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

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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.

Keywords: Spinach, *Spinacia oleracea* L., leaf initiation, leaf expansion, leaf size, nitrogen nutrition

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 1 summarizes experimental details.

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 tem-

Table 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

perature 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). 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). 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). 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). 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). 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 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 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

Table 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.

Time (DAE)	Experiment 1					Experiment 2					Experiment 3			Experiment 4			Experiment 5			Experiment 6		
	N(0)	N(75+50)	N(125/5)	N(120+80)	N(200/5)	N(0)	N(50+25)	N(75/5)	N(90+60)	N(150/5)	N(0)	N(200/1)	N(200/5)	N(0)	N(200/1)	N(200/5)	N(1.12)	N(1.68;L)	N(3.36)	N(1.68)	N(3.36;L)	N(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

independent variable. The expansion rate was characterised by the slope of the regression (tan α in Figure 1). The 'effective duration of expansion' (d; length of line 'a' in Figure 1) is defined as the maximum area of a leaf pair (cm^2; length of line 'b' in Figure 1) divided by its expansion rate (cm^2 d^{-1}) (cf. Vos & Biemond, 1992).

Destructive sampling

Plants were sampled six, seven or eight times in each experiment at approximately weekly intervals after emergence until the final date (Table 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.

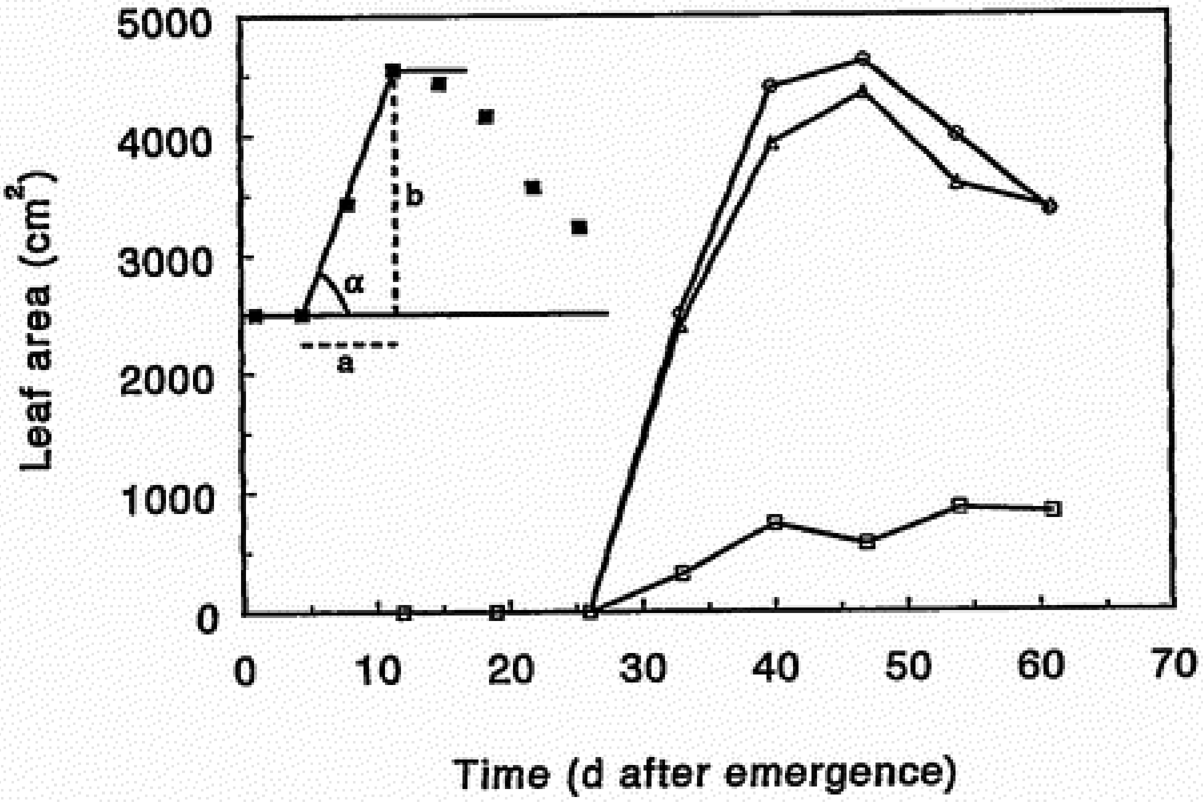

Figure 1. The change over time of the leaf area of leaves 13+14, expressed per m^2 soil surface (Experiment 3). (□) N(0); (○) N(200/1); (△) N(200/5). The upper left part explains the method of calculating the rate and duration of leaf expansion. The length of line 'a' represents the effective duration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

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 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.

The highest rates of leaf appearance were observed in Experiments 3 and 5; the lowest rates in Experiments 4 and 6 (Table 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 3) was significantly different (LSD-test, P=0.05) among treatments in Experiments 4 and 6, with more leaves at the higher nitrogen applications.

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

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

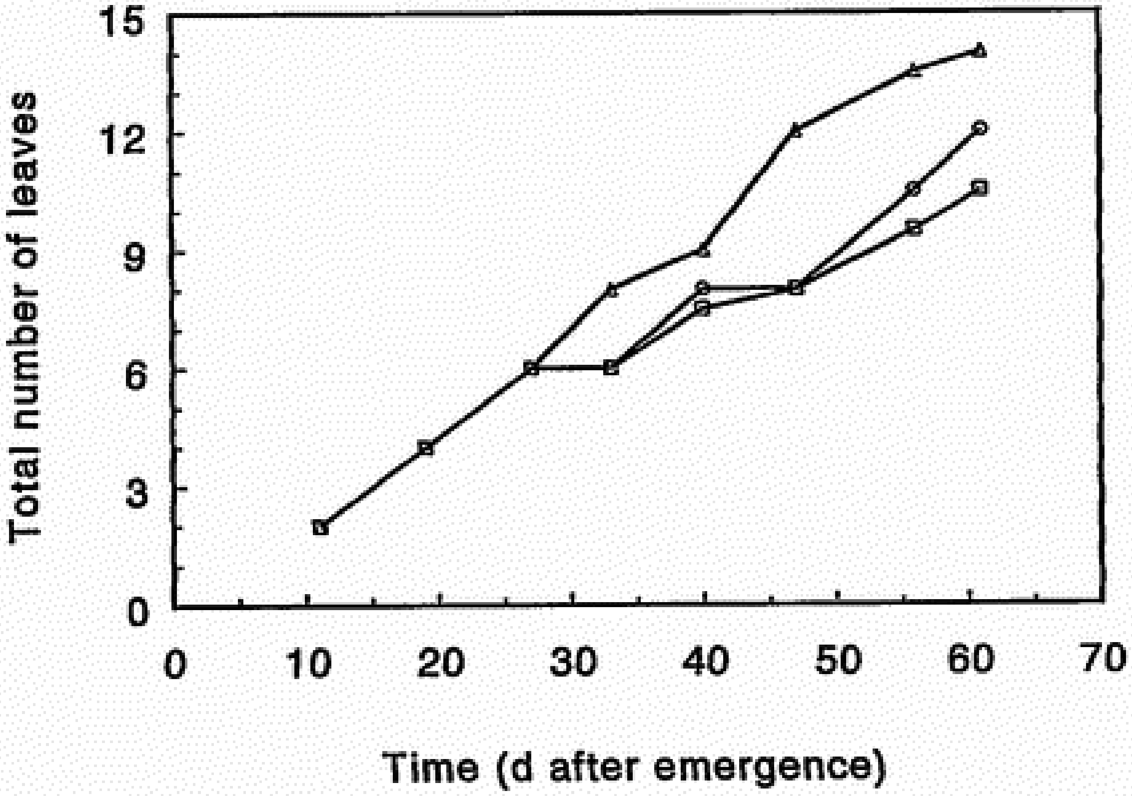

Figure 2. The total number of leaves for Experiment 6 as a function of time and nitrogen treatment. (□) N(1.68); (○) N(3.36;L); (△) N(5.04).

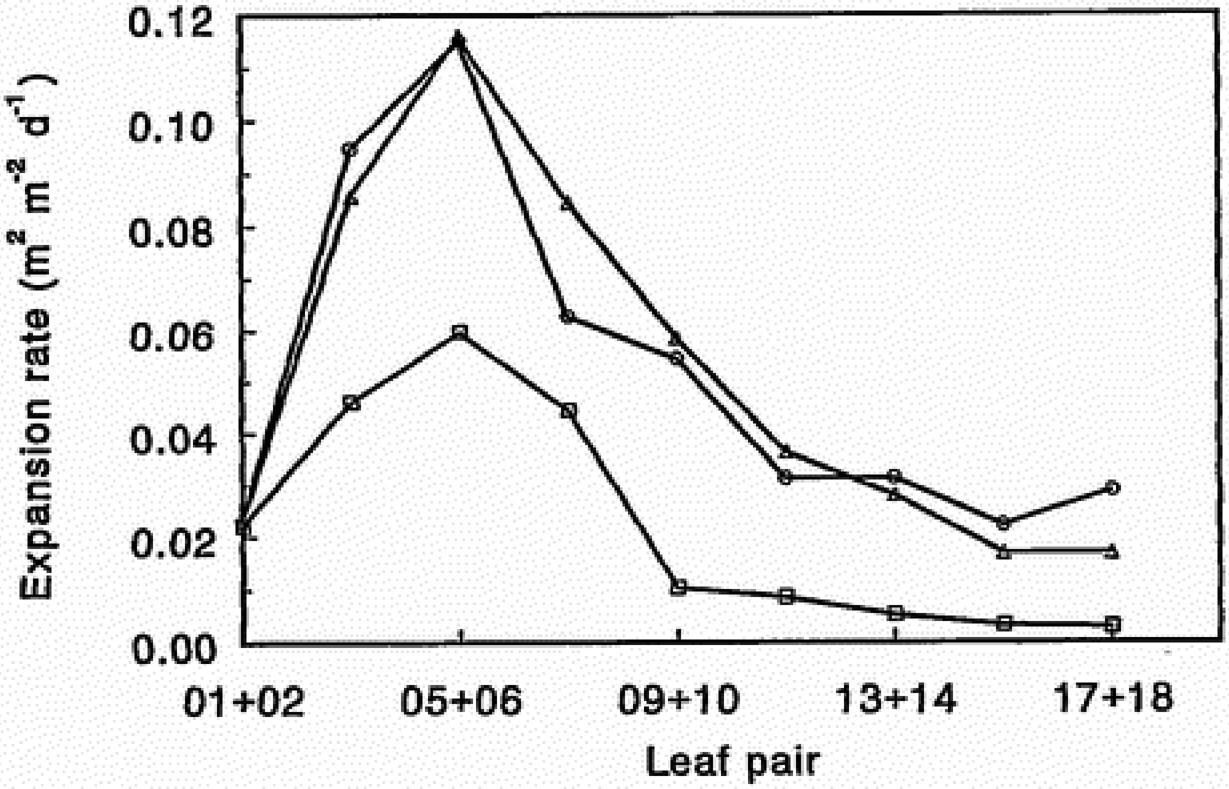

Figure 3. The rate of leaf expansion of successive leaf pairs in relation to nitrogen supply for Experiment 3. The rate of leaf expansion is expressed per m^2 surface area. (Plant density in this experiment: 386 plants m^{-2}.) (□) N(0); (○) N(200/1); (△) N(200/5).

3+4 or 5+6 and subsequently decreased (Figure 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 4, to illustrate the differences among the experiments: it varied from 1.45 to 3.60 cm^2 d^{-1}.

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 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 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 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 6) was nearly constant at 15 d, irrespective of leaf number and nitrogen treatment, except for leaf pair 1+2.

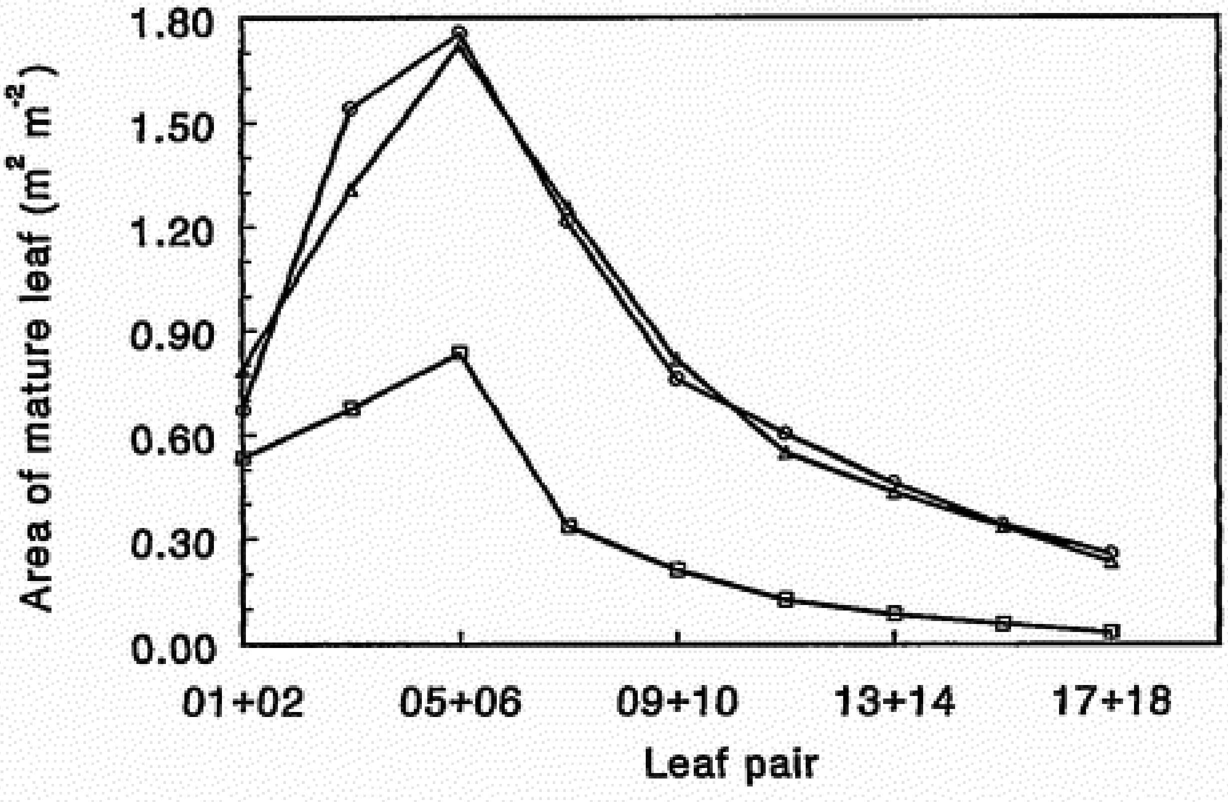

Figure 4. Mature leaf area of leaf pairs of Experiment 3 in relation to nitrogen supply. The mature leaf area is expressed per m^2 soil surface. (Plant density in this experiment: 386 plants m^{-2}.) (□) N(0); (○) N(200/1); (△) N(200/5).

Table 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)

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 7. It

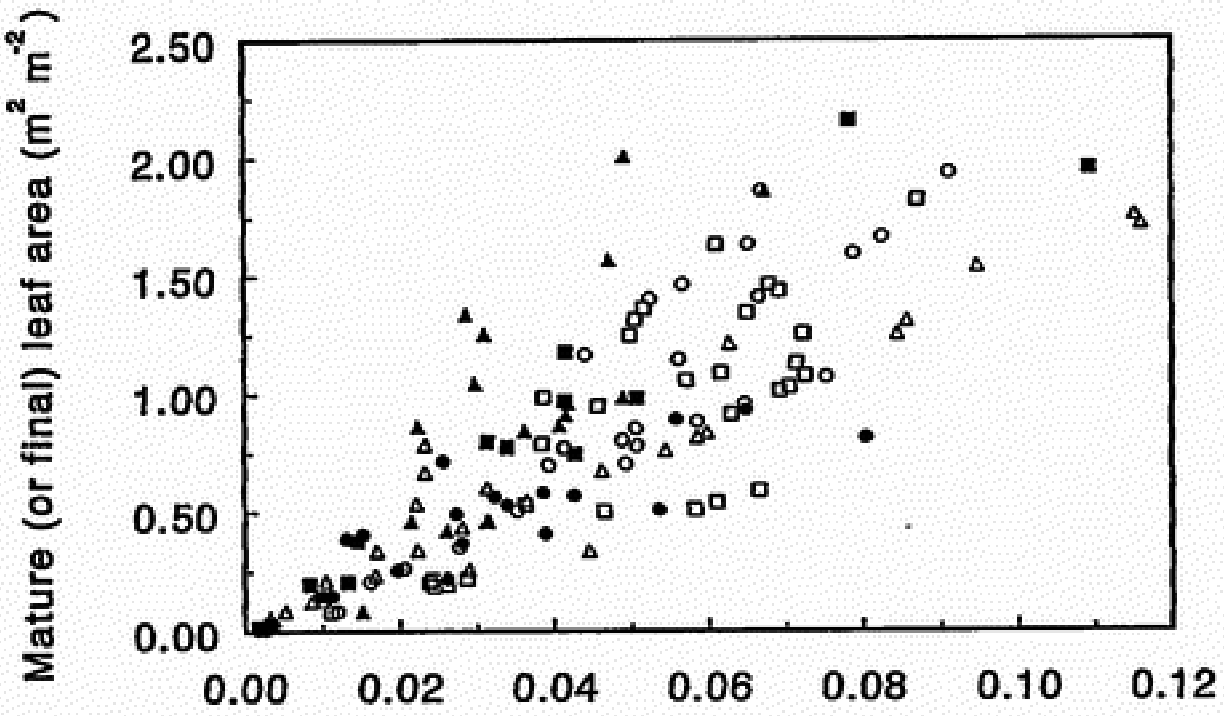

Figure 5. The relation between mature leaf area and rate of leaf expansion, irrespective of leaf position. (□) Experiment 1; (○) Experiment 2; (△) Experiment 3; (■) Experiment 4; (●) Experiment 5; (▲) Experiment 6.

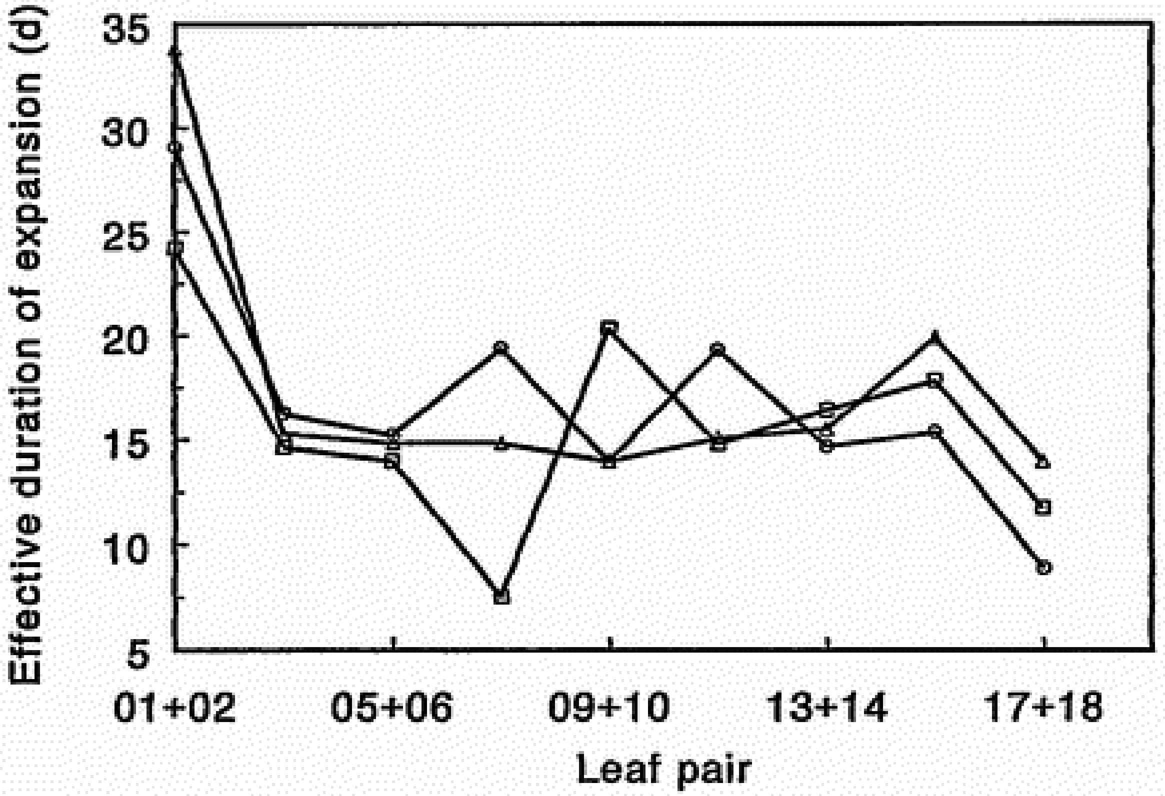

Figure 6. The effective duration of expansion as a function of leaf position and nitrogen treatment (Experiment 3). (□) N(0); (○) N(200/1); (△) N(200/5).

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.

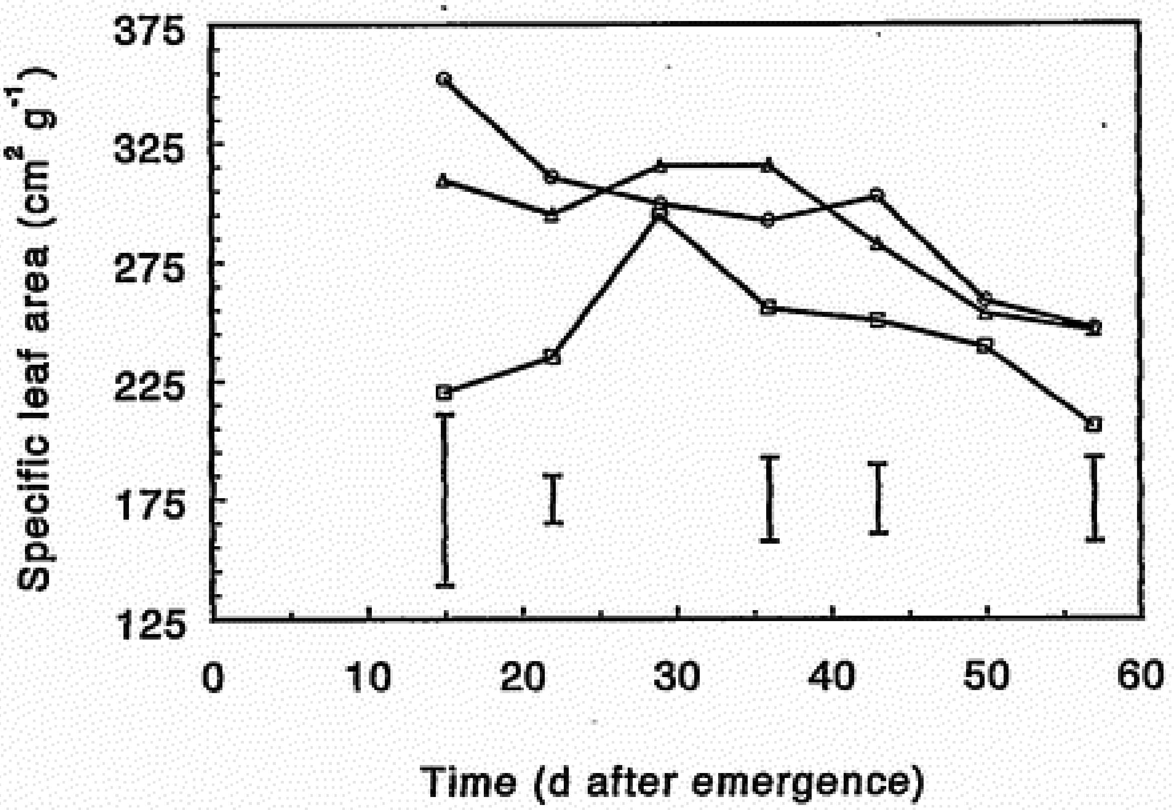

Figure 7. Specific leaf area over all green leaves of a plant for Experiment 4 as a function of time and nitrogen treatment. The vertical bars represent LSD values (P=0.05). (□) N(0); (○) N(200/1); (△) N(200/5).

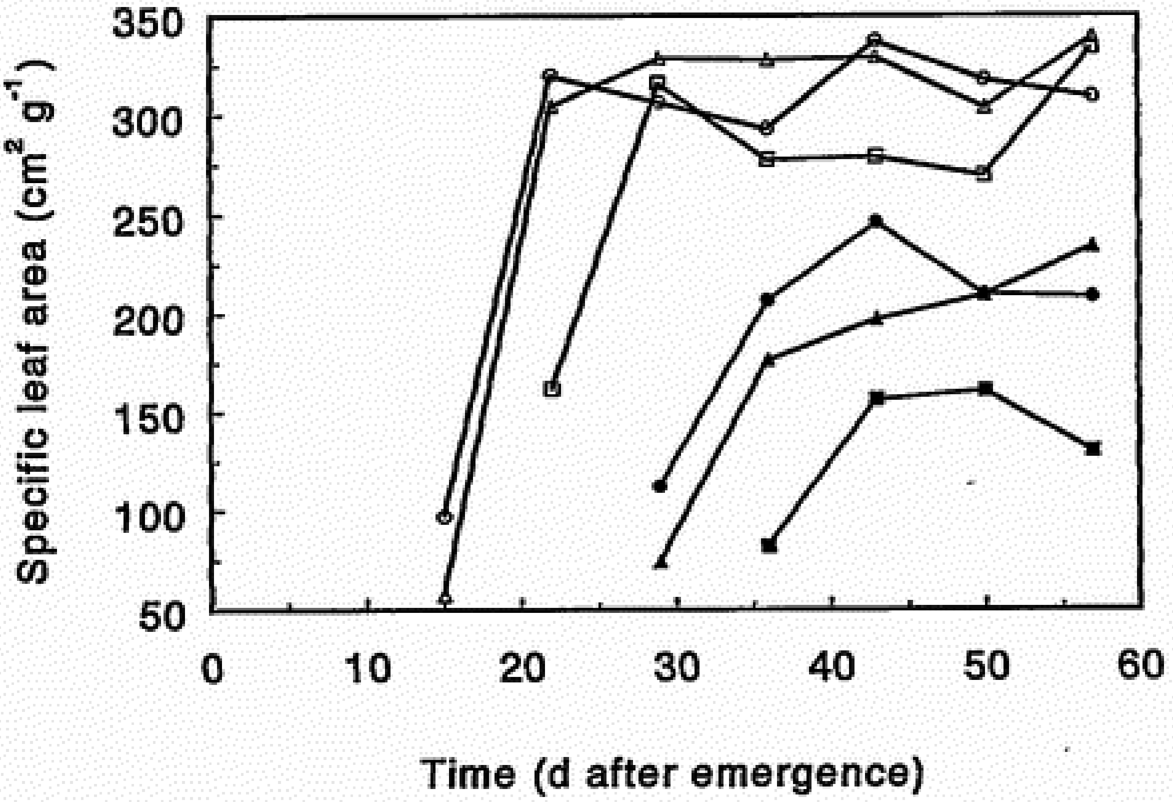

Figure 8. Specific leaf area of leaf pairs 3+4 and 7+8 against days after emergence (Experiment 4). Leaf pair 3+4: (open markers; (□) N(0); (○) N(200/1); (△) N(200/5)); leaf pair 7+8: (closed markers; (■) N(0); (●) N(200/1); (▲) N(200/5)).

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 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.

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 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.

Total green leaf area

Except in Experiments 3 and 4, total green leaf area increased more or less linearly

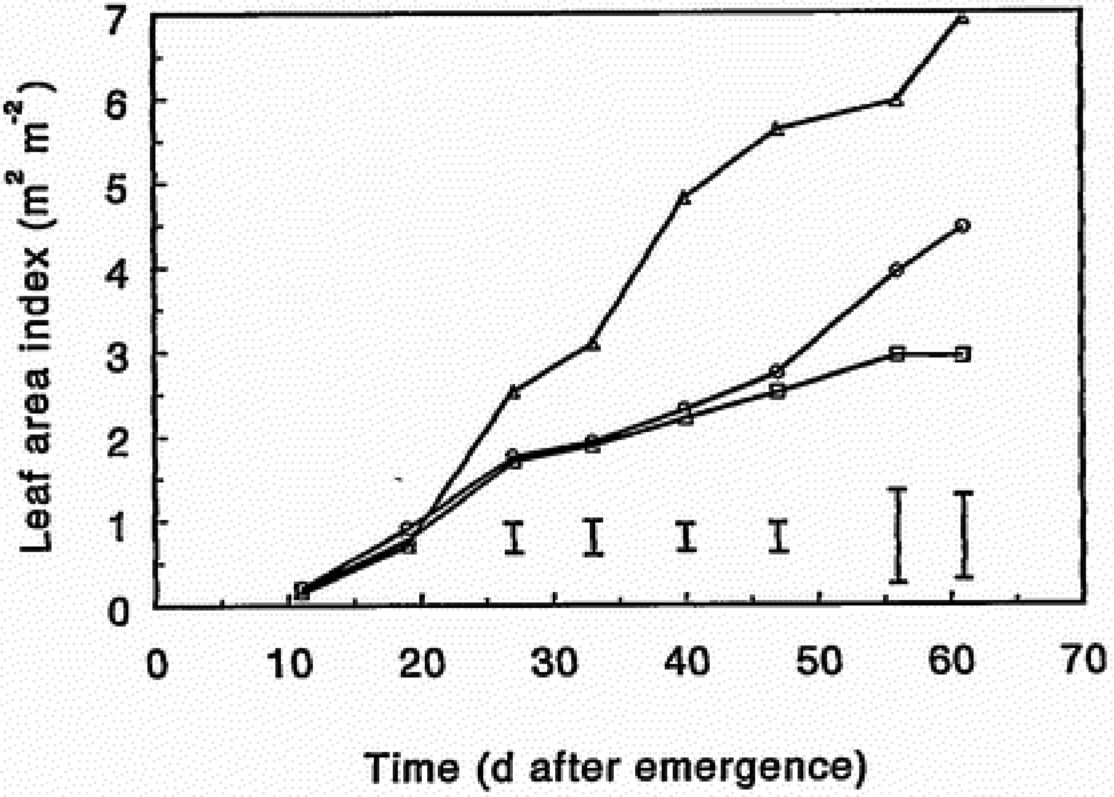

Figure 9. Total leaf area per m^2 for Experiment 6 as a function of time and nitrogen treatment. The vertical bars represent LSD values (P=0.05). (□) N(1.68); (○) N(3.36;L); (△) N(5.04).

until the end of each experiment (Figure 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.

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 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.

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