Netherlands Journal of Agricultural Science 44 (1996) 227-239

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

H. BIEMOND, J. VOS* AND P.C. STRUIK

Department of Agronomy, Wageningen Agricultural University, P.O. Box 341, NL-6700 AH Wageningen, The Netherlands
* Corresponding author (fax: +31-317-484575; e-mail: jan.vos@users.agro.wau.nl)

Received 11 May 1995; accepted 20 September 1996

Abstract

Six experiments were carried out with different amounts and different dates of application of nitrogen to analyse the dynamics of dry matter and nitrogen accumulation in spinach. 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. More nitrogen increased yield of dry matter and nitrogen, whereas splitting nitrogen application had much smaller effects. However, the partitioning of dry matter or nitrogen in spinach proved insensitive to nitrogen treatments. Harvest indices for dry matter (about 0.67) or nitrogen (about 0.74) of crops in a marketable stage were fairly constant over treatments and experiments. Increasing or splitting the nitrogen application affected the nitrogen accumulation more than the dry matter production resulting in large effects on N concentrations. The lack of variation in response to N for different N regimes facilitates the development of N application techniques aiming at high yield, high quality and reduced emission. 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 date. It was higher when nitrogen was abundant. High yields in autumn crops were associated with high nitrate concentrations but also with potentially high losses of nitrogen.

Key words: Spinach, *Spinacia oleracea* L., nitrogen nutrition, dry matter production, dry matter distribution, nitrogen uptake, nitrogen concentration, nitrogen distribution.

Introduction

Spinach (*Spinacia oleracea* L.) 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 is a crop that requires considerable amounts of nitrogen to allow the crop to grow rapidly and to acquire the dark green colour desired by consumers. Like most other vegetables, spinach is not very efficient in nitrogen use. In practice, the combination of strong positive responses to additional N and a low N use efficiency readily results in large losses of nitrogen by leaching or undesirably high levels of nitrate in the marketable produce, particularly when the crop is grown late in the season.

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 the negative effects mentioned above.

Effects of nitrogen on leaf development and growth of spinach were described by Biemond (1995). More nitrogen increased the total green leaf area mainly by increasing the size of individual leaves. This resulted from an increase in the rate of leaf expansion; the duration of expansion was not affected. The number of leaves remained constant.

Since most of the gain by applying N is brought about by stimulating one single process and since this process is partly separated in time for the different leaves of a plant, the continuous supply of adequate amounts of nitrogen is crucial.

Theoretically adequate N supply can also be obtained by supplying the nitrogen when it is needed or when it can be taken up by the crop. Such a dynamic strategy of nitrogen fertilization reduces the risk of nitrogen emission to the environment but increases the risk of temporary shortage of nitrogen. The feasibility of dynamic nitrogen application therefore depends on the plasticity of the response of the crop to temporary changes in supply. Vos *et al.* (1996) have analysed this in detail for Brussels sprouts, a vegetable crop with a long growth cycle. This paper tries to establish the potential of dynamic nitrogen application for a short cycle vegetable; it focuses on the effects of nitrogen application strategy (different rates and different ways of splitting this rate) on 1. accumulation of dry matter and nitrogen, 2. partitioning of dry matter and nitrogen to different plant parts (leaf blades, petioles, stem) and 3. vertical distribution of nitrogen and nitrate within the plant.

Materials and methods

Four field and two glasshouse experiments are described. All experiments were designed to study effects of different amounts of nitrogen applied and different ways of splitting the nitrogen application. Table 1 summarizes when and where each experiment was carried out.

Plant culture – field

Experiments 1–4 (the field experiments) were conducted on a sandy soil with about 3% organic matter. Cv. Trias (C.W. Pannevis B.V., Enkhuizen (NL)) 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.

Table 1. General information about six experiments with spinach: year, type of experiment (field/glasshouse), growing period (summer/autumn), sowing date, available amount of mineral nitrogen in the soil layer 0–60 cm at sowing (kg ha^{-1}; only field experiments) and date of final observations.

Experiment	Year	Type of experiment	Growing period	Sowing date	Mineral nitrogen	Final observations
1	1991	field	summer	25 Apr	94	25 Jun
2	1991	field	autumn	5 Aug	158	25 Sep
3	1992	field	summer	7 May	77	14 Jul
4	1992	field	autumn	19 Aug	66	20 Oct
5	1993	glasshouse	summer	18 May	–	5 Jul
6	1993	glasshouse	autumn	4 Aug	–	12 Oct

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. Seventy-five seeds of cv. Trias were sown in this medium, 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. On sunny days, the capacity of the cooling system of the glasshouse was insufficient, which resulted in temperatures of 2–3 °C above the set temperatures. 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.

Treatments and experimental design

The treatments consisted of different total amounts of nitrogen and different dates of application of nitrogen. Table 2 shows these amounts; see Biemond (1995) for dates of application. In the four field experiments, treatments were designed to compare different total amounts given either in few large doses or in many small doses. The different experiments reflect different ecological conditions (two years and two growing seasons per year). Per year the set-up of the two experiments was basically similar. In Experiments 5 and 6 the purposes of the treatments 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. Nitrogen supply for treatments with nitrogen stress was based on a comparison of actual growth (which was regularly measured at destructive harvests) with growth and nitrogen uptake in Experiments 1–4.

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 experi-

Table 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[1]	Total	Code	Amount[1]	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[1]	Total	Code	Amount[1]	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[1]	Total	Code	Amount[1]	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

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

mental 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 1). In the case of summer spinach, this final date was (far) beyond the marketable 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 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: replicates were treated separately) and subsequently analysed for total nitrogen and nitrate (see Biemond & Vos (1992) for methods of nitrogen and nitrate analysis).

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

Yields were higher when nitrogen supply was more abundant; effects of splitting the application of N on yield were small. Figure 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 (for summer spinach the last harvest before development of the flower stalk). 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 were linear and ran more or less parallel. The slopes indicate that relative treatment effects on total nitrogen uptake were much larger than those on total dry matter production in each experiment, resulting in large differences in total nitrogen concentration. 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 (about 0.67) did not differ among experiments or treatments.

The lines from all experiments in the third quadrant ran parallel to each other, similar to those in the first quadrant: large differences in leaf blade nitrogen concentrations occurred. The relationships between total and leaf blade nitrogen uptake (fourth quadrant) were similar for all experiments: the fraction nitrogen in leaf blades (on average 0.74) was almost constant over experiments and treatments.

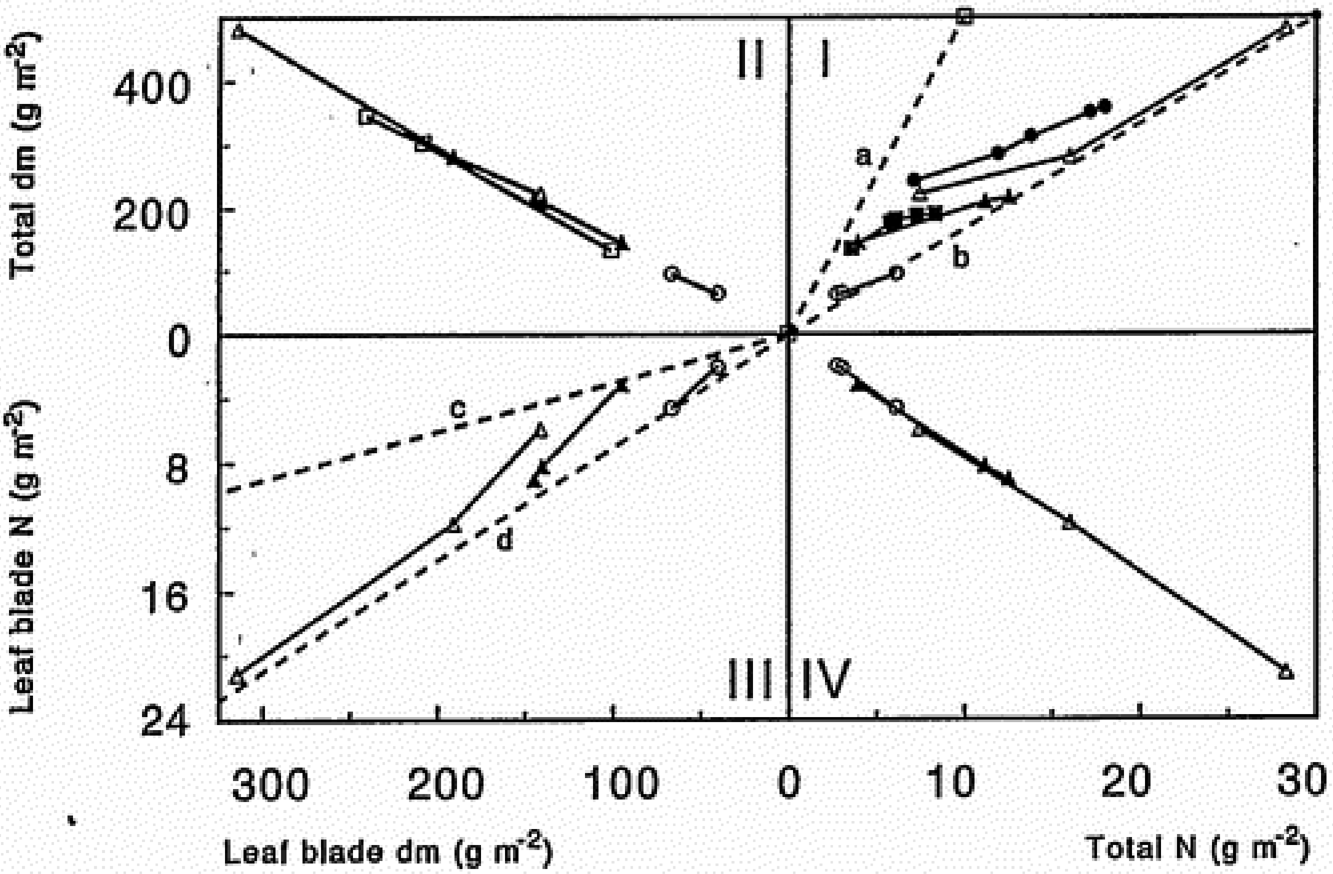

Figure 1. Relations between total dry matter production (Total dm), leaf blade dry matter production (Leaf blade dm), total nitrogen uptake (Total N) and leaf blade nitrogen uptake (Leaf blade N) at 35 DAE (days after emergence) for Experiment 1, at 42 DAE for Experiment 2, at 26 DAE for Experiment 3, 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}. The dotted lines in quadrants I and III represent 2 ("a"), 6 ("b"), 3 ("c") and 7 ("d") % nitrogen in the total or leaf blade dry matter. (■) Experiment 1; (●) Experiment 2; (▲) Experiment 3; (□) Experiment 4; (○) Experiment 5; (△) Experiment 6.

Partitioning of dry matter to different plant organs

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; only available for Experiments 3–6) were significantly affected by treatments in two experiments, but effects were inconsistent.

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 all treatments in the relative partitioning rates of dry matter increase to leaf blades, petioles and stems (comprising hypocotyl, complete stem, plus leaves, flower initials and flowers above leaf number 18) (Figures 2A, B and C: Experiment 5) were small. Similar patterns as in Figure 2 were observed in Experiment 3 (the other experiment with summer spinach; data not shown). In autumn spinach, however, the relative partitioning rates to leaf blades and petioles remained constant from about three weeks after emergence onwards, while no stem elongation took place.

Table 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 superscript letters indicate that there was a significant difference between treatments. Where available, LSD values are included. 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)	$0,654^{a}$	N(0)	0.756^{b}	N(1.12)	0.645^{ab}	N(1.68)	0.632^{a}
N(200/1)	0.671^{a}	N(200/1)	0.696^{a}	N(1.68;L)	0.632^{a}	N(3.36;L)	0.678^{b}
N(200/5)	0.671^{a}	N(200/5)	0.686^{a}	N(3.36)	0.699^{b}	N(5.04)	0.652^{ab}
LSD	0,035	LSD	0.046	LSD	0.056	LSD	0.030

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

Partitioning of nitrogen to different plant organs

The nitrogen concentration of leaf blades was higher than the average nitrogen concentration in the whole plant (see Figure 1, first and third quadrant). Therefore, the harvest indices for nitrogen were always higher than the harvest indices for dry matter (Table 3). The differences between treatments in these harvest indices were small, although the average nitrogen concentration in the whole plant of treatments with the largest amount of nitrogen applied, was 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 2D, E and F show the data from Experiment 5 as an example. The relative partitioning rates of nitrogen increase 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 in this experiment.

Concentration of nitrogen and nitrate in plant organs of different nodes

Figure 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 (data not shown). 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 2% at that same moment (Figure 3B). The highest organic nitrogen concentrations were not observed

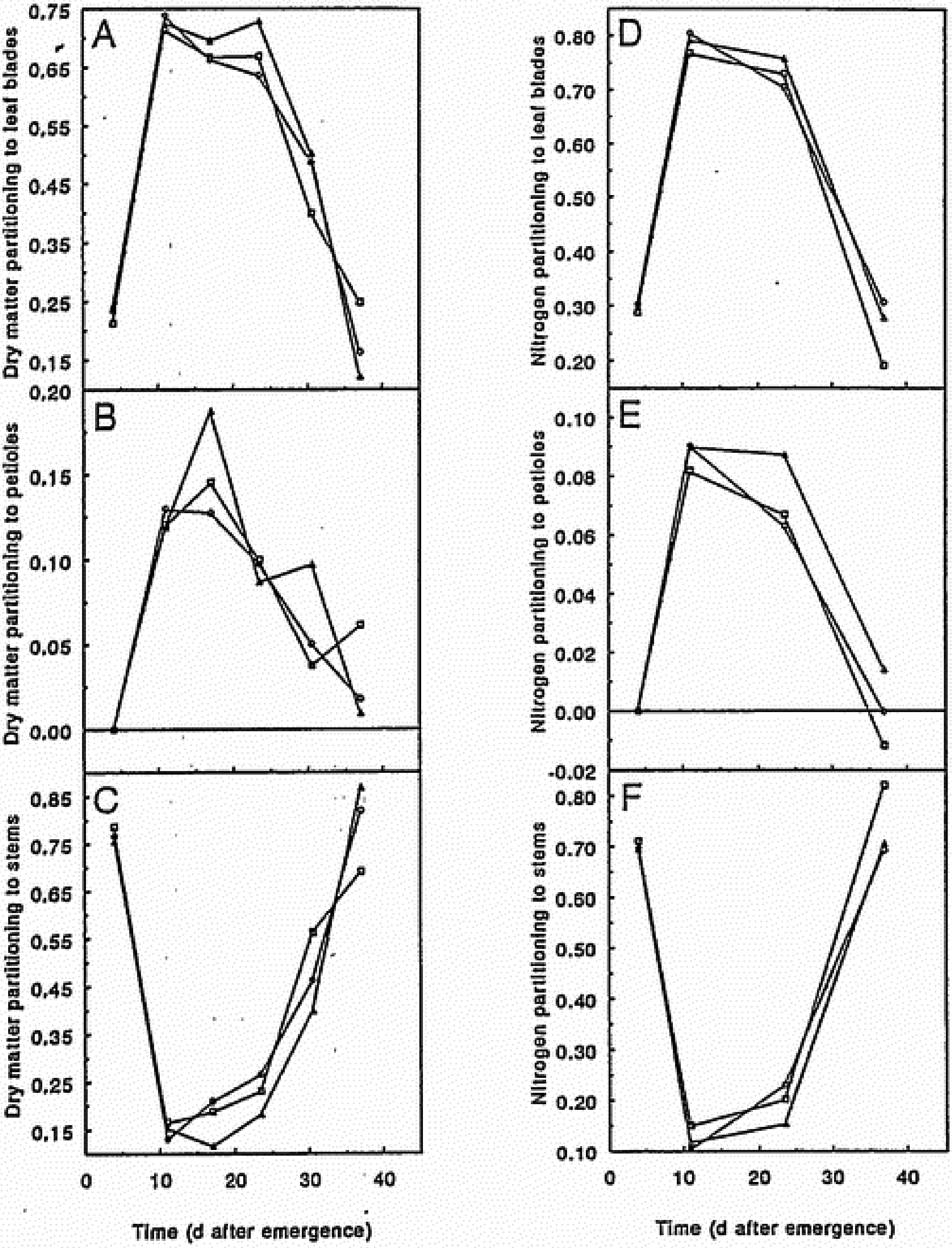

Figure 2. Changes with time in the relative partitioning rates of dry matter increase to the leaf blades (A), the petioles (B) and the stem (comprising hypocotyl, complete stem, and leaves, flower initials and flowers above leaf number 18; C) and changes with time in the relative partitioning rates of 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).

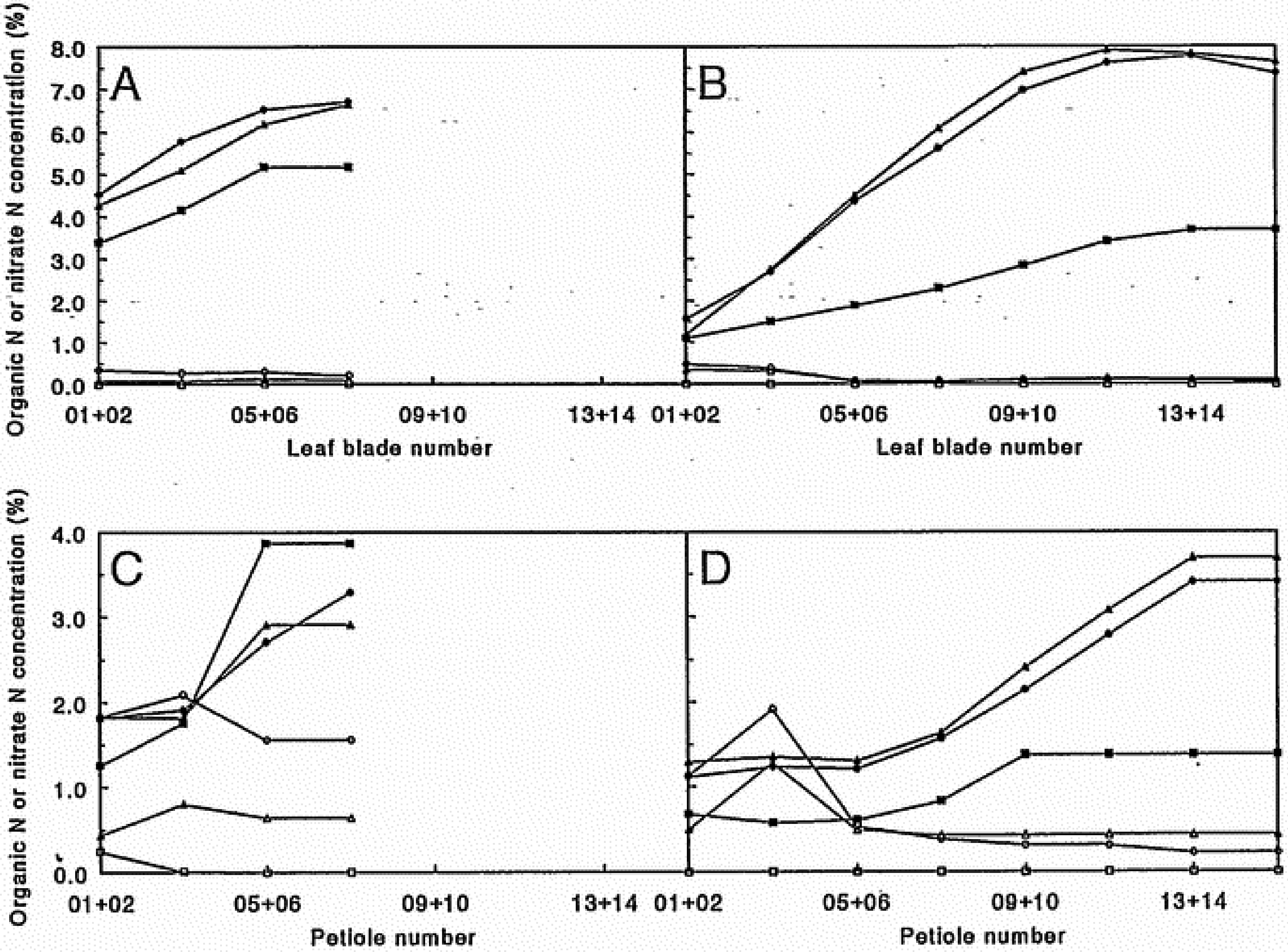

Figure 3. The concentration of organic nitrogen (closed symbols; (■) N(0); (●) N(200/1); (▲) N(200/5)) and nitrate nitrogen (open symbols; (□) N(0); (○) N(200/1); (△) N(200/5)) in the dry matter of the different pairs of leaf blades (A and B) and petioles (C and D) in Experiment 3 at 19 (A and C) and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

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). 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 3D. For all plant parts, no clear trend of nitrate concentration with time was observed (see e.g. Figure 4B). When the available amount of nitrogen could expected to be larger, nitrate concentrations were higher: e.g. when comparing N(200/1) of Experiments 3 and 4 to N(200/5), the first treatment had higher nitrate concentrations in the beginning of the growing season, but the second treatment at the end of the growing season.

Figure 4 shows examples of the changes with leaf age of the organic nitrogen and nitrate nitrogen concentrations of leaf blades. This leaf pair (consisting of leaf blades 03 and 04) appeared about 18 DAE (see Biemond, 1995). The decrease with leaf age in the organic nitrogen concentrations (Figure 4A) during (the first half of) the leaf's life explains why the organic nitrogen concentrations in Figure 3 increased with increasing leaf number. In most experiments, for leaf numbers lower than 03+04, the decrease in nitrogen concentration was larger and continued during a larger part of the leaf's life than for higher leaf numbers. As a result of the application of extra nitrogen to the N(3.36;L) plants during the second half of the 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 (Figure 4A) and the amount of dry matter (Figure 4D) of the N(1.68) and N(3.36;L) leaves were more or less constant after 20 days after appearance. This indicates that no redistribution of nitrogen from this leaf pair (Figure 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).

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. Firstly, different amounts of nitrogen resulted in large differences in dry matter and nitrogen accumulation. Secondly, the differences in nitrogen accumulation were relatively larger than those in dry matter accumulation especially when comparing different timing of N fertilisation. When more nitrogen was applied, a higher total nitrogen concentration was attained. Compared to a single dose, split applica-

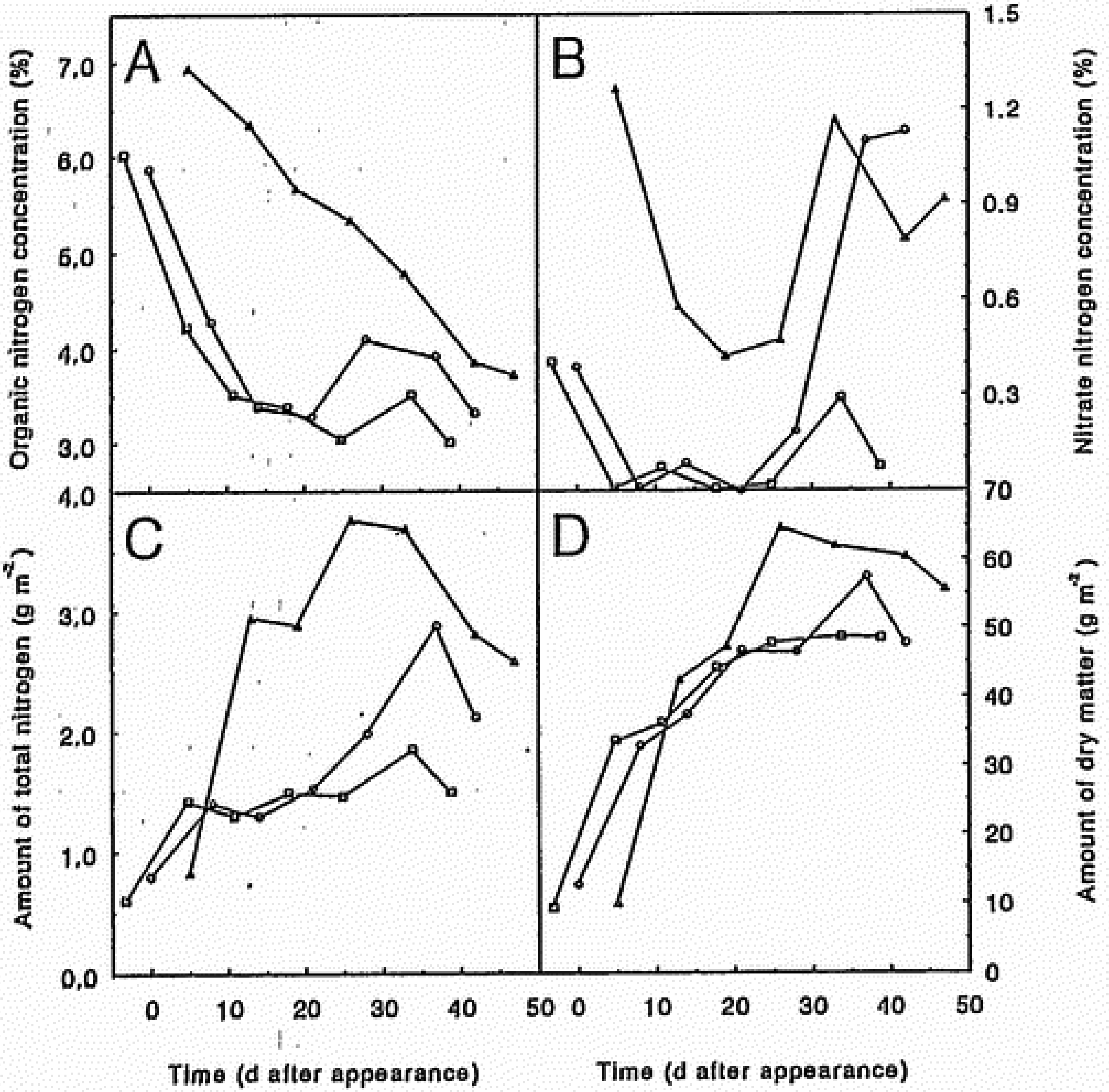

Figure 4. Changes with average leaf age in the concentrations (%) of organic nitrogen (A) and nitrate nitrogen (B) in the dry matter, total amount (g m^{-2}) of nitrogen (C) and dry matter (D) of leaf blades 03+04 of Experiment 6. (□) N(1.68); (○) N(3.36;L); (△) N(5.04).

tions of nitrogen reduced the nitrate concentrations in the lowest petioles. Niers (1994) reported an average nitrogen uptake (excluding roots) of 125 kg ha^{-1}, which is close to the current results.

In our experiments nitrogen treatments did not affect the partitioning of dry matter and nitrogen. Figure 2 suggests that one function can describe dry matter and nitrogen allocation, irrespective of nitrogen availability. Effects of other factors on the partitioning of dry matter and nitrogen also seemed to be small since differences between experiments in harvest indices were minor.

Apparently, the spinach crop is not very versatile in its response to nitrogen treatments or to the other (uncontrolled) factors involved in this research. The predictable partitioning of nitrogen and dry matter and the absence of significant changes in patterns of growth and development allows farmers to maintain a suitable supply of N, based on yielding ability, quality of the produce and environmental objectives. Variation in response to N is limited.

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 4C), growing new leaves requires nitrogen which needs to be 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. Nitrate nitrogen concentrations increased when more N was available. However, 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 (see also Breimer (1982)).

Acknowledgements

The authors thank the students A. Fehmi Soygenis, G. van Dijk and L. Vreugdenhil (who participated in the activities of the Department of Agronomy) for assistance with data collection and data processing, H.D. Halm (Department of Agronomy, Wageningen Agricultural University) for carrying out chemical analyses.

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.

Vos, J., H. Biemond & P.C. Struik, 1996. Dynamics of change of leaf attributes of Brussels sprouts in response to switches between high and low supply of nitrogen. *Netherlands Journal of Agricultural Science*, 44: 31–42.