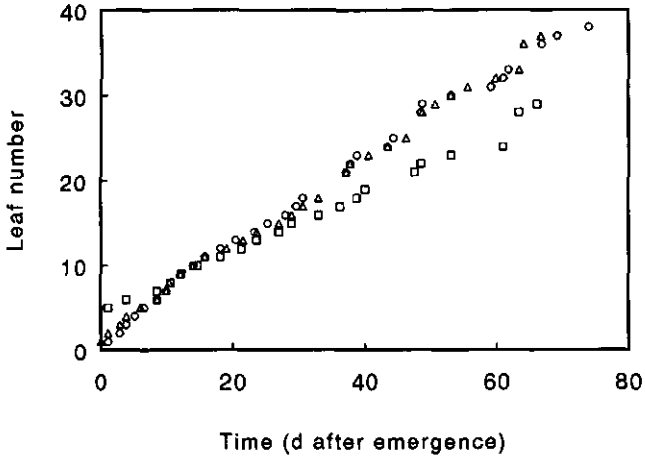

Figure 2.1.2. The number of leaves as a function of time and nitrogen treatment. (□) N1; (△) N2; (○) N3. Leaf number 1-20, main stem; 21-26, first order apical lateral branch (ALB); 27-34, second order ALB; 35 >, third order ALB.

Table 2.1.2. The mean number of leaves on main stems and apical lateral branches of different order (n=15) and the occurrence of the the specified apical lateral branches (ALBs) expressed as percentage of the maximally possible number (=15). Means of number of leaves, followed by different letters, indicate a statistically significant difference (Tukey-test; P=0.05).

Stem part	Nitrogen treatment					
	N1		N2		N3	
	number	%	number	%	number	%
Main stem	17.2 a	100	17.7 a	100	17.5 a	100
(n-1) 1st order ALB*	4.1 a	92	4.5 a	100	5.2 a	100
(n-2) 1st order ALB	4.8 a	77	8.0 b	100	8.5 b	100
(n-1)(n-1) 2nd order ALB	1.4 a	38	5.1 b	93	4.5 b	100
(n-1)(n-2) 2nd order ALB	1.3 a	31	3.8 a	87	6.5 b	100
(n-2)(n-1) 2nd order ALB	0.0 a	0	3.7 b	80	4.6 b	67
(n-2)(n-2) 2nd order ALB	0.0 a	0	2.5 b	60	4.3 b	67
All third order ALBs	0.1 a	0	2.2 ab	40	2.8 b	67
Total mean number of leaves	28.8 a		47.5 b		53.9 b	

* '(n-1)' and '(n-2)' indicate the leaf number below an inflorescence from which axil a branch emerged; the notation is in ascending order: (n-1)(n-2) 2nd order ALB indicates the second order apical lateral branch emerging from the '(n-2)-leaf position' on the first order ALB which in turn emerged from the '(n-1) leaf position' on the main stem.

appearance. The rate of leaf appearance, i.e. the slope of the curve in Figure 2.1.2, was typically 0.53 d^{-1}. Converted to thermal time (Gallagher, 1979; Ong & Baker, 1985) this means one leaf per 28 °C d (degree day; assumed base temperature 0 °C). Expressed in phyllochrons the following values apply: 1.88 d or 28.2 °C d.

The mean number of leaves that was ultimately formed on the main stem and on each of the ALBs is presented in Table 2.1.2. The number of above-ground main stem leaves ranged between 17.2 and 17.7, without statistically significant effects of treatments. Since all the N2 and N3 plants formed second order ALBs, the 'typical' number of leaves on the (n-1) 1st order ALB is 4 to 5, whereas it is 8 to 9 on the (n-2) 1st order ALB. 'Typical' numbers of leaves on the second order ALBs can not be derived from Table 2.1.2, since this number can only be determined if all plants produce the next higher order of ALBs, and this was not the case. The low N treatment produced less leaves on all ALBs, except for the (n-1) 1st order ALB. The total average number of leaves, excluding leaves on basal branches, was 28.8 for the N1 treatment, and 47.5 and 53.9 for the N2 and N3 treatments, respectively. Nitrogen affected branching in the sense that the occurrence of 1st, 2nd and 3rd order ALBs was generally higher for a higher rate of N supply.

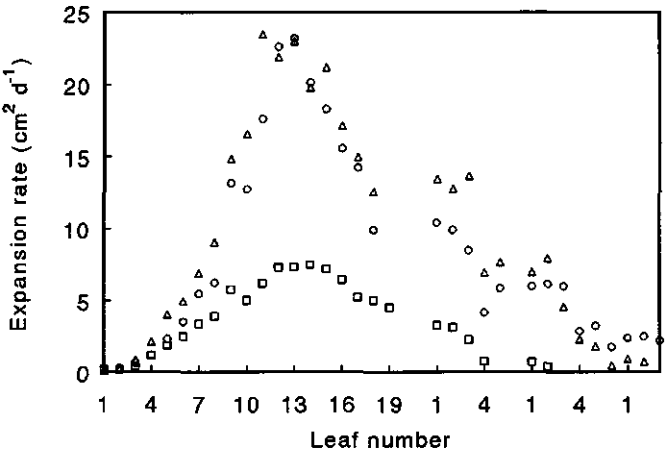

Figure 2.1.3. The rate of expansion of leaves versus leaf number as affected by nitrogen supply. The first sequence of leaf numbers starting with 1 pertains to the main stem; the second series pertains to the (n-1) first order apical branch, the third series pertains to the (n-1)(n-1) second order apical lateral branch and the fourth series pertains to the (n-1)(n-1)(n-1) third order apical lateral branch. (□) N1; (△) N2; (○) N3.

The rate of leaf expansion and sizes of fully expanded leaves

The rate of leaf expansion was influenced by leaf number and by the nitrogen treatment (Figure 2.1.3). The rate of expansion of main stem leaves increased with leaf number till leaf 13, and decreased gradually with further increase in leaf number. The first leaves on the ALBs differed little in expansion rates, but rates declined for higher leaf numbers. There was little effect of the nitrogen treatment on the rate of expansion of the lowest seven leaves on the main stem, but for higher leaf numbers the leaves of the N1 treatment showed substantially lower expansion rates.

The features noted for the rate of leaf expansion also apply to the areas of fully expanded leaves (Figure 2.1.4). Leaf expansion rate appears therefore as an important determinant of leaf size. Mature leaf sizes from all treatments and leaf numbers fell on one curvilinear line when plotted against leaf expansion rates (Figure 2.1.5). This curvilinearity implies that the duration of expansion was somewhat shorter for the larger leaves than for the smaller leaves. Still, Figure 2.1.5 indicates that the leaf expansion rate is an important determinant of mature leaf size.

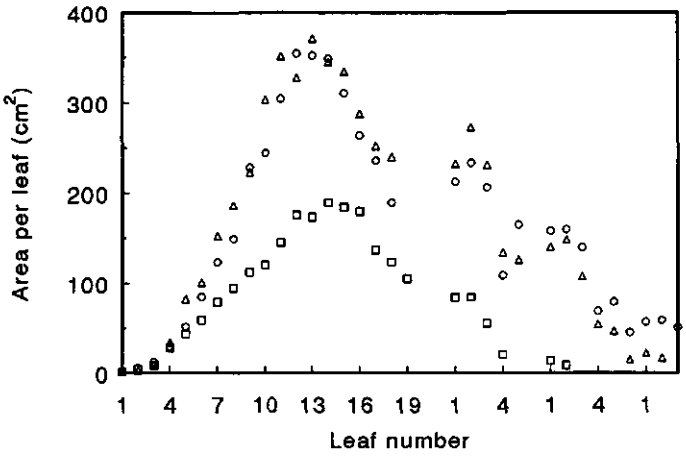

Figure 2.1.4. Mature leaf area of the leaves in relation to leaf number and nitrogen treatment. On the abscissa main stem leaf numbers are followed by leaf numbers on (n-1) apical lateral branches of the first, second and third order. (□) N1; (△) N2; (○) N3.

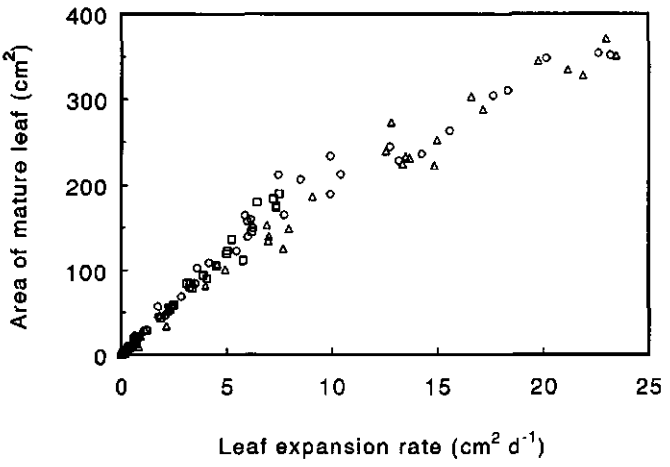

Figure 2.1.5. The association between mature leaf size and the leaf expansion rate, irrespective of leaf position. (□) N1; (△) N2; (○) N3.

A parameter which could be called 'effective duration of expansion' can be defined as the time lapse that is obtained by inserting the full grown leaf area and zero area into the regression equation that relates leaf area to time. These 'effective durations' appeared to

attain values between about 15 and 25 days, without showing systematic effects of stem section, leaf number, or nitrogen treatment (data not presented). This finding underlines the dominant effect of the leaf expansion rate on the areas of full grown leaves.

The rate of leaf senescence and the life spans of leaves

There were no differences between the N1 and N2 treatments in increase with time in the number of senesced leaves; leaf senescence proceeded somewhat slower in the N3 treatment. A linear regression gave death rates of 0.11 to 0.15 leaves per day (as opposed to a rate of leaf appearance of 0.53 d^{-1}). Expressed in thermal units these death rates amounted to 136 and 100 °C d per leaf.

The calculated life spans of the leaves on the apical lateral branches (Figure 2.1.6) varied between 30 and 100 days. The important features are that life spans were systematically influenced by leaf position and nitrogen treatment. The dependency of life span on leaf number was qualitatively similar to that of full grown leaf area on leaf number (Figure 2.1.4): the leaves with the largest full grown area showed the longest life spans, i.e. the middle leaves on the main stem. Except for the last formed leaves there were no differences in life spans between the N1 and the N2 treatments. Except for the leaves up to number 10 on the main stem, life spans of leaves of the N3 treatment were systematically longer (three weeks) than comparable leaves of the N1 and N2 treatments.

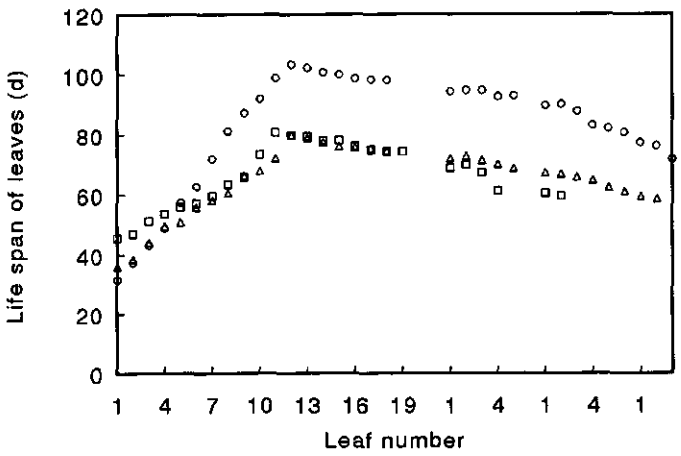

Figure 2.1.6. The calculated life spans of leaves in relation to leaf position and nitrogen treatment. The successive sequences of leaf numbers relate to the main stem, the (n-1) first order apical lateral branch (ALB), the (n-1)(n-1) second order ALB and the (n-1)(n-1)(n-1) third order ALB, respectively. (□) N1; (△) N2; (○) N3.

Total leaf area and the fractional composition of the total leaf area per plant

Figure 2.1.7 shows that the total green leaf area per plant was initially similar for the N2 and N3 treatments. From 100 DAE onwards statistically significant differences (Tukey-test; P=0.05) were found between these two treatments: the N3 plants showed continued increase in leaf area, whereas the N2 plants started to senesce. In the N1 treatments the low availability of nitrogen severely restricted the development of green area.

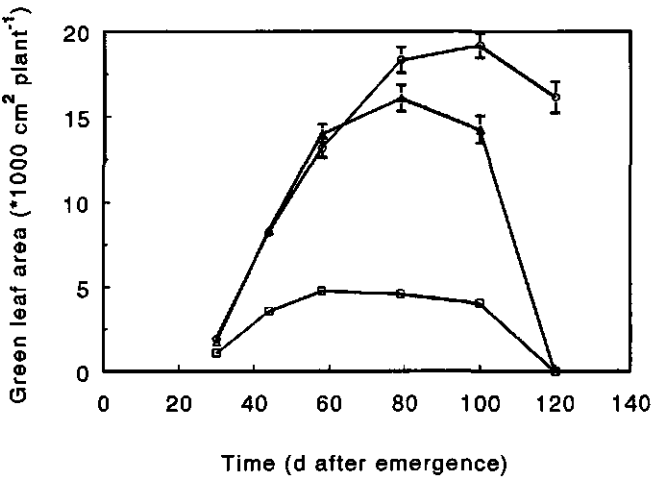

Figure 2.1.7. The changes with time in total green leaf area per plant as affected by nitrogen treatment. Error bars represent twice the standard error of the mean. If no error bar is visible, it fitted inside the marker. (□) N1; (△) N2; (○) N3.

The fractional distribution of total leaf area over main stems and apical and basal branches changed with time and was clearly different between the N treatments (Figures 2.1.8A-C). Main stem leaves were the dominant component of total leaf area early in the growing season. This proportion remained above 50% in the N1 treatment throughout growth, but declined to about 20% near maturity in the N2 and N3 treatments. The second highest contribution to total leaf area was made by the leaves on both first order ALBs. At 100 DAE the leaves of the second order ALBs represented 20 and 30% of the total leaf area in the N2 and N3 treatment, respectively. Leaves of third order ALBs, although present in the N2 and N3 treatments, contributed few percentages only to total leaf area. Basal lateral branches contributed only very little, if anything at all, at any time to total leaf area in the N1 treatment. In the N2 and N3 treatments BLBs always represented less than 15% of the total leaf area.

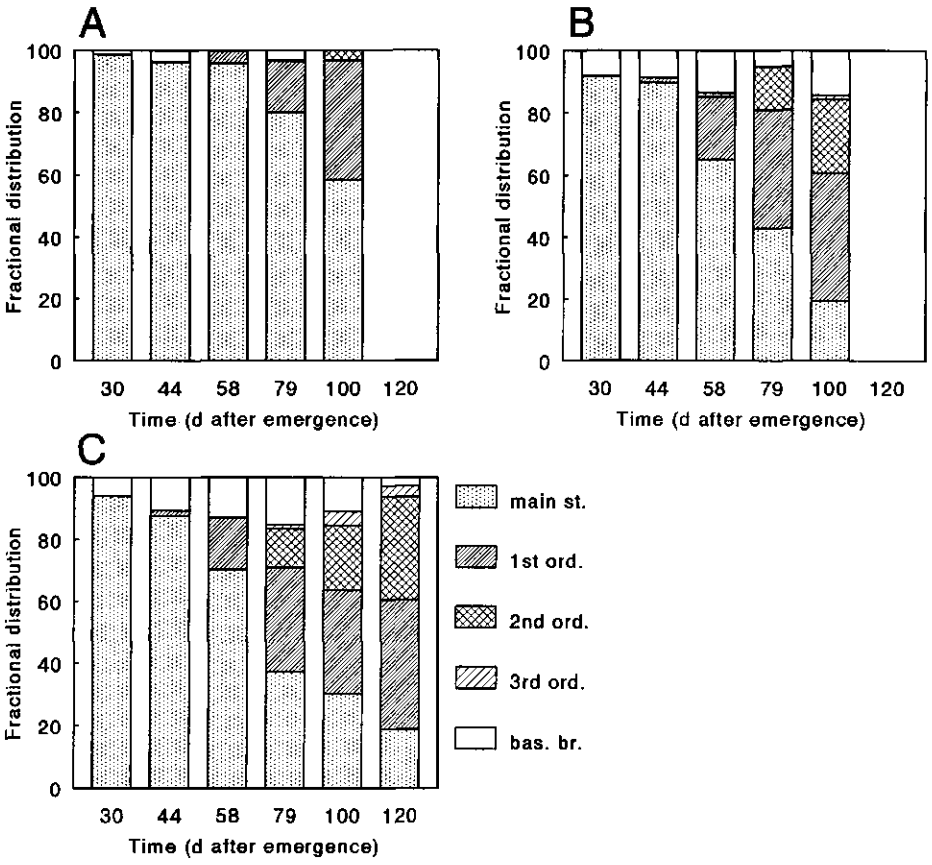

Figure 2.1.8. The fractional distribution of the total leaf area of the plant over morphologically different stem sections as observed at different harvests; (A) (B) and (C) pertain to the N1, N2 and N3 treatments, respectively. main st. refers to main stem; 1st ord. etc. refers to first order apical lateral branches etc.; bas. br. refers to basal lateral branches.

The specific leaf area

The specific leaf area, determined on bulked green leaves (Table 2.1.3) gradually increased from 170-180 $cm^2\ g^{-1}$ at 30 DAE to values between 420 and 500 $cm^2\ g^{-1}$ at 100 DAE. Higher rates of nitrogen supply resulted in lower SLAs, but not always were the differences between treatments statistically significant.

Table 2.1.3. Mean SLA (in $cm^2\ g^{-1}$) of green leaves for all N-levels. Different letters indicate that there was a significant difference (Tukey-test; P=0.05) in SLA between the N-levels.

Days	Nitrogen treatment		
after EM50	N1	N2	N3
30	183 a	169 a	173 a
44	349 c	324 b	296 a
58	422 b	405 ab	366 a
79	413 b	385 ab	368 a
100	491 ab	504 b	423 a
120	-	-	310

The time trend observed for bulked leaves is explained by a similar change with time for individual leaves (Figure 2.1.9). Somewhat higher SLAs for lower nitrogen dressings were also found for individual leaves. The data in Figures 2.1.1 and 2.1.9 pertain to the same leaves, and it is striking that SLA increased continuously during leaf expansion; SLA even tended to increase somewhat after cessation of expansion growth (cf. Vos, 1995).

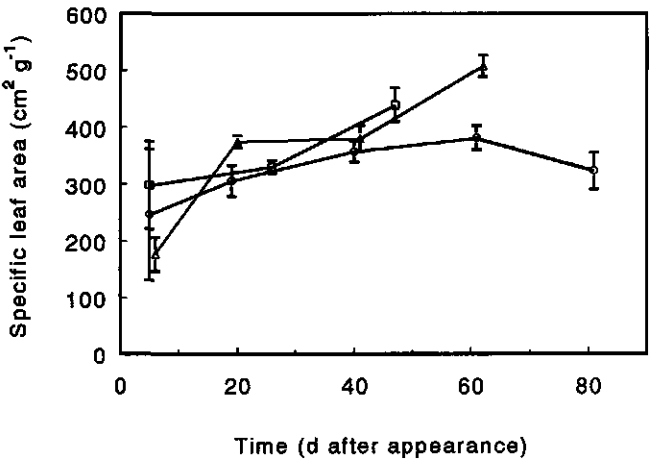

Figure 2.1.9. The change with leaf age in the specific leaf area (SLA) for the third leaf on the (n-1) first order apical lateral branch as affected by nitrogen treatment. Error bars represent twice the standard error of the mean. If no error bar is visible, it fitted inside the marker. (□) N1; (△) N2; (○) N3.

Stem length

The length of the mature N1 main stem and of any N1 ALBs was significantly (Tukey-test; $P=0.05$) shorter than the stem lengths for the N2 and the N3 treatments (Table 2.1.4). The length of stem segments tended to be shorter in the N3 than in the N2 treatment, except for second order ALBs that were inserted on the (n-2) ALB of the main stem. The latter is associated with the fact that those second order branches developed in a higher proportion of the plants in the N3 than in the N2 treatment (Table 2.1.2). The greater length of the (n-2) 1st order ALB in comparison to its (n-1) companion agrees with its larger number of leaves (Table 2.1.2).

Table 2.1.4. Mean length (cm) of stem sections (n=15), measured at 120 DAE. Means followed by different letters indicate statistically significant differences (Tukey-test; $P=0.05$).

Stem section	Nitrogen treatment		
	N1	N2	N3
main stem	83 a	104 b	96 b
(n-1) 1st order ALB*	20 a	44 b	44 b
(n-2) 1st order ALB	19 a	70 b	63 b
(n-1)(n-1) 2nd order ALB	2 a	29 b	23 b
(n-1)(n-2) 2nd order ALB	0 a	14 a	36 b
(n-2)(n-1) 2nd order ALB	0 a	9 a	12 a
(n-2)(n-2) 2nd order ALB	0 a	4 a	10 a

* '(n-1)' and '(n-2)' indicate the leaf number below an inflorescence from which axil a branch emerged; the notation is in ascending order: (n-1)(n-2) 2nd order ALB indicates the second order apical lateral branch emerging from the '(n-2)-leaf position' on the first order ALB which in turn emerged from the '(n-1) leaf position' on the main stem.

The number of basal lateral branches

Basal lateral branches emerged in most cases from the axils of node number 1 to 8 (below ground nodes not included). An average of two BLBs was present at early stages of growth in the N2 and N3 treatments (Figure 2.1.10), compared to only 0.8 in the N1 treatment. In all treatments the number of BLBs decreased with time. In the N3 treatment little over one basal branch survived throughout the growth period, whereas this number dropped to values around 0.1 in the N1 and N2 treatments.

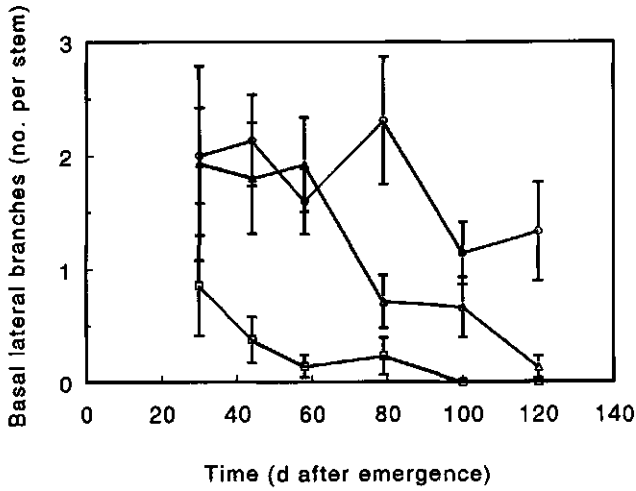

Figure 2.1.10. The number of basal lateral branches as function of time and nitrogen treatment. Error bars represent twice the standard error of the mean. If no error bar is visible, it fitted inside the marker. (□) N1; (△) N2; (○) N3.

Discussion

The current results and those described by Millard & McKerron (1986) allow the conclusion that the positive effects of higher rates of nitrogen supply on total leaf area and on leaf area duration are mainly explained by larger leaves and stimulation of apical branching. The N3 treatment attained a higher maximum total leaf area per plant than the N2 treatment mainly because the leaves on the second order apical lateral branches grew to larger sizes. The fact that mature leaf areas of main stem leaves in the N2 and N3 treatment were similar indicates that there is a maximum in the response to nitrogen of leaf size, beyond which other factors limit further expansion.

The rate of leaf expansion was the most important determinant of leaf size, irrespective nitrogen supply or leaf number. In his analysis of the response of maize and sorghum to nitrogen Muchow (1988) also found that the main effect was on leaf expansion.

Although the curvature in Figure 2.1.5 indicates that the duration of expansion was not constant across nitrogen treatments and leaf positions, Figure 2.1.5 still illustrates that variations in mature leaf areas are primarily brought about by variation in the rate of expansion. Humphries & French (1963) found no effect of nitrogen supply on cell size, and therefore the effect of nitrogen on the rate of leaf expansion is probably based on a

stimulation of the rate of cell division.

Although we did not accurately look at the first appearance of leaves, the current results indicate that the rate at which leaves and apical branches appear is hardly affected by nitrogen supply. This is in agreement with observations in maize and sorghum (Muchow, 1988).

We found no effect of nitrogen on the number of main stem leaves. Muchow (1988) concluded that nitrogen effects on leaf numbers were numerically small in maize and sorghum. The number of leaves or nodes on the main stem of potato and on each of the apical branches is a conservative property. In this study as well as in other experiments (Vos, unpublished) we found 17 or 18 main stem leaves in cv. Bintje. The number of leaves on the 'n-1' and 'n-2' first order ALBs is apparently 5 to 6 and 8 to 9, respectively. Unfortunately we cannot tie values to the number of leaves on higher orders of apical branches since these were not present in all cases. The smaller number of leaves on ALBs emerging from 'n-1' positions than on branches emerging from 'n-2' positions results in an asymmetric pattern of development: the second order ALBs on the 'n-2' first order branch appear later in time than those on the 'n-1' first order branch.

Although the distinction between green and yellow is subjective, the current results show that the active life span of leaves, i.e. the number of days that a leaf is considered green, was not smaller for the N1 plants than for the N2 plants, whereas leaves of the N3 plants lived longer. Unlike the other features discussed, this result may not apply to field conditions, because the lower leaves in well fertilized plants may senesce earlier at the stem densities used in practice (Millard & MacKerron, 1986; Firman & Allen, 1988). In sugar beet Hodánová (1981) observed a pattern of change in life spans of leaves with leaf number similar to that currently observed in potato (Figure 2.1.6). So this pattern could be a more common feature among crop plants.

The specific leaf areas in this experiment were fairly high. Data from field experiments show values typically in the range between 250 and 300 $cm^2\ g^{-1}$ (Spitters, 1990, personal communication). In glasshouses the radiation levels are substantially lower than in the field (in spite of additional artificial light) and low light levels are known to result in high specific leaf areas (Sale, 1973).

References

Biemond, H. & J. Vos, 1992. Effects of nitrogen on the development and growth of the potato plant. 2. The partitioning of dry matter, nitrogen and nitrate. *Annals of Botany* 70: 37-45.

Clutterbuck, B.J. & K. Simpson, 1978. The interactions of water and fertilizer nitrogen in effects on growth

pattern and yield of potatoes. *Journal of Agricultural Science* (Cambridge) 91: 161-172.

Datema, P., H. Niers & A. de Jager, 1986. Comparison of three methods of watering in pot experiments. Rapport IB 4-86. Institute for Soil Fertility, Haren, 121 pp.

Dyson, P.W. & D.J. Watson, 1971. An analysis of the effects of nutrient supply on the growth of potato crops. *Annals of Applied Biology* 69: 47-63.

Firman, D.M. & E.J. Allen, 1988. Field measurements of photosynthetic rate of potatoes grown with different amounts of nitrogen fertilzer. *Journal of Agricultural Science* (Cambridge) 111: 85-90.

Gallagher, J.N., 1979. Field studies of cereal leaf growth. 1. Initiation and expansion in relation to temperature and ontogeny. *Journal of Experimental Botany* 30: 625-636.

Gunasena H.P.M & P.M. Harris, 1968. The effect of the time of application of nitrogen and potassium on the growth of the second early potato, variety Craig's Royal. *Journal of Agricultural Science* (Cambridge) 71: 283-296.

Hodánová, D., 1981. Photosynthetic capacity, irradiance and sequential senescence of sugar beet leaves. *Biologia Plantarum* (Praha) 23: 58-67.

Humphries, E.C. & S.A.W. French, 1963. The effects of nitrogen, phosphorus, potassium and gibberellic acid on leaf area and cell division in Majestic potato. *Annals of Applied Biology* 52: 149-172.

Millard, P. & D.K.L. MacKerron, 1986. The effects of nitrogen application on growth and nitrogen distribution within the potato canopy. *Annals of Applied Biology* 109: 427-437.

Muchow, R.C., 1988. Effect of nitrogen supply on the comparative productivity of maize and sorghum in a semi-arid tropical environment. 1. Leaf growth and leaf nitrogen. *Field Crops Research* 18: 1-16.

Ng, E. & R.S. Loomis, 1984. Simulation of the growth and yield of the potato crop. Simulation Monographs. Pudoc, Wageningen, 147 pp.

Ong, C.K. & C.K. Baker, 1985. Temperature and leaf growth. In: N.R. Baker, W.J. Davies & C.K. Ong (Eds.), Control of leaf growth. Cambridge University Press, Cambridge, pp. 175-200.

Sale, P.J.M., 1973. Productivity of vegetable crops in a region of high solar input. 1. Growth and development of the potato (*Solanum tuberosum* L.). *Australian Journal of Agricultural Research* 24: 733-749.

Vos, J., 1995. Foliar development of the potato plant and modulations by environmental factors. In: P. Kabat, B. Marshall, B.J. van den Broek, J. Vos & H. van Keulen (Eds.), Modelling and parameterization of the soil-plant-atmosphere system. Wageningen Pers, Wageningen (in press).

SECTION 2.2

Effects of nitrogen on development and growth of the leaves of vegetables. 1. Appearance, expansion growth and life span of leaves of Brussels sprouts plants

H. Biemond, J. Vos and P.C. Struik

Abstract

Leaf growth and development of Brussels sprouts (*Brassica oleracea* L. var *gemmifera* DC) were studied in three experiments, as affected by amount and timing of nitrogen application. Rate of leaf appearance, leaf expansion, leaf size and leaf senescence were extensively recorded.

The rate of leaf appearance ranged between 0.39 and 0.72 d^{-1}. It was significantly increased by more nitrogen. The rate of leaf expansion and mature leaf area depended on leaf number and nitrogen treatment: they increased with leaf number, reaching a maximum between leaf numbers 10 and 20 and decreased subsequently. Leaf expansion rate was the main factor determining mature leaf area, but the duration of expansion also played a role: it was shorter for larger leaves. Plants receiving more nitrogen had a higher total green leaf area per plant, because of more and larger green leaves. Specific leaf area of all leaves declined gradually from 130-230 cm^2 g^{-1} (depending on experiment) at about 30 days after planting to 60 at the end of the experiments and was usually significantly increased by more nitrogen.

Introduction

Brussels sprouts is a biennial plant with a vegetative phase in the first year and a generative phase in the second year. Early vegetative growth consists of leaf, stem and root growth. Sprout growth starts when the main part of the leaf area is formed.

Nitrogen strongly affects leaf growth and development. Firstly, it affects the final number of leaves (Dale, 1982). That is an important characteristic for this crop, as it determines the potential number of sprouts: in each leaf axil one sprout may develop. Moreover, it affects growth and development of individual leaves: more nitrogen usually leads to faster increase

of leaf length and a larger final length and area (Terry *et al.*, 1981). Milford *et al.* (1985) observed in sugar beet that differences in leaf size usually were associated with differences in leaf expansion rate and not with differences in duration of expansion. Wilcockson & Abuzeid (1991) observed that in Brussels sprouts leaf size and sprout size were closely related. However, little information is available on effects of nitrogen on development and growth of the leaves of Brussels sprouts. This paper reports three glasshouse experiments on the effects of nitrogen on leaf growth by analyzing rates and durations of leaf appearance and expansion growth, rates of senescence and life spans of leaves, with the aim of understanding the dynamics of total green leaf area.

Materials and methods

Plant culture

Young Brussels sprouts plants (cv. Icarus SG2004; Experiment 1: with 6 leaves; Experiment 2: with 3 leaves; Experiment 3: with 5 leaves) were planted in 20-l pots (one plant per pot), containing sand, free from organic matter. Experiment 1 was planted on 1 May 1991, Experiment 2 on 4 Nov 1991 and Experiment 3 on 14 Apr 1992. The pots were placed in a glasshouse, set to maintain a day (12 h) temperature of 18 °C and a night temperature of 12 °C. Natural light was supplemented with 400 Watt Philips SON-AGRO-T lamps at a density of 0.7 lamps m^{-2}. In Experiment 1, initial density of the pots was 3.9 m^{-2}, after the first intermediate harvest (29 DAP (days after planting)), the spacing became wider (3.5 pots m^{-2}). In Experiment 2 initial density was 4.5 pots m^{-2}. As pots were removed at intermediate harvests, the remaining pots were spaced wider, up to 3.9 pots m^{-2} from 84 DAP onwards. In Experiment 3 initial density was 4.5 pots m^{-2}. The remaining pots were spaced wider following intermediate harvests until a density of 3.2 pots m^{-2} was reached at 70 DAP. Water was administered from the top of the pots until the plants were well established. Subsequently, a 5-cm-high saucer under the pots was filled daily with water to its brim. The pots were covered with polyethylene granules (Experiments 1 and 3) to prevent accumulation of salts in the top layer of the soil. Once every three weeks the plants were allowed to absorb all the water from the saucers. Then water was administered from the top once. Subsequently the standard way of supplying water via the bottom saucer was resumed (Datema *et al.*, 1986).

Treatments

Each experiment had four different treatments (Tables 2.2.1-2.2.3), consisting of different amounts of nitrogen and different dates of application. Other nutrients were supplied in equal

amounts in all treatments.

Table 2.2.1. Amounts and dates of application of nitrogen in the different treatments of Experiment 1.

	Amount of nitrogen (g per pot)			
Time (DAP)	N(4.5/3)	N(4.5/9)	N(13.5/3)	N(13.5/9)
0	1.5	0.5	4.5	1.5
14	-	0.5	-	1.5
30	-	0.5	-	1.5
43	1.5	0.5	4.5	1.5
55	-	0.5	-	1.5
66	-	0.5	-	1.5
72	-	0.5	-	1.5
82	-	0.5	-	1.5
89	1.5	0.5	4.5	1.5
Total	4.5	4.5	13.5	13.5

Table 2.2.2. Amounts and dates of application of nitrogen in the different treatments of Experiment 2.

	Amount of nitrogen (g per pot)			
Time (DAP)	N(6)	N(9)	N(12)	N(18)
7	1	1	1	1
21	1	1	1	1
35	2	2	2	2
42	2	2	2	2
49	-	3	3	3
63	-	-	3	3
79	-	-	-	3
122	-	-	-	3
Total	6	9	12	18

In Experiment 1, treatments were: N(4.5/3): 4.5 g N per pot, supplied in three splits; N(4.5/9): 4.5 g N per pot (nine splits); N(13.5/3): 13.5 g N per pot (three splits); N(13.5/9): 13.5 g N per pot (nine splits). In the statistical analyses, these four treatments were split into two factors, viz. the amount of nitrogen and the number of applications. In Experiment 2, nitrogen supply was discontinued at various stages of growth, resulting in total additions of 6 (N(6)), 9 (N(9)), 12 (N(12)) or 18 (N(18)) g N per pot. In Experiment 3, treatments were: N(5.6): 5.6 g N per pot, with nitrogen limitation throughout the experiment; N(9.8;E(arly)): 9.8 g N per pot, with non-limiting supply in the early stages of growth and limiting supply later; N(9.8;L(ate)): 9.8 g N per pot, with limiting supply

in the early stages of growth and non-limiting supply later; N(19.6): 19.6 g N per pot; non-limiting nitrogen supply throughout the experiment.

Table 2.2.3. Amounts and dates of application of nitrogen in the different treatments of Experiment 3.

	Amount of nitrogen (g per pot)			
Time (DAP)	N(5.6)	N(9.8;E)	N(9.8;L)	N(19.6)
10	0.98	1.96	0.56	1.96
35	0.14	1.96	0.56	1.96
51	0.56	1.96	0.56	1.96
57	0.56	0.56	0.56	1.96
65	1.12	1.12	1.12	3.92
79	0.56	0.56	0.56	1.96
85	0.56	0.56	1.96	1.96
99	0.56	0.56	1.96	1.96
118	0.56	0.56	1.96	1.96
Total	5.60	9.80	9.80	9.60

Each experiment was laid out in a randomized complete block design with four blocks, with each pot regarded as one experimental unit. At the start of the experiment, each block consisted of 28 (Experiment 1) or 32 (Experiments 2 and 3) pots, to allow (non-)destructive observations. In the statistical analyses, each harvest (see *Destructive sampling*) of each experiment was analysed separately.

Determination of leaf characteristics

In Experiments 1 and 3 the dynamics of the area of individual leaves were derived from frequent measurements of leaf length. From the moment a new expanding leaf could be distinguished at the apex, i.e. when it reached a length of about 5 cm, the length of the leaf blade (from the first lateral vein to the tip) was recorded, usually at intervals of 2-3 days, until final size was reached. Data were collected on eight stems per treatment (two stems per block).

In this study 'leaf appearance' was defined as the date of first record of leaf length. In Experiment 2 leaf length was not recorded but total number of leaves and number of dead leaves were counted weekly. In Experiment 1 the number of dead leaves was counted each time leaf lengths were measured; in Experiment 3 this number was counted weekly.

In Experiments 1 and 3 destructive sampling was applied (see *Destructive sampling*) at regular intervals during growth to obtain paired data on leaf length, and leaf area (determined

with a Li-Cor model 3100 electronic area meter; Li-Cor, Lincoln, Nebraska). These data were fitted to a logarithmic regression (Table 2.2.4), applied to each treatment separately, because analysis of variance showed significant (Tukey-test; P=0.05) effects of treatment. The regressions were used to calculate leaf areas from routinely measured leaf lengths.

Table 2.2.4. Results of regression analysis, relating leaf area (Y; cm^2) to leaf length (X; mm), using the model $\ln(Y) = a + b \ln(X)$. Standard error of a and b in parentheses.

Parameter	Nitrogen treatment (Experiment 1)			
	N(4.5/3)	N(4.5/9)	N(13.5/3)	N(13.5/9)
a	-5.41 (0.175)	-5.80 (0.153)	-5.07 (0.166)	-5.35 (0.165)
b	2.16 (0.0164)	2.24 (0.0167)	2.08 (0.0113)	2.14 (0.0126)
r^2	0.97	0.97	0.98	0.98
n	512	497	856	717
	Nitrogen treatment (Experiment 3)			
	N(5.6)	N(9.8;E)	N(9.8;L)	N(19.6)
a	-5.72 (0.143)	-5.42 (0.130)	-4.90 (0.153)	-5.03 (0.171)
b	2.20 (0.0235)	2.13 (0.0146)	2.02 (0.0238)	2.05 (0.0169)
r^2	0.93	0.97	0.92	0.95
n	618	609	664	739

Figure 2.2.1 presents typical examples of the time course of leaf area. The largest part of the leaf area is formed during a short period of time with practically linear expansion. Therefore, for each leaf, linear regressions were fitted to these data and the expansion rate characterized as the slope of the regression (= tan α in Figure 2.2.1). The 'effective duration of expansion' (d; length of line "a" in Figure 2.2.1) is defined as the final area of a leaf (cm^2; length of line "b" in Figure 2.2.1) divided by its expansion rate ($cm^2\ d^{-1}$) (cf. Vos & Biemond, 1992). The day of leaf senescence was defined as the day on which more than 90% of the leaf area was yellow. Life spans of leaves were calculated by subtracting day of leaf appearance from day of leaf senescence.

Destructive sampling

Destructive sampling of plants took place in Experiment 1 on six occasions between 29 and 152 DAP, in Experiment 2 on nine occasions between 28 and 169 DAP and in Experiment 3 on eight occasions between 29 and 175 DAP. Usually, at each sampling date one plant per treatment was sampled from each block, i.e. in total 16 plants. However, at the first two intermediate harvests in Experiment 2, only one plant from two

treatments per block was sampled, as no differences were expected between the treatments.

Measurements included leaf area, fresh and dry weight of leaf blades, petioles, sprouts, stem and top (= cluster of young leaves at the top of the stem). In the analysis of growth, leaf blades, petioles and sprouts from three (Experiment 1) or five (Experiments 2 and 3) nodes were combined.

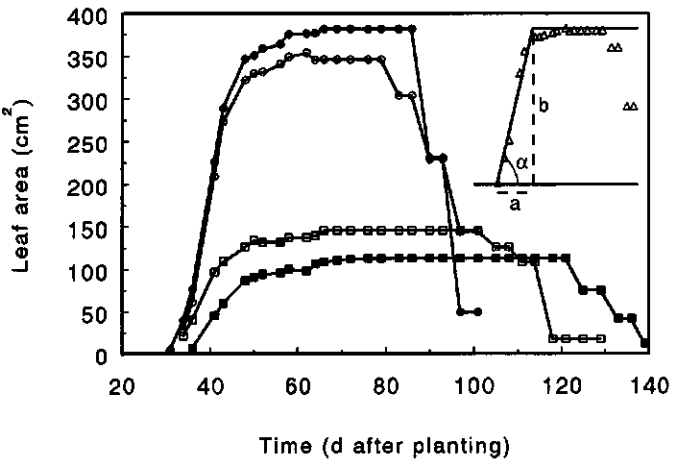

Figure 2.2.1. The change with time in leaf area of leaf 15, Experiment 3. (□) N(5.6); (○) N(9.8;E); (■) N(9.8;L); (●) N(19.6). The insert at the upper right explains the method of calculation of the rate and duration of leaf expansion. The length of line "a" represents the duration of leaf expansion (d), the length of line "b" the mature leaf area (cm^2) and tan α the rate of leaf expansion ($cm^2\ d^{-1}$).

Results

Rate of leaf appearance and number of leaves

Figure 2.2.2 shows the number of leaves as a function of time in Experiment 1. The rate of leaf appearance, based on linear regression over the whole growing season (Table 2.2.5), was significantly affected (Tukey-test; $P=0.05$) by the amount of nitrogen, but not by the number of applications (Experiment 1). The curves for leaf appearance in Experiments 2 and 3 (not presented) also had a somewhat sigmoidal shape: at the beginning and at the end of the growing period the rates of leaf appearance were lower. In Experiment 2 the rate of leaf appearance for N(6) was slightly lower than in the other treatments at the end of the growing season. In Experiment 3 modification of nitrogen availability caused a shift in rate of leaf appearance.

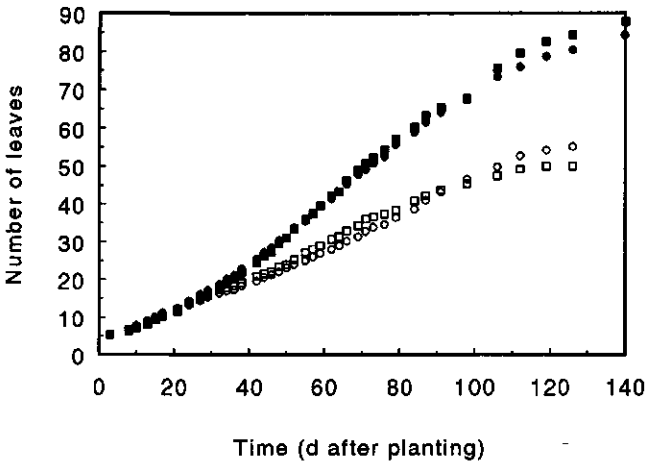

Figure 2.2.2. The number of leaves as a function of time and nitrogen treatment for Experiment 1. (□) N(4.5/3); (○) N(4.5/9); (■) N(13.5/3); (●) N(13.5/9).

The difference in amount of nitrogen in Experiment 1 caused large differences in total number of leaves (Table 2.2.5). Across experiments, plants in Experiment 1 attained the highest number of leaves, although they did not receive the largest amount of nitrogen. The differences between treatments in total number of leaves in Experiment 2 were small, but in Experiment 3 number of leaves and amount of nitrogen were well correlated. In Experiment 1 more than half of the leaves had died at 139 DAP, irrespective of treatment. In Experiment 2 the number of dead leaves (162 DAP) was similar in all treatments. In Experiment 3 total number of leaves in N(9.8;E) and N(9.8;L) were identical, but the number of dead leaves was different.

Rate of leaf expansion and size of fully expanded leaves

The rate of leaf expansion was influenced by leaf number and by nitrogen treatment (Experiments 1 and 3). In Experiment 1, the rate of leaf expansion increased with leaf number until leaf 12-17, depending on treatment and gradually decreased for higher leaf numbers (Figure 2.2.3). The maximum rate of leaf expansion in Experiment 3 was reached at slightly lower leaf numbers than in Experiment 1 and was nearly 30 $cm^2\ d^{-1}$ in N(19.6), compared to values exceeding 40 $cm^2\ d^{-1}$ for N(13.5/3) of Experiment 1. The expansion rate of the first ten leaves was similar for all treatments in Experiment 1. For the lower leaves in Experiment 3 expansion rates were equal for N(5.6) and N(9.8;L) on the one hand and N(9.8;E) and N(19.6) on the other; for the higher leaf numbers expansion rates were equal

for N(5.6) and N(9.8;E) on the one hand and for N(9.8;L) and N(19.6) on the other.

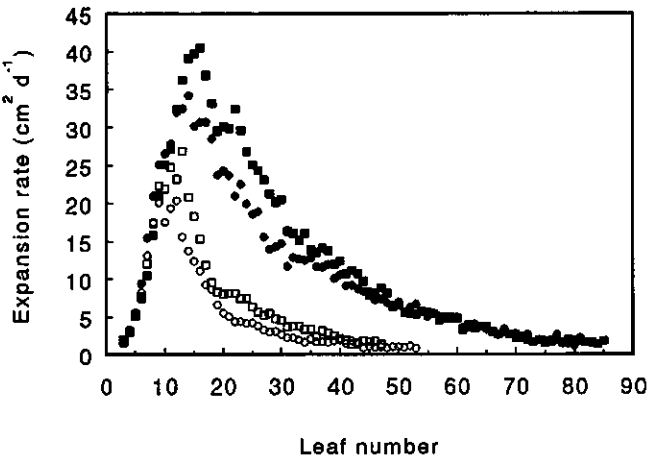

Figure 2.2.3. The rate of leaf expansion versus leaf insertion number as affected by nitrogen supply for Experiment 1. (□) N(4.5/3); (○) N(4.5/9); (■) N(13.5/3); (●) N(13.5/9).

The features noted for the rate of leaf expansion also apply to the area of fully expanded leaves in both experiments (Figure 2.2.4: Experiment 1). Leaf expansion rate therefore seems to be the major determinant of leaf size. The relation between leaf size and leaf expansion rate in Experiment 1 shows two distinct lines: one for N(4.5/3) and N(4.5/9) and a second and steeper one for N(13.5/3) and N(13.5/9) (Figure 2.2.5). Hence, leaves with the same expansion rate reached a smaller mature leaf size, when less nitrogen was available. The curvilinearity (which is more distinct for leaves in Experiment 3 than for those in Experiment 1) implies that the duration of expansion was somewhat shorter for the largest leaves. The mature size for leaves in Experiment 3 (data not shown) was about two-thirds of that in Experiment 1 (for the same leaf number from comparable nitrogen treatments).

The effective duration of expansion decreased from leaf 1 till leaf 12 from 30 to 15 days and subsequently increased nearly linearly with increasing leaf number (Figure 2.2.6: Experiment 1). The increase was faster for N(4.5/3) and N(4.5/9) than for N(13.5/3) and N(13.5/9), i.e. a certain leaf of N(13.5/3) or N(13.5/9) needed less time to reach mature size than the same leaf of N(4.5/3) or N(4.5/9). In Experiment 3 the curves of the effective duration of expansion followed the same pattern: longer duration with less nitrogen.

Table 2.2.5. Leaf appearance rate over the whole growing season, total number of leaves, number of dead leaves and number of green leaves per plant in Experiment 1 at 139 DAP, in Experiment 2 at 162 DAP and in Experiment 3 at 153 DAP. Different letters indicate a significant difference (LSD-test; P=0.05) between treatments.

Leaf appearance rate (d^{-1})

Experiment 1*		Experiment 2		Experiment 3	
N(4.5/3)	0.48	N(6)	0.42	N(5.6)	0.39
N(4.5/9)		N(9)	0.47	N(9.8;E)	0.47
N(13.5/3)	0.72	N(12)	0.49	N(9.8;L)	0.47
N(13.5/9)		N(18)	0.51	N(19.6)	0.54

Total number of leaves

Experiment 1*		Experiment 2		Experiment 3	
N(4.5/3)	52.1 a	N(6)	68.0 a	N(5.6)	59.7 a
N(4.5/9)		N(9)	76.8 ab	N(9.8;E)	71.5 b
N(13.5/3)	86.1 b	N(12)	79.3 b	N(9.8;L)	71.3 b
N(13.5/9)		N(18)	82.3 b	N(19.6)	82.8 c

Number of dead leaves

Experiment 1*		Experiment 2		Experiment 3	
N(4.5/3)	29.8 a	N(6)	31.5 a	N(5.6)	31.4 a
N(4.5/9)		N(9)	32.5 a	N(9.8;E)	44.0 b
N(13.5/3)	47.3 b	N(12)	34.0 a	N(9.8;L)	32.0 a
N(13.5/9)		N(18)	30.0 a	N(19.6)	50.0 b

Number of green leaves

Experiment 1*		Experiment 2		Experiment 3	
N(4.5/3)	22.3 a	N(6)	36.5 a	N(5.6)	28.4 a
N(4.5/9)		N(9)	44.2 ab	N(9.8;E)	27.5 a
N(13.5/3)	38.8 b	N(12)	45.2 ab	N(9.8;L)	39.2 a
N(13.5/9)		N(18)	52.2 b	N(19.6)	32.7 a

* mean value of N(4.5/3) and N(4.5/9) or N(13.5/3) and N(13.5/9)

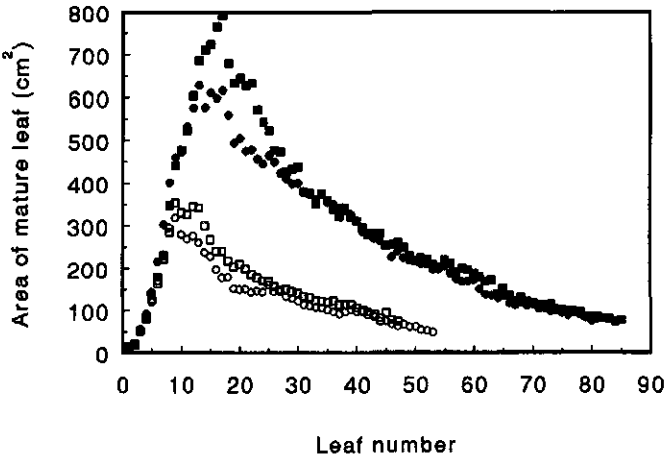

Figure 2.2.4. Mature leaf area of leaves of Experiment 1 in relation to leaf insertion number and nitrogen treatment. (□) N(4.5/3); (○) N(4.5/9); (■) N(13.5/3); (●) N(13.5/9).

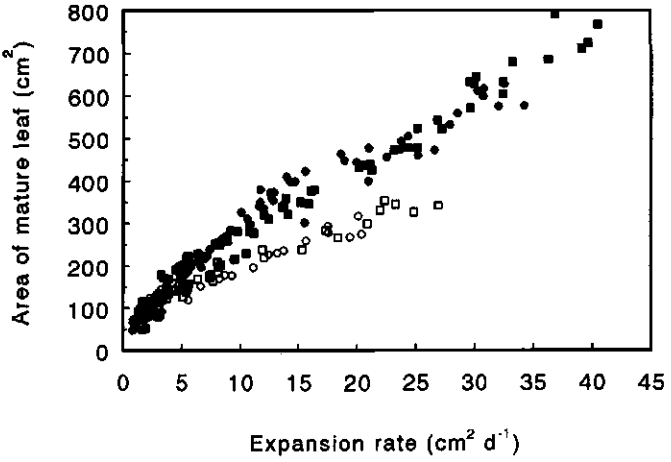

Figure 2.2.5. The relation between mature leaf size and leaf expansion rate, irrespective of leaf position (Experiment 1). (□) N(4.5/3); (○) N(4.5/9); (■) N(13.5/3); (●) N(13.5/9).

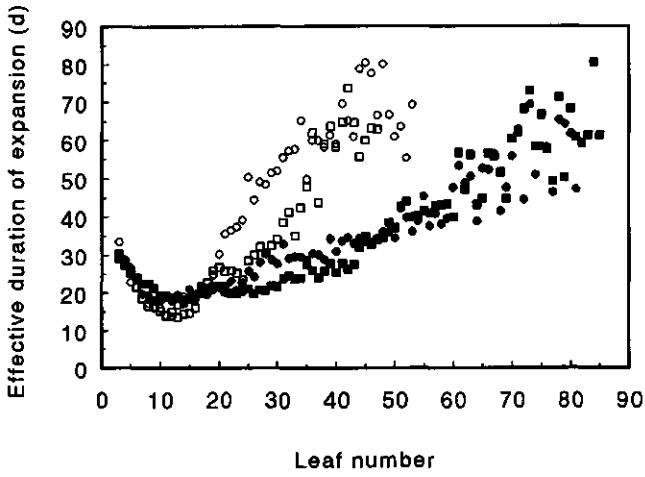

Figure 2.2.6. The effective duration of expansion as a function of leaf position and nitrogen treatment (Experiment 1). (□) N(4.5/3); (○) N(4.5/9); (■) N(13.5/3); (●) N(13.5/9).

Rate of leaf senescence and life span of leaves

In Experiments 1 and 2 the increase in the number of dead leaves was more or less exponential, starting at 20 and 60 DAP, respectively. In Experiment 1 the curves for N(4.5/3) and N(4.5/9) were different from those of N(13.5/3) and N(13.5/9). The number of dead leaves in Experiment 2 never differed among treatments. In Experiment 3 (Figure 2.2.7) it started from zero at 30 DAP and increased sigmoidally. During the first and last part of the growing period the differences between N(5.6) and N(9.8;L) were negligible, but between 80 and 130 DAP the number of dead leaves was lower in N(9.8;L): additional nitrogen delayed leaf senescence. After 120 DAP differences developed between N(9.8;E) and N(19.6), because leaf senescence was delayed more in N(9.8;E) than in N(19.6).

For leaves that were dead at the end of the experiment, life span was calculated (Figure 2.2.8: Experiment 3). Those for the first five leaves are not included, because their dates of appearance are unknown. The life span for the first leaves of N(5.6) and N(9.8;L) increased with leaf number and slowly decreased after reaching a maximum of 95 days between leaf number 15 and 20. The life span for leaves of N(9.8;E) and N(19.6) slowly increased from 60 days for leaf number 10 to 65 or 75 days for the highest leaf numbers of N(9.8;E) and N(19.6), respectively. The curves for Experiments 1 and 2 followed more or less the same pattern as those for N(5.6) and N(9.8;L) in Experiment 3: the life span increased with leaf number to a maximum of approximately 90 days between leaf number 20 and 30, and subsequently slowly decreased with increasing leaf number. The correlation between mature

leaf area and life span was not strong, although the largest leaves tended to have the longest life span.

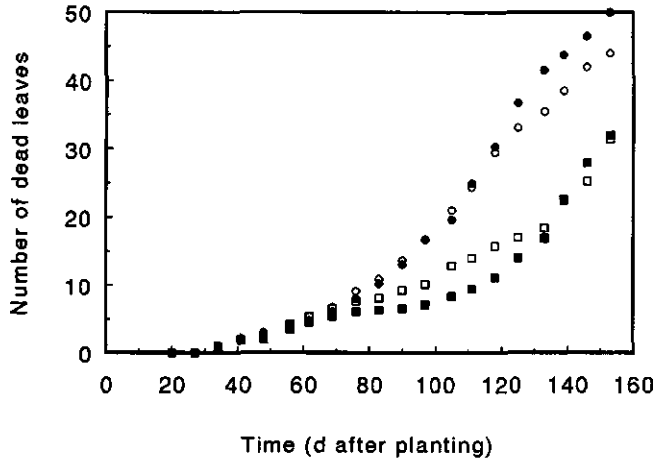

Figure 2.2.7. The number of dead leaves as a function of time and nitrogen treatment for Experiment 3. (□) N(5.6); (○) N(9.8;E); (■) N(9.8;L); (●) N(19.6).

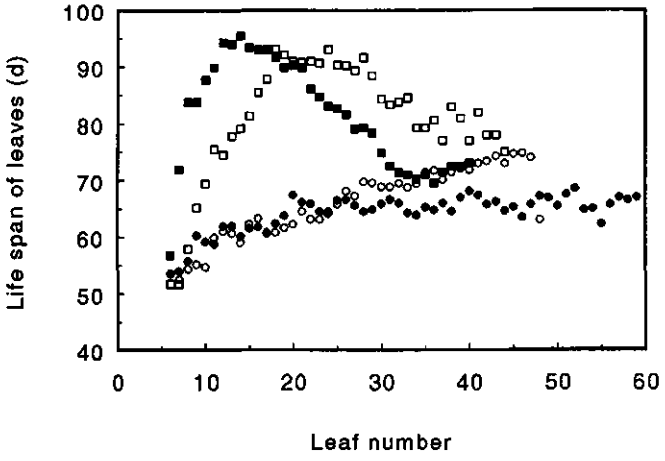

Figure 2.2.8. The life span of leaves of Experiment 3 as a function of leaf insertion number and nitrogen treatment. (□) N(5.6); (○) N(9.8;E); (■) N(9.8;L); (●) N(19.6).

Specific leaf area

Specific leaf area (SLA) was calculated over all green leaves of a plant at each (intermediate) harvest. In Experiment 3 (Figure 2.2.9) specific leaf area gradually decreased from 180 cm^2 g^{-1} at 29 DAP to 60 at 175 DAP, with significant differences (LSD-test, P=0.05) among the nitrogen treatments at all harvests, except at 70 DAP. At higher nitrogen availability, the SLA is higher. In N(9.8;L) upon the transition from limiting N-supply to high N-supply SLA increased and was significantly above the other treatments from 90 DAP onwards, except at 175 DAP. The changes in SLA with age of leaves were relatively small, compared to SLA of all green leaves. Figure 2.2.10 (Experiment 3) shows SLA of leaf numbers 6-10 and 36-40 as a function of days after mean appearance date. The differences between both groups were considerable, SLA being lower for leaves later appearing. Effects of nitrogen treatment for each group were similar to the effects on overall SLA: higher SLA at higher nitrogen availability. SLA of all green leaves in Experiment 1 decreased from 130 and 150 cm^2 g^{-1} (4.5 and 13.5 g N per plant, respectively) at 29 DAP to about 60 cm^2 g^{-1} at 152 DAP. The high nitrogen treatment had a significantly higher (Tukey-test, P=0.05) SLA at each (intermediate) harvest. Applying the nitrogen in three instead of nine splits resulted in higher SLA at 29, 103 and 152 DAP. SLA of Experiment 2 decreased nearly linearly from 230 cm^2 g^{-1} at 28 DAP to 60 at 169 DAP. Only at 169 DAP a significant treatment effect existed (LSD-test, P=0.05): SLA in N(18) was higher than in the other three treatments.

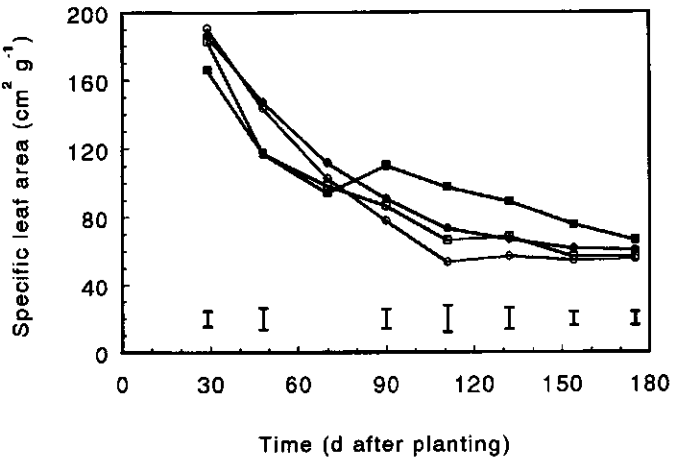

Figure 2.2.9. Specific leaf area over all leaves of a plant for Experiment 3 as a function of time and nitrogen treatment. The vertical bars represent LSD values (P=0.05). (□) N(5.6); (○) N(9.8;E); (■) N(9.8;L); (●) N(19.6).

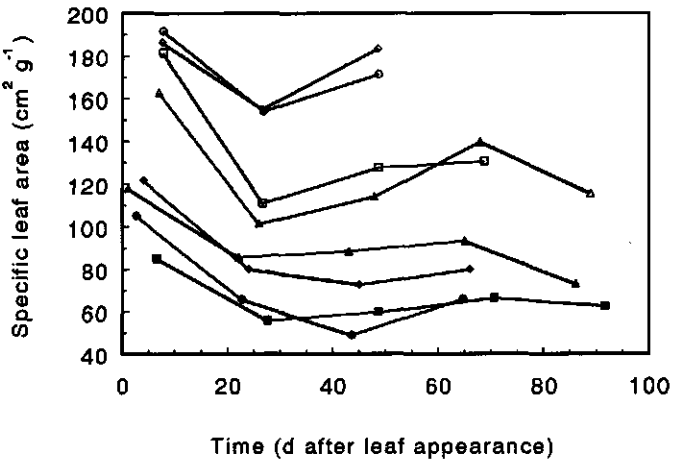

Figure 2.2.10. Specific leaf area of two groups of five leaves against days after leaf appearance (Experiment 3). Leaf blades 6-10: (open markers; (□) N(5.6); (○) N(9.8;E); (△) N(9.8;L); (◇) N(19.6)); leaf blades 36-40: (closed markers; (■) N(5.6); (●) N(9.8;E); (▲) N(9.8;L); (◆) N(19.6)).

Total leaf area per plant

Figure 2.2.11 shows total green leaf area per plant in Experiment 3. Significant differences (LSD-test, P=0.05) were observed from 48 DAP till 90 DAP; subsequently the differences were not significant. In plants of N(5.6) and N(9.8;L) leaf area increased over a long period of time. Leaf area in N(19.6) plants expanded much faster and reached the highest value at 90 DAP, but subsequently decreased rapidly. Early application (N(9.8;E)) resulted in a fast increasing leaf area at the beginning of the growing period and an early start of senescence, while late application (N(9.8;L)) resulted in increasing leaf area during a very large part of the growing season, i.e. until 132 DAP. The period that N(19.6) plants had a leaf area of more than 90% of the maximum value, was rather short, compared to N(13.5/3) and N(13.5/9) of Experiment 1 and N(12) and N(18) of Experiment 2, treatments that received high amounts of nitrogen (data not shown). In Experiment 1 N(4.5/3) and N(4.5/9) reached a maximum leaf area of about 4000 cm^2 $plant^{-1}$ and N(13.5/3) and N(13.5/9) 15000 and 12000, respectively. A leaf area of more than 90% of these maximum values was already reached at 50 DAP, which is much earlier than in Experiment 3. In Experiment 2 N(6) reached a maximum leaf area of 8000 cm^2 $plant^{-1}$ and N(9), N(12) and N(18) about 10000. These peak values (in Experiments 2 and 3) were usually maintained over a period of at least 30 days. The highest LAI (leaf area index) of about 5.2 was reached by N(13.5/3) in Experiment 1.

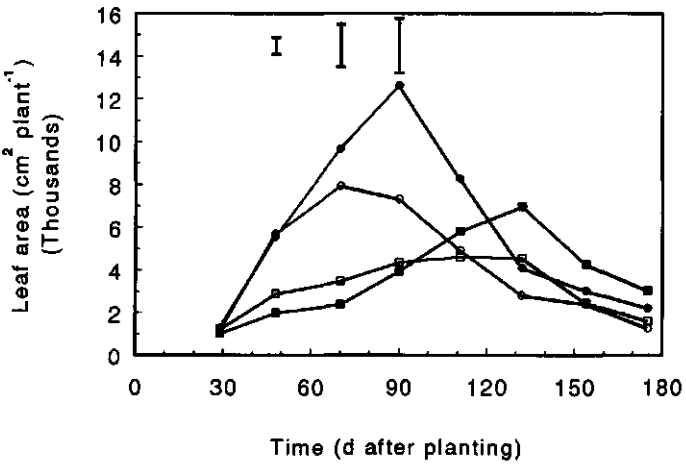

Figure 2.2.11. Total leaf area per plant for Experiment 3 as a function of time and nitrogen treatment. The vertical bars represent LSD values (P=0.05). (□) N(5.6); (○) N(9.8;E); (■) N(9.8;L); (●) N(19.6).

Discussion

Total green leaf area per plant is the result of the rate of leaf appearance, the rate and duration of leaf expansion and the life span of leaves. Treatment effects on any of these components will affect total leaf area. The rate of leaf appearance increased with more nitrogen, especially in Experiments 1 and 3. In Experiment 2, carried out during winter, radiation intensity may have been too low to allow expression of the N-effect. Terry (1970) found effects of nitrogen supply on the rate of leaf appearance in sugar beet. These effects were, however, quite small, compared to the differences in available nitrogen, similarly to the effects in the present study. Muchow (1988) found that nitrogen supply influenced the rate of leaf appearance in maize and sorghum, but final leaf number was not affected. Dale (1982), however, shows that the availability of nutrients affects final number of leaves. Steer & Hocking (1983) observed no effects of nitrogen supply on the duration of leaf production in glasshouse-grown sunflower, but the rate of leaf production was highest at the highest nitrogen supply. Final number of leaves was thus significantly affected by nitrogen supply. Effects on final number of leaves in Brussels sprouts plants in our experiments were large, because of the effects on rate of appearance.

In most cases, the number of dead leaves was closely related to total number of leaves. Treatment N(9.8;L) in Experiment 3, however, had a relatively small number of dead leaves.

Late application of nitrogen delayed leaf senescence in this treatment. This was also, more or less, the case for N(18) in Experiment 2.

Both, the rate of leaf expansion and the effective duration of expansion were different among nitrogen treatments and leaf number. Both characteristics contributed to differences in mature leaf size, but were negatively correlated. Leaves with a high rate of expansion had a relatively short effective duration of expansion and vice versa. The main effect of nitrogen on mature leaf size was through its effect on the rate of expansion, confirming results of Muchow (1988) for maize and sorghum. Also Dale (1982) and Terry *et al.* (1981) report leaf size increased at higher nutrient availability. Positive effects of nitrogen on leaf expansion rate were found by Terry (1970) for sugar beet leaves and by Radin & Boyer (1982) and Steer & Hocking (1983) for sunflower leaves. More nitrogen also had positive effects on the size of sugar beet leaves by increasing the duration of expansion (Terry, 1970) but in sunflower leaves the duration was not affected (Steer & Hocking, 1983).

The expansion rates of the lowest leaf numbers were probably not affected by nitrogen treatment, as these leaves did not experience different nitrogen availabilities.

It is not clear, why the number of dead leaves was higher and, hence, the life span shorter with more nitrogen. The number of dead leaves was higher with a higher total number of leaves (Table 2.2.5). Muchow (1988) found in maize and sorghum that leaf senescence during grain filling was faster at low rates of applied N. Wolfe *et al.* (1988) observed that leaves of maize plants, grown with insufficient nitrogen, had a shorter life span. These differential effects of nitrogen are possibly due to the different characteristics of the plant species.

In all three experiments, SLA decreased with time, apparently due to a gradual change in leaf morphology. Higher leaf numbers exhibit a much denser vein structure than the lower leaf numbers, resulting in lower SLA. The effect on overall SLA of all green leaves is a gradual decline in time.

Total green leaf area of N(19.6) in Experiment 3 rapidly declined after reaching its peak value, while treatments in Experiments 1 and 2, with high amounts of nitrogen, maintained a high leaf area over a longer period. This was not caused by a faster rate of leaf senescence in Experiment 3, but by a much slower increase in total green leaf area. Early in the growing period total leaf area of this crop depended on much fewer leaves than at the end of the growing period. The mature area of the (early) leaves in Experiment 3 was, however, much lower than in Experiment 1. In Experiment 3 this was compensated later in the growing period by a higher number of green leaves (data not shown).

References

Dale, J.E., 1982. The growth of leaves. The Institute of Biology's Studies in Biology no. 137. Edward Arnold, London, 60 pp.

Datema, P., H. Niers & A. de Jager, 1986. Comparison of three methods of watering in pot experiments. Rapport IB 4-86. Institute for Soil Fertility, Haren, 121 pp.

Milford, G.F.J., T.O. Pocock, J. Riley & A.B. Messem, 1985. An analysis of leaf growth in sugar beet. III. Leaf expansion in field crops. *Annals of Applied Biology* 106: 187-203.

Muchow, R.C., 1988. Effect of nitrogen supply on the comparative productivity of maize and sorghum in a semi-arid tropical environment I. Leaf growth and leaf nitrogen. *Field Crops Research* 18: 1-16.

Radin, J.W. & J.S. Boyer, 1982. Control of leaf expansion by nitrogen nutrition in sunflower plants. Role of hydraulic conductivity and turgor. *Plant Physiology* 69: 771-775.

Steer, B.T. & P.J. Hocking, 1983. Leaf and floret production in sunflower (*Helianthus annuus* L.) as affected by nitrogen supply. *Annals of Botany* 52: 267-277.

Terry, N., 1970. Developmental physiology of sugar beet. II. Effects of temperature and nitrogen supply on the growth, soluble carbohydrate content and nitrogen content of leaves and roots. *Journal of Experimental Botany* 21: 477-496.

Terry, N., L.J. Waldron & S.E. Taylor, 1981. Environmental influences on leaf expansion. In: J.E. Dale & F.L. Milthorpe (Eds.), The growth and functioning of leaves. Cambridge University Press, Cambridge, pp. 179-193.

Vos, J. & H. Biemond, 1992. Effects of nitrogen on the development and growth of the potato plant. 1. Leaf appearance, expansion growth, life spans of leaves and stem branching. *Annals of Botany* 70: 27-35.

Wilcockson, S.J. & A.E. Abuzeid, 1991. Growth of axillary buds of Brussels sprouts (*Brassica oleracea* var. *bullata* subvar. *gemmifera*). *Journal of Agricultural Science* (Cambridge) 117: 207-212.

Wolfe, D.W., D.W. Henderson, T.C. Hsiao & A. Alvino, 1988. Interactive water and nitrogen effects on senescence of maize. I. Leaf area duration, nitrogen distribution, and yield. *Agronomy Journal* 80: 859-864.

SECTION 2.3

Effects of nitrogen on development and growth of the leaves of vegetables. 2. Appearance, expansion growth and life span of leaves of leek plants

H. Biemond

Abstract

Leaf growth and development of leek (*Allium porrum* L.) were examined in three experiments, with different amounts and dates of application of nitrogen as treatments. Rates of leaf appearance, leaf expansion, leaf size and leaf senescence were measured.

The rate of leaf appearance was not affected by N treatments and almost constant across experiments at 0.15 d^{-1}. The rate of leaf expansion and the mature leaf area increased with leaf number, reached a maximum between leaf numbers 11 and 14 and decreased with higher leaf numbers. Both variables increased with more nitrogen. The duration of leaf expansion was more or less constant across leaf numbers and not influenced by nitrogen treatments; the leaf expansion rate was the main factor determining mature leaf area. The rate of leaf senescence was not influenced by N treatments. Differences in total green leaf area per plant were caused by differences in (mature) leaf area of individual leaves and not by the differences in the number of leaves. The specific leaf area of all leaves was more or less constant at 100 cm^2 g^{-1}.

Introduction

Leek is a biennial plant with a vegetative phase in the first year and a reproductive phase in the second year. Leaves are produced in the vegetative phase. The flower stalk usually grows during the second year, although sometimes already during the first year (Dragland, 1972). Development of the flower stalk indicates the end of leaf initiation.

Total leaf area per unit soil area is important for crop production, because it determines how much radiation is intercepted by the crop. For leek, also the number of leaves is relevant, because this number (and the thickness of the leaves) determines the diameter of the shaft, which is a quality characteristic. Leaf area at any time of crop development is

determined by several processes and variables, such as the rates of leaf appearance and leaf expansion, the area of mature leaves and the rate of leaf senescence, all of which are influenced by the availability of nutrients, especially nitrogen. Some data on development and growth of leaves of leek have been reported, but information on effects of nitrogen is scarce. Hay & Brown (1988) analysed development and expansion of leek leaves and concluded that leaf appearance was dependent on temperature. Hay & Kemp (1992) developed a simple model of leaf canopy expansion in leek, based on primordial development. This paper reports on one glasshouse and two field experiments with leek, designed to quantify the effects of nitrogen supply on rate and duration of leaf appearance, expansion and senescence.

Materials and methods

Plant culture - glasshouse

Young, pencil-thick leek plants (cv. Albana), with five leaves, were planted on 1 May 1991 in 20-l pots (four plants per pot), containing sand, free from organic matter. The pots were placed in a glasshouse, set to maintain a day (12 h) temperature of 18 °C and a night temperature of 12 °C. Natural light was supplemented with 400 Watt Philips SON-AGRO-T lamps at a density of 0.7 lamps m^{-2}. The density of the pots was 5.0 m^{-2} during the whole experiment, i.e. 20 plants m^{-2}. Water was administered from the top of the pot until the plants were well established. Subsequently, a 5-cm-high saucer under the pots was filled daily with water to its brim. The pots were covered with polyethylene granules to reduce evaporation and thus the accumulation of salts in the top layer of the soil. Once every three weeks the plants were allowed to absorb all the water from the saucers. Then water was administered from the top once. Subsequently the standard procedure of supplying water via the saucer was resumed (Datema *et al.*, 1986).

Plant culture - field

The two field experiments were conducted on a sandy soil with about 3% organic matter. Plants comparable to those of Experiment 1 were planted at about 10 cm depth in rows, at a row-spacing of 25 cm and a plant distance within the row of 20 cm, i.e. a plant density of 20 plants m^{-2}. Experiment 2 was planted on 12 May 1992 and Experiment 3 on 7 May 1993. Irrigation was applied immediately after planting and later on whenever necessary.

Treatments

Treatments consisted of different amounts and different timing of nitrogen application. Other nutrients were supplied in equal amounts to all treatments. Experiment 1 had four different

treatments: N(1.8/2): 1.8 g N per pot, supplied in two equal splits; N(1.8/6): 1.8 g N per pot, in six equal splits; N(5.4/2): 5.4 g N per pot, in two equal splits; N(5.4/6): 5.4 g N per pot, in six equal splits (Table 2.3.1). The experiment was laid out in a randomized complete block design with five blocks. In the statistical analyses the four treatments were split into two factors: 1. amount of nitrogen and 2. number of applications.

Table 2.3.1. Amounts and dates of application of nitrogen in the different treatments of Experiment 1. (DAP = days after planting.)

	Amount of nitrogen (g per pot)			
Time (DAP)	N(1.8/2)	N(1.8/6)	N(5.4/2)	N(5.4/6)
0	0.9	0.3	2.7	0.9
13	-	0.3	-	0.9
27	-	0.3	-	0.9
37	0.9	-	2.7	-
41	-	0.3	-	0.9
55	-	0.3	-	0.9
69	-	0.3	-	0.9
Total	1.8	1.8	5.4	5.4

The two field experiments had the same three treatments: N(0): no application of fertilizer nitrogen; N(200/1): 200 kg N per ha, applied shortly after planting; N(200/5): 200 kg N per ha, applied in five equal splits (Table 2.3.2). These two experiments were laid out in a split-plot design with nitrogen treatment as main factor and harvest date (see *Destructive sampling*) as split factor. Both experiments had four blocks. In the statistical analyses of each experiment, each harvest was analysed separately.

Determination of leaf characteristics

In Experiment 1 the dynamics of leaf area of individual leaves were derived from frequent measurements of leaf length. From the moment that a new expanding leaf could be distinguished at the apex, i.e. when it was visible in the sheath of the older leaves, the length of the leaf was recorded, usually at intervals of 2-3 days, until further extension was negligible. The data were collected throughout the growth period on 20 plants per treatment (two plants of two pots per block). A leaf was considered 'appeared' on the date of its first record of leaf length. In Experiment 1 the number of dead leaves was recorded each time leaf lengths were measured. The day of leaf senescence was defined as the day on which more than 90% of the leaf area was yellow.

experiments.

Table 2.3.3. Leaf appearance rate over the whole growing season; total number of leaves, number of dead leaves and number of green leaves per plant in Experiment 1 at 117 DAP, in Experiment 2 at 134 DAP and in Experiment 3 at 122 DAP.

Leaf appearance rate (d^{-1})

Experiment 1		Experiment 2		Experiment 3	
N(1.8/2)	0.15	N(0)	0.14	N(0)	0.16
N(1.8/6)	0.16	N(200/1)	0.15	N(200/1)	0.16
N(5.4/2)	0.16	N(200/5)	0.15	N(200/5)	0.16
N(5.4/6)	0.15				

Total number of leaves

Experiment 1		Experiment 2		Experiment 3	
N(1.8/2)	21	N(0)	23	N(0)	24
N(1.8/6)	23	N(200/1)	24	N(200/1)	24
N(5.4/2)	23	N(200/5)	25	N(200/5)	24
N(5.4/6)	22				

Number of dead leaves

Experiment 1		Experiment 2		Experiment 3	
N(1.8/2)	8	N(0)	12	N(0)	8
N(1.8/6)	9	N(200/1)	12	N(200/1)	8
N(5.4/2)	8	N(200/5)	13	N(200/5)	8
N(5.4/6)	8				

Number of green leaves

Experiment 1		Experiment 2		Experiment 3	
N(1.8/2)	13	N(0)	11	N(0)	16
N(1.8/6)	14	N(200/1)	13	N(200/1)	16
N(5.4/2)	15	N(200/5)	12	N(200/5)	16
N(5.4/6)	14				

At planting, usually one or two leaves per plant were dead. In all experiments the increase in the number of dead leaves was nearly linear (Figure 2.3.2: Experiment 1). At 62 DAP N(1.8/2) had significantly more dead leaves than N(1.8/6) and N(5.4/2) (Experiment 1); at 76 DAP treatments with two nitrogen applications had significantly more dead leaves than treatments with six applications (LSD-test, P=0.05). As death rate

was lower than leaf appearance rate, the number of green leaves increased with time in all experiments till the end.

Treatments did not affect final number of leaves, number of dead leaves or number of green leaves per plant in either of the experiments (Table 2.3.3). Even the differences among experiments were small: at the end of each experiment dead leaves represented between one-third and half of the total number of leaves.

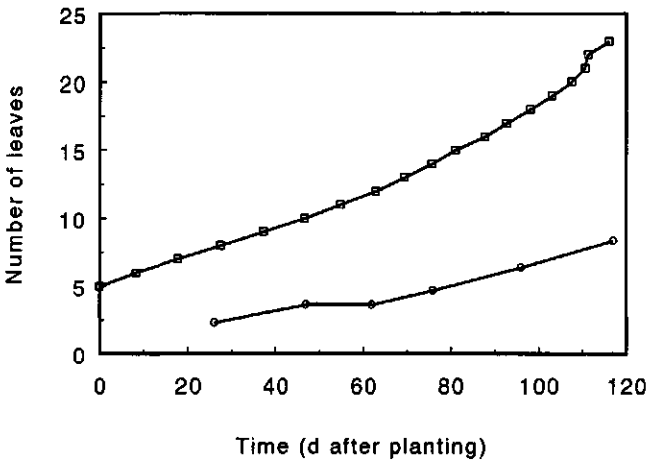

Figure 2.3.2. The mean total number of leaves (□) and mean number of dead leaves (○) as a function of time and averaged over nitrogen treatments for Experiment 1.

Rate of leaf expansion and size of mature leaves

The rate of leaf expansion was influenced by leaf number in all experiments. It increased in Experiment 1 (Figure 2.3.3) with leaf number, until it reached a maximum value of about 4.2 $cm^2\ d^{-1}$ at leaf 11 and decreased again for higher leaf numbers. The curves for leaf expansion rate in Experiments 2 and 3 had similar shapes as in Experiment 1 (data not shown), but the maximum was reached at slightly higher leaf numbers, viz. leaf pair 13+14 (in Experiments 2 and 3 two leaves were combined). The maximum rates were 9.1 and 8.2 $cm^2\ d^{-1}$ in Experiment 2 and 3, respectively, i.e. more than twice as high as in Experiment 1. In Experiment 1 the rate of leaf expansion was not affected by nitrogen treatment, while especially in Experiment 3 it was lower for most leaf pairs in N(0), than in N(200/1) and N(200/5).

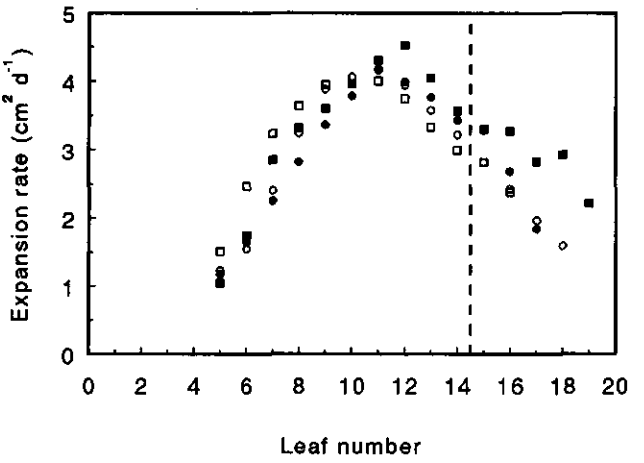

Figure 2.3.3. The rate of leaf expansion in relation to leaf number and nitrogen supply for Experiment 1. Data from not full-grown leaves are included. The dotted line is the border between full-grown (left) and not full-grown leaves (right). (□) N(1.8/2); (○) N(1.8/6); (■) N(5.4/2); (●) N(5.4/6).

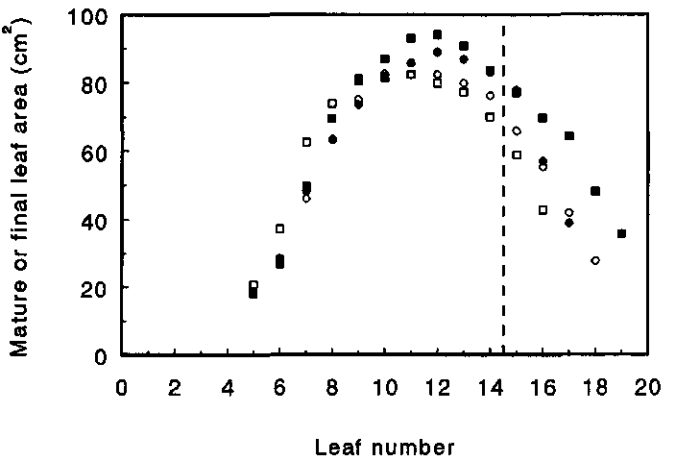

Figure 2.3.4. Mature leaf area of full-grown leaves or final leaf area of not full-grown leaves of Experiment 1 in relation to leaf number and nitrogen supply. The dotted line is the border between full-grown (left) and not full-grown leaves (right). (□) N(1.8/2); (○) N(1.8/6); (■) N(5.4/2); (●) N(5.4/6).

The curves relating mature leaf area (and final leaf area of not full-grown leaves) to leaf number had similar shapes as the corresponding curves for leaf expansion rate (Figure 2.3.4: Experiment 1). These similarities imply that the leaf expansion rate is the major determinant of mature leaf size. Mature (or final) leaf sizes in Experiment 1 fell on one line when plotted against leaf expansion rate (Figure 2.3.5), including the data of not full-grown leaves. This also applies to the data of Experiments 2 and 3, although in those experiments treatment effects on leaf expansion rate and mature leaf size were observed. The largest leaves in Experiments 2 and 3 had a mature area of about 335 (Experiment 2) or 400 cm^2 (Experiment 3), which is much larger than in Experiment 1 (maximum about 95 cm^2). N(0) leaves in Experiments 2 and 3 above leaf number 10 had about half the mature size of N(200/5) leaves.

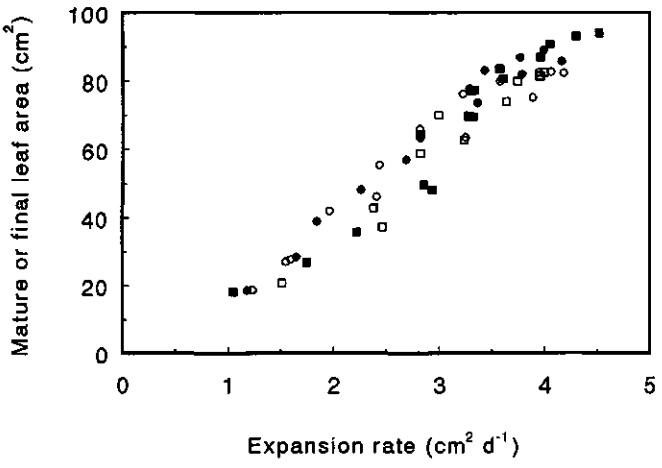

Figure 2.3.5. The relation between mature or final leaf area and leaf expansion rate, irrespective of leaf position (Experiment 1). Data from not full-grown leaves are included. (□) N(1.8/2); (○) N(1.8/6); (■) N(5.4/2); (●) N(5.4/6).

The effective duration of expansion in Experiment 1 (Figure 2.3.6) increased with leaf number from 14 d to 24 d. This variable decreased slowly with leaf number in Experiments 2 and 3 (data not shown). Mean value for Experiment 2 was nearly 40 d and for Experiment 3 nearly 50 d, which is much longer than in Experiment 1. Nitrogen treatments had no effects in any experiment.

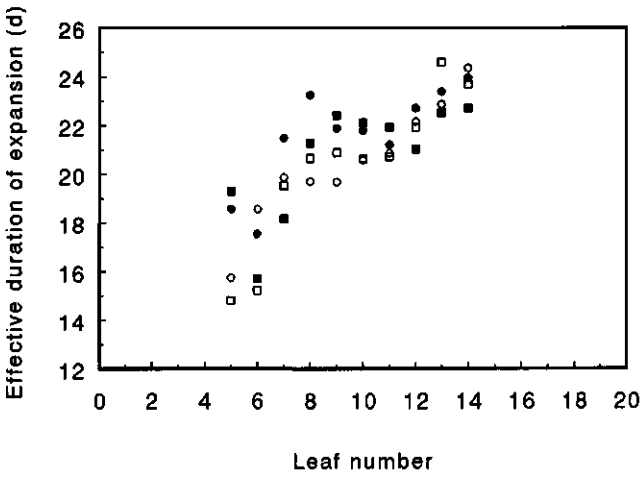

Figure 2.3.6. The effective duration of expansion as a function of leaf position and nitrogen treatment (Experiment 1). Data from not full-grown leaves are not included. (□) N(1.8/2); (○) N(1.8/6); (■) N(5.4/2); (●) N(5.4/6).

Specific leaf area

Specific leaf area (SLA) for all green leaves in Experiment 3 decreased for all treatments from about 116 $cm^2\ g^{-1}$ at 38 DAP to about 103 at 60 DAP and continued to decrease gradually for N(0) plants to reach 91 $cm^2\ g^{-1}$ at 122 DAP (Figure 2.3.7). SLA for N(200/1) and N(200/5) fluctuated after 60 DAP until it reached a final value of 102 for N(200/1) and 112 $cm^2\ g^{-1}$ for N(200/5). At 38 DAP N(0) and N(200/1) were significantly different, at 122 DAP all differences between treatments were significant (LSD-test, P=0.05). SLA of individual leaf pairs was as variable as that over all green leaves (Figure 2.3.8). Differences between leaf pairs were small and all curves were similar in shape: SLA decreased at the onset of the life of a leaf, then remained constant for some time and increased at the end of the leaf life.

SLA over all green leaves in Experiment 2 rapidly decreased from about 145 $cm^2\ g^{-1}$ at 35 DAP to 110 at 56 DAP, but subsequently only slowly until it reached a final value of about 90 $cm^2\ g^{-1}$. It never differed significantly among treatments. SLA in Experiment 1 was hardly influenced by treatments; however, applying nitrogen in two (compared to six) splits resulted in a significantly higher SLA at 47 DAP; 1.8 g N per pot (compared to 5.4) resulted in a significantly (P=0.05) higher SLA at 62 DAP. SLA in this experiment fluctuated between 80 and 100 $cm^2\ g^{-1}$.

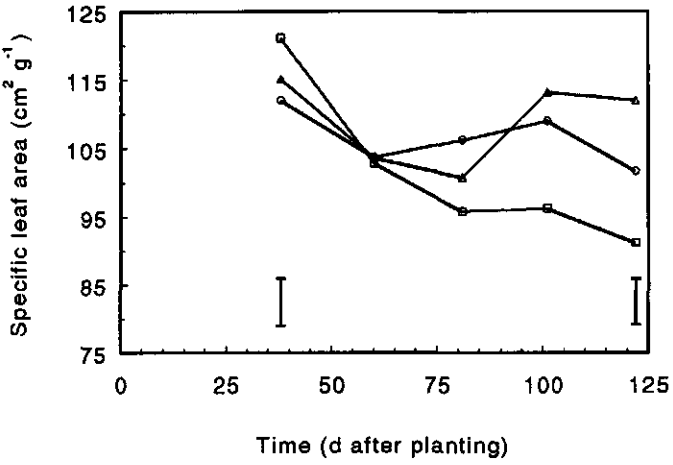

Figure 2.3.7. Specific leaf area over all leaves of a plant for Experiment 3 as a function of time and nitrogen treatment. The vertical bars represent LSD values (P=0.05). (□) N(0); (○) N(200/1); (△) N(200/5).

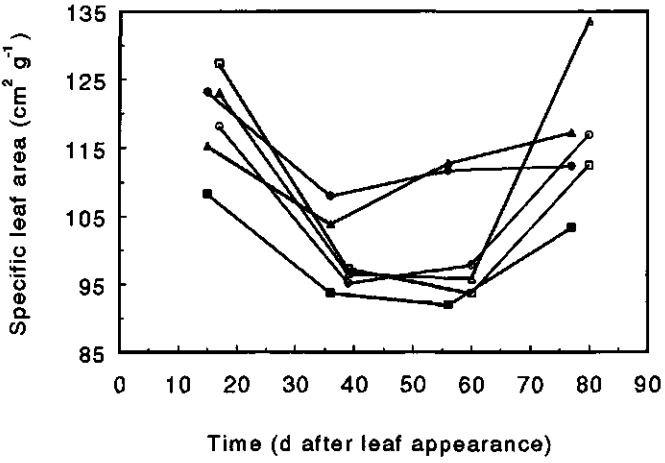

Figure 2.3.8. Specific leaf area of leaf pairs 7+8 and 11+12 against days after leaf appearance (Experiment 3). Leaf pair 7+8: (open markers; (□) N(0); (○) N(200/1); (△) N(200/5)); leaf pair 11+12: (closed markers; (■) N(0); (●) N(200/1); (▲) N(200/5)).

Total leaf area

Leaf area index (LAI) in Experiment 2 (Figure 2.3.9) increased until 91 DAP and subsequently decreased for N(0) and N(200/5) but not for N(200/1). At most occasions total leaf area for the N(200/1) and N(200/5) treatments was significantly (LSD-test, P=0.05) higher than for the N(0) treatment, but N(200/5) was never higher than N(200/1). LAI in Experiment 1 increased over the whole experimental period until N(1.8/2) and N(1.8/6) reached a final LAI of about 1.2 and N(5.4/2) and N(5.4/6) of about 1.8. LAI was significantly higher (LSD-test, P=0.05) for 5.4 g N at 96 and 117 DAP. LAI in Experiment 3 also increased monotonously over the growth period. N(0) reached a final value of 3.16, N(200/1) of 5.24 and N(200/5) of 5.03. LAI in N(0) was significantly (LSD-test, P=0.05) lower than in N(200/1) and N(200/5) at 101 and 122 DAP.

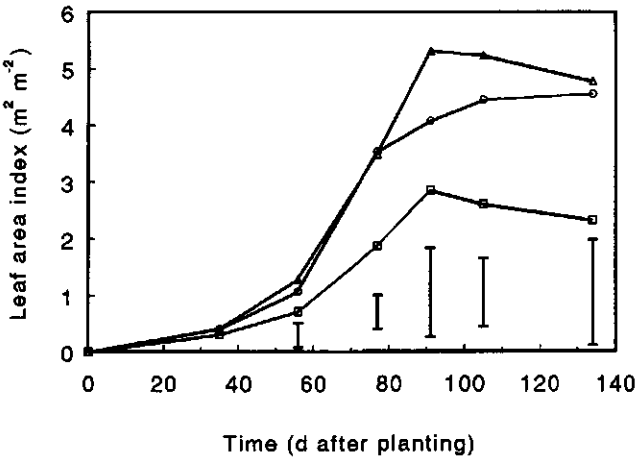

Figure 2.3.9. Total leaf area per plant for Experiment 2 as a function of time and nitrogen treatment. The vertical bars represent LSD values (P=0.05). (□) N(0); (○) N(200/1); (△) N(200/5).

LAI increased exponentially during a large part of the growing period (Table 2.3.4). RGR during this period was calculated from the first harvest until the end of the phase of exponential growth. The largest difference in length of the exponential phase across treatments and experiments, was that between N(200/1) in Experiment 2 and N(200/1) and N(200/5) in Experiment 3 and amounted to 24 d. The largest difference in RGR of the LAI was that between N(1.8/2) in Experiment 1 and N(200/1) in Experiment 2 and amounted to 0.0216 d^{-1}.

Table 2.3.4. Length of the period of exponential increase of the LAI (leaf area index) and the relative growth rate of the LAI, calculated over the period from the first harvest until the end of the phase of exponential growth.

Length of period of exponential increase of LAI (d)

Experiment 1		Experiment 2		Experiment 3	
N(1.8/2)	96	N(0)	91	N(0)	81
N(1.8/6)	96	N(200/1)	77	N(200/1)	101
N(5.4/2)	96	N(200/5)	91	N(200/5)	101
N(5.4/6)	96				

Relative growth rate of LAI (d^{-1})

Experiment 1		Experiment 2		Experiment 3	
N(1.8/2)	0.0297	N(0)	0.0395	N(0)	0.0353
N(1.8/6)	0.0334	N(200/1)	0.0514	N(200/1)	0.0449
N(5.4/2)	0.0406	N(200/5)	0.0452	N(200/5)	0.0411
N(5.4/6)	0.0390				

Discussion

Total green leaf area per plant is the integrated value of the number of green leaves and the size of each leaf. The number of green leaves is the resultant of the rate of leaf appearance and the life span of leaves. The size of each leaf is determined by the rate and duration of leaf expansion. Total leaf area is influenced by treatment effects on any of these components.

The rate of leaf appearance was not affected by amount and timing of nitrogen fertilization. Hay & Brown (1988) and Hay & Kemp (1992) found a linear relation between accumulated temperature and leaf appearance rate. However, the results of Experiment 1 show an increase in leaf appearance rate at the end of the experiment, although the temperature was constant. The rate of leaf appearance over the first 60 days (0.74 leaves per 100 degree days) was in accordance with the results of Hay & Brown (1988) and Hay & Kemp (1992), who found values of 0.76 and 0.74 leaves per 100 degree days, respectively. Mean temperature was lower at the end of Experiments 2 and 3, which resulted in a lower leaf appearance rate. Muchow (1988) found for maize and sorghum (also monocotyledons, but determinate plants) positive effects of nitrogen supply on rate of leaf appearance, but not on the final number of leaves. In experiments with sugar beet (a dicotyledonous plant), Terry (1970) found positive effects of nitrogen supply on the rate of leaf appearance.

Leaf expansion rates and mature leaf sizes in Experiment 1 were not affected by N

treatment. This is surprising, because nitrogen effects on these variables are often found, e.g. by Muchow (1988) for maize and sorghum. In Experiments 2 and 3 the amount of nitrogen influenced both variables positively. Nitrogen enhances mature leaf size by increasing the rate of leaf expansion and not its duration, as observed by Terry (1970) and Milford & Riley (1980) for sugar beet and Radin & Boyer (1982) for sunflower. Hay & Brown (1988) found a constant rate of leaf extension for leek leaves, independent of leaf number, which is not in agreement with the present results. Hay & Brown concluded that leaf length increased with increasing leaf number, because the number of leaves per plant, which were expanding at the same time, increased progressively. The number of expanding leaves per plant increased, because the duration of the linear phase of leaf expansion increased with increasing leaf number.

As a result of the nearly constant rate of leaf appearance and constant, but lower, rate of leaf senescence, the number of green leaves per plant increased linearly, confirming results for leek from Hay & Kemp (1992).

The change in SLA with time was mainly due to changes in SLA of individual leaves. The differences in SLA among leaf numbers were relatively small, compared to the changes in time. More nitrogen and/or more applications of nitrogen affected SLA positively.

Although leaf characteristics such as rate of leaf appearance, rate of leaf expansion and duration of expansion were almost equal for all treatments in Experiment 1, there was a large difference in final LAI between treatments with 1.8 and 5.4 g N per pot, as a result of small differences in the mature area of mainly higher leaf numbers. In Experiments 2 and 3 the differences in LAI among the treatments resulted mainly from differences in the area of mature leaves. The differences in final LAI between Experiment 1 on the one hand and Experiments 2 and 3 on the other also mainly resulted from the differences in the area of mature leaves. The differences in mature leaf area, however, cannot be explained. Differences in mature area per leaf within an experiment were always the result of differences in the amount of nitrogen and not of the timing of nitrogen fertilization.

Leek has, compared to other crops, a relatively low growth rate at the start of the growing season, which appears e.g. from Figure 2.3.9: in Experiment 2 an LAI of three was reached in about 70 days, while a crop as potato reaches this LAI under Dutch circumstances 30-40 days after emergence (Van der Zaag, 1984). A low growth rate implies a small demand for nitrogen. Therefore it was expected that leek would benefit from split nitrogen fertilization, as with this treatment nitrogen was applied when the growth rate (and the demand for nitrogen) was higher. However, splitting (N(200/5) treatments in Experiments 2 and 3) had insignificant effects.

References

Datema, P., H. Niers & A. de Jager, 1986. Comparison of three methods of watering in pot experiments. Rapport IB 4-86. Institute for Soil Fertility, Haren, 121 pp.

Dennett, M.D., B.A. Auld & J. Elston, 1978. A description of leaf growth in *Vicia faba* L. *Annals of Botany* 42: 223-232.

Dragland, S., 1972. Effect of temperature and day-length on growth, bulb formation and bolting in leeks (*Allium porrum* L.). Agricultural University of Norway, Department of Vegetable Crops, Report No. 46. Agricultural University of Norway, Oslo, 23 pp.

Hay, R.K.M. & J.R. Brown, 1988. Field studies of leaf development and expansion in the leek (*Allium porrum*). *Annals of Applied Biology* 113: 617-625.

Hay, R.K.M. & D.R. Kemp, 1992. The prediction of leaf canopy expansion in the leek from a simple model dependent on primordial development. *Annals of Applied Biology* 120: 537-545.

Milford, G.F.J. & J. Riley, 1980. The effects of temperature on leaf growth of sugar beet varieties. *Annals of Applied Biology* 94: 431-443.

Muchow, R.C., 1988. Effect of nitrogen supply on the comparative productivity of maize and sorghum in a semi-arid tropical environment I. Leaf growth and leaf nitrogen. *Field Crops Research* 18: 1-16.

Radin, J.W. & J.S. Boyer, 1982. Control of leaf expansion by nitrogen nutrition in sunflower plants. Role of hydraulic conductivity and turgor. *Plant Physiology* 69: 771-775.

Terry, N., 1970. Developmental physiology of sugar beet. II. Effects of temperature and nitrogen supply on the growth, soluble carbohydrate content and nitrogen content of leaves and roots. *Journal of Experimental Botany* 21: 477-496.

Vos, J. & H. Biemond, 1992. Effects of nitrogen on the development and growth of the potato plant. 1. Leaf appearance, expansion growth, life spans of leaves and stem branching. *Annals of Botany* 70: 27-35.

Zaag, D.E. van der, 1984. Reliability and significance of a simple method of estimating the potential yield of the potato crop. *Potato Research* 27: 51 - 73.

SECTION 2.4

Effects of nitrogen on development and growth of the leaves of vegetables. 3. Appearance and expansion growth of leaves of spinach

H. Biemond

Abstract

Leaf development in spinach (*Spinacia oleracea* L.) was studied in six field and glasshouse experiments. Treatments consisted of different amounts and different ways of fractioning nitrogen supply. Rates of leaf appearance and leaf expansion were recorded, as well as final leaf size.

The rate of leaf appearance varied between 0.16 and 0.57 d^{-1} across experiments, but was hardly affected by nitrogen treatment. The rate of leaf expansion and the mature leaf area increased with leaf number, reached a maximum at leaf pair 3+4 or 5+6 and decreased subsequently. Both characteristics were positively correlated to nitrogen supply. The duration of expansion was not influenced by nitrogen treatments and varied between 15 and 30 days in most experiments. The rate of leaf expansion was the main factor determining mature leaf size.

Specific leaf area over all green leaves slowly decreased with time in most experiments and was around 300 cm^2 g^{-1}. As the differences in the number of leaves were small, the differences in total green leaf area per plant resulted from differences in (mature) leaf area of individual leaves.

Introduction

Spinach is an annual plant with a short growth cycle, compared to other vegetable crops. The vegetative growth consists of the production of a leaf rosette. The crop is usually harvested for fresh consumption at the end of the vegetative phase, before the elongation of a flower stalk starts.

The size of the leaf rosette is very important, because it determines crop yield: the leaf blades and part of the petioles are harvested and used. The size of the leaf area per plant is determined by rate and duration of leaf appearance, rate and duration of leaf expansion,

mature leaf area and rate of leaf senescence. Nitrogen nutrition can have large effects on some of these variables. Positive effects of nitrogen on the rate of leaf appearance were reported by Terry (1970) for sugar beet (sugar beet belongs to the same family (Chenopodiaceae) as spinach). The main effects of nitrogen are usually on the rate of leaf expansion: more nitrogen usually leads to faster increase of leaf area and a larger final area (Terry *et al.*, 1981). Milford *et al.* (1985) observed for sugar beet that differences in leaf size were usually associated with differences in leaf expansion rate and not with differences in duration of expansion.

Information available on nitrate accumulation in spinach and photosynthesis of spinach leaves is abundant, but quantitative information on the effects of nitrogen on development and growth of spinach leaves is scarce. This paper reports on field and glasshouse experiments with spinach, designed to quantify the effects of nitrogen supply on rate and duration of leaf appearance, expansion and senescence.

Materials and methods

Four field and two glasshouse experiments are described. Table 2.4.1 summarizes experimental details.

Table 2.4.1. General information about six experiments with spinach: type of experiment, growing period, year, sowing date and date of final observations.

Experiment	Type of experiment	Growing period	Year	Sowing date	Final observations
1	field	summer	1991	25 Apr	25 Jun
2	field	autumn	1991	5 Aug	25 Sep
3	field	summer	1992	7 May	14 Jul
4	field	autumn	1992	19 Aug	20 Oct
5	glasshouse	summer	1993	18 May	5 Jul
6	glasshouse	autumn	1993	4 Aug	12 Oct

Plant culture - field

The field experiments (Experiments 1-4) were conducted on a sandy soil, on the experimental farm of the Department. Cv. Trias was sown at 5.5 g seed per m^2, ca 1.5 cm deep, in rows with a spacing of 12.5 cm, resulting in a plant density of about 400 plants m^{-2}. Irrigation was applied whenever necessary.

Plant culture - glasshouse

Cv. Trias was also used in the glasshouse experiments (Experiments 5 and 6). Rectangular trays were used with dimensions: length 45, width 30 and depth 15 cm. After filling these with sand, free from organic matter, seventy-five seeds were sown, ca 1.5 cm deep in three rows, resulting in a plant density similar to that in Experiments 1-4. The trays were placed in a glasshouse, set to maintain a day temperature of 18 °C (12 h) and a night temperature of 12 °C. In Experiment 5, natural light was supplemented with 400 Watt Philips SON-AGRO-T lamps at a density of 0.7 lamps m^{-2}. Water was administered from the top of the tray until the plants were well established. Subsequently, a 8-cm-high saucer under the trays was filled daily with water to its brim. Once every three weeks the plants were allowed to absorb all the water from the saucers. Then water was administered from the top once. Subsequently, the standard way of supplying water via the bottom saucer was resumed (Datema *et al.*, 1986).

Treatments

The treatments consisted of different total amounts and different dates of application of nitrogen. Other nutrients were supplied in equal amounts to all treatments. Experiment 1 had five treatments: N(0): no fertilizer nitrogen; N(75+50): 125 kg N per ha, applied in two splits; N(125/5): 125 kg N per ha, in five splits; N(120+80): 200 kg N per ha, in two splits; N(200/5): 200 kg N per ha, in five splits (Table 2.4.2). Experiment 2 also had five treatments: N(0): no fertilizer nitrogen; N(50+25): 75 kg N per ha, applied in two splits; N(75/5): 75 kg N per ha, in five splits; N(90+60): 150 kg N per ha, in two splits; N(150/5): 150 kg N per ha, in five splits (Table 2.4.2). Experiment 3 and 4 had the same three treatments: N(0): no fertilizer nitrogen; N(200/1): 200 kg N per ha, applied shortly after sowing; N(200/5): 200 kg N per ha, applied in five splits (Table 2.4.2). Experiments 1-4 were laid out in a split-plot design with nitrogen treatment as main factor and harvest date (see *Destructive sampling*) as split factor. All four field experiments had four blocks. In the statistical analyses, each harvest was analysed separately.

In Experiment 5, treatments were: N(1.12): 1.12 g N per tray, with nitrogen limitation throughout; N(1.68;L(ate)): 1.68 g N per tray, with limiting supply in the early stages of growth followed by non-limiting supply; N(3.36): 3.36 g N per tray; non-limiting nitrogen supply throughout (Table 2.4.2). In Experiment 6, treatments were: N(1.68): 1.68 g N per tray, with nitrogen limitation throughout; N(3.36;L(ate)): 3.36 g N per tray, with limiting supply in the early stages of growth followed by non-limiting supply; N(5.04): 5.04 g N per tray; non-limiting nitrogen supply throughout (Table 2.4.2). Experiments 5 and 6 were laid out in a randomised complete block design with four blocks. Each tray was regarded as one experimental unit. In the statistical analyses, each harvest (see *Destructive sampling*) was

Table 2.4.2. Amounts (in kg per ha (Experiments 1-4) or g per tray (Experiments 5 and 6)) and dates (in days after emergence (DAE)) of application of fertilizer nitrogen in two field experiments in 1991 (Experiments 1 and 2), two field experiments in 1992 (Experiments 3 and 4) and two glasshouse experiments in 1993 (Experiments 5 and 6) with spinach. A negative time means application before emergence (emergence = day 0), usually at sowing date. N(...) is omitted from treatment codes.

	Experiment 1					Experiment 2					Experiment 3			Experiment 4			Experiment 5			Experiment 6		
Time																						
(DAE)	0	75+50	125/5	120+80	200/5	0	50+25	75/5	90+60	150/5	0	200/1	200/5	0	200/1	200/5	1.12	1.68;L	3.36	1.68	3.36;L	5.04
-15																	0.28	0.28	0.84			
-12	0	75	25	120	40																	
-8						0	50	15	90	30	0	200	40									
-7														0	200	40				0.28	0.28	0.84
6						0	0	15	0	30												
7														0	0	40	0.28	0.28	0.84	0.28	0.28	0.84
10											0	0	40									
13														0	0	40						
14						0	0	15	0	30												
15	0	0	25	0	40																	
17											0	0	40									
20						0	25	15	60	30				0	0	40						
21																	0.28	0.28	0.84			
25											0	0	40									
26																	0.28	0.84	0.84			
27						0	0	15	0	30				0	0	40						
28	0	0	25	0	40																	
31											0	0	40									
32																				0.28	0.28	0.84
34	0	50	25	80	40																	
41	0	0	25	0	40																	
42																				0.28	0.84	0.84
49																				0.56	1.68	1.68
Total	0	125	125	200	200	0	75	75	150	150	0	200	200	0	200	200	1.12	1.68	3.36	1.68	3.36	5.04

analysed separately.

Estimating leaf variables

In all experiments, information about leaf growth was deduced from data, collected during weekly intermediate harvests, on leaf appearance, leaf senescence and increase of leaf area with time. Figure 2.4.1 presents typical examples of the dynamics of leaf expansion. The largest part of the leaf area is formed during a phase with almost linearly increasing area. Therefore, for the linear phase of each leaf pair, linear regression analysis was carried out with leaf area as dependent and time as independent variable. The expansion rate was characterised by the slope of the regression (tan α in Figure 2.4.1). The 'effective duration of expansion' (d; length of line "a" in Figure 2.4.1) is defined as the maximum area of a leaf pair (cm^2; length of line "b" in Figure 2.4.1) divided by its expansion rate ($cm^2\ d^{-1}$) (cf. Vos & Biemond, 1992).

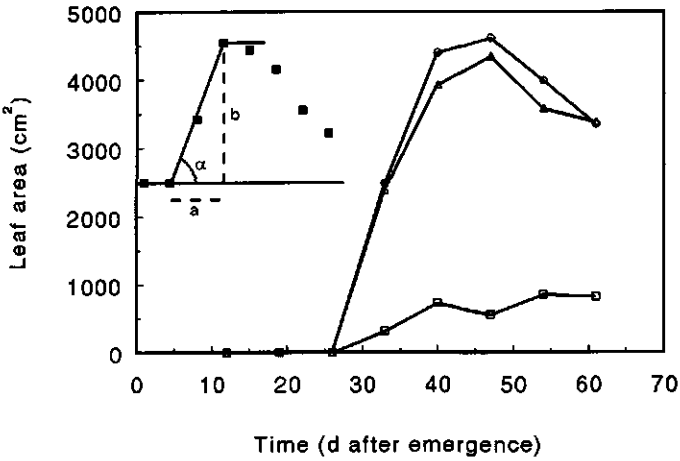

Figure 2.4.1. The change over time of the leaf area of leaves 13+14, expressed per m^2 soil surface (Experiment 3). (□) N(0); (○) N(200/1); (△) N(200/5). The upper left part explains the method of calculating the rate and duration of leaf expansion. The length of line "a" represents the effective duration of leaf expansion (d), the length of line "b" the maximum leaf area (cm^2) and tan α the rate of leaf expansion ($cm^2\ d^{-1}$).

Destructive sampling

Plants were sampled six, seven or eight times in each experiment at approximately weekly intervals after emergence until the final date (Table 2.4.1). In Experiments 1-4 at each sampling date 0.25 m^2 per treatment was harvested from each block. In Experiments 5 and 6 at each sampling date one tray (= 0.135 m^2) per treatment was used from each block.

The measurements included leaf area, fresh and dry weights of leaf blades, petioles, hypocotyl + stem (= stem until leaf number 18) and top (= stem + leaves + flowers from leaf number 19; only observed in summer spinach). In Experiments 3-6 leaves were split into leaf blades and petioles. Leaves, leaf blades and petioles were sampled until leaf number 18. In all experiments material from two successive leaf positions on the stem was pooled.

Results

Rate of leaf appearance and number of leaves

The differences in total number of leaves between treatments were small in most experiments. Figure 2.4.2 shows the number of leaves of Experiment 6 as a function of time. N(5.04) (N non-limiting) had significantly more leaves than N(1.68) (N limiting) and N(3.36;L) (transition from limiting to non-limiting N supply) at 33, 47 and 56 DAE; at 61 DAE, all treatments were significantly (LSD-test, P=0.05) different. The rate of leaf appearance was constant in N(5.04), but decreased in N(1.68) and N(3.36;L) about four weeks after emergence.

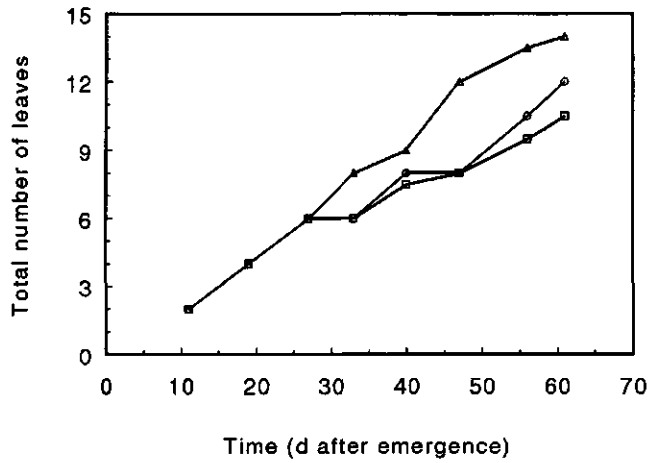

Figure 2.4.2. The total number of leaves for Experiment 6 as a function of time and nitrogen treatment. (□) N(1.68); (○) N(3.36;L); (△) N(5.04).

The highest rates of leaf appearance were observed in Experiments 3 and 5; the lowest rates in Experiments 4 and 6 (Table 2.4.3). The differences among experiments were very large: the lowest rate was 0.16 d^{-1} for N(1.68) in Experiment 6; the highest 0.57 d^{-1} for

N(200/1) and N(200/5) in Experiment 4. The total number of leaves per plant (Table 2.4.3) was significantly different (LSD-test, $P=0.05$) among treatments in Experiments 4 and 6, with more leaves at the higher nitrogen applications.

Table 2.4.3. Mean rate of leaf appearance (values for Experiments 1, 3 and 5 over the period, that the plants had less than 18 leaves) and total number of leaves per plant in Experiment 1 at 42 DAE, Experiment 2 at 35 DAE, Experiment 3 at 33 DAE, Experiment 4 at 57 DAE, Experiment 5 at 27 DAE and Experiment 6 at 61 DAE; this was in Experiments 1, 3 and 5 the last sampling date, before the plants had 18 leaves. Different letters indicate significant differences (LSD-test; $P=0.05$) between treatments.

Mean rate of leaf appearance (d^{-1})

Experiment 1		Experiment 2		Experiment 3		Experiment 4		Experiment 5		Experiment 6	
N(0)	0.42	N(0)	0.38	N(0)	0.51	N(0)	0.19	N(1.12)	0.43	N(1.68)	0.16
N(75+50)	0.44	N(50+25)	0.40	N(200/1)	0.57	N(200/1)	0.20	N(1.68;L)	0.44	N(3.36;L)	0.18
N(125/5)	0.44	N(75/5)	0.40	N(200/5)	0.57	N(200/5)	0.20	N(3.36)	0.48	N(5.04)	0.25
N(120+80)	0.44	N(90+60)	0.38								
N(200/5)	0.44	N(150/5)	0.40								

Total number of leaves per plant

Experiment 1		Experiment 2		Experiment 3		Experiment 4		Experiment 5		Experiment 6	
N(0)	14a	N(0)	12a	N(0)	15a	N(0)	10a	N(1.12)	11a	N(1.68)	11a
N(75+50)	14a	N(50+25)	12a	N(200/1)	16a	N(200/1)	12b	N(1.68;L)	11a	N(3.36;L)	12b
N(125/5)	14a	N(75/5)	12a	N(200/5)	16a	N(200/5)	12b	N(3.36)	12a	N(5.04)	14c
N(120+80)	14a	N(90+60)	12a								
N(200/5)	14a	N(150/5)	12a								

Rate of leaf expansion and maximum size of leaves

The rate of leaf expansion varied with leaf number and was influenced by nitrogen in all experiments. Usually it increased with leaf number until a maximum at leaf pair 3+4 or 5+6 and subsequently decreased (Figure 2.4.3: Experiment 3). In N(0) (N limiting) in Experiment 3 it was lower than in N(200/1) (N non-limiting, single application) and N(200/5) (N non-limiting, five splits) for all leaf pairs, except 1+2. In all experiments leaf pairs of treatments that received higher nitrogen applications had higher rates of leaf expansion. The maximum rate of leaf expansion per leaf pair in each experiment is shown in Table 2.4.4, to illustrate the differences among the experiments: it varied from 1.45 to 3.60 $cm^2\ d^{-1}$.

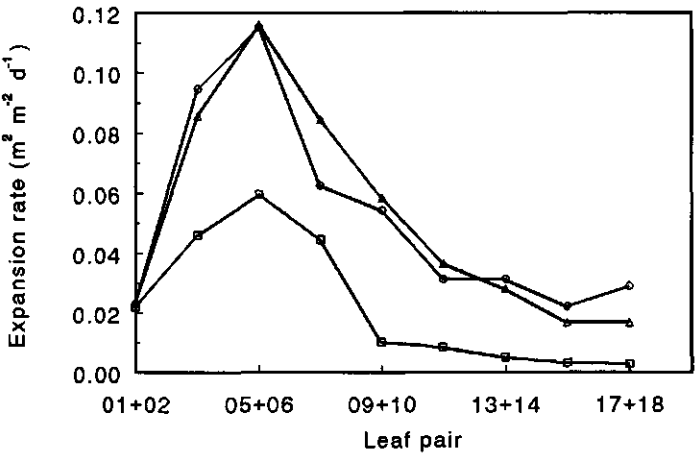

Figure 2.4.3. The rate of leaf expansion of successive leaf pairs in relation to nitrogen supply for Experiment 3. The rate of leaf expansion is expressed per m^2 surface area. (Plant density in this experiment: 386 plants m^{-2}.) (□) N(0); (○) N(200/1); (△) N(200/5).

Table 2.4.4. Maximum rate of leaf expansion per leaf pair and maximum area per leaf pair with the leaf pairs and treatments, to which they refer. Plant density in each experiment is taken into account, when calculating these figures.

Experiment	Maximum rate of leaf expansion (cm^2 d^{-1})	Leaf pair and treatment to which maximum rate refers
1	2.06	5+6, N(120+80)
2	2.99	5+6, N(150/5)
3	3.01	5+6, N(200/5)
4	3.60	3+4, N(200/1)
5	1.87	9+10, N(3.36)
6	1.46	3+4, N(5.04)
	Maximum leaf area (cm^2)	**Leaf pair and treatment to which maximum leaf area refers**
1	43.1	5+6, N(120+80)
2	63.7	5+6, N(150/5)
3	45.6	5+6, N(200/1)
4	61.8	5+6, N(200/1)
5	21.6	7+8, N(3.36)
6	43.5	5+6, N(5.04)

The shape of the curves relating mature leaf areas (and final leaf area of not full-grown leaves) to leaf number was similar to that of the corresponding curves for rate of leaf expansion (Figure 2.4.4: Experiment 3; in Experiment 3, all leaf pairs were full-grown). This suggests that the rate of leaf expansion is a major determinant of mature leaf size. Mature (or final) leaf size for all experiments was uniformly related to leaf expansion rate even when not full-grown leaves were included (Figure 2.4.5). The values of Experiment 6 show the largest deviation. The maximum final leaf area in each experiment, expressed per leaf pair, is presented in Table 2.4.4, with the corresponding leaf pair. This maximum was reached by the same or a nearby leaf pair, exhibiting maximum rate of leaf expansion.

The effective duration of expansion in most experiments increased with leaf number until leaf pair 3+4 or 5+6 and subsequently decreased. It varied between 15 and 30 days, except in Experiment 6, where it varied between 20 and 50 days. Nitrogen treatments did not influence effective duration of expansion in any experiment. The effective duration of expansion in Experiment 3 (Figure 2.4.6) was nearly constant at 15 d, irrespective of leaf number and nitrogen treatment, except for leaf pair 1+2.

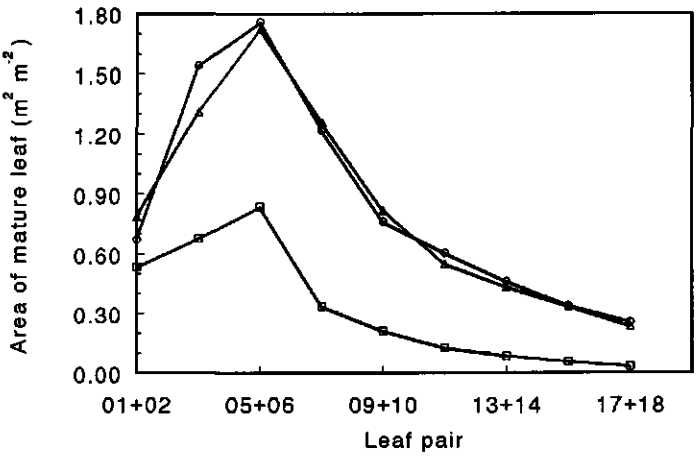

Figure 2.4.4. Mature leaf area of leaf pairs of Experiment 3 in relation to nitrogen supply. The mature leaf area is expressed per m^2 soil surface. (Plant density in this experiment: 386 plants m^{-2}.) (□) N(0); (○) N(200/1); (△) N(200/5).

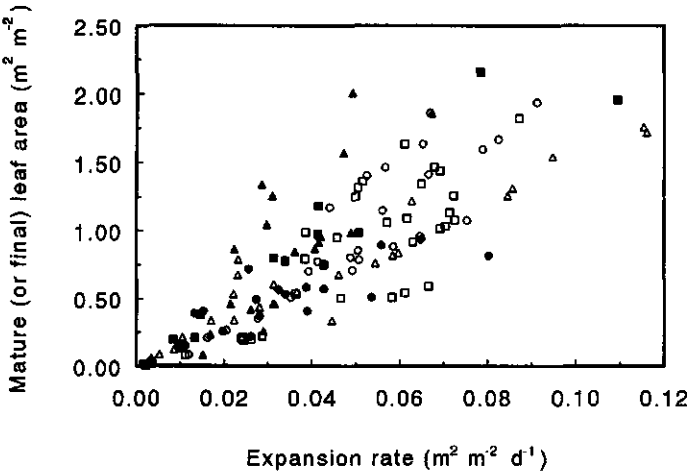

Figure 2.4.5. The relation between mature leaf area and rate of leaf expansion, irrespective of leaf position. (□) Experiment 1; (○) Experiment 2; (△) Experiment 3; (■) Experiment 4; (●) Experiment 5; (▲) Experiment 6.

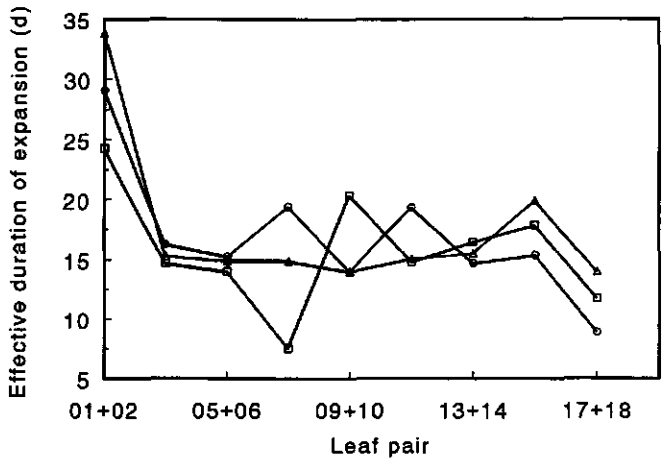

Figure 2.4.6. The effective duration of expansion as a function of leaf position and nitrogen treatment (Experiment 3). (□) N(0); (○) N(200/1); (△) N(200/5).

Specific leaf area

SLA (specific leaf area, calculated as the ratio between the total green area of all leaves and the sum of their dry weights) in Experiment 4 is presented in Figure 2.4.7. It increases from 220 $cm^2\ g^{-1}$ at 15 DAE for N(0) (N limiting) to 295 $cm^2\ g^{-1}$ at 29 DAE and subsequently decreased to reach a final value of 206 $cm^2\ g^{-1}$ at 57 DAE. SLA of N(200/1) (N non-limiting, single application) and N(200/5) (N non-limiting, five splits) was much higher than that of N(0) at 15 DAE, i.e. 352 and 310 $cm^2\ g^{-1}$, respectively, but decreased continuously during the growing season, to reach a final value of 247 $cm^2\ g^{-1}$ at 57 DAE. SLA of the N(0) plants was significantly (LSD-test, $P=0.05$) lower than that of N(200/5) plants at five and lower than that of N(200/1) plants at four (intermediate) harvests (Figure 2.4.7). This trend of decreasing SLA in the course of the growing period was observed in all other experiments, except in Experiment 5, although in Experiment 1 it increased again at the end of the growing period and in Experiment 3 the decrease was observed only in N(0). In Experiment 5 SLA increased in the course of the growing period. Significant (LSD-test, $P=0.05$) treatment effects were observed in all experiments. The differences were sometimes small, but clear differences were always observed among treatments supplied with different amounts of nitrogen: less fertilizer nitrogen resulted in a lower SLA. SLA was around 300 $cm^2\ g^{-1}$ in all experiments.

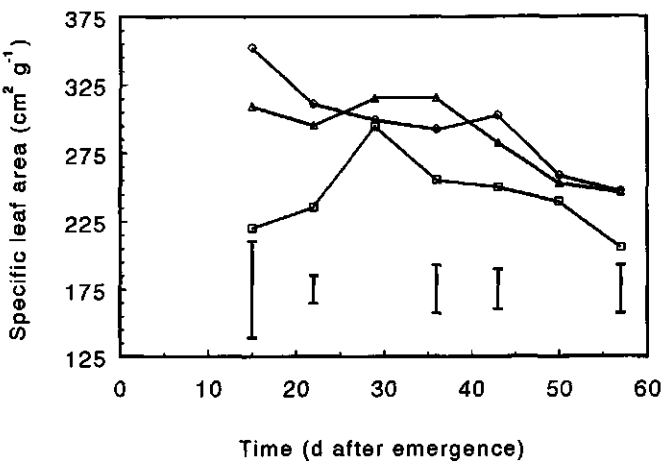

Figure 2.4.7. Specific leaf area over all green leaves of a plant for Experiment 4 as a function of time and nitrogen treatment. The vertical bars represent LSD values ($P=0.05$). (□) N(0); (○) N(200/1); (△) N(200/5).

In the course of the life span of individual leaves, SLA did not show a decrease, but a fast increase at the beginning of the leaf's life and a more or less constant SLA subsequently (Figure 2.4.8: leaves 3+4 and 7+8 of Experiment 4). The differences among leaf pairs were considerable, SLA being much lower for higher leaf numbers. These trends in SLA of leaf pairs were also observed in other experiments.

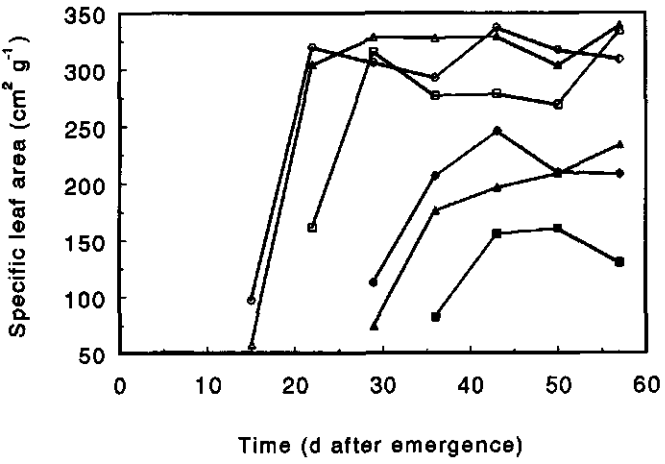

Figure 2.4.8. Specific leaf area of leaf pairs 3+4 and 7+8 against days after emergence (Experiment 4). Leaf pair 3+4: (open markers; (□) N(0); (○) N(200/1); (△) N(200/5)); leaf pair 7+8: (closed markers; (■) N(0); (●) N(200/1); (▲) N(200/5)).

Total green leaf area

Except in Experiments 3 and 4, total green leaf area increased more or less linearly until the end of each experiment (Figure 2.4.9: Experiment 6). In Experiment 3 it increased until 33 DAE and subsequently decreased until the end of this experiment. In Experiment 4 it increased until 36 DAE and was more or less constant further on. The maximum (usually final) values of the LAI (leaf area index), attained in each experiment, were between 5.0 and 7.0. In Experiment 6, the LAI of N(1.68) and N(3.36;L) was significantly (LSD-test, P=0.05) below that of N(5.04) from 27 till 56 DAE; at 61 DAE LAI was significantly different among all treatments. In Experiments 1-4, LAI of N(0) (limiting N throughout) was significantly (LSD-test, P=0.05) below that of the other treatments at most (intermediate) harvests, while the differences in LAI among the other treatments were small and mostly insignificant; in Experiment 5 treatment N(3.36) showed significantly higher LAI values than N(1.12) and N(1.68;L) for most (intermediate) harvests, while differences between N(1.12) and N(1.68;L) were insignificant. Generally, LAI for the treatments with no (Experiments

1-4) or a small supply of fertilizer nitrogen (Experiments 5 and 6) was less than half of that for the treatments with a high amount of fertilizer nitrogen.

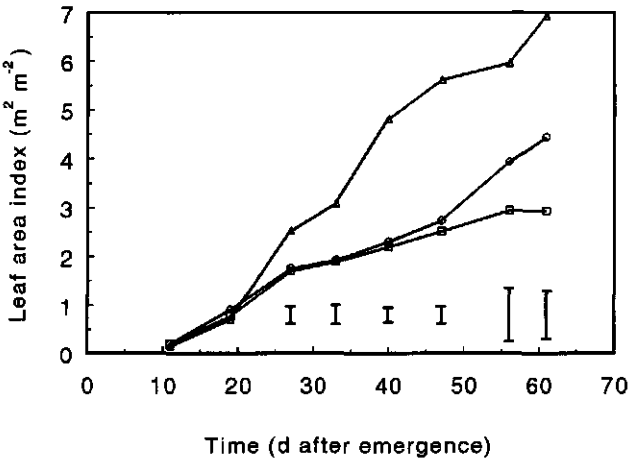

Figure 2.4.9. Total leaf area per m^2 for Experiment 6 as a function of time and nitrogen treatment. The vertical bars represent LSD values (P=0.05). (□) N(1.68); (○) N(3.36;L); (△) N(5.04).

Discussion

Total green leaf area (a variable with important effects on the yield of fresh spinach) is determined by the number of leaves and their size and is affected by treatment effects on either of these characteristics.

The rate of leaf appearance, which mainly determines the final number of leaves, was hardly influenced by nitrogen treatment in any of the experiments. Nitrogen effects on the rate of leaf appearance are common, but usually small, compared to the differences in available nitrogen. Terry (1970) reports positive effects of nitrogen supply on the rate of leaf appearance in sugar beet as does Muchow (1988) for maize and sorghum, although the final number of leaves was not affected. The differences in rate of leaf appearance among our experiments were large: in most experiments with autumn spinach (except Experiment 2) it was much lower than in the experiments with summer spinach. These differences were partly the result of higher average temperatures in the experiments with summer spinach. Temperature influences the rate of development of spinach (Parlevliet, 1967). This does not give a complete explanation, because temperatures in Experiments 5 and 6 were equal. A second reason is that increasing daylength increases the rate of development (Parlevliet,

1967).

The maximum size of leaves was mainly determined by the rate of leaf expansion and hardly by the duration of leaf expansion. Nitrogen treatments only affected the rate and not the duration of leaf expansion, confirming results of Milford & Riley (1980) for sugar beet and of Radin & Boyer (1982) for sunflower. Splitting fertilizer nitrogen application, compared to a single application, did not affect the rate of leaf expansion. The relatively large mature leaf area at a given expansion rate in Experiment 6 (Figure 2.4.5) was caused by the relatively long effective duration of expansion of those leaves.

Overall SLA for all green leaves decreased as a result of differences in SLA between successive leaf pairs and not as a result of changes in SLA for any given leaf pair with time. More nitrogen positively affected SLA. A young leaf is bubbled: this results in a low SLA at the beginning of its life.

Differences in total green leaf area among nitrogen treatments mainly resulted from differences in leaf size, associated with differences in the rate of leaf expansion; differences in the number of leaves were small. Senescence of leaves was not observed, except in Experiment 3, even though most experiments continued far beyond the normal harvest date. As a consequence, no decrease in LAI was observed at the end of an experiment.

The results of these experiments suggest that sufficient nitrogen must be available at the beginning of the growing period for optimal growth of a spinach crop, because effects of nitrogen shortage are instantaneous: leaves of N limited plants were already smaller at the first sampling date after emergence. The spinach crop, with its short growing cycle, did not benefit from splitting the nitrogen fertilisation: applying nitrogen in five splits instead of one single application rarely resulted in differences in number and size of the leaves of spinach plants.

The present findings elucidate the reactions of the plant to nitrogen fertilisation. This enables us to develop modules on plant development in crop simulation models and to fine-tune nitrogen fertilisation.

References

Datema, P., H. Niers & A. de Jager, 1986. Comparison of three methods of watering in pot experiments. Rapport IB 4-86. Institute for Soil Fertility, Haren, 121 pp.

Milford, G.F.J., T.O. Pocock, J. Riley & A.B. Messem, 1985. An analysis of leaf growth in sugar beet. III. Leaf expansion in field crops. *Annals of Applied Biology* 106: 187-203.

Milford, G.F.J. & J. Riley, 1980. The effects of temperature on leaf growth of sugar beet varieties. *Annals of Applied Biology* 94: 431-443.

Muchow, R.C., 1988. Effect of nitrogen supply on the comparative productivity of maize and sorghum in a

semi-arid tropical environment I. Leaf growth and leaf nitrogen. *Field Crops Research* 18: 1-16.

Parlevliet, J.E., 1967. The influence of external factors on the growth and development of spinach cultivars (*Spinacia oleracea* L.). Meded. Landbouwhogeschool 67-2. Veenman, Wageningen, 75 pp.

Radin, J.W. & J.S. Boyer, 1982. Control of leaf expansion by nitrogen nutrition in sunflower plants. Role of hydraulic conductivity and turgor. *Plant Physiology* 69: 771-775.

Terry, N., 1970. Developmental physiology of sugar beet. II. Effects of temperature and nitrogen supply on the growth, soluble carbohydrate content and nitrogen content of leaves and roots. *Journal of Experimental Botany* 21: 477-496.

Terry, N., L.J. Waldron & S.E. Taylor, 1981. Environmental influences on leaf expansion. In: J.E. Dale & F.L. Milthorpe (Eds.), The growth and functioning of leaves. Cambridge University Press, Cambridge, pp. 179-193.

Vos, J. & H. Biemond, 1992. Effects of nitrogen on the development and growth of the potato plant. 1. Leaf appearance, expansion growth, life spans of leaves and stem branching. *Annals of Botany* 70: 27-35.

CHAPTER 3

Effects of nitrogen on accumulation and partitioning of dry matter, nitrogen and nitrate of some vegetable crops

SECTION 3.1

Effects of nitrogen on the development and growth of the potato plant. 2. The partitioning of dry matter, nitrogen and nitrate

H. Biemond and J. Vos

Abstract

Potatoes (*Solanum tuberosum* L.) were planted in pots in a temperature controlled glasshouse. The treatments consisted of three levels of nitrogen supply, i.e. 2.5, 8 and 16 g nitrogen per pot (treatments called N1, N2 and N3). The accumulation rates of dry matter and nitrogen showed an upper limit of response to nitrogen supply; N3 plants continued to accumulate dry matter and N at a constant rate for a longer period of time than N2 and N1 plants. The uptake of nitrogen slowed earlier in time than the rate of dry matter accumulation in all treatments. The proportion of the dry matter in tubers of mature plants was not affected by nitrogen treatment, but the start of tuber bulking was delayed in the N3 plants. The final proportion of total plant nitrogen in the tubers was similar for all treatments. The concentration of nitrogen in the dry matter of mature plants increased with the level of N supply. Maximum haulm weight increased with the level of N supply. Apical lateral branches of the first and second order made up larger proportions of the total haulm dry weight and total leaf area as more nitrogen was supplied. Yet, the distribution of dry matter over stems and leaves was not different between nitrogen treatments. Stems were the most responsive to N treatment in terms of N concentrations.

In each of the component organs (stems, leaves, tubers) the concentration of nitrogen declined with time. Fairly strong associations were observed between the concentrations of N in component organs. The concentration of nitrate in leaves usually increased initially with leaf age, peaked and declined. A substantial part of the differences between treatments in the concentrations of N in leaf dry matter were attributable to differences in nitrate concentration. Nitrate in stems and tubers fell virtually below the limit of detection at total nitrogen concentration of less than 1%, but increased in proportion to total N above that threshold, especially in stems.

Introduction

Nitrogen affects the yield of potato (*Solanum tuberosum* L.) crops mainly by its effects on leaf area duration (e.g. Gunasena & Harris, 1968). It is our objective to analyze and understand that response. With our study we seek to contribute to the knowledge of the physiological aspects of nitrogen nutrition, but the analysis is dominated by our aim to provide material for the construction of simulation models of potato that take the nitrogen economy into account. We have an 'explanatory approach' of modelling in mind. That means that the behaviour of a crop is explained by an integration of knowledge of the processes at the level of the individual plant and its component organs. In this context we reported about developmental aspects, notably leaf growth and stem branching, in the companion paper (Vos & Biemond, 1992). This paper focusses primarily on plant growth, nitrogen uptake and aspects of partitioning of dry matter, nitrogen and nitrate and asks the question how these aspects are influenced by nitrogen supply. It appears from the analysis that the regulation of nitrogen uptake and the termination of further development (apical branching) in response to declining uptake are important blank spots in our insight of the responses to nitrogen of potato.

Materials and methods

The design of the experiment was described in detail in Vos & Biemond (1992). Therefore, a brief description suffices here.

Plant culture

Seed potatoes (cv. Bintje) were planted on March 28, 1988 in 20 litre pots, containing sand, free from organic matter. The pots were placed in a glasshouse, kept at 18 °C during the day (12 h) and at 12 °C during the night (12 h). Natural light was supplemented with 400 Watt Philips SON-T fluorescent lamps, installed at a density of 0.7 lamps m^{-2}. The date of emergence was April 10. The number of stems per plant was reduced to three (one plant per pot).

Treatments

The treatments consisted of three different amounts of nitrogen. The other nutrients were supplied in equal amounts to all treatments. Nutrient solutions were supplied on seven occasions at two weeks intervals, starting at one week after emergence. In total 2.5 g N per pot was supplied to the low N treatment (N1), 8.0 g N per pot to the intermediate N

treatment (N2), and 16.0 g N per pot to the high N treatment (N3). One pot of each treatment was regarded as a block.

Sampling and plant analyses

Destructive analyses of plants were made on six occasions (30, 44, 58, 79, 100 and 120 DAE (days after emergence)). Five randomly selected blocks of plants were used at each sampling date. The measurements included leaf area and fresh weight and dry weights of leaves, branches, stolons and tubers.

The 15 blocks of plants on which non-destructive measurements of leaf growth were made (see Vos & Biemond, 1992) were harvested after plant senescence (at 127 DAE for the N1 and N2 treatment and at 148 DAE for the N3 treatment). At these final harvests plants were dissected into above-ground haulms, below-ground stems plus stolons, and tubers.

Dried and weighed samples were ground. To reduce the number of samples for chemical analysis, we bulked the samples from the replicates, mixed them thoroughly, and took one subsample which was subsequently analysed for nitrogen and nitrate. However, samples from replicates at the final harvest were not bulked, but chemical analysis was done for all samples.

Plant samples were digested for the determination of total nitrogen, using salicylic acid as an additive to bind and reduce nitrate (cf. Novozamsky *et al.*, 1983). Total nitrogen determinations were made with an auto-analyzer, using a procedure based on the so-called reaction of Berthelot. It is described in the Manuals of Technicon Autoanalyzer II, Industrial Method No. 334-74B/W + (released January 1976/ revised March 1977. Technicon Industrial Systems/Tarrytown N.Y.).

Nitrate was extracted from the samples by shaking 500 mg material in 25 ml water for 30 minutes, followed by filtration. In the automated procedure, nitrate in solution is reduced to nitrite at pH 7.5 in a copper-cadmium reductor cell. The nitrite subsequently reacts with sulfanilamide under acidic conditions to form a diazo compound which couples with N-1-Naphtylethylenediamide dihydrochloride to form a dye that is measured at 520 nm (AutoAnalyzer II method NL 211-91WT; Technicon Industrial Systems/Tarrytown N.Y.).

Organic nitrogen is in this paper the difference between the total nitrogen concentration and the nitrogen present as nitrate.

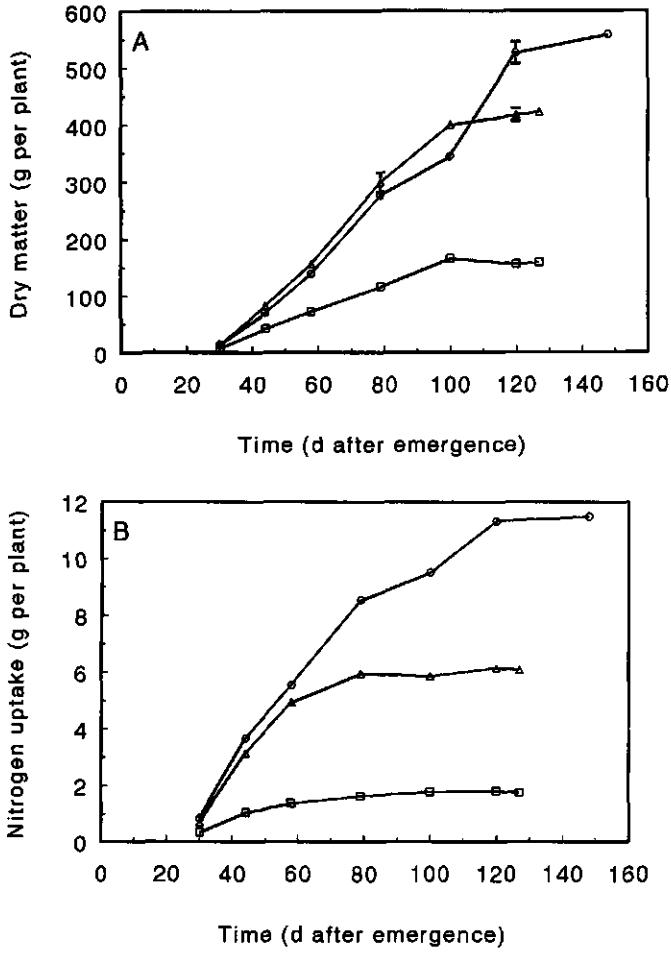

Figure 3.1.1. Changes with time in total dry weight per plant (A) and in the amount nitrogen per plant (B). Error bars (only in A) represent twice the standard error of the mean. If no error bar is visible, it fitted inside the marker. (□) N1; (△) N2; (○) N3.

Results

Changes with time in total dry matter and nitrogen uptake

There were large, significant effects (Tukey-test; $P=0.05$) of nitrogen supply on plant dry weight and nitrogen uptake at maturity (Figure 3.1.1). The N1 treatment showed a slower rate of dry matter accumulation throughout growth (Figure 3.1.1A) than the N2 and N3

treatments, resulting in significantly (Tukey-test; $P=0.05$) lower dry matter per plant at each harvest. Plants of the N3 treatment initially accumulated dry matter at a slightly slower rate than plants of the N2 treatment. However, the N3 plants continued to accumulate dry matter at an almost constant rate for a longer period of time than the N2 plants, leading to significant differences (Tukey-test; $P=0.05$) in total dry matter per plant between N2 and N3 from 120 DAE onwards.

The accumulation of total nitrogen was initially only slightly larger in the N3 treatment than in the N2 treatment (Figure 3.1.1B). N uptake in the N2 treatment showed a fairly constant initial phase, but clearly levelled off beyond 58 DAE, whereas N uptake levelled off much later in the N3 treatment. In the N1 treatment N uptake versus time did not show a linear phase, in contrast to dry matter accumulation.

It is mentioned in passing that the treatments hardly differed in total nitrogen uptake when expressed as a proportion from the amount supplied. These proportions amounted to 0.69, 0.76 and 0.72 for the N1, N2 and N3 treatments, respectively. This finding of an apparently conservative 'efficiency of uptake' implies that it seems possible to set targets for total N uptake in studies on N nutrition in pots, using an N-free medium.

The partitioning of dry matter and nitrogen between haulm and tubers

With increasing rate of N supply plants initially allocated relatively more dry matter to the haulms than to the tubers (once tubers were set) (Figure 3.1.2A). The proportion of the total dry weight in tubers (i.e. 'harvest index') amounted to 0.66, 0.53 and 0.38 at 44 DAE for the treatments N1, N2 and N3, respectively. These values differed statistically significantly between treatments (Tukey-test; $P=0.05$). The differences between the treatments in 'harvest index' persisted for much of the growing period, but had nearly disappeared at maturity: 0.87, 0.86 and 0.87 were the respective harvest indices for mature N1, N2 and N3 plants, respectively.

The fraction of total nitrogen allocated to tubers ('harvest index for nitrogen'; Figure 3.1.2B) showed basically similar treatment effects and time changes as were observed for dry matter. At maturity the proportions of total N in tubers amounted to 0.87, 0.85 and 0.85, respectively for N1, N2 and N3 plants. These similar proportions of dry matter and nitrogen in tubers imply that the nitrogen concentrations of mature haulms and tubers were similar and therefore equal to the average nitrogen concentration in total dry matter, at least within each treatment. These weighted mean concentrations of nitrogen in the dry matter of mature plants were very much different between treatments and amounted to 1.1%, 1.4% and 2.1% for N1, N2 and N3 plants.

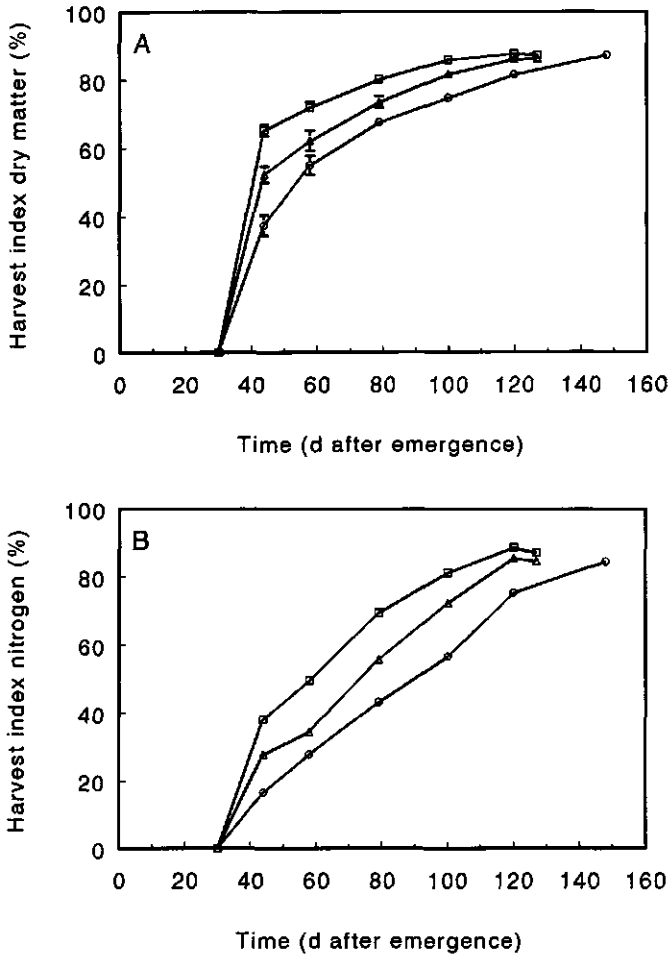

Figure 3.1.2. Changes with time in the proportion of total plant dry matter in tubers, i.e. harvest index (A), and in the proportion of total plant nitrogen in tubers, i.e. nitrogen harvest index (B). Error bars (only in A) represent twice the standard error of the mean. If no error bar is visible, it fitted inside the marker. (□) N1; (△) N2; (○) N3.

Haulm growth and the partitioning of dry matter and nitrogen within the haulm

Initial haulm (stems and leaves) growth was about four times faster in the N2 and N3 treatments than in the N1 treatment (Figure 3.1.3A). The differences between the N2 and N3 treatments were significant (Tukey-test; P=0.05) from 79 DAE onwards. The decline in haulm dry weight in the last part of the growing season represents a net relocation of dry matter (care was taken to prevent losses via leaf shedding). In the N3 treatment this

relocation started later in time than in the N2 treatment.

Not only did haulm dry weights differ significantly between nitrogen treatments (Figure 3.1.3A), but the composition of the haulms also differed in terms of the contributions by main stem, basal lateral branches and apical lateral branches of the first, second and third order (Figure 3.1.3B) (see Vos & Biemond (1992) for morphological definitions).

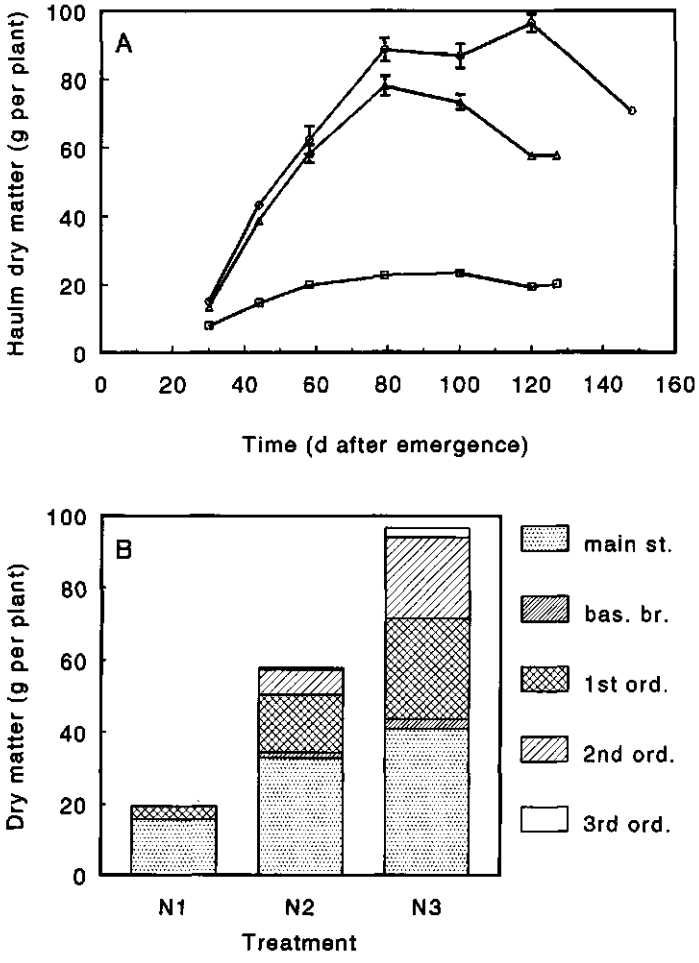

Figure 3.1.3. Changes with time in the dry weight of the haulms (shoots) (A; (□) N1; (△) N2; (○) N3), and stacked bars showing the dry weights of stem plus leaf material of morphologically different sections, i.e. the main stem (main st.), the basal lateral branches (bas. br.), and the apical lateral branches of the first, second and third order (1st ord., 2nd ord., 3rd ord.), respectively, for the three nitrogen treatments at 120 days after emergence (B). Error bars (only in A) represent twice the standard error of the mean. If no error bar is visible, it fitted inside the marker.

In spite of large differences in total haulm dry weight and in morphological composition of haulms, there were only small effects of nitrogen treatments on the distribution of haulm dry weight between leaves and stems (Figure 3.1.4A). Young plants showed a higher proportion of leaves than stems in all treatments, but beyond 44 DAE the proportion of leaves in haulm dry weight remained between 60 an 65% for the N1 and N3 treatments. At most intermediate harvests the value for the N2 treatment was intermediate between those of the N1 and N3 treatments, but dropped to lower values at the last two sampling dates.

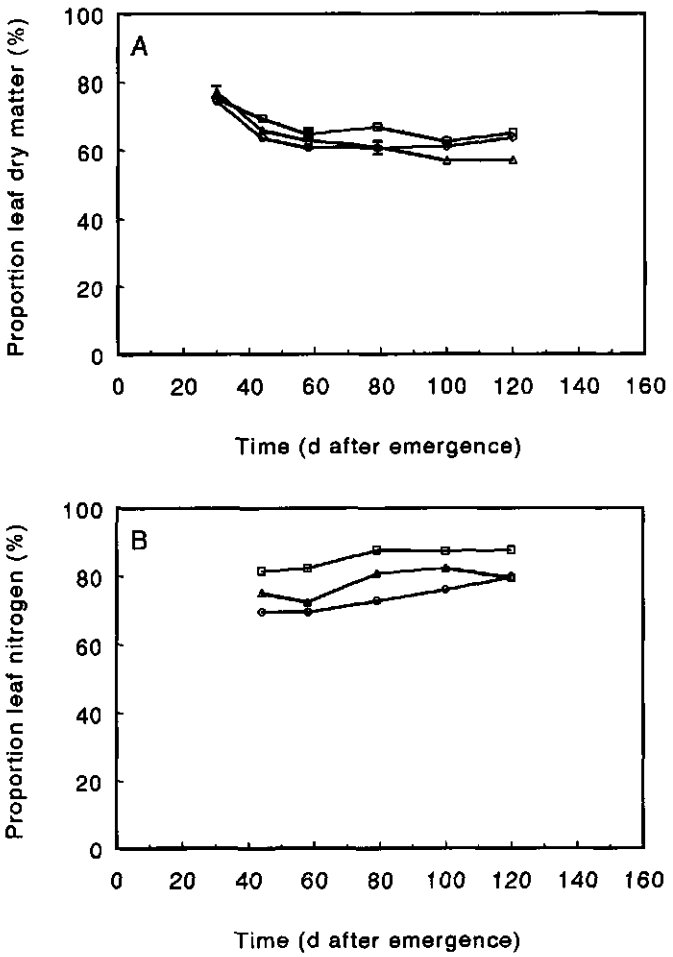

Figure 3.1.4. Changes with time in the dry weight of all leaves, expressed in % of the total dry weight of the haulm (A), and in the amount of nitrogen in leaves, expressed in % of the total amount of nitrogen in the haulm (B). (□) N1; (△) N2; (○) N3.

The proportion of the nitrogen in the haulms that was present in leaves ranged from 70 to 85% (Figure 3.1.4B), and was higher than the proportion of dry matter in leaves (Figure 3.1.4A), implying on average higher nitrogen concentrations in leaves than in stems. The effects of nitrogen treatments were obvious: greater rates of N supply resulted in relatively less N in leaves and more in stems, suggesting that the N concentration of stems is more responsive to N supply than the N concentration of leaves. In all treatments the proportion of N in leaves tended to increase with time and this suggests that relocation of N from stems is relatively more important than relocation from leaves.

Dry matter and nitrogen in leaves

Figure 3.1.5A represents an example of the changes with time of the dry weight of a leaf as affected by nitrogen treatment: leaf dry weight increased for approximately three weeks after leaf appearance, i.e. during the period of leaf expansion (Vos & Biemond, 1992), then a phase followed of decline in leaf weight, resulting in the total loss of 40 to 50% of the maximum weight. The leaves of the N1 treatment weighed consistently and significantly less than leaves of the N2 and N3 treatments.

The concentration of total N in leaf dry matter declined continuously throughout the life span of the leaf (Figure 3.1.5B). However, in spite of the continuous decline in N concentration, the absolute amount of nitrogen per leaf still increased during leaf expansion because of increase in leaf mass (i.e. in the period between 29 and 44 DAE in the example in Figure 3.1.5A). The concentration of total nitrogen was consistently higher for higher rates of nitrogen supply. The concentration of nitrate N (i.e. nitrogen present in the form of nitrate, so not the nitrate concentration!) typically showed an increase with time during the first part and a decline with time during the last part of the life span of a leaf, with a higher maximum concentration for higher rates of nitrogen supply (Figure 3.1.5B). The data imply that a substantial part of the differences between treatments in total nitrogen were attributable to differences in nitrate concentration in leaves. This point is further illustrated in Figure 3.1.6, comparing gradients with leaf number in the concentrations of total N and organic N. The first 10 to 12 leaves on the main stem showed no systematic differences in the concentration of organic N at 44 DAE, whereas systematically higher total N concentrations were observed for higher rates of N supply. The differences in total N concentrations between the N2 treatment and the N3 treatment at higher levels of insertion on the main stem and on the first order apical lateral branch were not explained by differences in nitrate concentration. These leaves were still expanding or approaching their final leaf area at 44 DAE. Late in the season (i.e. at 120 DAE; Figure 3.1.6B) the concentration of organic nitrogen in leaves was clearly higher in leaves of N3 plants than in leaves of N1 and N2 plants. Again, higher total N concentrations in leaves of N3 plants were partly attributable

to higher nitrate concentrations in leaves of N3 plants than in leaves of N2 plants. However, near maturity there was hardly a gradient left in the concentration of organic N with leaf number.

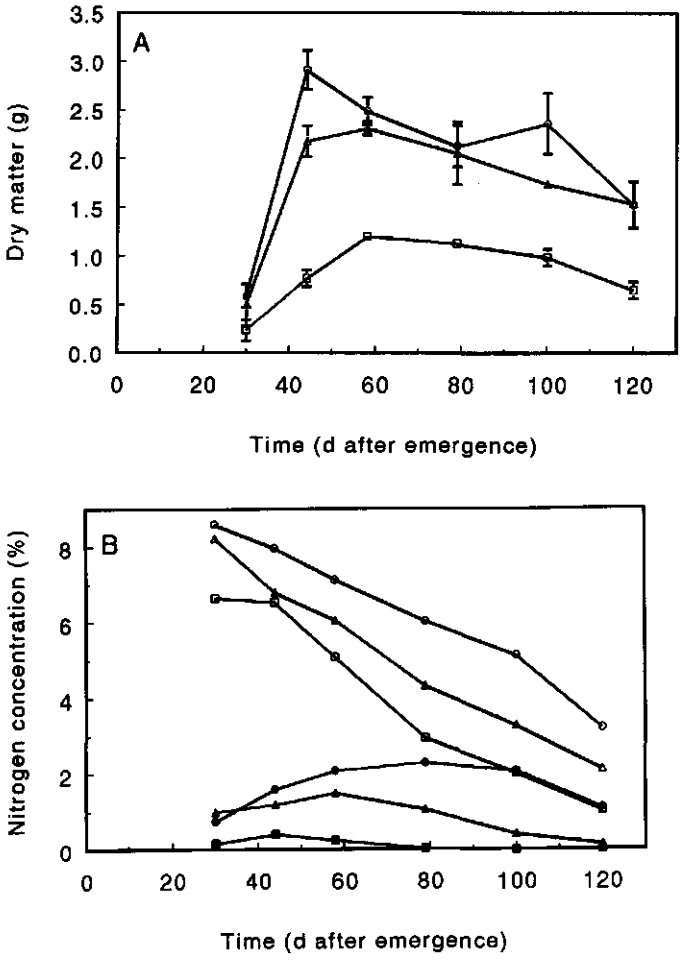

Figure 3.1.5. Changes in dry weight of leaf number 13 on the main stem (A), and changes with time in the concentrations of total nitrogen (open markers; (□) N1; (△) N2; (○) N3) and nitrate N (i.e. N present as nitrate; closed markers; (■) N1; (▲) N2; (●) N3) in the dry matter of that leaf (B). Error bars (only in A) represent twice the standard error of the mean. If no error bar is visible, it fitted inside the marker.

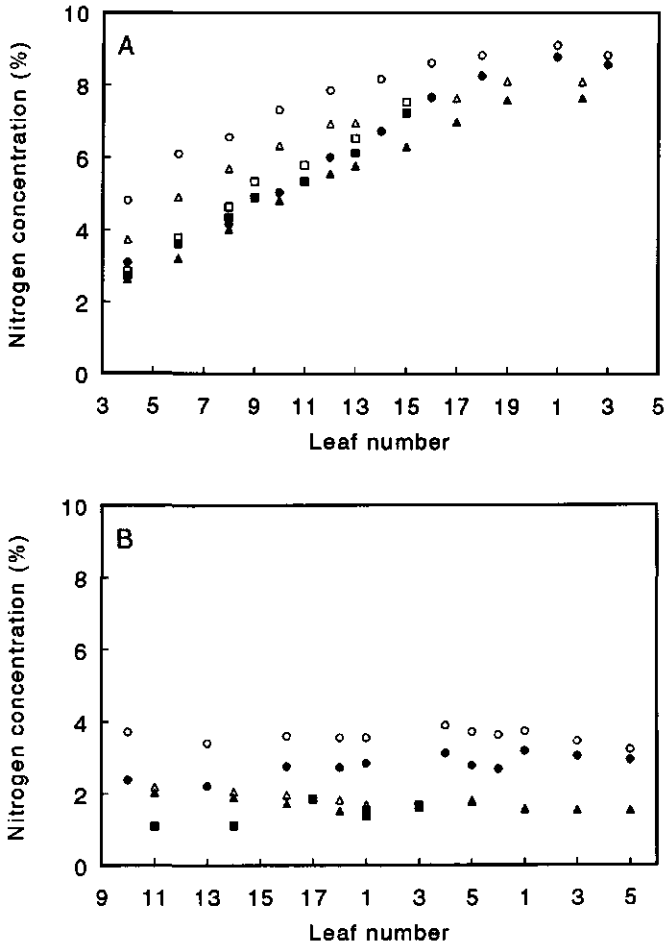

Figure 3.1.6. The concentrations of total nitrogen (open markers; (□) N1; (△) N2; (○) N3) and organic nitrogen (closed markers; (■) N1; (▲) N2; (●) N3) in leaf dry matter in relation to leaf number; the first sequence of numbers on the abscissa relates to the main stem, the second series to the (n-1) 1st order apical lateral branch and the third series to the (n-1)(n-1) 2nd order apical lateral branch (see Vos & Biemond (1992) for morphological definitions); data collected at 44 days after emergence (A) and at 120 days after emergence (B).

Associations between nitrogen concentrations in stems, tubers and leaves

The concentration of total nitrogen in stems declined with time and was systematically higher for higher rate of nitrogen supply. The same held for the nitrogen concentration in tubers, although the decline in concentration with time was only slight. Thus, the nitrogen concentration in each type of organ appeared to be affected in a similar fashion by time and

treatment, and therefore we plotted the weighted mean nitrogen concentration of all stem material, and that of tubers, against the weighted mean N concentration of all leaf material. This plot (Figure 3.1.7) showed fairly strong associations between nitrogen concentrations in component plant parts, regardless of time of sampling or nitrogen treatment. The association between the N concentration in stems and leaves and tubers and leaves can be represented by a quadratic function (r^2=0.99 and r^2=0.70, respectively).

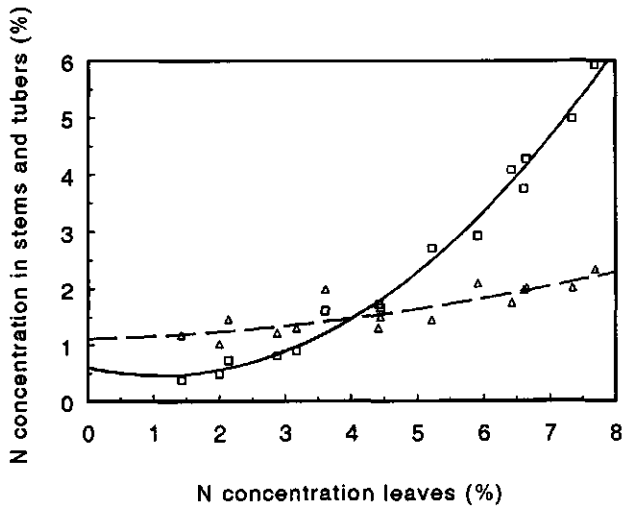

Figure 3.1.7. The concentrations of nitrogen in the dry matter of stems and tubers plotted versus the concentration of nitrogen in leaves; data include all treatments and sampling dates. (□) N concentration stems; full drawn line = quadratic regression between N concentration of leaves and stems; (▵) N concentration tubers; broken line = quadratic regression between N concentration of leaves and tubers.

Associations between total nitrogen and nitrate concentrations

Unlike the total nitrogen concentration, the nitrate N concentration in leaves did not continuously decline with leaf age (Figure 3.1.5). Therefore, a conservative association between total nitrogen and nitrate can not be expected for leaves. In stems and tubers, however, such associations appeared to exist. Nitrate was hardly detectable in stems at total nitrogen concentrations lower than about 1% (regardless time or N treatment) (Figure 3.1.8A). Nitrate N concentrations increased above that threshold in proportion to the concentration of total nitrogen. The slope of a linear regression is 0.58, indicating that substantial amounts of nitrate are present in stems with high total nitrogen concentrations.

In tubers (Figure 3.1.8B) nitrate was hardly detectable at total nitrogen concentrations of about 1.0 to 1.2%. Above that threshold nitrate N concentrations increased with total nitrogen, but the slope of a linear regression is small (0.05), indicating that nitrate

accumulation in tubers is comparatively and biologically insignificant.

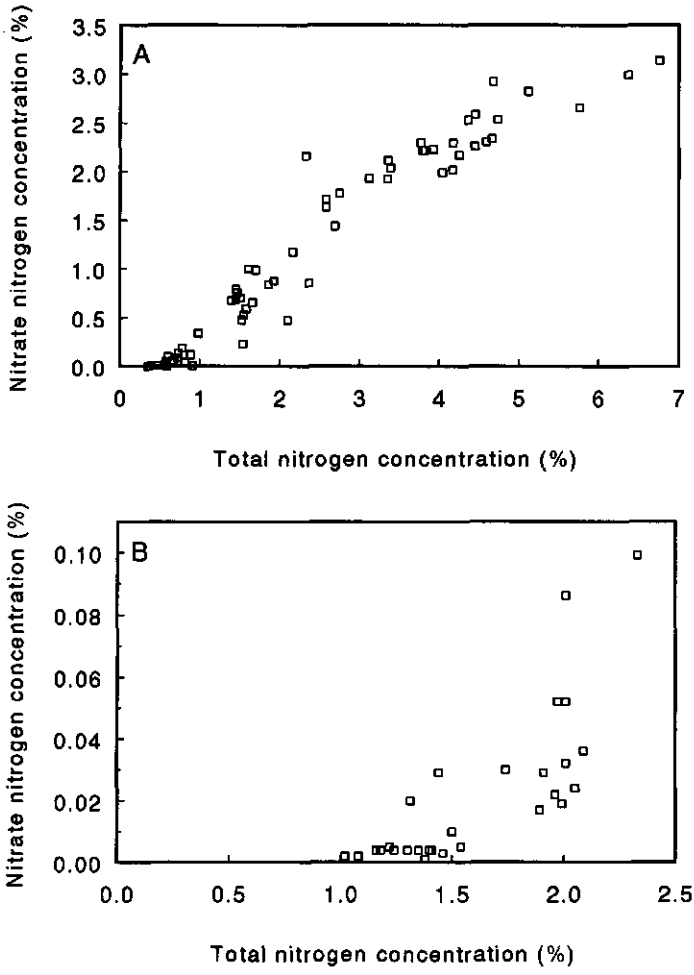

Figure 3.1.8. The concentration of nitrate N (i.e. N present as nitrate) plotted against the concentrations of total nitrogen, (A) stem material, (B) tuber material. The data include all treatments and sampling dates.

Discussion

The availability of nitrogen can be too low, optimal or in excess of what is required for unrestricted plant growth. The N1 treatment in the current experiment clearly represents a case with limiting availability of N. Growth characteristics of the plants of the N2 and N3 treatments were similar in many respects during early stages of growth and therefore the N3 treatment represents a case where N was initially available above the amount needed for unrestricted growth. The N2 treatment was somewhere between sufficient and excessive supply, at least during the earlier stages; at later stages more nitrogen would have stimulated growth and development and then N was clearly limiting in the N2 treatment. Relevant questions are how growth and development are modified when N availability is limiting, more or less optimal, or in excess of the requirement.

It was discussed in the companion paper (Vos & Biemond, 1992) that rates of appearance of organs are generally not affected by N supply, but that more apical branches and leaves were produced when more nitrogen was supplied. Nitrogen thus affected the duration and not the rate of appearance of successive organs. Also it was shown that the rate of leaf expansion, being an important determinant of mature leaf size, responded to N supply, but not any more so beyond an optimal level of supply.

The current data (Figure 3.1.1) indicate that the rate of dry matter accumulation is reduced when N is absolutely limiting, most likely via limited leaf expansion and interception of radiaton (Vos & Biemond, 1992). Since the N2 and N3 plants grew initially at approximately similar rates (Figure 3.1.1) there was clearly also an upper limit of response of plant growth to nitrogen, perhaps also because leaf numbers and leaf sizes were not different between the N2 and N3 plants during the initial stages of growth (Vos & Biemond, 1992). The initially excessive availability of nitrogen in the N3 treatment led to only slightly faster nitrogen uptake than in the N2 treatment (Figure 3.1.1B). The plant clearly regulated its maximum uptake and one can hypothesize that the N3 plants did not absorb more nitrogen than the N2 plants in the initial stages, because the upper response of leaf growth to nitrogen had been reached. However, this proposition still leaves open the nature of the mechanism of regulation of uptake as such.

In all treatments the rate of nitrogen accumulation slowed down earlier in time than the rate of dry matter accumulation (Figure 3.1.1). The decline in the rate of N uptake in the N2 treatment did not bring about a simultaneous decline in the sizes of the leaves that were expanding at that time (Vos & Biemond, 1992).

The earlier decline in nitrogen accumulation than in dry matter accumulation supports the idea that some measure of the N status can be found that heralds future nitrogen limitation. Monitoring the N status of a crop is of practical importance as a basis for

decisions on the application of supplementary nitrogen fertilizer. A measure of the N status cannot be an absolute one, however, since the concentrations of nitrogen and nitrate change with developmental stage and thus with time (Greenwood *et al.*, 1986, 1990). The concentration of nitrate in petioles or stem bases is currently used as a measure of the N status (Van Loon *et al.*, 1987; Williams & Maier, 1990). The current results confirm that the nitrate concentration of stems (and probably also of petioles, although we did not look at petioles separately) is fairly closely linked with total nitrogen (Figure 3.1.8A). Still, the interpretation of test results would benefit from further analysis of the interrelations between the nitrogen regime of the plant and its developmental and growth processes and internal nitrogen economy.

The treatments resulted in plants that differed considerably in final dry weight, in nitrogen uptake, and in foliar development (Figure 3.1.1; Figure 3.1.3B; Vos & Biemond, 1992). The final distributions of dry matter and nitrogen over haulms and tubers were not affected by nitrogen treatment, but the time paths leading to that end results were different: we also observed retarded tuber bulking for increased rates of N supply (Dyson & Watson, 1971). Nitrogen is apparently withdrawn from each successive leaf (cf Figures 3.1.5 and 3.1.6). Relocation of nitrogenous compounds, and perhaps also other compounds, to other destinations in the plant is therefore a continuous process. However, massive net decline of total haulm weight, due to the relocation of constituents to tubers, was not observed under continuous N limitation (the N1 treatment, Figure 3.1.3A), and occurred later in time in the N3 treatment than in the N2 treatment. Still, the temporal pattern of the distribution of dry matter between stems and leaves within the haulm was (although statistically significantly) numerically only slightly affected by N.

The N concentrations in mature plants differed almost twofold between the N1 and the N3 treatments. Still, the final distribution of N was not affected by N supply. This finding and the associations between the concentrations of N in component parts (Figure 3.1.7) suggest that there is some regulatory mechanism active that results in a conservative pattern of N allocation. It is noted however, that stems act as the most flexible component. Interestingly, Vos (1981) found a similar behaviour of stems of an unrelated species such as *Triticum aestivum*.

Potato cultivars are usually classified in maturity classes. Early maturing cultivars stop leaf growth earlier than late cultivars. Consequently early cultivars develop fewer orders of apical lateral branches than late cultivars. Cv. Bintje is classified in the middle of the spectrum. Although the maturity class influences the branching pattern, the distribution of dry matter and nitrogen over component haulm parts and over haulm and tubers is probably fairly conservative.

In conclusion it is stated that the current study indicates that if one were to predict plant

growth from a given amount of nitrogen, it would not be too difficult to predict the rates of appearance of leaves and branches, but it would be hard to predict the final stage of development (order of apical branching) that the plants would attain. The distributions of dry matter and nitrogen over component plants can be estimated, but the difficulty would be to define the maximum rate of N uptake and to predict how and when the transition is made between excessive availability of nitrogen and nitrogen limitation. Although the distribution of nitrogen within the plant seems fairly fixed, it would also be difficult to predict how much dry matter would be ultimately produced per unit of N taken up, i.e. to explain the nitrogen concentration in the dry matter at plant maturity.

References

Dyson, P.W. & D.J. Watson, 1971. An analysis of the effects of nutrient supply on the growth of potato crops. *Annals of Applied Biology* 69: 47-63.

Greenwood, D.J., G. Lemaire, G. Gosse, P. Cruz, A. Draycott & J.J. Neeteson, 1989. Decline in percentage N of C3 and C4 crops with increasing plant mass. *Annals of Botany* 66: 425-436.

Greenwood, D.J., J.J. Neeteson & A. Draycott, 1986. Quantitative relationships for the dependence of growth rate of arable crops on their nitrogen content, dry weight and aerial environment. *Plant and Soil* 91: 281-301.

Gunasena, H.P.M. & P.M. Harris, 1968. The effect of the time of application of nitrogen and potassium on the growth of the second early potato, variety Craig's Royal. *Journal of Agricultural Science* (Cambridge) 71: 283-296.

Loon, C.D. van, J.H.G. Slangen & J.H. Houwing, 1987. Nitrate content of leaf petioles as a guide to optimalization of N-fertilization of ware potatoes. EAPR Abstracts of Conference Papers and Posters. 10th Triennial Conference of the EAPR, Aalborg, 26-31 July, 1987, pp. 146-147.

Novozamsky, I., V.J.G. Houba, R. van Eck & W. van Vark, 1983. A novel digestion technique for multi-element plant analysis. *Communications in Soil Science and Plant Analysis* 14: 239-249.

Vos, J., 1981. The effects of temperature and nitrogen on post-floral growth of wheat; measurements and simulations. Agricultural Research Reports 911. Pudoc, Wageningen, 164 pp.

Vos, J. & H. Biemond, 1992. Effects of nitrogen on the development and growth of the potato plant. 1. Leaf appearance, expansion growth, life spans of leaves and stem branching. *Annals of Botany* 70: 27-35.

Williams, C.J.M. & N.A. Maier, 1990. Determination of the nitrogen status of irrigated potato crops. I. Critical nutrient ranges for nitrate-nitrogen in petioles. *Journal of Plant Nutrition* 13: 971-984.

SECTION 3.2

Effects of nitrogen on accumulation and partitioning of dry matter and nitrogen of vegetables. 1. Brussels sprouts

H. Biemond, J. Vos and P.C. Struik

Abstract

Brussels sprouts (*Brassica oleracea* L. var *gemmifera* DC) accumulates large amounts of nitrogen and the nitrogen harvest index is low. Detailed information on nitrogen effects on crop development and growth, however, is scarce. Therefore, four experiments were carried out in which treatments consisted of different amounts and different dates of application of nitrogen. Dry matter and nitrogen accumulation of stem, apical bud and groups of leaf blades, petioles and sprouts were measured frequently throughout growth.

Total amounts of accumulated dry matter and nitrogen were affected by amount of nitrogen applied and date of application. Sprout growth started halfway the growing period. The final harvest index for dry matter ranged from 0.10-0.35 and for nitrogen from 0.20-0.55; both were not significantly affected by treatments in most experiments. Nitrate nitrogen concentrations were only high (maximally about 2%) shortly after planting. The total nitrogen concentration of leaf blades and petioles increased with increasing leaf number. This increase resulted from a decreasing nitrogen concentration during the leaf's life. The total nitrogen concentration in sprouts changed little with leaf number.

Introduction

Brussels sprouts is a biennial plant species with a vegetative phase in the first year and a generative phase in the second year. During vegetative growth, leaves, stem, sprouts and roots develop.

Although some information is available on accumulation and partitioning of fresh and dry matter and nitrogen at harvest in relation to nitrogen nutrition, information on the dynamics of these characteristics during crop growth is scarce. Such information, however, is important for a proper understanding of the nitrogen uptake and for fine-tuning of the nitrogen nutrition to the demand of the crop.

The harvest index of Brussels sprouts is relatively low, compared to other crops. Fisher & Milbourn (1974) examined the partitioning of dry matter as affected by plant density, date of stopping (removal of the apical bud) and leaf removal; Abuzeid & Wilcockson (1989) analysed effects of different plant densities and different sowing dates in three years. In both sets of experiments it was observed that rapid sprout growth did not begin until approximately five months after sowing in May, while about three months later harvest indices between 25 and 40% were observed.

Another scientific question is whether sprout growth results only from photosynthesis occurring during sprout growth or also from relocation of dry matter from the dying leaves to the sprouts, as hypothesized by Verheij (1970). Fisher & Milbourn (1974) concluded from a leaf removal experiment that remobilisation of dry matter from other plant parts is unimportant, confirming results from a ^{14}C tracing experiment, carried out by Wilcockson & Abuzeid (1991).

Neuvel (1990) examined the effects of several amounts and dates of application of fertiliser nitrogen. When sown in April or transplanted in May and with an application of 300 kg ha^{-1} fertiliser nitrogen, the total dry matter production in October was, on average, 14.5 ton ha^{-1} and N uptake about 335 kg ha^{-1}. Maximum sprout yield of about 4.5 ton ha^{-1} dry matter was usually attained at the largest amount of nitrogen applied, i.e. 300 or 375 kg ha^{-1}.

Effects of nitrogen nutrition on leaf development and growth of Brussels sprouts are reported in Biemond *et al.* (1995). A larger total green leaf area, attained with more nitrogen, resulted mainly from larger leaves; the number of leaves increased only slightly. Larger leaves resulted from larger leaf expansion rates. However, amount and timing of nitrogen nutrition also affects plant growth and nitrogen uptake: these aspects are analysed in this paper. The relative partitioning rates of dry matter and nitrogen increase over different plant organs are derived from sequential harvest data.

Materials and methods

A brief description of the design of three glasshouse experiments, described in this paper, suffices here, because details were given by Biemond *et al.* (1995). The fourth experiment was a field experiment and not described before. Experiment 1 was carried out from May 1991 - September 1991, Experiment 2 from November 1991 - April 1992, Experiment 3 from April 1992 - October 1992 and Experiment 4 (the field experiment) from May 1991 - November 1991.

Plant culture - glasshouse

Young Brussels sprouts plants (cv. Icarus SG2004; with six, three and five leaves in Experiments 1, 2 and 3, respectively) were planted in 20-l pots (one plant per pot), containing sand, free from organic matter. The pots were placed in a glasshouse, set to maintain a day (12 h) temperature of 18 °C and a night temperature of 12 °C. Natural light was supplemented with 400 Watt Philips SON-AGRO-T lamps at a density of 0.7 lamps m^{-2}.

Plant culture - field

The field experiment (Experiment 4) was conducted on a clay soil. Plants of the same cultivar and of the same plant size as in Experiment 1 were planted (on 2 May 1991) about 7.5 cm deep in rows, with a row-spacing of 75 cm and a distance between plants within the row of 40 cm, resulting in a plant density of 3.3 plants m^{-2}.

Treatments and experimental design

Each glasshouse experiment had four different treatments (Figure 3.2.1), consisting of different total amounts and different dates of application of nitrogen. One treatment in each experiment was expected to have no nitrogen stress. Its nitrogen supply was based on previous experience. In Experiment 1 treatments were: N(4.5/3): 4.5 g N per pot, supplied in three splits of 1.5 g; N(4.5/9): 4.5 g N per pot, (nine equal splits); N(13.5/3): 13.5 g N per pot (three equal splits); N(13.5/9): 13.5 g N per pot (nine equal splits). In the statistical analyses, treatments were split into two factors, viz. amount of nitrogen (4.5 vs 13.5 g per pot) and number of applications (three vs nine splits). In Experiment 2, nitrogen was applied in splits of one, two or three g N per pot on such dates that nitrogen shortage could only occur after discontinuing nitrogen supply. Total amounts of nitrogen applied to each treatment were 6 N(6), 9 N(9), 12 N(12) or 18 g N N(18) per pot. In Experiment 3, treatments were: N(5.6): 5.6 g N per pot, with nitrogen limitation throughout the experiment; N(9.8;E(arly)): 9.8 g N per pot, with non-limiting supply in the early stages of growth and limiting supply later; N(9.8;L(ate)): 9.8 g N per pot, with limiting supply in the early stages of growth and non-limiting supply later; N(19.6): 19.6 g N per pot; non-limiting nitrogen supply throughout the experiment. Other nutrients were supplied in equal amounts to all treatments.

The glasshouse experiments were laid out in a randomised complete block design with four blocks, with each pot regarded as one experimental unit. At the start of the experiments, each block consisted of 28 (Experiment 1) or 32 (Experiments 2 and 3) pots (see *Sampling and plant analyses*).

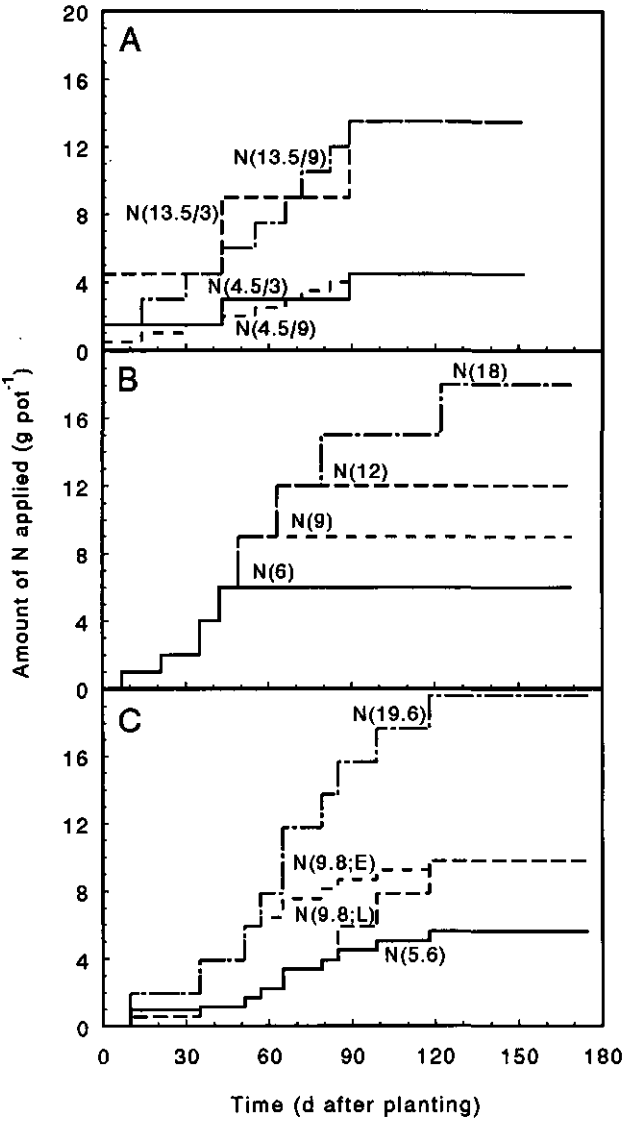

Figure 3.2.1. Accumulated amounts (in g per pot) of nitrogen, applied to the different treatments of Experiment 1 (A), Experiment 2 (B) and Experiment 3 (C).

Experiment 4 had three treatments: N(0): no application of fertiliser nitrogen; N(120+80): 200 kg N per ha, applied in two splits (120 kg ha^{-1} at 1 and 80 at 95 days after planting (DAP)); N(200/5): 200 kg N per ha, applied in five equal splits (at 1, 32, 61, 81 and 95 DAP). A week before planting 168 kg ha^{-1} mineral N was available in the soil layer

0-90 cm. This experiment was laid out in a split-plot design with four blocks; nitrogen treatment was main factor and harvest date (see *Sampling and plant analyses*) split factor. Statistical analyses of each experiment were separate for each harvest, since we were interested in differences among treatments and not specifically among harvests. The significance of differences is assessed with an LSD-test (P=0.05), after an analysis of variance.

Sampling and plant analyses

Destructive analyses of plants were carried out several times: in Experiment 1 on six occasions between 29 and 152 DAP (days after planting), in Experiment 2 on nine occasions between 28 and 169 DAP, in Experiment 3 on eight occasions between 29 and 175 DAP and in Experiment 4 on three occasions (68, 126 and 186 DAP). In Experiments 1-3 one plant per treatment was used from each block on each sampling date; in Experiment 4 on each sampling date four plants per treatment were used from each block.

The measurements in Experiments 1-3 included leaf area, and fresh and dry weights of leaf blades, petioles, sprouts, stem and top (= cluster of young leaves, shorter than 5 cm, at the top of the stem). In the growth analysis, leaf blades, petioles and sprouts from three (Experiment 1) or five (Experiments 2 and 3) nodes were pooled. Dead leaf blades and petioles were collected. The measurements in Experiment 4 included leaf area, and fresh and dry weights of all leaf blades, all petioles, all sprouts and stem. Dead leaves and petioles were not collected in this experiment. With the term "leaf number" always the leaf insertion number is meant; otherwise the term "number of leaves" is used.

Dried samples were ground. To reduce the number of samples for chemical analysis, the samples from the replicates of Experiments 1-3 were pooled and mixed thoroughly. One subsample from this pooled sample was subsequently analysed for total nitrogen and nitrate (Biemond & Vos, 1992). However, at the final harvest the replicates were not pooled; leaf blades, petioles and sprouts from all nodes of one plant were pooled after drying and subsequently analysed for total nitrogen and nitrate (Biemond & Vos, 1992). All samples of Experiment 4 were analysed, the replicates were never pooled.

In this paper organic nitrogen is the difference between the total nitrogen concentration and the nitrogen present as nitrate.

Results

Accumulation of dry matter and nitrogen

Figure 3.2.2 shows the relations between total dry matter production, total nitrogen uptake, dry matter in sprouts, and nitrogen in sprouts at the final harvest of each experiment; each datum point represents one of the treatments. Especially the relation between total dry matter production and total nitrogen uptake differed considerably between experiments. The relations depicted in the other three quadrants were fairly conservative, as data from most experiments and treatments fell on a common line, except that the field experiment deviated somewhat from the general pattern, because shed dead leaf blades and petioles were not included. In most cases the total dry matter production or sprout nitrogen uptake still increased with increasing sprout dry matter production or total nitrogen uptake. Linear regression lines would practically pass through the origin. This means that harvest indices for dry matter and nitrogen and total and sprout nitrogen concentrations were fairly constant over ranges of dry matter production and nitrogen uptake.

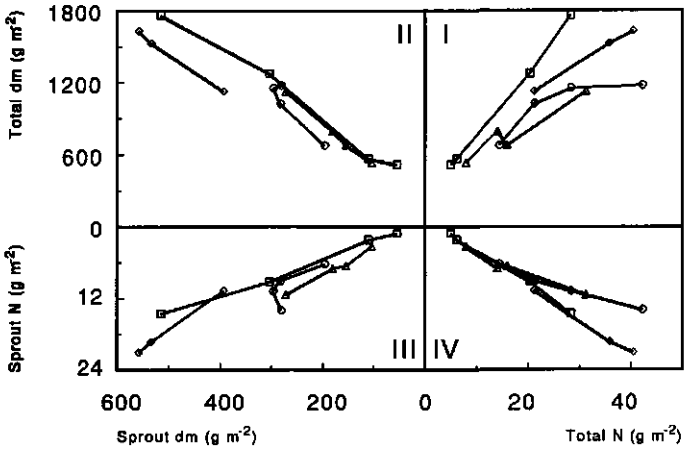

Figure 3.2.2. Relations between total dry matter production (Total dm), sprout dry matter production (Sprout dm), total nitrogen uptake (Total N) and sprout nitrogen uptake (Sprout N) at the final harvest of each experiment. All variables are in g m^{-2}. (□) Experiment 1; (○) Experiment 2; (△) Experiment 3; (◇) Experiment 4.

Partitioning of dry matter to different plant organs

In all experiments sprout growth started between 70 and 100 DAP. In Experiments 1 and 3 sprout growth started three weeks earlier in the two treatments with a large supply of

nitrogen shortly after planting, compared to the two treatments with a low initial rate of nitrogen supply. The final harvest indices are shown in Table 3.2.1. They were higher in Experiment 4, compared to the glasshouse experiments, because shed dead leaf blades and petioles were not included in total dry matter. When these plant parts were excluded from the total dry matter in Experiment 1 (which was carried out in the same year and period as Experiment 4, with the same cultivar), the harvest index was on average 0.32, stressing the importance of leaf shedding. Except in Experiment 1, amount or date of application of nitrogen did not significantly affect the harvest indices.

Table 3.2.1. The harvest index for dry matter and for nitrogen at 152 DAP for Experiment 1, at 169 DAP for Experiment 2, at 175 DAP for Experiment 3 and at 186 DAP for Experiment 4. In Experiment 4 dead leaf blades and petioles were not included in total dry matter and total nitrogen uptake.

Harvest index for dry matter

Experiment 1*		Experiment 2**		Experiment 3**		Experiment 4**	
N(4.5/3)	0.19	N(6)	0.28	N(5.6)	0.19	N(0)	0.35
N(4.5/9)	0.10	N(9)	0.27	N(9.8;E)	0.23	N(120+80)	0.35
N(13.5/3)	0.29	N(12)	0.25	N(9.8;L)	0.23	N(200/5)	0.34
N(13.5/9)	0.23	N(18)	0.24	N(19.6)	0.22		

Harvest index for nitrogen

Experiment 1*		Experiment 2**		Experiment 3**		Experiment 4**	
N(4.5/3)	0.35	N(6)	0.43	N(5.6)	0.40	N(0)	0.50
N(4.5/9)	0.22	N(9)	0.43	N(9.8;E)	0.50	N(120+80)	0.54
N(13.5/3)	0.51	N(12)	0.38	N(9.8;L)	0.41	N(200/5)	0.52
N(13.5/9)	0.45	N(18)	0.33	N(19.6)	0.36		

* applying 13.5 instead of 4.5 g N per pot and in three times instead of nine resulted in significantly higher harvest indices for dry matter and for nitrogen.

** in Experiments 2, 3 and 4 treatment differences were not significant.

Figure 3.2.3 shows the changes with time in the relative partitioning rates of dry matter increase to the stem, the leaf blades (including dead ones and top of the plant), petioles (including dead ones) and sprouts. The relative partitioning rates are calculated as the increase in dry matter of a particular plant part over a period of time between harvests, divided by the total increase of dry matter in the same period. The trends as shown for Experiment 3 in Figure 3.2.3 were similar in Experiments 1 and 2; the data of Experiment 4 are not comparable, because dead leaf blades and petioles were not sampled in this experiment. After a slow increase for a longer period, the relative partitioning rate to the

stem usually decreased at the end of each experiment. The relative partitioning rate to the leaf blades decreased until the penultimate harvest. The relative partitioning rate to the petioles increased until about 50 DAP and usually decreased thereafter. A negative value for the relative partitioning rate to leaf blades or petioles resulted from a decreasing amount of dry matter in these plant parts: reallocation (to the sprouts) probably has occurred there. The relative partitioning rate to the sprouts increased after sprout initiation in nearly all cases until the penultimate harvest. In Experiments 1 and 2 the relative partitioning rate to sprouts was never above 1.0. The only effect of nitrogen treatments on the pattern of distribution of dry matter was the delayed sprout growth for treatments with a low availability of nitrogen shortly after planting.

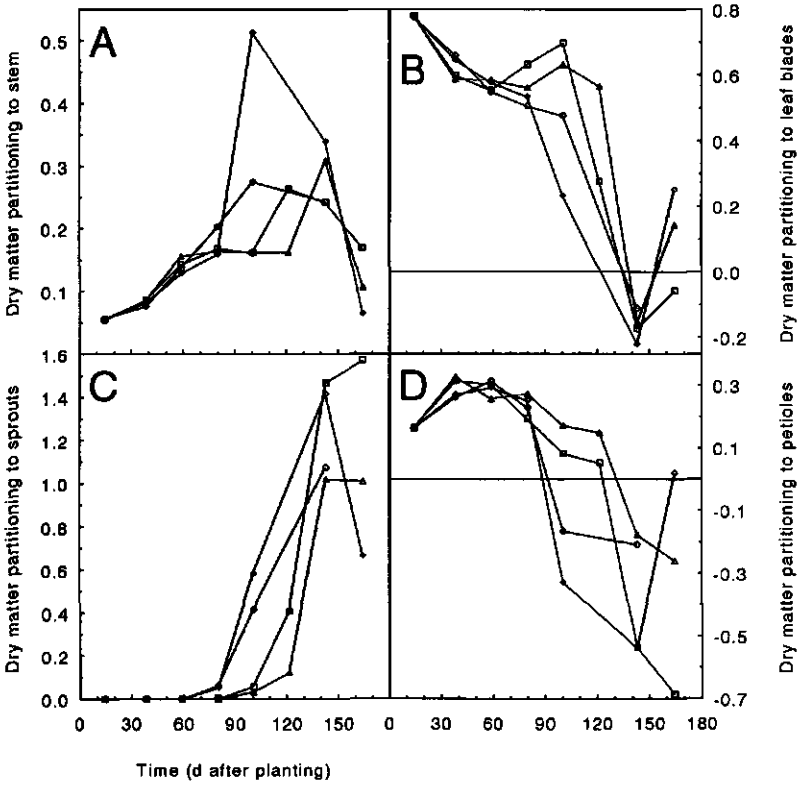

Figure 3.2.3. Changes with time in the relative partitioning rates of dry matter increase to the stem (A), the leaf blades (including dead ones and top of the plant; B), the sprouts (C) and the petioles (including dead ones; D) of Experiment 3. Mind differences in scales of y-axes. (□) N(5.6); (○) N(9.8;E); (△) N(9.8;L); (◇) N(19.6).

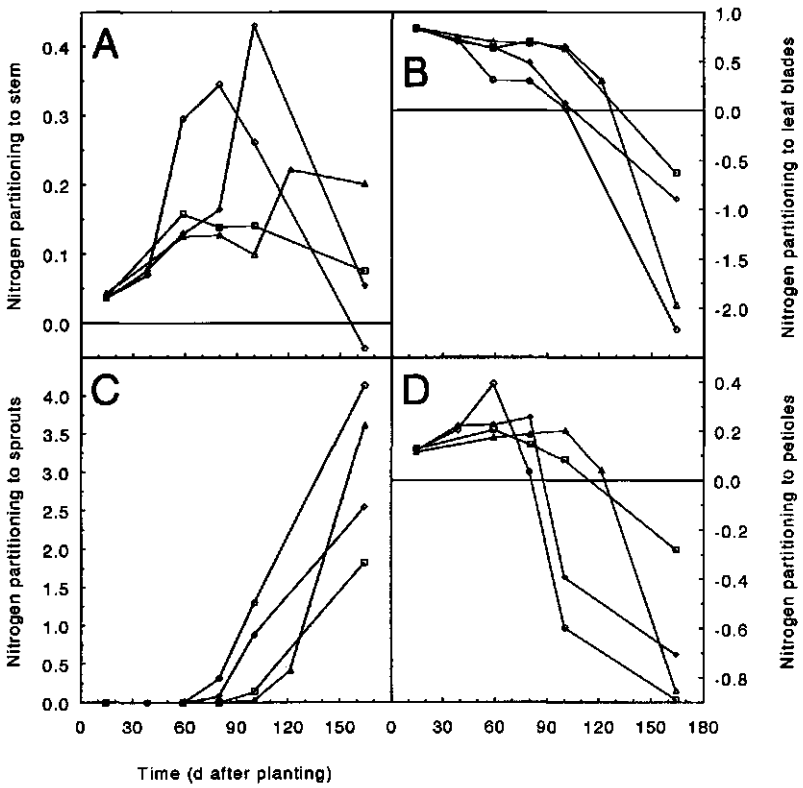

Figure 3.2.4. Changes with time in the relative partitioning rates of nitrogen increase to the stem (A), the leaf blades (including dead ones and top of the plant; B), the sprouts (C) and the petioles (including dead ones; D) of Experiment 3. Mind differences in scales of y-axes. (□) N(5.6); (○) N(9.8;E); (△) N(9.8;L); (◇) N(19.6).

Partitioning of nitrogen to different plant organs

The harvest index for nitrogen was always higher than the harvest index for dry matter, because the nitrogen concentration in sprouts was higher than that of the whole plant. Final values of the harvest index for nitrogen are shown in Table 3.2.1. Although significant effects were only observed in Experiment 1, higher amounts of nitrogen in Experiment 2 resulted in slightly lower harvest indices for nitrogen. The harvest indices for dry matter in N(9.8;E) (the treatment with the early nitrogen application) and N(9.8;L) (the treatment with the late nitrogen application) of Experiment 3 were similar, but the harvest index for nitrogen was 25% higher for N(9.8;E). When dead leaf blades and petioles in Experiment 1 were excluded from the calculation of the harvest index for nitrogen, it was on average 0.44; Experiment 4 had a value of on average 0.52, also with excluding dead leaf blades and petioles.

The change with time in the relative partitioning rate of nitrogen increase to the distinguished component plant parts is shown in Figure 3.2.4 for Experiment 3. The overall pattern is similar to the one observed for the relative dry matter partitioning (Figure 3.2.3). The treatments of the different glasshouse experiments all showed similar patterns, except that there was no decrease at the end of Experiment 2 in the relative partitioning rate of nitrogen increase to the stem. Variation in the proportion of additional N allocated to the stem suggests that the pool of N in stems acts as a buffer to store N taken up in excess of current requirements. The relative partitioning rate to leaf blades decreased during the whole growing period and was negative at the end, as a result of reallocation of nitrogen, apparently to sprouts. The relative partitioning rate to petioles increased slightly during the first half of the growing periods and decreased during the second part. High harvest indices for nitrogen (Table 3.2.1) were generally associated with high relative partitioning rates to sprouts and low relative partitioning rates to leaf blades and petioles at the end of the growing periods. The treatments with the lowest amount of nitrogen in each experiment did not always comply with this rule, especially not N(6) of Experiment 2: the final harvest index for nitrogen was high, but a relatively small fraction of nitrogen was reallocated from the leaf blades and the petioles to the sprouts.

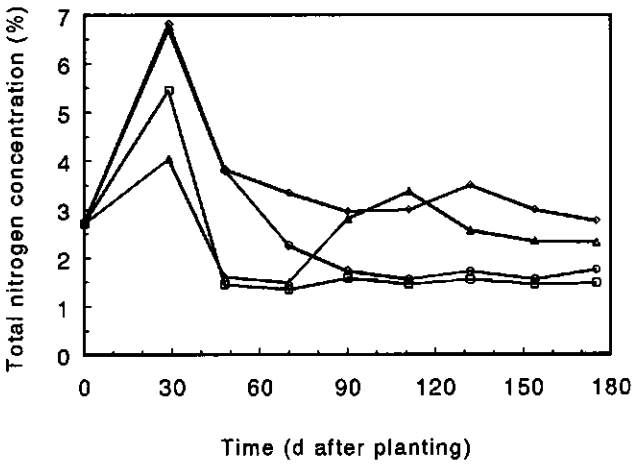

Figure 3.2.5. Changes with time in the concentration of total nitrogen in the dry matter of all above-ground plant parts for Experiment 3. (□) N(5.6); (○) N(9.8;E); (△) N(9.8;L); (◇) N(19.6).

Concentration of nitrogen and nitrate

The average nitrogen concentration over all above-ground plant parts was lower at planting time than at the first intermediate harvest in all experiments, except in Experiment 2. Usually this concentration subsequently decreased continuously until the end of each experiment (Figure 3.2.5). This general pattern was affected by changes in the nitrogen availability; in this way the average nitrogen concentration could increase, as happened to N(9.8;L) of Experiment 3 after the addition of extra nitrogen.

The nitrate nitrogen concentration over all above-ground plant parts (data not shown) was below 0.1% at most harvests, except for Experiment 2 and in the other experiments shortly after planting. In Experiments 1, 3 and 4 concentrations between 0.7 and 2.0% were observed at the first intermediate harvests for treatments which received a high amount of nitrogen shortly after planting, but this nitrate had disappeared after 50 DAP. The nitrate nitrogen concentration in Experiment 2 fluctuated around 1.75% until 100 DAP, whereafter it decreased for all treatments; final values were below 0.25%.

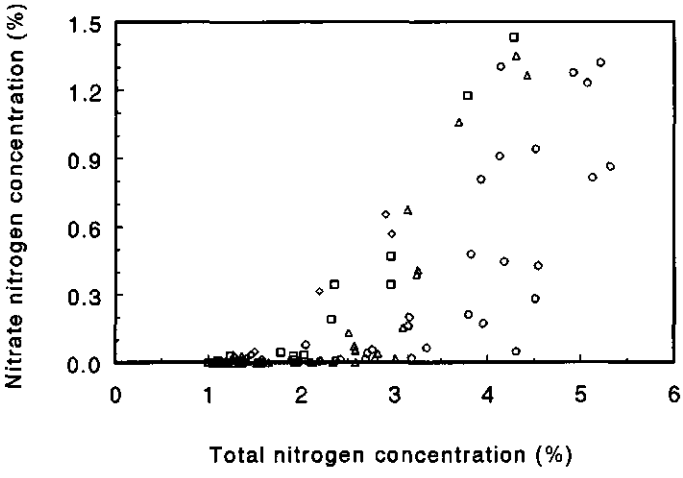

Figure 3.2.6. The concentrations of nitrate nitrogen (i.e. N present as nitrate) in the dry matter of stems plotted against the concentrations of total nitrogen in the dry matter. Data from all harvests from all treatments from all experiments. (□) Experiment 1; (○) Experiment 2; (△) Experiment 3; (◇) Experiment 4.

The nitrate nitrogen concentration in leaf blades and petioles had no clear relation with their total nitrogen concentration. Figure 3.2.6 shows a positive relation between the nitrate nitrogen and the total nitrogen concentration of the stems for data from all treatments at all harvests of all experiments. A linear regression for all data from Experiments 1, 3 and 4 where the nitrate nitrogen concentration was above 0.1% was carried out. The data from

Experiment 2 were excluded, because the relation seemed to be different for this experiment, which was carried out in winter. The regression equation ($n=17$; $r^2=0.80$) revealed that nitrate started to accumulate at a rate of 0.57% nitrate nitrogen per 1.00% total nitrogen above 2.07% total nitrogen. This equation represents the upper limit of possible nitrate nitrogen concentrations.

Concentration of nitrogen and nitrate in plant organs of different nodes

The total nitrogen concentrations of different groups of leaf blades, petioles and sprouts are shown in Figure 3.2.7 for Experiment 3 at 90 and 154 DAP. The lowest concentrations for green leaf blades (Experiment 3: about 1.0%) or petioles (Experiment 3: about 0.5%) were similar at all harvests of a certain experiment, but the gradient in total nitrogen concentration with leaf number declined as the canopy aged. Petioles always had lower concentrations than the corresponding leaf blades. The total nitrogen concentration of a certain group of leaf blades or petioles decreased with time, but it was more or less constant for sprouts. The trends as observed in Figure 3.2.7 were similar in all three glasshouse experiments. In Experiment 1 the differences between treatments were very small at all harvests. In Experiment 2 differences between treatments developed during the growing season, treatments with a larger amount of fertiliser nitrogen having higher concentrations. This sometimes resulted in differences between treatments in total nitrogen concentration of the same leaf blade numbers of more than 4% (nitrate nitrogen was negligible). In N(18) of Experiment 2 the increase with leaf number was very slow at 154 and 169 DAP. This was observed over a longer period in N(19.6) of Experiment 3 (see Figure 3.2.7): the increase in total nitrogen concentration with leaf number disappeared with increasing plant age, while this increase stayed with the other three treatments.

In Experiments 1 and 3 differences in total nitrogen concentration between different groups of sprouts were small (see Figure 3.2.7), mainly because lower sprout numbers had much higher concentrations than lower leaf numbers. In Experiment 2 there was a decrease in total nitrogen concentration with increasing sprout number, especially at the first harvests where sprouts were present. At 169 DAP the concentrations were nearly equal for all sprout numbers.

In Experiments 1 and 3 the highest nitrate nitrogen concentrations, found in leaf blades, were about 2%. These concentrations decreased with increasing leaf number. In petioles maximum concentrations of about 4% were found, which also decreased with increasing leaf number. The period in which nitrate was present in important concentrations in these two experiments, was until about 50 DAP. In Experiment 2 nitrate was present over a longer period, until about 125 DAP. Maximum concentrations, found in leaf blades, were about 3% and about 4% in petioles. They decreased with increasing leaf number. The nitrate nitrogen

concentration in sprouts was always negligible in all experiments.

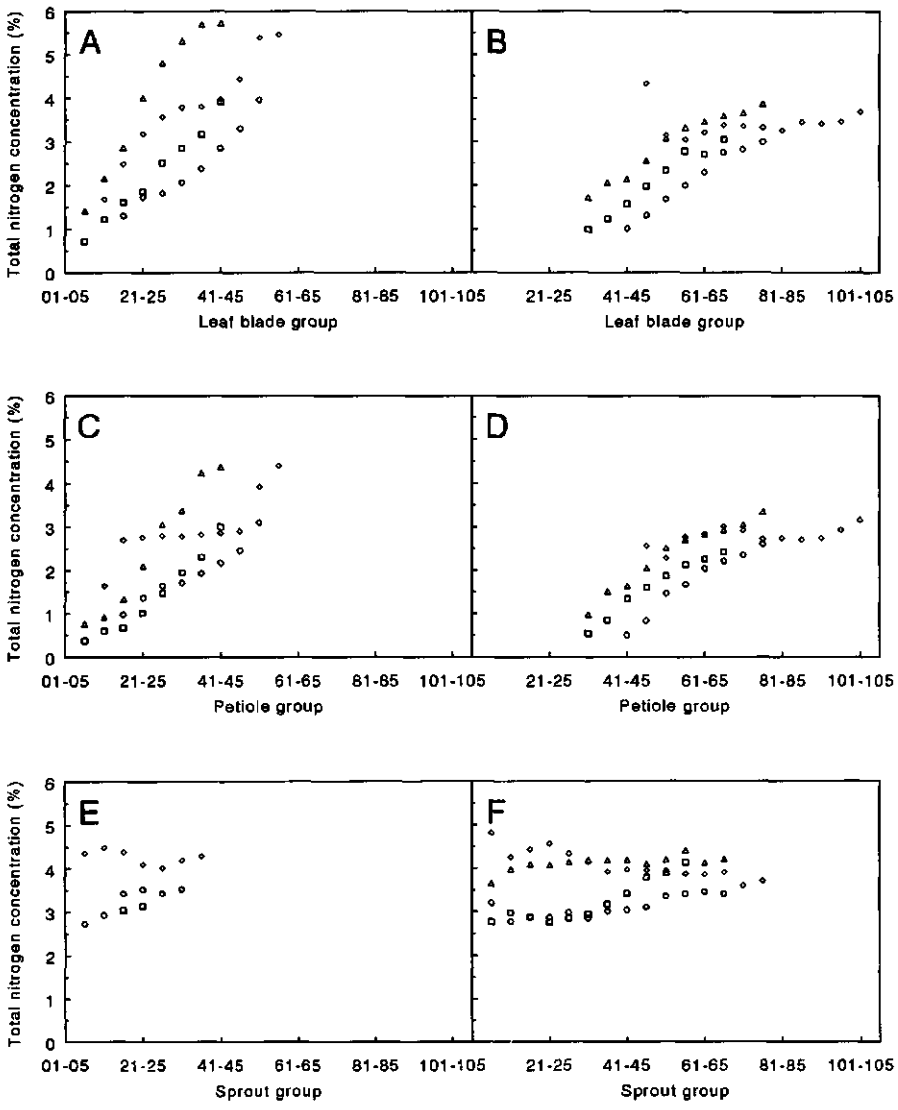

Figure 3.2.7. The concentration of total nitrogen in the dry matter of the different groups of leaf blades (A and B), petioles (C and D) and sprouts (E and F) for Experiment 3 at 90 (A, C and E) and 154 DAP (B, D and F). (□) N(5.6); (○) N(9.8;E); (△) N(9.8;L); (◇) N(19.6).

Discussion

Both amount of nitrogen applied and timing of the nitrogen fertilisation affected the accumulation and partitioning of dry matter and nitrogen of Brussels sprouts in various ways. In the tested ranges of applied amounts of fertiliser nitrogen, there were always positive effects on dry matter production.

A larger amount of nitrogen led to an earlier start of sprout growth, but effects on the harvest index for dry matter were insignificant (except in Experiment 1), confirming results of J.J. Neuvel (pers. comm.). Abuzeid & Wilcockson (1989) found a final harvest index between 25 and 40% (including easy recoverable roots and dead leaves in total dry matter) in three field experiments with Brussels sprouts, the variation being the result of different sowing dates. Fisher & Milbourn (1974) found similar values with varying plant densities, dates of stopping and several cultivars, excluding dead leaves and roots from total dry matter.

A higher harvest index for nitrogen compared to the harvest index for dry matter was also observed by J.J. Neuvel (pers. comm.) in experiments with various cultivars.

Usually the total nitrogen concentration of a crop decreases with increasing plant mass (Greenwood *et al.*, 1986, 1990). In Experiments 1, 3 and 4, this concentration was relatively low at planting time and increased until the first intermediate harvest. This low concentration of transplants, also observed by J.J. Neuvel (pers. comm.), was apparently due to the poor nutritional status before planting (= 0 DAP), which was not the case for the transplants of Experiment 2. The total nitrogen concentration in Experiment 2 was high over a long period, (in contrast with Experiments 1, 3 and 4, where it decreased quickly after the first intermediate harvest) as a result of the low light intensity during this winter period. This was also the cause of the prolonged high nitrate nitrogen concentrations in Experiment 2: a low light intensity results in a higher nitrate concentration (Van Diest, 1986).

An increasing total nitrogen concentration of leaves with increasing height in the plant is often found. It is usually explained as an effect of leaf age and as being more efficient for the plant, because the leaves with the higher nitrogen concentration are in the upper canopy layers. There they receive more light, which can be utilised more effectively by leaves with a high than by leaves with a low nitrogen concentration (Hirose & Werger, 1987). An increasing total nitrogen concentration with leaf number was, however, not found in all cases. Sometimes even lower leaf numbers had high nitrogen concentrations (N(19.6) in Figure 3.2.7B). A large part of this nitrogen was apparently luxury consumption (this phenomenon occurred only in treatments which received a large amount of nitrogen), but still the question arises, why this nitrogen was present as organic nitrogen and hardly as nitrate nitrogen. The higher total nitrogen concentration in lower sprout numbers, compared to

lower leaf numbers, was usually no luxury consumption, because it was observed for all treatments.

Whenever nitrate was present, its concentration was highest in the lowest (= oldest) leaf blades or petioles. Darwinkel (1975) observed for crops as turnip and rape the highest nitrate concentration in the oldest leaves and explained this as a result of the low nitrate reductase activity in these leaves.

The results from our experiments show that it is important for the production of a Brussels sprouts crop that, especially at the beginning of the growing season, sufficient nitrogen for unrestricted growth is available. When this is not the case, the sprout growth is delayed, which is apparently the cause of a lower final harvest index for dry matter (which means the absolute amount of dry matter in sprouts is lower because the total amount of dry matter was also lower). The high final harvest index for nitrogen for treatments with sufficient nitrogen for unrestricted growth at the beginning of the growing season, but not at the end, is interesting from an environmental point of view. With a high harvest index for nitrogen, a relatively low amount of nitrogen is left on the field in crop residues at harvest. This means less nitrogen can leach after mineralization of the crop residues.

References

Abuzeid, A.E. & S.J. Wilcockson, 1989. Effects of sowing date, plant density and year on growth and yield of Brussels sprouts (*Brassica oleracea* var. *bullata* subvar. *gemmifera*). *Journal of Agricultural Science* (Cambridge) 112: 359-375.

Biemond, H. & J. Vos, 1992. Effects of nitrogen on the development and growth of the potato plant. 2. The partitioning of dry matter, nitrogen and nitrate. *Annals of Botany* 70: 37-45.

Biemond, H., J. Vos & P.C. Struik, 1995. Effects of nitrogen on development and growth of the leaves of vegetables. 1. Appearance, expansion growth and life span of leaves of Brussels sprouts plants. *Netherlands Journal of Agricultural Science* 43: 217-232.

Darwinkel, A., 1975. Aspects of assimilation and accumulation of nitrate in some cultivated plants. Dissertation Wageningen Agricultural University. Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen, 64 pp.

Diest, A. van, 1986. Means of preventing nitrate accumulation in vegetable and pasture plants. In: H. Lambers, J.J. Neeteson & I. Stulen (Eds.), Fundamental, ecological and agricultural aspects of nitrogen metabolism in higher plants. Martinus Nijhoff, Dordrecht, pp. 455-471.

Fisher, N.M. & G. Milbourn, 1974. The effect of plant density, date of apical bud removal and leaf removal on the growth and yield of single-harvest Brussels sprouts (*Brassica oleracea* var. *gemmifera* D.C.). 1. Whole plant and axillary bud growth. *Journal of Agricultural Science* (Cambridge) 83: 479-487.

Greenwood, D.J., G. Lemaire, G. Gosse, P. Cruz, A. Draycott & J.J. Neeteson, 1990. Decline in percentage N of C3 and C4 crops with increasing plant mass. *Annals of Botany* 66: 425-436.

Greenwood, D.J., J.J. Neeteson & A. Draycott, 1986. Quantitative relationships for the dependence of growth

rate of arable crops on their nitrogen content, dry weight and aerial environment. *Plant and Soil* 91: 281-301.

Hirose, T. & M.J.A. Werger, 1987. Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. *Physiologia Plantarum* 70: 215-222.

Neuvel, J.J., 1990. Nitrogen fertilization of Brussels sprouts (in Dutch). Report 102. Research Station for Arable Farming and Field Production of Vegetables, Lelystad, 35 pp.

Verheij, E.W.M., 1970. Spacing experiments with Brussels sprouts grown for single-pick harvest. *Netherlands Journal of Agricultural Science* 18: 89-104.

Wilcockson, S.J. & A.E. Abuzeid, 1991. Growth of axillary buds of Brussels sprouts (*Brassica oleracea* var. *bullata* subvar. *gemmifera*). *Journal of Agricultural Science* (Cambridge) 117: 207-212.

SECTION 3.3

Effects of nitrogen on accumulation and partitioning of dry matter and nitrogen of vegetables. 2. Leek

H. Biemond

Abstract

Information about the nitrogen demand and response of leek (*Allium porrum* L.) is scarce, but relevant for optimisation of nitrogen fertilisation strategies. The purpose of the current study was to analyse the dynamics of accumulation of dry matter and nitrogen in leek. Therefore four experiments were carried out with different amounts and different dates of application of nitrogen. Observations included frequent measurements of dry matter and nitrogen accumulation of leaf blades, leaf sheaths and, if present, scape.

Both the amount of nitrogen applied and the time of application affected the total accumulation of dry matter and nitrogen in the plant. The relative partitioning rates of dry matter increase to the shaft were affected in such a way that the final harvest indices for dry matter (which ranged from 0.32-0.53) were significantly lower at higher amounts of nitrogen applied. The final harvest indices for nitrogen ranged from 0.21-0.35 and were not significantly affected by amount or timing of fertiliser applications. The total nitrogen concentrations of leaf blades and leaf sheaths decreased with increasing leaf age. Average nitrate nitrogen concentrations over all plant parts were always below 0.4%.

Introduction

Leek is a biennial plant. During the vegetative phase, in the first year, growth of leaf blades, leaf sheaths and roots takes place. Usually the scape develops during the second year, but sometimes scapes are observed at harvest in the first year, which are only visible after dissecting the plants (Dragland, 1972).

Information about accumulation and partitioning of fresh and dry matter and nitrogen at harvest in relation to nitrogen fertilisation is scarce, just as information on the dynamics of these characteristics during crop growth. Such information, however, is important for a proper understanding of the nitrogen requirement and for fine-tuning the nitrogen fertilisation

to the demand of the crop, and to minimise nitrogen losses to the environment. Such information is also indispensable for the development of simulation models on plant growth and the use of inputs. Therefore this study aims to acquire this information.

The potential dry matter yield of leek in Western Europe is around 1200 g m^{-2}, when early planted, well-watered and well-fertilised (Brewster, 1994). The highest yields are usually achieved with about 60 plants m^{-2}, but to produce large leeks, densities of 15-20 plants m^{-2} are used. The optimal total nitrogen supply (mineral nitrogen in soil layer 0-30 cm plus fertiliser nitrogen) is about 270 kg ha^{-1} under Dutch circumstances.

Based on Experiments 1, 3 and 4 of the current study, Biemond (1995) described effects of nitrogen on leaf development and growth of leek. More nitrogen increased the total green leaf area by increasing the area of individual leaves. The rate of leaf appearance, the rate of leaf senescence and therefore the number of green leaves were not affected. Larger individual leaves resulted from larger leaf expansion rates, the duration of leaf expansion being unaffected. In this paper, the plant dry matter and nitrogen accumulation and the relative partitioning of dry matter and nitrogen over leaf blades, leaf sheaths and other organs are analysed.

Materials and methods

Two glasshouse experiments (Experiments 1 and 2) and two field experiments (Experiments 3 and 4) are described. A brief description of the design of Experiments 1, 3 and 4 suffices here, because details were given by Biemond (1995). Experiment 2 was not described before.

Plant culture - glasshouse

Young, pencil-thick leek plants (cv. Albana; in Experiment 1 with five and in Experiment 2 with six leaves) were planted (Experiment 1: 1 May 1991; Experiment 2: 30 September 1992) in 20-l pots (containing sand, free from organic matter), four plants per pot in Experiment 1 and five plants per pot in Experiment 2. The glasshouse, in which the pots were placed, was set to maintain a day (12 h) temperature of 18 °C (Experiment 1) or 20 °C (Experiment 2) and a night temperature of 12 °C (both experiments). Natural light was supplemented with 400 Watt Philips SON-AGRO-T lamps at a density of 0.7 lamps m^{-2}. With a pot density of 5.0 m^{-2} in Experiment 1 and 4.0 pots m^{-2} in Experiment 2, a similar plant density of 20 plants m^{-2} was attained in both experiments.

Plant culture - field

Experiments 3 and 4 were conducted on a sandy soil with about 3% organic matter. Plants of the same cultivar and plant size as in Experiment 1 were planted about 10 cm deep in rows, with a row-spacing of 25 cm and a distance between plants within the row of 20 cm, resulting in a plant density of 20 plants m^{-2}. Experiment 3 was planted on 12 May 1992; Experiment 4 on 7 May 1993. Irrigation was applied immediately after planting and later on whenever necessary.

Treatments and experimental design

Each glasshouse experiment had four different treatments, each field experiment three different treatments (see Figure 3.3.1), consisting of different total amounts and dates of application of nitrogen. In Experiment 1 treatments were: N(1.8/2): 1.8 g N per pot, supplied in two splits of 0.9 g; N(1.8/6): 1.8 g N per pot (six equal splits); N(5.4/2): 5.4 g N per pot (two equal splits); N(5.4/6): 5.4 g N per pot (six equal splits). In Experiment 2 treatments were: N(1.0): 1.0 g N per pot, with nitrogen limitation throughout the experiment; N(2.4;E(arly)): 2.4 g N per pot, with non-limiting supply in the early stages of growth and limiting supply later; N(2.4;L(ate)): 2.4 g N per pot, with limiting supply in the early stages of growth and non-limiting supply later; N(5.9): 5.9 g N per pot; non-limiting supply throughout the experiment. In both glasshouse experiments, other nutrients were supplied in equal amounts to all treatments. The experiments were laid out in a randomised complete block design, Experiment 1 with five blocks and Experiment 2 with four blocks. In the statistical analyses of Experiment 1 the four treatments were split into two factors, viz. amount of nitrogen (1.8 vs 5.4 g per pot) and number of applications (two vs six splits).

Experiments 3 and 4 had the same three treatments: N(0): no application of fertiliser nitrogen; N(200/1): 200 kg N per ha, applied shortly after planting; N(200/5): 200 kg N per ha, applied in five splits of 40 kg. At planting 77 kg ha^{-1} mineral N was available in the soil layer 0-60 cm in Experiment 3; this was 75 kg ha^{-1} for Experiment 4. These two experiments were laid out in a split-plot design with nitrogen treatment as main factor and harvest date (see *Sampling and plant analyses*) as split factor. Both experiments had four blocks. Statistical analyses of each experiment were made on separate data for each harvest, since we were interested in differences among treatments and not specifically among harvests. The significance of differences is assessed with an LSD-test (P=0.05), following an analysis of variance.

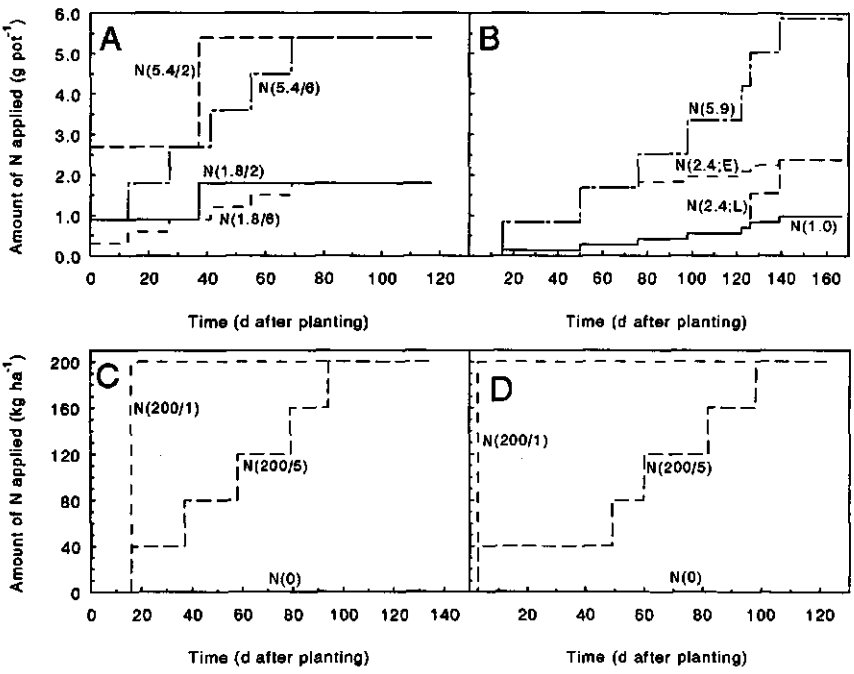

Figure 3.3.1. Accumulated amounts of nitrogen (in g pot^{-1} or kg ha^{-1}), applied to the different treatments of Experiment 1 (A), Experiment 2 (B), Experiment 3 (C) and Experiment 4 (D).

Sampling and plant analyses

Destructive analyses of plants were carried out several times: in Experiment 1 on six occasions between 26 and 117 DAP (days after planting), in Experiment 2 on six occasions between 54 and 167 DAP, in Experiment 3 on six occasions between 35 and 134 DAP and in Experiment 4 on five occasions between 38 and 122 DAP. In Experiments 1 and 2 on each sampling date one pot per treatment was used from each block. In Experiments 3 and 4 1.0 m^2 per treatment was harvested from each block on each sampling date.

The measurements included leaf area, and fresh and dry weights of leaf blades, leaf sheaths and scape (when present). In the growth analysis, each leaf blade and each leaf sheath were taken separately in Experiment 1, but in Experiments 2, 3 and 4 two successive leaf blades or leaf sheaths were pooled. Dead leaf blades and leaf sheaths were collected in Experiments 2, 3 and 4, although this was especially difficult in Experiment 4, because of the wet growing season. In this paper the shaft is defined as being the total of living leaf sheaths. (The marketable part comprises mainly the shaft.) With the term "leaf number" always the leaf insertion number is meant; otherwise the term "number of leaves" is used.

Dried samples were ground. To reduce the number of samples for chemical analysis, the samples from the replicates of all harvests, except the final ones, were pooled and mixed thoroughly. One subsample from this pooled sample was subsequently analysed for total nitrogen and nitrate (Biemond & Vos, 1992). At the final harvests the samples from the individual replicates were analysed separately, after leaf blades and leaf sheaths from all nodes of one plant were pooled.

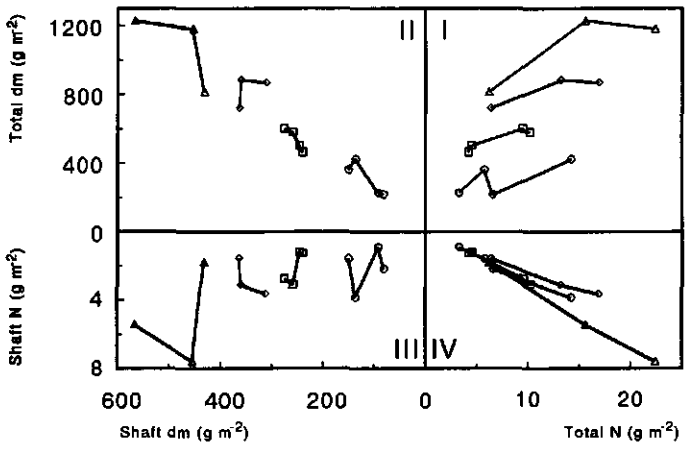

Figure 3.3.2. Relations between total dry matter production (Total dm), shaft dry matter production (Shaft dm), total nitrogen uptake (Total N) and shaft nitrogen uptake (Shaft N) at the final harvest of each experiment. All variables are in g m^{-2}. (□) Experiment 1; (○) Experiment 2; (△) Experiment 3; (◇) Experiment 4.

Results

Accumulation of dry matter and nitrogen

Figure 3.3.2 shows the relations between total dry matter production, shaft dry matter production, total nitrogen uptake and shaft nitrogen uptake at the final harvest of each experiment; each datum point represents one of the treatments. There was a close relation between amount of nitrogen applied and nitrogen uptake. The first quadrant shows large differences among experiments in dry matter production and nitrogen uptake across experiments. These differences will partly have resulted from differences in the amount of incident radiation, which increased in the sequence Experiment 2 - Experiment 1 - Experiment 4 - Experiment 3 (data not shown). The relative differences in total dry matter production within one experiment were much smaller than the relative differences in total

nitrogen uptake, resulting in large differences in total nitrogen concentration. In the second and third quadrant large differences existed between experiments as a result of large differences in absolute dry matter yield. The fourth quadrant, however, shows that the relations between total and shaft nitrogen were fairly conservative. A linear regression line for each experiment would practically pass the origin, indicating that harvest indices for nitrogen were fairly constant over ranges of dry matter production and nitrogen uptake.

Table 3.3.1. The harvest index for dry matter and for nitrogen at 117 DAP for Experiment 1, at 167 DAP for Experiment 2, at 134 DAP for Experiment 3 and at 122 DAP for Experiment 4. Different letters indicate that there was a significant difference between treatments.

Harvest index for dry matter

Experiment 1*		Experiment 2		Experiment 3		Experiment 4	
N(1.8/2)	0.49	N(1.0)	0.40 b	N(0)	0.53 c	N(0)	0.50 c
N(1.8/6)	0.52	N(2.4;E)	0.41 b	N(200/1)	0.46 b	N(200/1)	0.41 b
N(5.4/2)	0.46	N(2.4;L)	0.38 b	N(200/5)	0.39 a	N(200/5)	0.36 a
N(5.4/6)	0.44	N(5.9)	0.32 a				

Harvest index for nitrogen

Experiment 1*		Experiment 2		Experiment 3		Experiment 4	
N(1.8/2)	0.27	N(1.0)	0.27 a	N(0)	0.29 a	N(0)	0.24 a
N(1.8/6)	0.29	N(2.4;E)	0.27 a	N(200/1)	0.35 a	N(200/1)	0.24 a
N(5.4/2)	0.29	N(2.4;L)	0.33 a	N(200/5)	0.33 a	N(200/5)	0.21 a
N(5.4/6)	0.30	N(5.9)	0.27 a				

* applying 5.4 instead of 1.8 g N per pot and in two times instead of six had no significant effects.

Partitioning of dry matter to different plant organs

The harvest indices for dry matter are calculated as the amount of dry matter in the living leaf sheaths (= the shaft), divided by the total amount of dry matter. At the first intermediate harvest of each experiment harvest indices were between 0.35 and 0.50. Except in Experiment 2 (carried out in winter) the harvest indices increased slightly with time: final values are shown in Table 3.3.1. In Experiment 1 the final harvest indices for dry matter were not significantly affected by treatments, but in Experiments 2-4 applying more nitrogen decreased the final harvest indices for dry matter significantly. Nitrogen stimulated leaf blade growth more than it stimulated leaf sheath growth. The lower harvest indices for dry matter in N(200/5) compared to N(200/1) of Experiments 3 and 4 can be explained as a result of a better nitrogen availability in N(200/5): although the total amount of nitrogen applied was the same, at the end of both experiments more nitrogen was available for the N(200/5) in the

upper 30 cm of the soil (data not shown), where most roots are found.

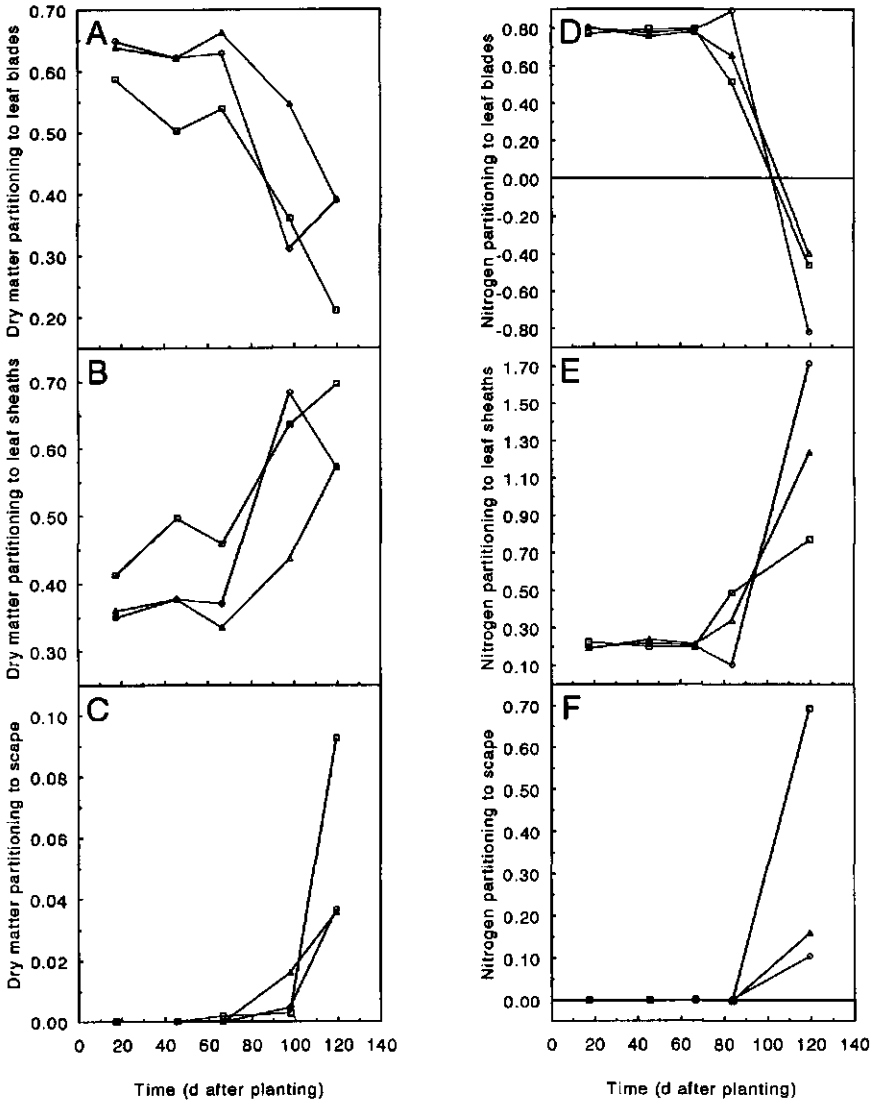

Figure 3.3.3. Changes with time in the relative partitioning rates of dry matter increase to the leaf blades (including dead ones; A), leaf sheaths (including dead ones; B) and scape (C) and changes with time in the relative partitioning rates of nitrogen increase to the leaf blades (including dead ones; D), leaf sheaths (including dead ones; E) and scape (F) of Experiment 3. Note differences in scales of y-axes. (□) N(0); (○) N(200/1); (△) N(200/5).

The relative partitioning rates of dry matter increase to leaf blades (including dead ones), leaf sheaths (including dead ones) and scape are calculated from the increase in dry matter of a particular plant part over the period of time between harvests, divided by the total increase of dry matter in the same period. Figures 3.3.3A, B and C show the relative partitioning rates in Experiment 3, which decreased for leaf blades and increased for leaf sheaths and scapes during the second half of the growing season. Similar patterns (not shown) were observed in Experiment 4; they indicate that the rate of storage of dry matter in leaf sheaths increased at the end of the growing season, probably because leek is a biennial. The relatively high final partitioning rate to scapes in Experiment 1 (about 0.30) was caused by the unusually high number of scapes observed in this experiment.

Partitioning of nitrogen to different plant organs

The harvest index for nitrogen was always lower than the harvest index for dry matter due to the lower than average nitrogen concentration of the shaft. The final harvest indices for nitrogen (Table 3.3.1) were not affected by treatments, indicating negligible effects of nitrogen nutrition on the nitrogen partitioning. This is also illustrated for Experiment 3 in Figures 3.3.3D, E and F: the relative partitioning rates of nitrogen increase to leaf sheaths during the first half of this experiment were similar in all treatments.

Although decreasing relative partitioning rates of nitrogen increase to leaf blades at the end of an experiment were also observed in Experiments 1 and 2, negative values (which indicate reallocation of nitrogen from leaf blades to other plant organs) were only found in Experiment 3. Nitrogen shortage as in N(0) of Experiments 3 and 4 stimulated the growth of the scape: the relative partitioning rates of dry matter and nitrogen increase to the scape at the end of an experiment (Figures 3.3.3C and F) were largest in N(0).

Concentration of nitrogen and nitrate in plant organs of different nodes

Figure 3.3.4 shows the total nitrogen concentrations of different pairs of leaf blades and leaf sheaths in Experiment 4 at 60 and 122 DAP. The pattern for the leaf blades of Experiment 3 was similar to that for the leaf blades of Experiment 4 in Figure 3.3.4, but in Experiments 1 and 2 the total nitrogen concentrations of leaf blades at each harvest were higher at higher leaf numbers; this was caused by a decrease of the total nitrogen concentration of leaf blades with time. In Experiments 1 and 2 the concentrations of the lowest leaf blade numbers were about 2.5% and the concentrations of the highest leaf blade numbers decreased with time from 5.5 to 2.5%. Although in Experiments 3 and 4 the differences in the total nitrogen concentrations between different leaf blade numbers were negligible, the total nitrogen concentration of each leaf blade pair still decreased with time: all leaf blade pairs had lower concentrations at later harvests (see Figure 3.3.5).

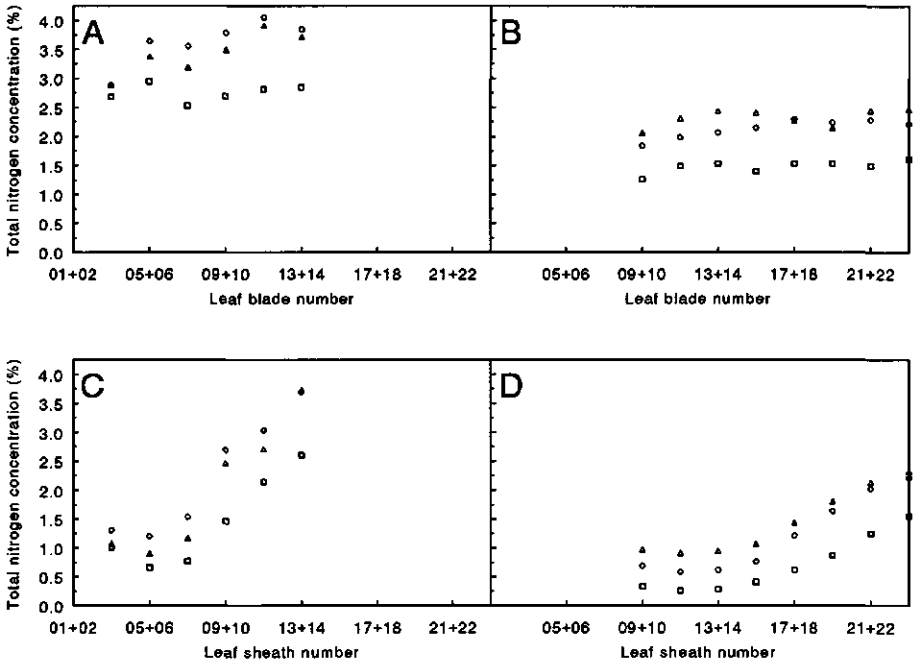

Figure 3.3.4. The concentration of total nitrogen in the dry matter of the different pairs of leaf blades (A and B) and leaf sheaths (C and D) for Experiment 4 at 60 (A and C) and 122 DAP (B and D). (□) N(0); (○) N(200/1); (△) N(200/5).

The pattern of the total nitrogen concentrations of different leaf sheaths was similar in all experiments (Figures 3.3.4C and D: Experiment 4): this concentration increased with increasing leaf sheath number. The concentration in a certain leaf blade (pair) was always higher than in the corresponding leaf sheath (pair) (see Figure 3.3.5 for an example). The differences in nitrogen concentration between similar leaf blade and leaf sheath number were highest for the lowest observed leaf number and nearly negligible for the highest observed leaf number at a certain harvest.

Figure 3.3.5 shows an example of the changes in the total nitrogen concentration of leaf blades and leaf sheaths with time. The differences in the rates of leaf appearance between the treatments were negligible (see Biemond, 1995); this leaf pair (9+10) appeared about 32 DAP. This implies that the starting value of the total nitrogen concentration was different between the treatments.

The nitrate nitrogen concentration of a leaf blade or leaf sheath (pair) was usually below 0.5% of the dry weight. At each harvest the highest concentrations were observed in the

middle leaf numbers. The nitrate nitrogen concentration of a leaf sheath was between one and two times the nitrate nitrogen concentration of the corresponding leaf blade.

The average nitrate nitrogen concentrations over all above-ground plant parts were below 0.4% at all harvests of all experiments; usually they decreased with time. Final values were below 0.05%, except for the N(2.4;L) and N(5.9) of Experiment 2: they had average concentrations of 0.07 and 0.19%, respectively.

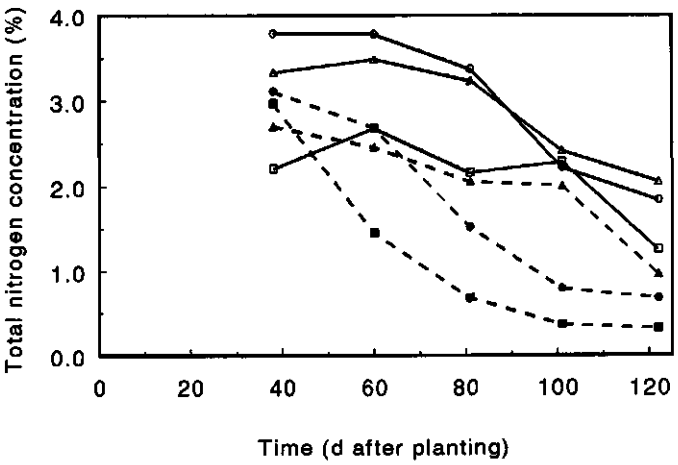

Figure 3.3.5. Changes with time in the concentration of total nitrogen in the dry matter of leaf blades 9+10 (open symbols; (□) N(0); (○) N(200/1); (△) N(200/5)) and leaf sheaths 9+10 (closed symbols; (■) N(0); (●) N(200/1); (▲) N(200/5)) of Experiment 4.

Discussion

Within experiments the accumulation of total dry matter and nitrogen of leek was affected in various ways by amount and timing of the nitrogen fertilisation. The differences between the experiments are probably attributable to other factors, such as the amount of incident radiation. The maximum total dry matter production was in accordance with the value mentioned by Brewster (1994), viz. 1200 g m^{-2}; the maximum total nitrogen uptake (about 22.5 g m^{-2}) was about 10% below the uptake observed by Booij *et al.* (1993) in a comparable experiment.

Although the partitioning of dry matter in the current experiments was clearly affected by the nitrogen treatments, Booij *et al.* (1993) observed, with nitrogen fertiliser rates varying from 0 to 250 kg N ha^{-1}, a similar harvest index for dry matter of about 0.54 for all nitrogen

treatments. The harvest index for nitrogen in the experiment of Booij *et al.* was equal to that for dry matter, while in this study much lower values were observed. A lower harvest index for dry matter at higher nitrogen rates, as observed in the current experiments, was observed by Greenwood *et al.* (1980) for crops as sugar beet, radish and swede, even when total dry matter was unaffected.

The final nitrate nitrogen concentrations observed are in accordance with the values, observed by Venter (1982) who found maximum values of about 0.05% of the dry weight.

The question arises why no increase in total nitrogen concentration with increasing leaf number was observed in leaf blades of Experiments 3 and 4. Such an increase is often found and explained by removal of nitrogen with leaf age. Allocation of nitrogen in the upper canopy layers is efficient for the plant since these leaves receive most of the light. (Hirose & Werger, 1987). However, assuming the light distribution in the canopy affects the nitrogen distribution (see e.g. Hikosaka *et al.* (1994), who observed this for a vine), different parts of one leek leaf may have different nitrogen concentrations. This results from the leaf orientation, because the tip of a leaf (which is the oldest part) is exposed to higher light intensities than the base of a leaf, which is more overshadowed by higher leaves. These specific properties of leaves of leek may result in an alternation of the gradient in nitrogen concentration with leaf number.

The differences in the total nitrogen concentrations in leaf sheaths were probably the result of the differences in age between the leaves, the younger leaf sheaths having higher total nitrogen concentrations. However, the total nitrogen concentrations in the oldest two leaf sheath pairs were higher than expected according to the trends, probably because these leaf sheaths were exposed to the light and will therefore have contained some chlorophyll. This can also be the reason, why the highest nitrate nitrogen concentrations were not observed in the oldest leaf sheaths, but in the middle leaf sheath numbers: in the oldest leaf sheaths more nitrogen could be reduced, because the nitrate reductase activity was higher as result of the exposure to light. Darwinkel (1975) observed the highest nitrate concentrations in crops as turnip and rape in the oldest leaves: he explained this as a result of the low nitrate reductase activity in these leaves. In accordance with current results, Venter (1982) observed the highest nitrate concentrations in the middle leaf sheath numbers of leek.

The results from the current experiments show that a larger amount of available nitrogen does not necessarily lead to a higher dry matter yield of the shaft, because the harvest index for dry matter decreases. The harvest index for nitrogen was equal at different amounts of available nitrogen; this means the amounts of nitrogen in crop residues, which can leach below the rooting depth of the next crop, after mineralization, are higher at higher crop yields.

References

Biemond, H., 1995. Effects of nitrogen on development and growth of the leaves of vegetables. 2. Appearance, expansion growth and life span of leaves of leek plants. *Netherlands Journal of Agricultural Science* 43: 233-246.

Biemond, H. & J. Vos, 1992. Effects of nitrogen on the development and growth of the potato plant. 2. The partitioning of dry matter, nitrogen and nitrate. *Annals of Botany* 70: 37-45.

Booij, R., C.T. Enserink, A.L. Smit & A. van der Werf, 1993. Effects of nitrogen availability on crop growth and nitrogen uptake of Brussels sprouts and leek. *Acta Horticulturae* 339: 53-65.

Brewster, J.L., 1994. Onions and other vegetable alliums. Crop production science in horticulture 3. CAB International, Wallingford, 236 pp.

Darwinkel, A., 1975. Aspects of assimilation and accumulation of nitrate in some cultivated plants. Dissertation Wageningen Agricultural University. Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen, 64 pp.

Dragland, S., 1972. Effect of temperature and day-length on growth, bulb formation and bolting in leeks (*Allium porrum* L.). Agricultural University of Norway, Department of Vegetable Crops, Report No. 46. Agricultural University of Norway, Oslo, 23 pp.

Greenwood, D.J., T.J. Cleaver, M.K. Turner, J. Hunt, K.B. Niendorf & S.H.M. Loquens, 1980. Comparison of the effects of nitrogen fertilizer on the yield, nitrogen content and quality of 21 different vegetable and agricultural crops. *Journal of Agricultural Science* (Cambridge) 95: 471-485.

Hikosaka, K., I. Terashima & S. Katoh, 1994. Effects of leaf age, nitrogen nutrition and photon flux density on the distribution of nitrogen among leaves of a vine (*Ipomoea tricolor* Cav.) grown horizontally to avoid mutual shading of leaves. *Oecologia* 97: 451-457.

Hirose, T. & M.J.A. Werger, 1987. Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. *Physiologia Plantarum* 70: 215-222.

Venter, F., 1982. Der Nitratgehalt in Porree. Der Einfluss von N-Menge, Erntezeitpunkt und Düngerform. *Gemüse* 18: 402-405.

SECTION 3.4

Effects of nitrogen on accumulation and partitioning of dry matter and nitrogen of vegetables. 3. Spinach

H. Biemond

Abstract

Much research has been done on the nitrate accumulation of spinach (*Spinacia oleracea* L.), but research on environmentally friendly fertiliser strategies for spinach is still scarce. The purpose of the current study was to analyse the dynamics of dry matter and nitrogen accumulation in spinach. Six experiments were carried out with different amounts and different dates of application of nitrogen. In these experiments frequent measurements were carried out on dry matter and nitrogen accumulation in leaf blades, petioles and stems.

The total accumulation of dry matter and nitrogen differed largely among and within experiments. However, the relative partitioning rates of dry matter increase for the different plant organs were unaffected by treatments. As a result the harvest indices for dry matter of a crop in the marketable stage were constant at about 0.67 in all experiments. The same applies to the harvest indices for nitrogen, which were on average 0.74. The organic nitrogen concentration of leaf blades and petioles decreased with leaf age, although in most experiments this decrease was smaller at higher leaf numbers. The nitrate nitrogen concentration decreased with increasing leaf number at any sampling harvest. Average nitrate nitrogen concentrations over all plant parts were maximally 1.5% of the dry mass.

Introduction

Spinach is an annual plant with a short growth cycle. During the vegetative growth a leaf rosette is produced. The harvest for fresh consumption usually takes place at the end of the vegetative phase, because flower stalks are unwanted in the marketable produce.

Spinach has often been used as a model crop to study the nitrate accumulation and its regulation (e.g. Breimer, 1982; Steingröver, 1986). However, information is still scarce about the effects of nitrogen fertilisation on the dynamics of accumulation and partitioning of fresh matter, dry matter and nitrogen. Such information is helpful to match the nitrogen

fertilisation to the demand of the crop, in order to minimise nitrogen losses to the environment. It can also be helpful to reduce the nitrate concentrations in the marketable produce.

Effects of nitrogen on leaf development and growth of spinach were described by Biemond (1995). More nitrogen increased the total green leaf area by increasing the size of individual leaves. This resulted from an increase of the rate of leaf expansion; the duration of expansion was not affected. The number of leaves remained constant. In this paper the accumulation of dry matter and nitrogen and the relative partitioning of dry matter and nitrogen over leaf blades, petioles and other organs are analysed.

Materials and methods

In this paper four field and two glasshouse experiments are described. Table 3.4.1 summarizes when and where each experiment was carried out.

Table 3.4.1. General information about six experiments with spinach: year, type of experiment (field/glasshouse), growing period (summer/autumn), sowing date and date of final observations.

Experiment	Year	Type of experiment	Growing period	Sowing date	Final observations
1	1991	field	summer	25 Apr	25 Jun
2	1991	field	autumn	5 Aug	25 Sep
3	1992	field	summer	7 May	14 Jul
4	1992	field	autumn	19 Aug	20 Oct
5	1993	glasshouse	summer	18 May	5 Jul
6	1993	glasshouse	autumn	4 Aug	12 Oct

Plant culture - field

Experiments 1-4 (the field experiments) were conducted on a sandy soil with about 3% organic matter. Cv. Trias was sown at 5.5 g seed per m^2, ca 1.5 cm deep, in rows with a row-spacing of 12.5 cm, resulting in a plant density of about 400 plants m^{-2}. Irrigation was applied whenever necessary.

Plant culture - glasshouse

In Experiments 5 and 6 rectangular trays with the following dimensions were used: length 45, width 30 and depth 15 cm. These trays were filled with sand, free from organic matter. After filling with sand, seventy-five seeds of cv. Trias were sown, about 1.5 cm deep in

three rows, which resulted in a plant density similar to the ones in Experiments 1-4. The glasshouse, in which the trays were placed, was set to maintain a day temperature of 18 °C (12 h) and a night temperature of 12 °C. In Experiment 5 natural light was supplemented with 400 Watt Philips SON-AGRO-T lamps at a density of 0.7 lamps m^{-2}. See Biemond (1995) for further details.

Table 3.4.2. Amounts per dressing and total amounts (in kg per ha (Experiments 1-4) or g per tray (Experiments 5 and 6)) of application of fertilizer nitrogen in two field experiments in 1991 (Experiments 1 and 2), two field experiments in 1992 (Experiments 3 and 4) and two glasshouse experiments in 1993 (Experiments 5 and 6) with spinach.

Experiment 1			Experiment 2		
Code	Amount*	Total	Code	Amount*	Total
N(0)	0	0	N(0)	0	0
N(75+50)	75+50	125	N(50+25)	50+25	75
N(125/5)	5*25	125	N(75/5)	5*15	75
N(120+80)	120+80	200	N(90+60)	90+60	150
N(200/5)	5*40	200	N(150/5)	5*30	150
Experiment 3			**Experiment 4**		
Code	Amount*	Total	Code	Amount*	Total
N(0)	0	0	N(0)	0	0
N(200/1)	200	200	N(200/1)	200	200
N(200/5)	5*40	200	N(200/5)	5*40	200
Experiment 5			**Experiment 6**		
Code	Amount*	Total	Code	Amount*	Total
N(1.12)	4*0.28	1.12	N(1.68)	6*0.28	1.68
N(1.68;L)	3*0.28+0.84	1.68	N(3.36;L)	3*0.28+3*0.84	3.36
N(3.36)	4*0.84	3.36	N(5.04)	6*0.84	5.04

* a+b means splits in a and b amounts;
c*d means c times an amount of d.

Treatments and experimental design

The treatments consisted of different total amounts of nitrogen and different dates of application of nitrogen. Table 3.4.2 shows these amounts; see Biemond (1995) for dates of application. In Experiment 1, 94 kg ha^{-1} mineral N was available in the soil layer 0-60 cm at sowing; this was 158 kg ha^{-1} in Experiment 2, 77 in Experiment 3 and 66 in Experiment 4. In Experiments 5 and 6 the purposes of the different amounts and dates of application were for N(1.12) and N(1.68) to have nitrogen limitation throughout the experiment, for

N(1.68;L(ate)) and N(3.36;L(ate)) to have limiting supply in the early stages of growth and non-limiting supply later and for N(3.36) and N(5.04) to have non-limiting nitrogen supply throughout the experiment. In all experiments other nutrients than nitrogen were supplied in equal amounts to all treatments.

Experiments 1-4 were laid out in a split-plot design with nitrogen treatment as main factor and harvest date as split factor. Experiments 5 and 6 were laid out in a randomised complete block design, in which each tray was regarded as one experimental unit. All experiments had four blocks. In the statistical analyses, every harvest was analysed separately. The significance of differences was assessed with an LSD-test (P=0.05), following an analysis of variance.

Sampling and plant analyses

Destructive analyses of plants were made six, seven or eight times in each experiment. These analyses were carried out more or less weekly from emergence until the final date (Table 3.4.1). In the case of summer spinach, this final date was (far) beyond the normal harvest date. In Experiments 1-4 at each sampling date 0.25 m^2 per treatment was harvested from each block. In Experiments 5 and 6 at each sampling date one tray (= 0.135 m^2) per treatment was used from each block.

Measurements included leaf area, and fresh and dry weights of leaf blades, petioles, hypocotyl + stem (= stem as far as leaf number 18) and top (= stem + leaves + flower initials + flowers above leaf number 18; only observed in summer spinach; the leaves above number 18 were very small). Leaves were divided into leaf blades and petioles in Experiments 3-6, but not in Experiments 1 and 2. Leaves or leaf blades and petioles were sampled separately up to leaf number 18. In all experiments material from two successive leaf positions on the stem was pooled. With the term "leaf number" always the leaf insertion number is meant; otherwise the term "number of leaves" is used.

Dried samples were ground, but to reduce the number of samples for chemical analysis the samples from the replicates were pooled and mixed thoroughly. One subsample from this pooled sample was subsequently analysed for total nitrogen and nitrate (Biemond & Vos, 1992). However, the samples of Experiment 4 were not analysed for total nitrogen or nitrate and at the final harvests of Experiments 1, 2 and 6, all plant parts of one treatment were pooled after drying (instead of the samples from the replicates) and subsequently analysed for total nitrogen and nitrate (Biemond & Vos, 1992).

In this paper organic nitrogen is the difference between the total nitrogen concentration and the nitrogen present as nitrate.

Results

Accumulation of dry matter and nitrogen

Figure 3.4.1 shows the relations between total dry matter production, total nitrogen uptake, dry matter production of leaf blades and nitrogen uptake of leaf blades, for each experiment at the harvest at which the crop was marketable. Each datum point represents one of the treatments; the treatments of one experiment are connected by a line (from Experiments 1, 2 and 4 half of the data are lacking (see **Materials and methods**)). In the first quadrant, lines from all experiments ran more or less parallel. However, the relative differences in total nitrogen uptake within an experiment were much larger than in total dry matter production, which indicates that large differences in total nitrogen concentrations existed. The relations between total and leaf blade dry matter production (second quadrant) were similar in all experiments. One linear regression line for all experiments would practically pass the origin, indicating that the fraction dry matter in leaf blades did not differ among experiments nor among treatments.

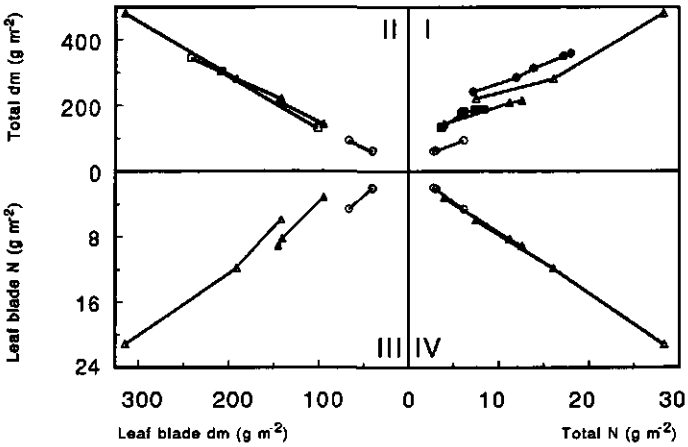

Figure 3.4.1. Relations between total dry matter production (Total dm), leaf blade dry matter production (Leaf blade dm), total nitrogen uptake (Total N) and leaf blade nitrogen uptake (Leaf blade N) at 35 DAE (days after emergence) for Experiment 1, at 42 DAE for Experiment 2, at 26 DAE for Experiment 3, at 57 DAE for Experiment 4, at 27 DAE for Experiment 5 and at 61 DAE for Experiment 6 (as far as data were available). All variables are in g m^{-2}. (■) Experiment 1; (●) Experiment 2; (▲) Experiment 3; (□) Experiment 4; (○) Experiment 5; (△) Experiment 6.

The lines from all experiments in the third quadrant ran parallel to each other, similar to those in the first quadrant: this indicates large differences in leaf blade nitrogen concentrations existed. The relations between total and leaf blade nitrogen uptake (fourth quadrant) were similar for all experiments: the fraction nitrogen in leaf blades was equal among experiments and treatments in the same way as the fraction dry matter in leaf blades in the second quadrant.

Partitioning of dry matter to different plant organs

In the present spinach experiments, the harvest indices were calculated by dividing the amount of dry matter in the leaf blades by the total amount of dry matter. The harvest indices for dry matter (Table 3.4.3; only available for Experiments 3-6) were significantly affected by treatments in two experiments, but there was no clear trend.

Table 3.4.3. The harvest index for dry matter and for nitrogen at 26 DAE for Experiment 3, at 57 DAE for Experiment 4, at 27 DAE for Experiment 5 and at 61 DAE for Experiment 6. Data of the harvest index for nitrogen were not available for Experiment 4. Different letters indicate that there was a significant difference between treatments. No statistical analyses could be carried out on the harvest indices for nitrogen (see section on *Sampling and plant analyses* in **Materials and methods**).

Harvest index for dry matter

Experiment 3		Experiment 4		Experiment 5		Experiment 6	
N(0)	0.65 a	N(0)	0.76 b	N(1.12)	0.64 a	N(1.68)	0.63 a
N(200/1)	0.67 a	N(200/1)	0.70 a	N(1.68;L)	0.63 a	N(3.36;L)	0.68 b
N(200/5)	0.67 a	N(200/5)	0.69 a	N(3.36)	0.70 a	N(5.04)	0.65 ab

Harvest index for nitrogen

Experiment 3		Experiment 5		Experiment 6	
N(0)	0.79	N(1.12)	0.71	N(1.68)	0.79
N(200/1)	0.72	N(1.68;L)	0.69	N(3.36;L)	0.73
N(200/5)	0.73	N(3.36)	0.75	N(5.04)	0.75

The relative partitioning rates of dry matter increase were calculated from the increase of dry matter of a particular plant part over the period between harvests, divided by the total increase of dry matter in the same period. The differences between treatments in the relative partitioning rates of dry matter increase to leaf blades, petioles and stems (comprising hypocotyl, complete stem, and leaves, flower initials and flowers above leaf number 18) (Figures 3.4.2A, B and C: Experiment 5) were small, which confirms that effects of nitrogen on the partitioning of dry matter were small. Similar patterns as in Figure 3.4.2 were

observed in Experiment 3 (the other experiment with summer spinach). In autumn spinach, however, no decrease in the relative partitioning rates to leaf blades and petioles was observed three of four weeks after emergence, while no stem elongation took place.

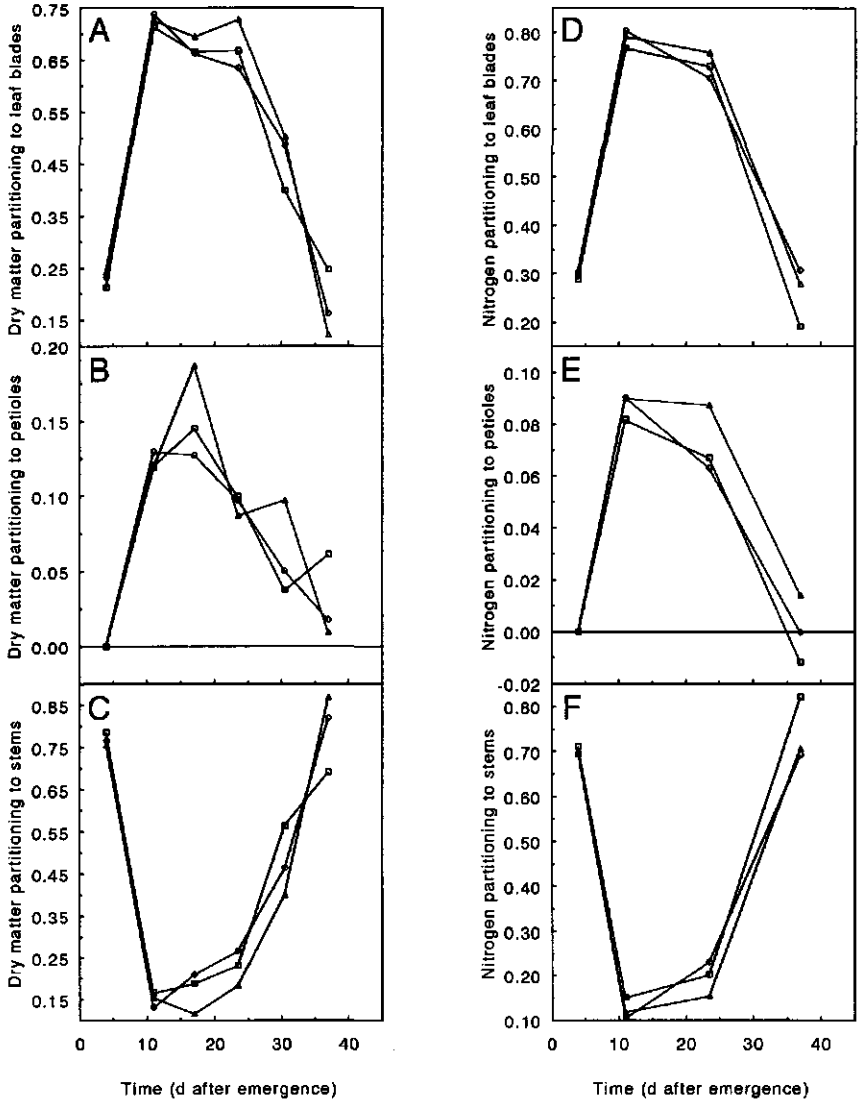

Figure 3.4.2. Changes with time in the relative partitioning rates of dry matter increase to the leaf blades (A), the petioles (B) and the stem (comprising hypocotyl, complete stem, and leaves, flower initials and flowers above leaf number 18; C) and changes with time in the relative partitioning rates of nitrogen increase to the leaf blades (D), the petioles (E) and the stem (comprising hypocotyl, complete stem, and leaves, flower initials and flowers above leaf number 18; F) of Experiment 5. Note differences in scales of y-axes. (□) N(1.12); (○) N(1.68;L); (△) N(3.36).

Partitioning of nitrogen to different plant organs

The nitrogen concentration of leaf blades was higher than the average nitrogen concentration in the whole plant. Therefore, the harvest indices for nitrogen were always higher than the harvest indices for dry matter (Table 3.4.3). The differences between treatments in these harvest indices were small, although the average nitrogen concentrations in the whole plant of treatments with the largest amount of nitrogen applied, were twice as high as in the treatments with the lowest amount of nitrogen applied (data not shown).

The relative partitioning rates of nitrogen increase followed a similar course over time as the relative partitioning rates for dry matter increase. Figures 3.4.2D, E and F show an example from Experiment 5. They were for leaf blades higher and for petioles and stems lower than those for dry matter, also reflected by the higher than average total nitrogen concentration of leaf blades.

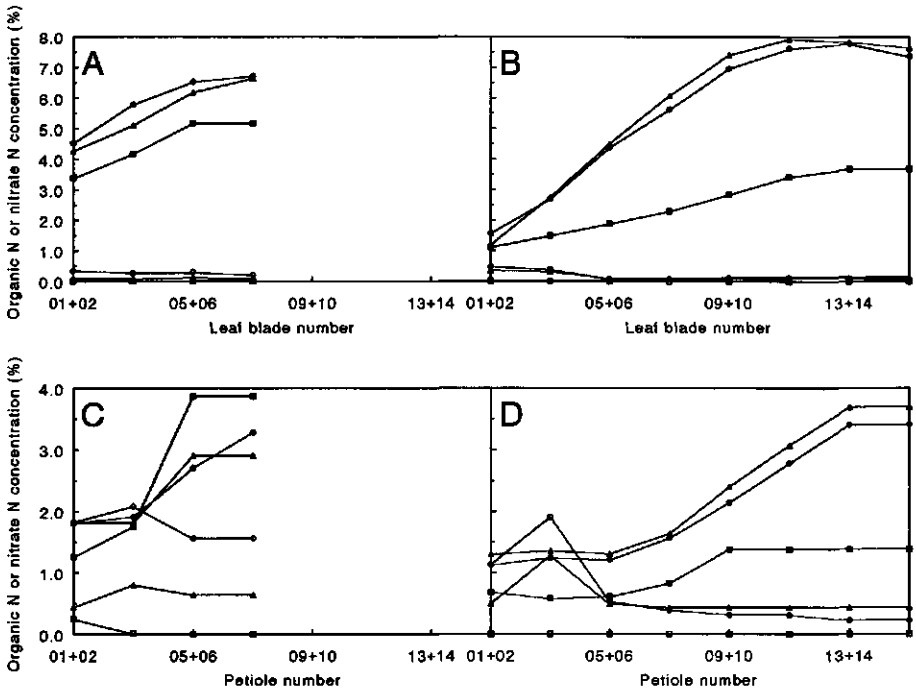

Figure 3.4.3. The concentration of organic nitrogen (closed symbols; (■) N(0); (●) N(200/1); (▲) N(200/5)) and nitrate nitrogen (open symbols; (□) N(0); (○) N(200/1); (△) N(200/5)) in the dry matter of the different pairs of leaf blades (A and B) and petioles (C and D) in Experiment 3 at 19 (A and C) and 33 DAE (B and D).

Concentration of nitrogen and nitrate in plant organs of different nodes

Figure 3.4.3 shows the organic nitrogen and nitrate nitrogen concentrations of successive pairs of leaf blades and petioles in Experiment 3 at 19 and 33 DAE. Similar patterns were observed in the other experiments. Organic nitrogen concentrations of young leaf blades were around 8.0% in many treatments, while the nitrogen concentration of the oldest leaf blades of the same plants was 6% lower at that same moment (Figure 3.4.3B). The highest organic nitrogen concentrations were not observed in the youngest leaves, but in the third youngest leaves. The organic nitrogen concentration in petioles was half that in leaf blades.

Usually higher nitrate concentrations were observed in autumn spinach than in summer spinach. Nitrate nitrogen concentrations decreased with increasing leaf number and were much higher in petioles than in leaf blades (Figure 3.4.3). In several cases the measured nitrate nitrogen concentrations in petioles were higher than the organic nitrogen concentrations, e.g. in the oldest petioles in Figure 3.4.3D.

Figure 3.4.4 shows examples of the changes with leaf age of the organic nitrogen and nitrate nitrogen concentrations of leaf blades. This leaf pair appeared about 18 DAE (see Biemond, 1995). The decrease with leaf age in the organic nitrogen concentrations (Figure 3.4.4A) during (the first half of) the leaf's life explains why the organic nitrogen concentrations in Figure 3.4.3 increased with increasing leaf number. In most experiments, for lower leaf numbers than 03+04, the decrease in nitrogen concentration was larger and lasted during a larger part of the leaf's life, while the opposite applies to higher leaf numbers. As a result of the application of extra nitrogen to the N(3.36;L) plants during the second half of this experiment, the nitrate nitrogen concentration and amount of total nitrogen increased during the second half of the leaf's life in the N(3.36;L) leaf blade pair. The organic nitrogen concentration and the amount of dry matter (Figure 3.4.4D) of the N(1.68) and N(3.36;L) leaves were more or less constant during the second half of the leaf's life. This indicates that no redistribution of nitrogen from this leaf pair (Figure 3.4.4C) occurred. For this leaf pair there were large differences in total nitrogen concentration among treatments, but the differences in dry matter accumulation were small.

The curves for the average nitrate nitrogen concentrations over all above-ground plant parts with time differed between experiments and within an experiment between treatments. Treatments with nitrogen deficiency had concentrations below 0.1%, except during the first three weeks after emergence, but treatments with a large amount of available nitrogen reached final values between 0.7 (Experiment 1) and 1.5% (Experiment 6).

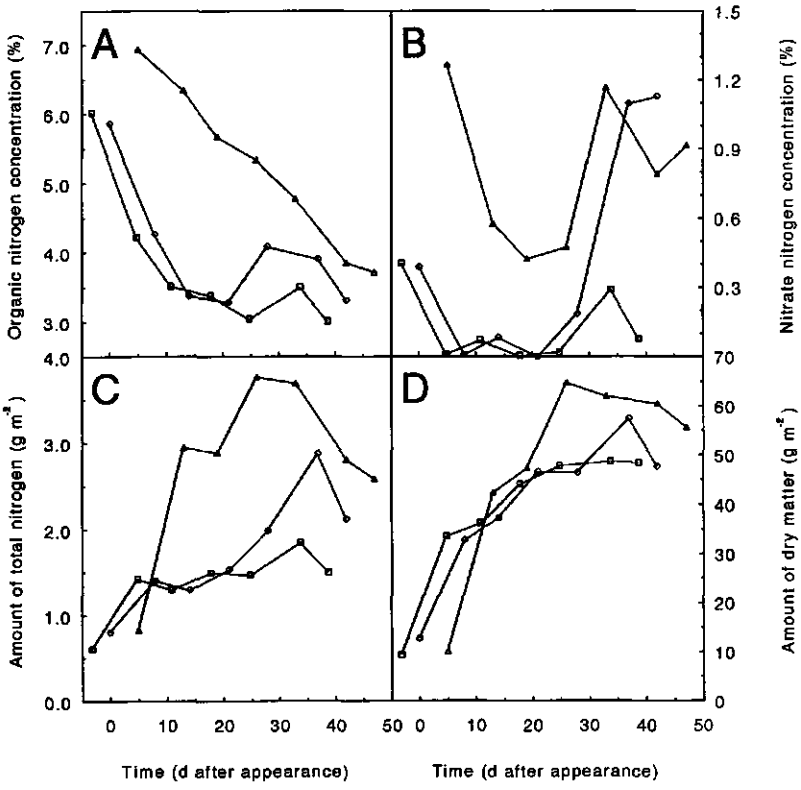

Figure 3.4.4. Changes with average leaf age in the concentrations (%) of organic nitrogen (A) and nitrate nitrogen (B) in the dry matter, total amount (g m^{-2}) of nitrogen (C) and dry matter (D) of leaf blades 03+04 of Experiment 6. (□) N(1.68); (○) N(3.36;L); (△) N(5.04).

Discussion

The accumulation of total and leaf blade dry matter and nitrogen was affected in different ways by the amount of nitrogen applied and the timing of the nitrogen fertilisation. In the first place different amounts of nitrogen resulted in large differences in dry matter and nitrogen accumulation. In the second place the differences in nitrogen accumulation were relatively larger than in dry matter accumulation; at a larger amount of nitrogen applied, a higher total nitrogen concentration was attained. Niers (1994) reported an average nitrogen uptake (excluding roots) of 125 kg ha^{-1}, which is close to the current results. However, in my experiments the partitioning of dry matter and nitrogen was unaffected by nitrogen treatments. The partitioning functions from Figure 3.4.2, both for dry matter and nitrogen,

can be used in simulation models without taking into account the amount of nitrogen which is available to the plant. Effects of other factors on the partitioning of dry matter and nitrogen, which should be taken into account in a simulation model, were also small, as differences between experiments in harvest indices were small.

The organic nitrogen concentrations in the lowest leaf blade numbers decreased with increasing leaf age. This decrease, which is also often observed for other plant species (e.g. by Hikosaka *et al.* (1994) for a vine), is explained by redistribution of nitrogen from older leaves to young leaves. Allocation of nitrogen to young leaves in the upper canopy layers is efficient for the plant in terms of dry matter production, since the upper leaves receive most of the light (Hirose & Werger, 1987). When no redistribution of nitrogen takes place (Figure 3.4.4), growing new leaves requires nitrogen, taken up by the roots.

The highest nitrate nitrogen concentrations were observed in the oldest leaves, confirming results of Breimer (1982). Darwinkel (1975), who observed this for crops as turnip and rape, explained this as a result of the low nitrate reductase activity in the oldest leaves. In most cases differences in nitrate nitrogen concentrations explained only part of the differences between treatments in total nitrogen concentrations. An acceptable yield in autumn spinach was usually accompanied by a high nitrate concentration. The amount of nitrogen, which had to be available to the crop to reach an acceptable yield, was high above the nitrogen uptake. Therefore it is unwise to grow autumn spinach, because it both leads to high nitrate concentrations in the marketable produce and high nitrogen losses from the field, after harvest.

References

Biemond, H., 1995. Effects of nitrogen on development and growth of the leaves of vegetables. 3. Appearance and expansion growth of leaves of spinach. *Netherlands Journal of Agricultural Science* 43: 247-260.

Biemond, H. & J. Vos, 1992. Effects of nitrogen on the development and growth of the potato plant. 2. The partitioning of dry matter, nitrogen and nitrate. *Annals of Botany* 70: 37-45.

Breimer, T., 1982. Environmental factors and cultural measures affecting the nitrate content in spinach. *Fertilizer Research* 3: 191-292.

Darwinkel, A., 1975. Aspects of assimilation and accumulation of nitrate in some cultivated plants. Dissertation Wageningen Agricultural University. Centre for Agricultural Publishing and Documentation (PUDOC), Wageningen, 64 pp.

Hikosaka, K., I. Terashima & S. Katoh, 1994. Effects of leaf age, nitrogen nutrition and photon flux density on the distribution of nitrogen among leaves of a vine (*Ipomoea tricolor* Cav.) grown horizontally to avoid mutual shading of leaves. *Oecologia* 97: 451-457.

Hirose, T. & M.J.A. Werger, 1987. Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of a *Solidago altissima* stand. *Physiologia Plantarum* 70: 215-222.

Niers, H., 1994. N-uptake and crop properties of spinach (*Spinacia oleracea* L.) in the field (in Dutch). Report 3. Institute for Agrobiological and Soil Fertility Research, Haren, 77 pp.

Steingröver, E.G., 1986. Nitrate accumulation in spinach. Ph.D. Thesis Rijksuniversiteit Groningen. Van Denderen, Groningen, 84 pp.

CHAPTER 4

General discussion

General discussion

In the first section of this chapter, the leaf development and growth of potato, Brussels sprouts, leek and spinach will be compared and discussed. In the second section this will be done for the accumulation and partitioning of dry matter and nitrogen. Also the relations between both aspects and some scientific and practical implications will be discussed.

Leaf development and growth

The total dry matter production of a crop mainly depends on the total leaf area duration of that crop. For potato and Brussels sprouts the leaf area is indirectly important for the amount of marketable produce, because not the leaves, but the tubers and sprouts, respectively, are consumed. However, the marketable produce of a leek crop consists of the lower parts of the leaves (the leaf sheaths) and in the case of spinach the leaves are consumed. Therefore, for leek and spinach, number, area and thickness of leaves are yield components.

The progress of the leaf area over time is the resultant of the following variables: rate of leaf appearance, rate of leaf senescence (= rate of leaf disappearance), expansion rate of each leaf and duration of expansion of each leaf. The dates of appearance and disappearance of a leaf determine the life span of a leaf. The expansion rate and duration of expansion of each leaf determine the area of a leaf at a certain moment. The total leaf area per unit soil area (e.g. per m^2) is the resultant of the leaf area per plant, multiplied by the plant density. It is important to keep this in mind, because the four crops were grown at very different plant densities. For each of the four crops Table 4.1 gives values for several leaf growth characteristics, which were observed when nitrogen was not limiting. Spinach is grown at much larger plant densities than the other crops, because spinach plants are much smaller than plants of potato, Brussels sprouts or leek.

The thickness of a leaf partly determines the fraction of the incoming radiation which is absorbed and the fraction which is transmitted per leaf layer. This leaf thickness partly depends on the specific leaf area (SLA).

Table 4.1. Typical values per plant or per leaf of several leaf growth characteristics of potato, Brussels sprouts, leek and spinach as observed under the described environmental conditions (see Chapter 2) in treatments without nitrogen stress. Plant densities used were 3.6 for potato, 3.3 for Brussels sprouts, 20 for leek and 400 plants m^{-2} for spinach. Most values are averages; "rate of leaf expansion" and "maximum leaf size" are maximum values.

leaf characteristic	potato	Brussels sprouts	leek	spinach
rate of leaf appearance (d^{-1})	0.53	0.59	0.15	0.35
rate of leaf appearance ($100\ ^{\circ}Cd^{-1}$)	3.53	3.93	1.00	2.33
life span of leaves (d)	70	70	70	?
total number of leaves ($plant^{-1}$)	55	85	25	14
rate of leaf expansion ($cm^2\ d^{-1}$)	25	40	9	1.8
duration of leaf expansion (d)	22	35	40	18
maximum leaf size (cm^2)	375	750	400	32
specific leaf area ($cm^2\ g^{-1}$)	400	100	100	300

?: value unknown for this crop

Rate of leaf appearance and number of leaves

Potato and spinach are annual plants, Brussels sprouts and leek are biennial plants. This may have its consequences for the period over which new leaves appear. The experiment with potato was continued until the foliage was dead. Leaf appearance was finished much earlier: at about 90 days after emergence (DAE). Watson (1963) explained the termination of leaf appearance by the strong competition for nutrients by the tubers and the inadequate uptake by the roots. However, according to Almekinders & Struik (1995), the cessation of shoot growth results from a shift of the assimilate partitioning from shoot to tubers. For the biennial crops and for spinach in the vegetative phase leaf appearance took place during the whole experimental period.

When comparing the rates of leaf appearance of the four crops, it can be observed that they were more constant for potato (over the period during which leaves appeared) and spinach, than for leek and Brussels sprouts (see Chapter 2). Burton (1972) also reported a constant rate of leaf appearance for potato.

Effects of nitrogen treatments on rate of leaf appearance (which were mainly attributable to amount of nitrogen and not to the timing of nitrogen fertilisation) were most pronounced in Brussels sprouts, while final total numbers of leaves in several experiments were more than one-third lower in nitrogen limited plants, compared to plants growing under non-limiting conditions. Positive effects of nitrogen on rates of leaf appearance are reported by Terry (1970) for sugar beet and by Steer & Hocking (1983) for glasshouse-grown sunflowers. Muchow (1988) observed no effects on the final total

number of leaves of maize and sorghum, although the rates of leaf appearance were affected. These differences between crops in effects on final total number of leaves may be associated with the determinate nature of maize and sorghum whereas sugar beet is an indeterminate crop in its first year of growth.

Rate and duration of leaf expansion and maximum sizes of leaves

For all crops, the rates of leaf expansion and the maximum sizes of leaves first increased with leaf number and gradually decreased beyond a certain leaf number. This suggests a common mechanism.

More nitrogen increased the rate of leaf expansion of individual leaves of all four crops, confirming results of Terry (1970) for sugar beet leaves and of Radin & Boyer (1982) and Steer & Hocking (1983) for sunflower leaves. This effect was the main cause of differences in total green leaf area among treatments. A small treatment effect on duration of expansion was observed in Brussels sprouts, where the highest leaf numbers had a shorter duration of expansion when more nitrogen was applied.

Differences among individual leaves in maximum areas were also mainly attributable to differences in rates of leaf expansion, confirming results of Muchow (1988) for maize and sorghum. Differences in the effective duration of expansion (within an experiment; see Section 2.1 for a definition of 'effective duration') were of minor importance in Brussels sprouts: the largest leaves had shorter effective durations of expansion. Differences in duration between individual leaves of potato, spinach and leek were negligible within an experiment, although not among experiments.

Large differences were observed among crop species in maximum sizes of leaves, rather resulting from differences in rates of leaf expansion than from differences in the effective durations of expansion. For potato, durations varied mainly between 15 and 30 days, for spinach between 15 and 40, for leek between 15 and 55 and for Brussels sprouts between 15 and 90 days. Although the maximum value of duration of expansion differed largely between crops, the minimum value was similar and most leaves had an effective duration of expansion between 15 and 50 days.

Rate of leaf senescence and life spans of leaves

Dead leaves were not observed in spinach. In the experiments with the other three crops, the number of dead leaves was very low during the first five weeks after planting or emergence, but increased thereafter in different ways. For potato it increased more or less linearly until the whole foliage started to senesce. The rate of leaf senescence increased slightly with time for Brussels sprouts and leek until about 50% and 40%, respectively, of the total number of leaves was dead at the final harvest. Clear effects of nitrogen treatments were only observed

in Brussels sprouts, where more nitrogen resulted in more dead leaves, due to shorter life spans. The late application of nitrogen in Experiment 3 (Section 2.2) delayed leaf senescence, compared to early application. Moorby & Milthorpe (1975) and Millard & MacKerron (1986) explained faster leaf senescence for potato leaves, when more nitrogen was applied, by more shading of the larger total leaf area. Firman & Allen (1988) observed a faster decrease in photosynthetic rate of potato leaves with leaf age when they were more shaded.

In most cases the life spans of leaves of potato and Brussels sprouts increased with leaf number until about the largest leaf and decreased gradually thereafter with increasing leaf number. During the experimental period of each leek experiment the life spans increased slightly with increasing leaf number. The average life span of leaves was about 70 days for all three crops.

Specific leaf area

Specific leaf area (SLA) of bulked leaves usually decreased with time, except in the experiment with potato, where it increased until 100 DAE. In potato and leek fluctuations in overall SLA with time were caused by fluctuations of the SLA of individual leaves with time. However, in Brussels sprouts and spinach fluctuations in overall SLA with time were caused by differences in SLA between successive leaf numbers: the SLA of the lower leaf numbers, which senesce, was higher than the SLA of new, appearing leaves. More nitrogen increased the SLA with 10-20%, except in potato.

Total leaf area

The main differences between crops in the progress of total green leaf area per plant with time were that during the second part of the growing period the total leaf area decreased for potato and Brussels sprouts, while it increased or remained more or less constant for leek and spinach. While in spinach no leaf senescence before the marketable harvest was observed (dead or yellowing leaves are unacceptable in the marketable produce!) a decreasing total leaf area could not occur during that period. In all other crops dead leaves were observed. The above mentioned differences in the progress of total green leaf area with time were due to the fact that the largest leaves of potato and Brussels sprouts died during the experimental period while in leek these were still green at final harvest.

In general it is important that a minimum leaf area index (LAI) of three is maintained over a prolonged period to attain a maximum yield, since light interception approaches 100% when the LAI is ≥ 3 (see e.g. Spitters, 1987; Haverkort *et al.*, 1991). Several authors (Ivins & Bremner, 1964; Bremner & Radley, 1966; Reestman, 1968; Burton, 1989) mentioned this for potato. Figure 4.1 shows the relation between LAI and light interception for a leek crop (Experiment 3, Section 2.3). The extinction coefficient was on average 0.46. With an LAI

of three, the fraction intercepted light was about 0.70; the relation between LAI and light interception differs between potato and leek, which may be attributable to the different leaf orientation of the two crops. When an LAI of three is never reached (which occurred in several experiments in nitrogen-limited treatments, due to restricted leaf growth) light interception and, as a result, dry matter accumulation was reduced. In the paragraph on *Accumulation of dry matter and nitrogen* the relation between leaf area and dry matter accumulation is discussed further.

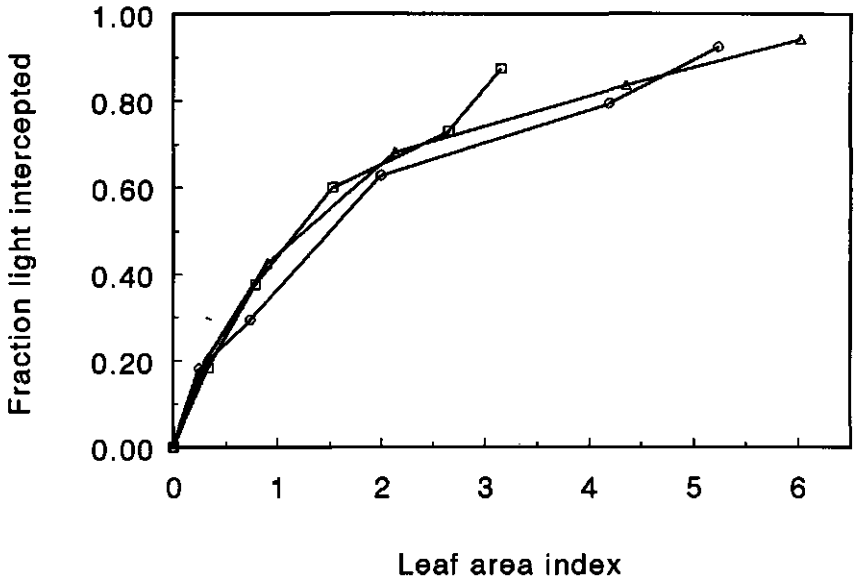

Figure 4.1. Fraction intercepted light against leaf area index for Experiment 3 of Section 2.3. (□) N(0), (○) N(200/1), (△) N(200/5).

Table 4.2 summarizes how the different leaf growth variables of the four crops responded to nitrogen fertilisation. Effects of nitrogen nutrition on leaf growth were most pronounced in Brussels sprouts, potato and spinach and were least pronounced in leek. Differences between treatments in total leaf area of a crop were more attributable to differences in the area of individual leaves than to differences in the number of leaves, while nitrogen nutrition had its largest effects on the rate of leaf expansion.

Table 4.2. Summary of effects of nitrogen fertilisation on different leaf growth variables of potato, Brussels sprouts, leek and spinach.

leaf characteristic	potato	Brussels sprouts	leek	spinach
rate of leaf appearance	0	+	0	+
total number of leaves	+	+	0	+
rate of leaf senescence	-	+	0	?
life span of leaves	+*	-	0	?
rate of leaf expansion	+	+	+	+
duration of leaf expansion	0	-	0	0
maximum leaf size	+	+	+	+
specific leaf area	-	+	+	+

+: positively affected with more nitrogen
0: not affected by nitrogen nutrition
-: negatively affected with more nitrogen
?: response to nitrogen nutrition unknown
* usually a typical result of '0' or '-' is found

Accumulation and partitioning of dry matter and nitrogen

The development and growth of the crop determines the demand for nitrogen. When we can predict the growth of the crop (with the help of a simulation model) and the amount of nitrogen, which is needed for this growth, we are able to predict the demand for nitrogen. Such calculations require quantitative information on effects of nitrogen on crop development and on accumulation and distribution of dry matter and nitrogen. Crops as Brussels sprouts usually leave large amounts of nitrogen rich crop residues on the field. It is relevant to know whether it is possible to lower the amount of nitrogen in these crop residues by manipulation of nitrogen fertilisation.

Accumulation of dry matter and nitrogen

Across the current experiments the total above-ground dry matter accumulation of a marketable crop was largest for potato and Brussels sprouts (the crops with the longest growing period) and lowest for spinach (which had the shortest growing period). The question arises whether these differences between crops were only attributable to differences in the length of the growing season or also to other factors, such as radiation use efficiency. Therefore the total above-ground dry matter production at the harvest when the crop was marketable was plotted against the leaf area duration (LAD). The latter was calculated as the integral of the leaf area index with time (Figure 4.2). Each

datum point represents one of the treatments; the treatments of one experiment are connected by a line. One experiment with Brussels sprouts seems aberrant, because the dry matter of dead leaves was not included. The four crops showed similar relations between total dry matter production and leaf area duration. There is no trend that treatments with an LAI above three over a long period had a relatively lower dry matter production, while the light interception was already supposed to be maximal at an LAI of about three. Effects of nitrogen on total dry matter accumulation resulted from differences in LAD; differences in Radiation Use Efficiency (RUE) were apparently unimportant. For Experiments 3 and 4 of Section 3.3 it was calculated that treatment effects on RUE were negligible, although the nitrogen concentrations at the final harvests in the green leaves of plants of the treatment with 200 kg N ha^{-1} were about 1.6 times higher than in the green leaves of plants of the treatment with no nitrogen application (data not shown). It also appears from Figure 4.2 that the relations for glasshouse experiments did not differ from those for field experiments, indicating that glasshouse and field experiments are comparable and conclusions from greenhouse experiments are relevant for field conditions.

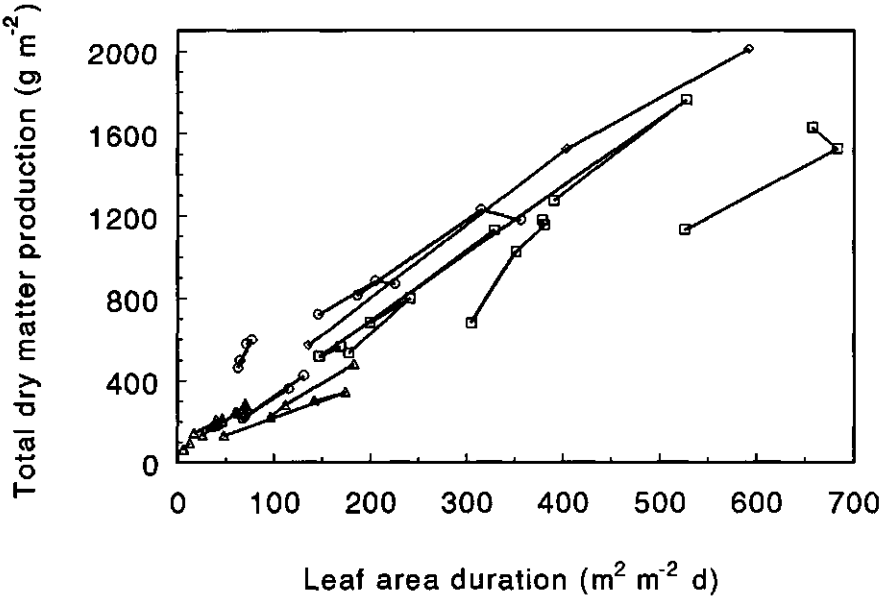

Figure 4.2. Total above-ground dry matter production (in g m^{-2}) of potato (◇), Brussels sprouts (□), leek (○) and spinach (△) against leaf area duration (in m^2 m^{-2} d) at the harvest when the crop was marketable.

It was already shown for Brussels sprouts, leek and spinach (Chapter 3) that no linear relation between total nitrogen uptake and total dry matter production existed, because

usually within an experiment the nitrogen concentration in the dry matter was higher when more dry matter was produced (comparing different treatments at the same moment; in general the nitrogen concentration decreases with increasing plant mass (Greenwood *et al.*, 1986, 1990)). The relation between total dry matter production and total nitrogen uptake differed greatly among different experiments of the same crop and among experiments of different crops, as shown in Figure 4.3. These results indicate that a certain amount of dry matter can be produced with widely varying nitrogen concentrations. This was illustrated for winter wheat by Justes *et al.* (1994), who not only calculated a critical nitrogen concentration curve (see Chapter 1), but also a minimum and a maximum nitrogen concentration curve: these two curves indicated that the nitrogen concentration could be 2.4 times below or 1.6 times above the critical nitrogen concentration, respectively.

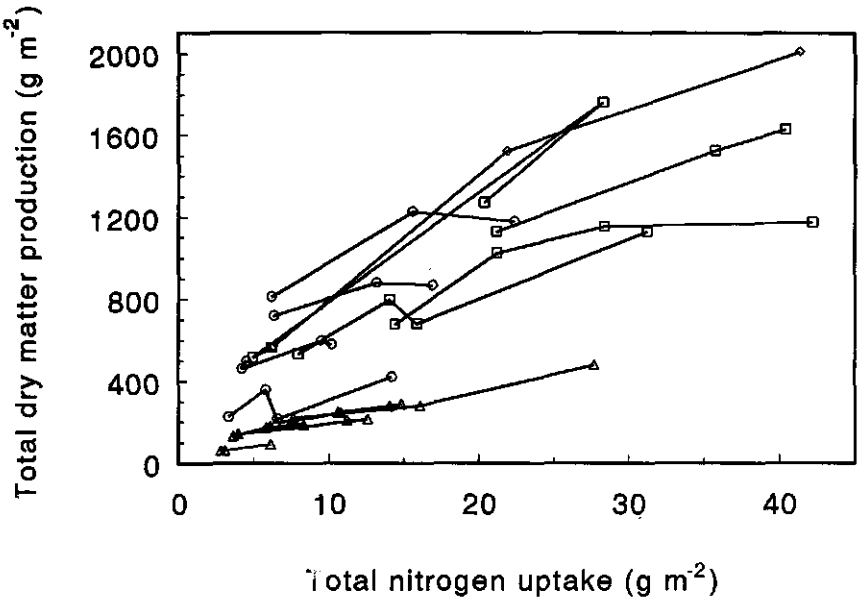

Figure 4.3. Relations between total dry matter production (in g m^{-2}) and total nitrogen uptake (in g m^{-2}) for potato (◇), Brussels sprouts (□), leek (○) and spinach (△) at the harvest when the crop was marketable.

For the sake of discussion, three different nitrogen pools can be distinguished in plants (see Figure 4.4): a storage pool (e.g. tubers of potato), structural nitrogen, e.g. in cell walls, and metabolic nitrogen, e.g. in Rubisco and other enzymes. Nitrogen, taken up from the soil, which flows into the metabolic pool, can be used again as part of this pool (e.g. for growth of new organs) or can be incorporated into the other pools subsequently. It is generally not possible to withdraw nitrogen from the storage and the structural pool; translocatable nitrogen

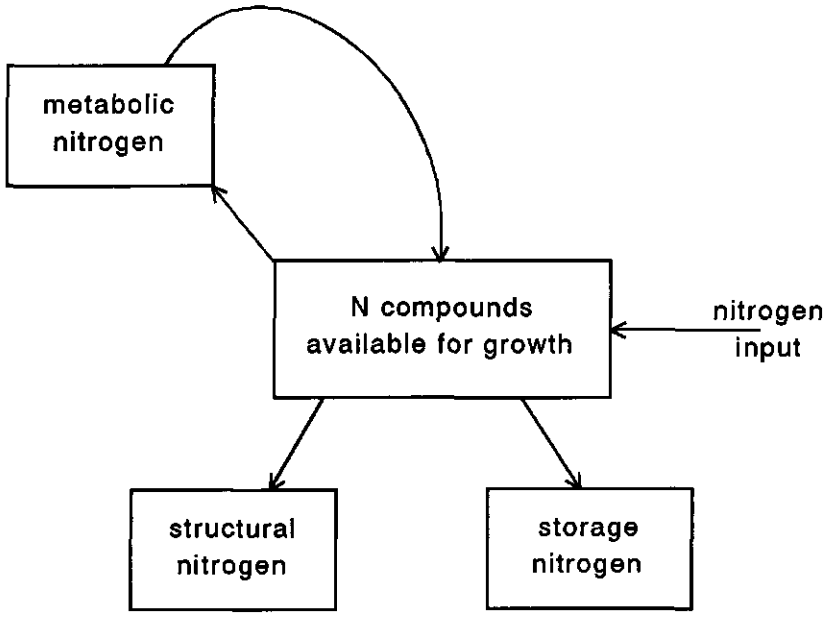

Figure 4.4. Nitrogen pools and their relations.

can be considered as metabolic nitrogen. The nitrogen concentration of the whole plant decreases with increasing plant mass, because the fraction structural tissue, with a relatively low nitrogen concentration, increases while the fraction tissue with a relatively high nitrogen concentration decreases (Greenwood *et al.*, 1986, 1990). The nitrogen concentration of storage organs is variable (but low in comparison with young vegetative tissue). For instance at the final harvest of the experiment with potato, the nitrogen concentration was 1.8 times higher in tubers of plants of the treatment with 16 g N $plant^{-1}$ than in tubers of plants with 2.5 g N $plant^{-1}$. The nitrogen concentration in dead material (e.g. dead leaves) represents more or less the concentration of structural, not translocatable, nitrogen. Also this concentration is variable; the fraction structural nitrogen of a plant organ is higher when more nitrogen is available during its growth. When nitrogen uptake stops because its supply is exhausted, leaf development and growth is possible as long as translocatable nitrogen is available. When all leaves have reached a minimum nitrogen concentration, leaf growth stops and, after a short period, also dry matter accumulation, because the photosynthesizing system cannot be renewed. When the sink strength of storage organs for nitrogen is high, the available amount of metabolic nitrogen is exhausted faster while less reinvestment in the metabolic pool takes place. This results in a shorter growing period, but the nitrogen concentration in storage organs is higher. When the sink strength of storage organs for

nitrogen is low, reinvestment of nitrogen in the metabolic pool takes place over a longer period, resulting in a larger dry matter yield of storage organs with a lower nitrogen concentration.

Partitioning of dry matter to different plant organs

An increase in marketable yield can be achieved by increasing the total yield, but also by increasing the harvest index. It was shown that the harvest index for dry matter increased in the sequence Brussels sprouts - leek - spinach - potato (Table 4.3). Nitrogen treatments had no or only small effects in potato and spinach, but large and significant effects in leek and Brussels sprouts. The direction of nitrogen effects was opposite in these two crops, since more nitrogen decreased the harvest index for dry matter in leek and increased it in Brussels sprouts, although for this last crop only in one experiment, out of four. A lower harvest index means more crop residues.

A higher final harvest index for dry matter resulted from higher partitioning rates of dry matter increase to the consumable plant parts. In Brussels sprouts, differences between treatments developed because nitrogen stress delayed sprout initiation. In leek differences between treatments in relative partitioning rates were present during the entire growing season.

Table 4.3. Average values of harvest indices for dry matter and nitrogen at final harvest of potato, Brussels sprouts, leek and spinach as observed under the described environmental conditions (see Chapters 2 and 3); effects of nitrogen fertilisation on these harvest indices.

plant characteristic	potato	Brussels sprouts	leek	spinach
harvest index for dry matter	0.87	0.25	0.43	0.67
nitrogen fertilisation effect	0	+	-	0
harvest index for nitrogen	0.86	0.42	0.28	0.74
nitrogen fertilisation effect	0	+	0	0

+: increased with more nitrogen
0: not affected by nitrogen nutrition
-: reduced with more nitrogen

Partitioning of nitrogen to different plant organs

The average harvest index for nitrogen increased in the sequence leek - Brussels sprouts - spinach - potato (Table 4.3). The effects of the nitrogen treatments on the partitioning of nitrogen to different plant organs were similar to those for dry matter in Brussels sprouts and potato. However, especially in leek, the nitrogen treatments affected the harvest index for dry matter whereas the harvest index for nitrogen was unaffected. For Brussels sprouts

and spinach, the nitrogen harvest indices were above the harvest indices for dry matter, for potato the two indices were similar and for leek the nitrogen harvest indices were below the harvest indices for dry matter. These differences between harvest indices resulted from lower (Brussels sprouts, spinach), similar (potato) or higher nitrogen concentrations (leek) in the crop residues, compared to the consumable produce.

The presence of differences in harvest indices for nitrogen implies that relative differences existed in amount of nitrogen in crop residues. However, no effects of treatments on harvest indices for nitrogen were observed in potato, leek or spinach and significant effects in Brussels sprouts in only one experiment out of four: the opportunities to reduce the relative amount of nitrogen in crop residues by manipulating the nitrogen fertilisation are limited. This is not so relevant for the two crops with a high harvest index for nitrogen, potato and spinach, but leek and Brussels sprouts have large amounts of nitrogen rich crop residues: the green leaf blades. In normal cropping practice a part of the leaf blades of leek is harvested together with the consumable part, the white shaft. However, the leaves of Brussels sprouts remain on the field after harvest.

Concentration of organic nitrogen in plant organs of different nodes

In nearly all cases the organic nitrogen concentrations in the dry matter of a leaf blade, petiole or leaf sheath decreased with increasing age of the organ. As a result the nitrogen concentration usually increased with leaf number, although in some experiments this last phenomenon was not observed, e.g. in two field experiments with leek (Experiments 3 and 4, Section 3.3). In some other experiments, no clear decrease of the nitrogen concentration with increasing age was observed, e.g. for the highest leaf numbers in spinach. The organic nitrogen concentration in the dry matter of sprouts differed in all cases only slightly between different sprout numbers and with sprout age.

The amount of nitrogen in a leaf increases until just before complete leaf expansion (Simpson, 1986). After a leaf reaches its maximum size, both import and export of nitrogen take place, but the export rate is higher (Simpson, 1986), which usually results in a continuous decline in leaf nitrogen concentration until the end of the leaf's life. This decline is affected by photon flux density (Hikosaka *et al.*, 1994). A declining nitrogen concentration with leaf age results usually in an increasing leaf nitrogen concentration with increasing leaf number (Chapter 3). In several cases the gradient in leaf nitrogen concentration was steeper at higher nitrogen rates. This may have resulted from a steeper gradient in photon flux density, because leaves had a larger area at higher nitrogen rates (Chapter 2): then higher leaf layers absorbed more light. This is a possible explanation for the faster leaf senescence of leaves of Brussels sprouts, when more nitrogen was applied.

Concentration of nitrate nitrogen

The nitrate nitrogen concentration is a variable, which is interesting for several reasons. In the first place it is a quality characteristic for the marketable produce. High concentrations are unwanted (forbidden!), because these can be harmful for consumers; about 75% of our total nitrate uptake originates from vegetables (Breimer, 1982). In the second place the nitrate concentration of certain plant parts are used as a criterion for nitrogen fertilisation, e.g. by potato growers (see e.g. Van Loon *et al.*, 1987; McMurdo *et al.*, 1988). In the current study the highest nitrate concentrations in dry matter were observed in spinach and the lowest in leek. In Brussels sprouts and potato the nitrate concentrations in the marketable produce were low, although this could be accompanied by high values in leaves and/or stems.

When observing the same type of plant organs (e.g. petioles) of different ages at a certain moment, the highest nitrate concentrations were usually observed in the oldest organ. However, this does not imply that the nitrate concentration of a certain plant organ (e.g. petiole number 10) always increased with increasing age; nitrogen shortage during the life cycle of that petiole could cause a decreasing (instead of an increasing) nitrate concentration with age. Justes *et al.* (1994) observed that the proportion of nitrate to total nitrogen in the shoot of winter wheat mainly depended on the crop stage of development; it was independent of the nitrogen nutrition level.

The nitrate concentrations in the marketable produce of leek, potato and Brussels sprouts, as observed in the current study, were low. In spinach, however, high concentrations were observed, except in treatments with nitrogen stress. Especially in autumn spinach, a good yield was unavoidably accompanied by high nitrate concentrations. At the final harvest of Experiment 6 with spinach, the nitrate concentrations in the fresh leaf blades of plants with the largest amount of nitrogen were 3000 ppm and in the fresh petioles 6000 ppm.

Associations between total nitrogen and nitrate nitrogen concentrations

Conservative associations between the total nitrogen and nitrate nitrogen concentrations in the dry matter of whole leaves (potato, spinach), leaf blades, petioles and leaf sheaths did not exist, because the nitrate nitrogen concentrations in these plant organs did not continuously decline with their age, in contrast with the total nitrogen concentrations. Only in stems of Brussels sprouts and stems and tubers of potato, such associations between nitrate nitrogen and total nitrogen concentrations were observed. In the stems of potato, nitrate started to accumulate above a total nitrogen concentration of about 1%, in Brussels sprouts above about 2% total nitrogen, but for both crops the nitrate nitrogen concentration increased with the same proportion of about 0.57% per percent total nitrogen.

An aspect of sustainable crop production is maximisation of utilisation of resources and minimisation of impact on environment. For nitrogen that means the availability and the demand need to be balanced. To achieve that goal one needs insight in the responses to nitrogen of processes determining growth and development. Expanding such insight was the focus of this work. Effects of nitrogen treatments, comprising various amounts and dates of application of nitrogen, were studied on leaf attributes and accumulation and partitioning of dry matter and nitrogen. Effects of amount of nitrogen were dominant; effects of date of application of nitrogen were negligible in most cases. This study showed that a plant never recovers from serious nitrogen stress by applying extra nitrogen. This implies that the grower has to avoid nitrogen stress, it cannot be recommended to reduce the amount of fertiliser nitrogen. Although this study does not give concrete answers to the question how much nitrogen has to be applied, it is a basis for further research. The study also shows how difficult it is to establish relations between nitrogen availability and development and growth of a crop. The data collected are suitable to be used in a simulation model, which includes developmental effects of nitrogen.

References

Almekinders, C.J.M. & P.C. Struik, 1995. Shoot development and flowering in potato (*Solanum tuberosum* L.). (submitted)

Breimer, T., 1982. Environmental factors and cultural measures affecting the nitrate content in spinach. *Fertilizer Research* 3: 191-292.

Bremner, P.M. & R.W. Radley, 1966. Studies in potato agronomy. II. The effects of variety and time of planting on growth, development and yield. *Journal of Agricultural Science* (Cambridge) 66: 253-262.

Burton, W.G., 1972. The response of the potato plant and tuber to temperature. In: A.R. Rees, K.E. Cockshull & D.W. Hand (Eds.), Crop processes in controlled environments. Academic Press, London, pp. 217-233.

Burton, W.G., 1989. The potato. 3th Edition. Longman Scientific & Technical, Harlow, 742 pp.

Firman, D.M. & E.J. Allen, 1988. Field measurements of the photosynthetic rate of potatoes grown with different amounts of nitrogen fertilizer. *Journal of Agricultural Science* (Cambridge) 111: 85-90.

Greenwood, D.J., G. Lemaire, G. Gosse, P. Cruz, A. Draycott & J.J. Neeteson, 1990. Decline in percentage N of C3 and C4 crops with increasing plant mass. *Annals of Botany* 66: 425-436.

Greenwood, D.J., J.J. Neeteson & A. Draycott, 1986. Quantitative relationships for the dependence of growth rate of arable crops on their nitrogen content, dry weight and aerial environment. *Plant and Soil* 91: 281-301.

Haverkort, A.J., D. Uenk, H. Veroude & M. van de Waart, 1991. Relationships between ground cover, intercepted solar radiation, leaf area index and infrared reflectance of potato crops. *Potato Research* 34: 113-121.

Hikosaka, K., I. Terashima & S. Katoh, 1994. Effects of leaf age, nitrogen nutrition and photon flux density

on the distribution of nitrogen among leaves of a vine (*Ipomoea tricolor* Cav.) grown horizontally to avoid mutual shading of leaves. *Oecologia* 97: 451-457.

Ivins, J.D. & P.M. Bremner, 1964. Growth, development and yield in the potato. *Outlook on Agriculture* 4: 211-217.

Justes, E., B. Mary, J.M. Meynard, J.M. Machet & L. Thelier-Huche, 1994. Determination of a critical nitrogen dilution curve for winter wheat crops. *Annals of Botany* 74: 397-407.

Loon, C.D. van, J.H.G. Slangen & J.H. Houwing, 1987. Nitrate content of leaf petioles as a guide to optimalisation of N-fertilization of ware potatoes. In: Abstracts of Conference Papers and Posters. 10th Triennial Conference of the European Association for Potato Research, Aalborg (Denmark), pp. 146-147.

McMurdo, W., R.K. Prange & R. Veinot, 1988. Nitrogen fertilization and petiole tissue testing in production of whole seed tubers of the potato cultivars Sebago and Atlantic. *Canadian Journal of Plant Science* 68: 901-905.

Millard, P. & D.K.L. MacKerron, 1986. The effects of nitrogen application on growth and nitrogen distribution within the potato canopy. *Annals of Applied Biology* 109: 427-437.

Moorby, J. & F.L. Milthorpe, 1975. Potato. In: L.T. Evans (Ed.), Crop physiology. Some case histories. Cambridge University Press, London, pp. 225-257.

Muchow, R.C., 1988. Effect of nitrogen supply on the comparative productivity of maize and sorghum in a semi-arid tropical environment I. Leaf growth and leaf nitrogen. *Field Crops Research* 18: 1-16.

Radin, J.W. & J.S. Boyer, 1982. Control of leaf expansion by nitrogen nutrition in sunflower plants. Role of hydraulic conductivity and turgor. *Plant Physiology* 69: 771-775.

Reestman, A.J., 1968. Iets over het produktiepatroon van de aardappel. In: Directie landbouwonderwijs van het MLV (Ed.), Landbouwplantenteelt 1968. Ministerie van Landbouw en Visserij, 's Gravenhage, pp. 88-121.

Simpson, R.J., 1986. Translocation and metabolism of nitrogen: whole plant aspects. In: H. Lambers, J.J. Neeteson & I. Stulen (Eds.), Fundamental, ecological and agricultural aspects of nitrogen metabolism in higher plants. Martinus Nijhoff, Dordrecht, pp. 71-96.

Spitters, C.J.T., 1987. An analysis of variation in yield among potato cultivars in terms of light absorption, light utilization and dry matter partitioning. *Acta Horticulturae* 214: 71-84.

Steer, B.T. & P.J. Hocking, 1983. Leaf and floret production in sunflower (*Helianthus annuus* L.) as affected by nitrogen supply. *Annals of Botany* 52: 267-277.

Terry, N., 1970. Developmental physiology of sugar beet. II. Effects of temperature and nitrogen supply on the growth, soluble carbohydrate content and nitrogen content of leaves and roots. *Journal of Experimental Botany* 21: 477-496.

Watson, D.J., 1963. Some features of crop nutrition. In: J.D. Ivins & F.L. Milthorpe (Eds.). The growth of the potato. Butterworths, London, pp. 223-247.

Summary

Nitrogen nutrition effects on development, growth and nitrogen accumulation of vegetables

Currently the cultivation of field grown vegetables in the Netherlands can be accompanied by large nitrogen losses to the environment. Nitrogen residues in the soil after the final vegetable crop in a growing season are often too large. Therefore, most of these losses occur as nitrate leaching to ground and surface water during winter. Large residues result from the application of large amounts of fertiliser nitrogen, together with inefficient nitrogen uptake in multiple cropping systems. Accurate, research-based recommendations for the nitrogen fertilisation of vegetable crops are scarce. Funding of the research required is not easy, because the cultivated acreage per crop is low. Growers often apply more fertiliser nitrogen than needed, to ensure a good yield and quality of the marketable produce.

There are several ways to reduce nitrogen losses, but this study aimed at obtaining insight in the responses to nitrogen of important processes of growth and development, which is necessary to be able to match nitrogen supply and nitrogen requirement of crops. Optimisation of the nitrogen fertilisation needs quantification of effects of amount of nitrogen applied and fractionation of nitrogen supply on 1. leaf attributes (e.g. leaf appearance, rate and duration of leaf expansion, specific leaf area; Chapter 2) and 2. accumulation and partitioning of dry matter and nitrogen (Chapter 3). Potato, Brussels sprouts, leek and spinach were studied. Both glasshouse and field experiments were carried out; glasshouses were set to maintain a day (12 h) temperature of 18 °C and a night temperature of 12 °C.

Leaf development and growth

In the one experiment with potato, the rate of leaf appearance was 0.53 leaves per day (one leaf per 28 °Cd) and was hardly affected by nitrogen supply. The rate of leaf expansion and the areas of mature leaves increased with leaf insertion number on the main stem to reach a maximum for leaf numbers 12-14, and declined for higher leaf numbers. Both leaf characteristics were enhanced by more nitrogen. The number of main stem leaves was not affected by nitrogen supply. Leaves on apical lateral branches declined in mature area with increase in leaf number. The expansion rate of leaves was the dominant factor that determined the mature leaf area, irrespective of leaf number or nitrogen treatment. The smallest leaves were observed at the lowest rate of nitrogen

supply. Nitrogen promoted apical branching and consequently the total number of leaves that appeared on a plant. The proportion of total leaf area contributed by leaves on apical branches increased with time and nitrogen supply. Active life span, i.e. the period of time between leaf appearance and yellowing of the leaf, showed a similar relation to leaf number as mature leaf area, at least in qualitative terms. Leaves of the treatment with 16 g N $plant^{-1}$ showed consistently longer life spans (about three weeks) than leaves of the treatments with 2.5 and 8 g N $plant^{-1}$.

In Brussels sprouts the rates of leaf appearance ranged between 0.39 and 0.72 d^{-1}. They were significantly increased by more nitrogen. The rates of leaf expansion and mature leaf area depended on leaf number and nitrogen treatment: they increased with leaf insertion number, reaching a maximum between leaf numbers 10 and 20 and decreased subsequently. Both leaf characteristics were enhanced by more nitrogen. Leaf expansion rate was the main factor determining mature leaf area, but the duration of expansion also played a role: it was shorter for larger leaves. Plants receiving more nitrogen had a higher total green leaf area per plant, because of more and larger green leaves. Specific leaf area of all leaves declined gradually from 130-230 $cm^2\ g^{-1}$ (depending on experiment) at about 30 days after planting to 60 at the end of the experiments and was usually significantly increased by more nitrogen.

In leek the rate of leaf appearance was not affected by nitrogen treatments and was almost constant across experiments at 0.15 d^{-1}. The rate of leaf expansion and the mature leaf area increased with leaf number, reached a maximum between leaf numbers 11 and 14 and decreased with higher leaf numbers. Both variables increased with more nitrogen. The duration of leaf expansion was more or less constant across leaf numbers and not influenced by nitrogen treatments; the leaf expansion rate was the main factor determining mature leaf area. The rate of leaf senescence was not influenced by nitrogen treatments. Differences in total green leaf area per plant were caused by differences in (mature) leaf area of individual leaves and not by differences in the number of leaves. The specific leaf area of all leaves was more or less constant at 100 $cm^2\ g^{-1}$.

In spinach the rates of leaf appearance varied between 0.16 and 0.57 d^{-1} across experiments, but were hardly affected by nitrogen treatments. Differences among experiments were not attributable to differences in temperature. The rate of leaf expansion and the mature leaf area increased with leaf number, reached a maximum at leaf pair 3+4 or 5+6 and decreased subsequently. Both characteristics were positively correlated to nitrogen supply. The duration of expansion was not influenced by nitrogen treatments and varied between 15 and 30 days in most experiments. The rate of leaf expansion was the main factor determining mature leaf size. Specific leaf area over all green leaves very slowly decreased with time in most experiments and was approximately 300 $cm^2\ g^{-1}$, averaged over the whole experimental period. As the differences in the number of leaves were small, the differences in total green

leaf area per plant resulted from differences in (mature) leaf area of individual leaves.

Accumulation and partitioning of dry matter and nitrogen

In potato, plants with 16 g N $plant^{-1}$ continued to accumulate dry matter and nitrogen at a constant rate for a longer period of time than plants with 2.5 and 8 g N $plant^{-1}$, although the accumulation rates in plants with 8 and 16 g N $plant^{-1}$ were similar during the first two-third of the experiment. In all treatments, the uptake of nitrogen slowed down earlier in time than the rate of dry matter accumulation. The proportion of dry matter in tubers of mature plants was not affected by nitrogen treatment, but the start of tuber bulking was delayed in plants with 16 g N $plant^{-1}$. The final proportion of total plant nitrogen in tubers was similar for all treatments. The concentration of nitrogen in the dry matter of mature plants increased with level of nitrogen supply. Maximum haulm weight increased with level of nitrogen supply. Apical lateral branches of the first and second order made up larger proportions of the total haulm dry weight and total leaf area as more nitrogen was supplied. Yet, the distribution of dry matter over stems and leaves was not different between nitrogen treatments. In each of the component organs (stems, leaves, tubers) the concentration of nitrogen declined with time. Stems were the most responsive to nitrogen treatment in terms of nitrogen concentrations. Fairly strong associations were observed between the concentrations of nitrogen in component organs. The concentration of nitrate in leaves usually increased initially with leaf age, peaked and declined. A substantial part of the differences between treatments in the concentrations of nitrogen in leaf dry matter was attributable to differences in nitrate concentration. Nitrate in stems and tubers fell virtually below the limit of detection at total nitrogen concentration of less than 1%, but increased in proportion to total nitrogen above that threshold, especially in stems.

In Brussels sprouts, total amounts of accumulated dry matter and nitrogen were affected by amount of nitrogen applied and date of application. Sprout growth started halfway the growing period. The final harvest index ranged from 0.10-0.35 for dry matter and from 0.20-0.55 for nitrogen; neither one was significantly affected by treatments in most experiments. Nitrate nitrogen concentrations were only high (maximally about 2%) shortly after planting. The total nitrogen concentration of leaf blades and petioles increased with increasing leaf number. This increase resulted from a decreasing nitrogen concentration during the leaf's life. The total nitrogen concentration in sprouts changed little with leaf number.

In leek, both amount of nitrogen applied and time of application affected the total accumulation of dry matter and nitrogen in the plant. The relative partitioning rates of dry matter increase to the shaft were affected in such a way that the final harvest indices for dry matter (which ranged from 0.32-0.53) were significantly lower at higher amounts of nitrogen

applied. The final harvest indices for nitrogen ranged from 0.21-0.35 and were not significantly affected by amount or timing of fertiliser applications. The total nitrogen concentrations of leaf blades and leaf sheaths decreased with increasing leaf age. Average nitrate nitrogen concentrations over all plant parts were always below 0.4%.

The total accumulation of dry matter and nitrogen in spinach differed largely among and within experiments. However, the relative partitioning rates of dry matter increase for the different plant organs were unaffected by treatments. As a result the harvest indices for dry matter of a crop in the marketable stage were constant at about 0.67 in all experiments. The same applies to the harvest indices for nitrogen, which were on average 0.74. The organic nitrogen concentration of leaf blades and petioles decreased with leaf age, although in most experiments this decrease was smaller at higher leaf insertion numbers. The nitrate nitrogen concentration decreased with increasing leaf number at any sampling date. Average nitrate nitrogen concentrations over all plant parts were maximally 1.5% of the dry mass.

In Chapter 4 the characteristics of the four different crops were compared and discussed. Spinach plants were smallest, because leaf attributes of spinach deviated most from those of the other crops. Nitrogen treatments had the smallest effects on leaves of leek and the largest effects on leaves of Brussels sprouts. In all crops, differences among nitrogen treatments in total green leaf area per unit soil area resulted mainly from the effects of nitrogen on rates of expansion of individual leaves.

Across experiments and crops, a clear association existed between leaf area duration and total dry matter production. However, the association between total dry matter production and total nitrogen uptake was vague, although more available nitrogen resulted in larger nitrogen uptake and larger total dry matter production. The partitioning of dry matter and nitrogen was not affected by nitrogen treatments in potato and spinach. In leek the nitrogen partitioning was not affected although treatments affected dry matter partitioning significantly. In all cases effects of amount of nitrogen were much larger than effects of fractionation of nitrogen supply. Nitrogen stress during the period in which the nitrogen uptake rate potentially was high, always led to yield reduction. A high nitrogen uptake rate occurred during the first half of the experimental period, except for leek where it was during the middle half of the experimental period.

Although this study does not give concrete answers as how much nitrogen has to be applied, it is a basis for further research. It also shows how difficult it is to establish relations between nitrogen availability and development and growth of a crop. The data, gathered in this study, are suitable to develop a simulation model, which includes developmental effects of nitrogen.

Samenvatting

Effecten van stikstofbemesting op ontwikkeling, groei en stikstofopname van groenten

Momenteel gaat de teelt van vollegrondsgroenten in Nederland gepaard met flinke stikstofemissies naar het milieu, alhoewel er gelukkig al diverse maatregelen getroffen worden om deze terug te dringen. Deze verliezen vinden meestal plaats doordat nitraat tijdens de winter naar grond- en oppervlaktewater uitspoelt. De oorzaak hiervan is dat na het laatste groentegewas in een seizoen de stikstofresten in de bodem te groot zijn. Deze grote resten zijn het resultaat van de toediening van grote hoeveelheden kunstmeststikstof en/of een inefficiënte stikstofopname van het betreffende gewas. Het telen van meerdere gewassen achter elkaar op hetzelfde perceel in één jaar vergroot de problemen. Goede, op onderzoek gebaseerde adviezen voor de stikstofbemesting van groentegewassen zijn schaars, omdat vanwege het kleine areaal per gewas er relatief weinig onderzoek wordt verricht. Telers bemesten vaak met meer kunstmeststikstof dan nodig, om verzekerd te zijn van een goede opbrengst en kwaliteit van het marktbare produkt.

Er zijn verschillende manieren om deze stikstofverliezen te verminderen; mijn onderzoek richtte zich op het verkrijgen van inzicht in de effecten van stikstof op belangrijke groei- en ontwikkelingsprocessen. Om de stikstofbemesting te kunnen optimaliseren is het nodig om de effecten van de hoeveelheid toegediende stikstof en van deling van stikstofgift op de gewasgroei te kennen. In Hoofdstuk 2 zijn de effecten van beide factoren op bladkenmerken (bijvoorbeeld bladverschijning, snelheid en duur van bladexpansie, specifieke bladoppervlakte) beschreven. Hoofstuk 3 gaat over de effecten op accumulatie en verdeling van drogestof en stikstof. Het onderzoek vond zowel in de kas als in het veld plaats met de gewassen aardappel, spruitkool, prei en spinazie. Gewoonlijk werd in de kasproeven een dag/nacht temperatuurregime van 18/12 °C aangehouden, met een thermofase van 12 uur.

Bladontwikkeling en groei

In de enige proef met aardappel was de bladverschijningssnelheid 0.53 bladeren per dag (een blad per 28 °Cd). Stikstofbemesting had hierop nauwelijks invloed. De snelheid van bladexpansie (van individuele bladeren) en de oppervlaktes van volwassen bladeren namen op de hoofdstengel toe met bladnummer tot een maximum bij bladnummer 12-14; bij nog hogere bladnummers namen beide kenmerken weer af. Beide kenmerken bereikten hogere waarden als meer stikstof was toegediend. Het aantal bladeren aan de hoofdstengel werd

niet door de behandelingen beïnvloed. De oppervlakte van volwassen bladeren op de apicale zijassen nam af met toenemend bladnummer. De bladexpansiesnelheid was onder alle omstandigheden de belangrijkste oppervlakte-bepalende factor. De kleinste bladeren werden waargenomen bij de behandeling met de kleinste hoeveelheid toegediende stikstof. Stikstof bevorderde het uitlopen van apicale zijassen en daarmee het totaal aantal bladeren dat verscheen aan een plant. De fractie oppervlakte blad aan apicale zijassen van de totale bladoppervlakte nam toe met de tijd en met hoeveelheid toegediende stikstof. De levensduur van bladeren, dat is de periode tussen bladverschijning en bladvergeling, vertoonde eenzelfde relatie met bladnummer als de oppervlakte van volwassen bladeren. Bladeren van de behandeling met 16 g N $plant^{-1}$ leefden ongeveer drie weken langer dan bladeren van de behandelingen met 2.5 en 8 g N $plant^{-1}$.

In spruitkool varieerde de snelheid van bladverschijning tussen 0.39 en 0.72 d^{-1}. Bij een grotere hoeveelheid toegediende stikstof was deze snelheid hoger. De snelheid van bladexpansie en de oppervlakte van volwassen bladeren hingen af van bladnummer en stikstofbehandeling: ze namen toe met bladnummer, bereikten tussen bladnummers 10 en 20 een maximum en namen daarna af. De maximale bladoppervlakte werd hoofdzakelijk door de bladexpansiesnelheid bepaald, maar de duur van expansie speelde wel een rol: deze was korter voor grotere bladeren. Planten die meer stikstof ontvangen hadden, hadden een grotere totale oppervlakte aan groen blad, vanwege meer en grotere bladeren. De specifieke bladoppervlakte van alle bladeren nam langzaam af van 130-230 cm^2 g^{-1} op ongeveer 30 dagen na planten tot 60 aan het eind van de proeven. Gewoonlijk was zij significant hoger als meer stikstof was toegediend.

In prei werd de bladverschijningssnelheid niet door stikstofbehandelingen beïnvloed en was deze voor alle proeven ongeveer 0.15 d^{-1}. De snelheid van bladexpansie en de maximale bladoppervlakte namen toe met bladnummer, bereikten een maximum tussen bladnummers 11 en 14 en namen af met hoger bladnummer. Beide variabelen bereikten hogere waarden bij meer stikstof. De duur van bladexpansie was min of meer constant over bladnummers en werd niet beïnvloed door stikstofbehandelingen: de maximale bladoppervlakte werd hoofdzakelijk door de bladexpansiesnelheid bepaald. De snelheid van bladafsterving werd niet door stikstofbehandelingen beïnvloed. Verschillen in de totale oppervlakte groen blad per plant werden veroorzaakt door verschillen in oppervlakte van individuele (volwassen) bladeren en niet door verschillen in aantal bladeren. De specifieke bladoppervlakte van alle bladeren was ongeveer constant op 100 cm^2 g^{-1}.

In spinazie varieerde de bladverschijningssnelheid over proeven tussen 0.16 en 0.57 d^{-1}, maar stikstofeffecten waren verwaarloosbaar. De snelheid van bladexpansie en de maximale bladoppervlakte namen toe met bladnummer, bereikten een maximum bij bladpaar 3+4 of 5+6 en namen daarna weer af. Beide variabelen waren positief gecorreleerd met de

hoeveelheid toegediende stikstof. De duur van expansie werd niet door stikstofbehandelingen beïnvloed en varieerde tussen 15 en 30 dagen. De maximale bladoppervlakte werd hoofdzakelijk door de snelheid van bladexpansie bepaald. De specifieke bladoppervlakte over alle groene bladeren nam in de meeste proeven langzaam af in de tijd en was ongeveer 300 $cm^2\ g^{-1}$. Aangezien de verschillen in aantallen bladeren per plant klein waren, kwamen de verschillen in totale oppervlakte groen blad vooral voort uit verschillen in (maximale) bladoppervlakte van individuele bladeren.

Accumulatie en verdeling van drogestof en stikstof

Aardappelplanten die 16 g stikstof toegediend kregen, accumuleerden over een langere periode drogestof en stikstof met een constante snelheid dan planten met 2.5 of 8 g toegediende stikstof, alhoewel de accumulatiesnelheden in planten met 8 en 16 g N $plant^{-1}$ tijdens tweederde deel van de proef gelijk waren. In alle behandelingen nam de opnamesnelheid van stikstof eerder af dan de accumulatiesnelheid van drogestof. In volwassen planten werd de fractie drogestof in de knollen niet door stikstofbehandeling beïnvloed, maar het begin van de knolvulling werd vertraagd in planten met 16 g N $plant^{-1}$. De uiteindelijke fractie stikstof in knollen was voor alle behandelingen gelijk. De stikstofconcentratie in de drogestof van volwassen planten nam toe met het niveau van stikstofbemesting, evenals het maximum gewicht van het loof. Apicale zijassen van de eerste en tweede orde maakten een groter deel van het totale loofgewicht en hun bladeren maakten een groter deel van de totale bladoppervlakte uit als meer stikstof was toegediend. De verdeling van drogestof over stengels en bladeren verschilde echter niet tussen de behandelingen. Zowel in de stengels, als in de bladeren en knollen nam de stikstofconcentratie af in de tijd. In termen van stikstofconcentratie waren de stengels het meest gevoelig voor de stikstofbehandelingen. De stikstofconcentraties in alle plantedelen toonden onderling vrij sterke verbanden. De nitraatconcentratie in bladeren nam gewoonlijk eerst toe met bladleeftijd tot een maximum en nam daarna weer af. Een substantieel deel van de verschillen tussen behandelingen in stikstofconcentraties in de drogestof van bladeren was toe te schrijven aan verschillen in nitraatconcentraties. Beneden een concentratie totaalstikstof van 1% was in stengels en knollen nitraat niet meer waarneembaar. Daarboven nam de nitraatconcentratie proportioneel toe met de concentratie totaalstikstof, vooral in stengels.

De totale hoeveelheden geaccumuleerde drogestof en stikstof in spruitkool werden zowel door hoeveelheid toegediende stikstof als door tijdstip van toediening beïnvloed. De groei van de spruiten begon halverwege de groeiperiode van het gewas. De uiteindelijke oogstindex voor drogestof varieerde van 0.10-0.35 en voor stikstof van 0.20-0.55. Beide werden in de meeste proeven niet significant door behandelingen beïnvloed. Alleen kort na planten waren de concentraties nitraatstikstof over de gehele plant vrij hoog (maximaal ongeveer 2%). De

concentratie totaalstikstof van bladschijven en bladstelen nam toe met toenemend bladnummer. Deze toename kwam voort uit een afname in de stikstofconcentratie met bladleeftijd. De concentratie totaalstikstof in spruiten verschilde weinig tussen verschillende spruitnummers.

Ook in prei hadden zowel hoeveelheid toegediende stikstof als tijdstip van toediening effecten op de totale hoeveelheid door de plant geaccumuleerde drogestof en stikstof. De verdeling van drogestof over de diverse plantedelen werd zodanig beïnvloed dat de uiteindelijke oogstindices voor drogestof (die varieerden van 0.32-0.54) significant lager waren als meer stikstof was toegediend. De uiteindelijke oogstindices voor stikstof varieerden van 0.21-0.35 en werden niet significant beïnvloed door hoeveelheid toegediende stikstof of toedieningstijdstip. De concentraties totaalstikstof van bladschijven en bladschedes namen af met toenemende bladleeftijd. De gemiddelde concentraties nitraatstikstof in de drogestof van de gehele plant waren altijd lager dan 0.4%.

Zowel tussen als binnen de spinazieproeven werden grote verschillen waargenomen in totale hoeveelheid geaccumuleerde drogestof en stikstof. Echter, de verdeling van drogestof naar de verschillende organen van de plant werd niet door behandelingen beïnvloed. Dit resulteerde erin dat de oogstindices voor drogestof van een gewas op het marktbare tijdstip in alle proeven ongeveer 0.67 waren. Ook de oogstindices voor stikstof waren ongeveer gelijk, namelijk 0.74. De concentratie organische stikstof van bladschijven en bladstelen nam af met bladleeftijd, alhoewel in de meeste proeven deze afname bij hogere bladnummers geringer was. Op een willekeurig oogsttijdstip nam de concentratie nitraatstikstof af met toenemend bladnummer. De gemiddelde concentraties nitraatstikstof in de drogestof van de gehele plant waren maximaal 1.5%.

In Hoofstuk 4 werden de waargenomen karakteristieken van de vier gewassen vergeleken en bediscussieerd. De bladkenmerken van spinazie weken het meest af van die van de andere gewassen. Stikstofbehandelingen hadden het minst effect op bladeren van prei en het meest effect op bladeren van spruitkool. In alle gewassen waren verschillen in totale oppervlakte groen blad tussen behandelingen hoofdzakelijk het gevolg van de effecten van stikstof op de bladexpansiesnelheden.

Er bestond een duidelijke positieve relatie tussen bladoppervlakteduur (dat is de integraal van de bladoppervlakte-index over de tijd) en de totale drogestofproduktie. De relatie tussen de totale drogestofproduktie en de totale stikstofopname was echter zwak, alhoewel een grotere hoeveelheid beschikbare stikstof altijd in een grotere stikstofopname en een grotere drogestofproduktie resulteerde. In aardappel en spinazie werd de verdeling van drogestof en stikstof niet door stikstofbehandelingen beïnvloed; in prei werd de verdeling van stikstof niet beïnvloed. In alle gevallen waren effecten van hoeveelheid stikstof veel groter dan effecten

van stikstofdeling. Stikstoftekorten tijdens de periode, waarin de opnamesnelheid van stikstof gewoonlijk het hoogst is, leidden altijd tot opbrengstreducties. Deze periode lag in de eerste helft van het groeiseizoen, behalve voor prei, daar lag deze in het midden van het groeiseizoen.

Dit onderzoek geeft geen concrete antwoorden, zoals gewenste hoeveelheden stikstofbemesting, maar is wel een basis voor verder onderzoek. Het laat eveneens zien hoe moeilijk het is om relaties tussen de beschikbaarheid van stikstof en de groei en ontwikkeling van een gewas vast te leggen. De verzamelde gegevens zijn geschikt om een simulatiemodel te ontwikkelen, waarin effecten van stikstof op ontwikkeling van een plant worden meegenomen.

Curriculum vitae

Hendrik Biemond werd op 5 april 1966 geboren te Stellendam. Na het behalen van het VWO-diploma aan de Reformatorische scholengemeenschap Guido de Brès te Rotterdam, begon hij in september 1984 aan de studie Landbouwplantenteelt aan de Landbouwuniversiteit (toen nog Landbouwhogeschool geheten) te Wageningen. In juni 1990 behaalde hij het doctoraalexamen met als afstudeervakken Akkerbouw, Produktkunde en Plantencytologie en -morfologie. Van januari 1991 tot en met december 1994 was hij als assistent in opleiding (AIO) in dienst van de vakgroep Landbouwplantenteelt en Graslandkunde (later Agronomie). In deze periode voerde hij het in dit proefschrift beschreven onderzoek uit. Van januari tot en met juni 1995 was hij gastmedewerker bij de vakgroep Agronomie. Vanaf juli 1995 is hij in dienst van Baan Development B.V. te Ede als junior application software engineer.