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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

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Abstract

Leaf growth and development of Brussels sprouts (*Brassica oleracea* L. var *gemmifera* DC) was 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.

Keywords: Brussels sprouts, *Brassica oleracea* L., leaf development, leaf expansion, nitrogen nutrition, leaf senescence

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 1–3), consisting of different amounts of nitrogen and different dates of application. Other nutrients were supplied in equal amounts in all treatments.

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

Table 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

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.

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

Table 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

Table 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	19.60

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

Table 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.

	Nitrogen treatment (Experiment 1)			
Parameter	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

area (determined with a Li-Cor model 3100 electronic area meter; Li-Cor, Lincoln, Nebraska). These data were fitted to a logarithmic regression (Table 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.

Figure 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 1). The 'effective duration of expansion' (d; length of line 'a' in Figure 1) is defined as the final area of a leaf (cm^2; length of line 'b' in Figure 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

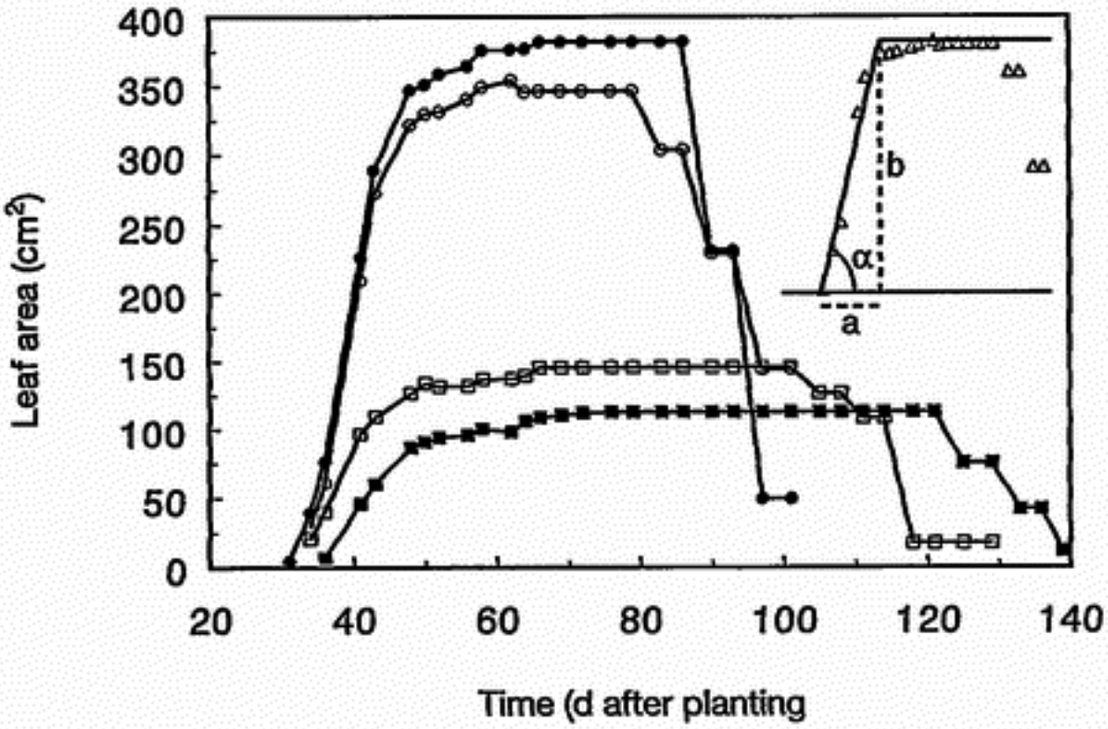

Figure 1. The change with time in leaf area of leaf 15, Experiment 3. (□) N(5.6); (○) N(9.8;E); (■) N(9.8;L); (●) N(19.6). The insert at the upper right explains the method of calculation of the rate and duration of leaf expansion. The length of line 'a' represents the duration of leaf expansion (d),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

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.

Results

Rate of leaf appearance and number of leaves

Figure 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 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.

The difference in amount of nitrogen in Experiment 1 caused large differences in total number of leaves (Table 5). Across experiments, plants in Experiment 1 at-

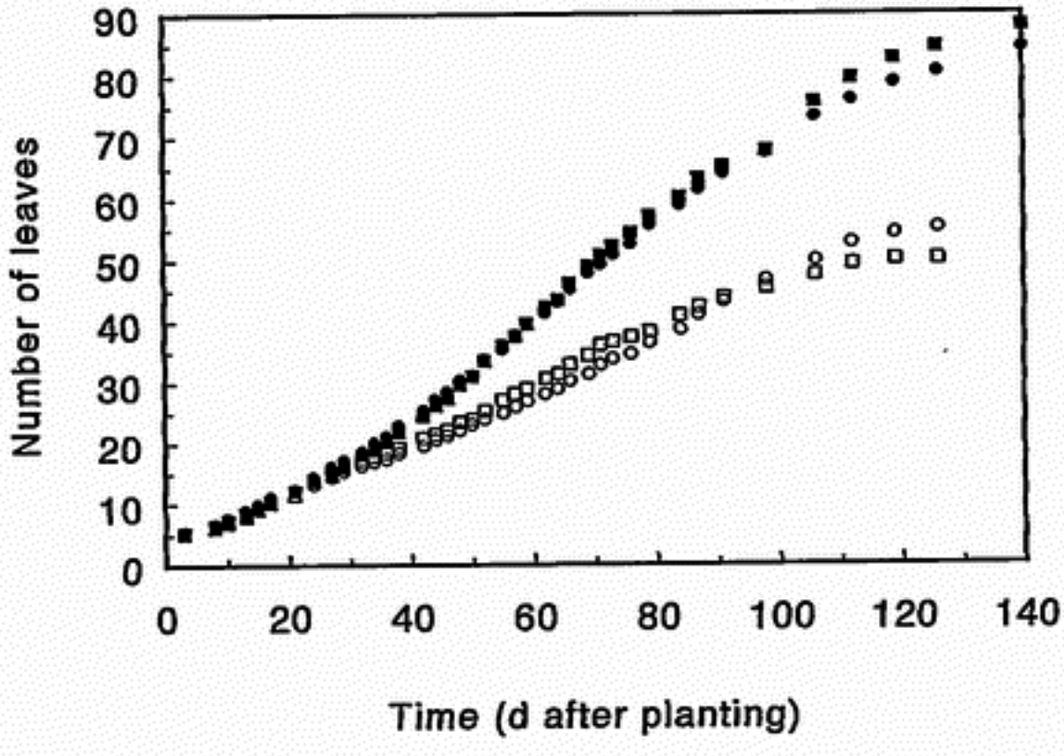

Figure 2. The number of leaves as a function of time and nitrogen treatment for Experiment 1. (□) N(4.5/3); (○) N(4.5/9); (■) N(13.5/3); (●) N(13.5/9).

Table 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 superscript 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)

tained 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 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.

The features noted for the rate of leaf expansion also apply to the area of fully expanded leaves in both experiments (Figure 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 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).

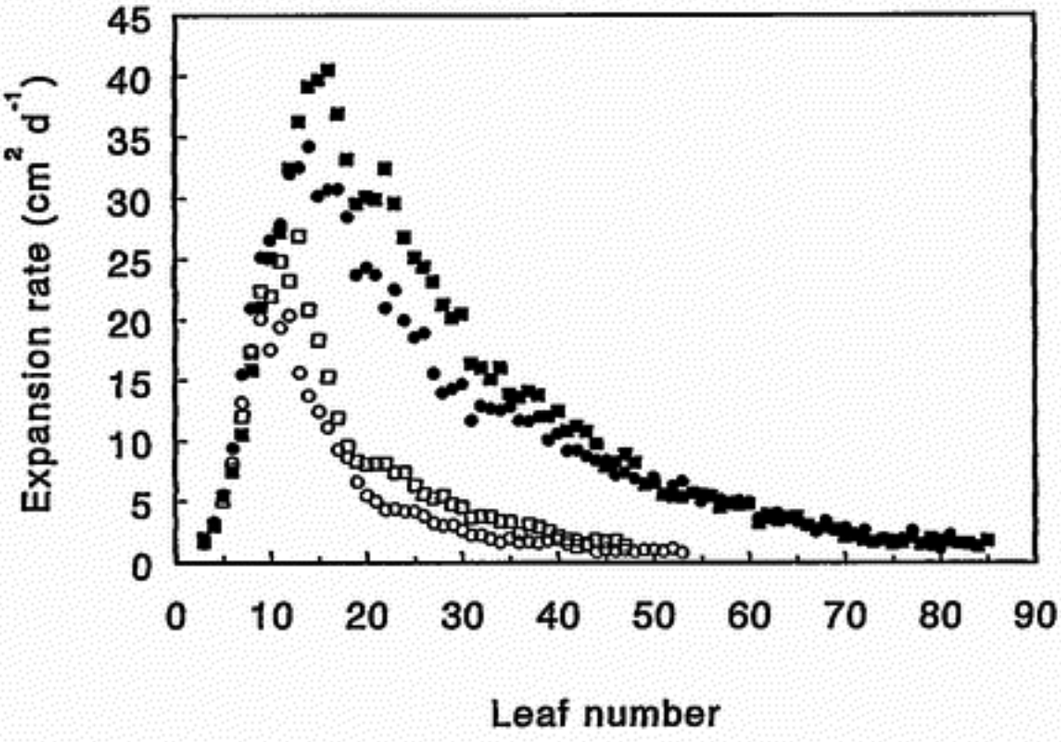

Figure 3. The rate of leaf expansion versus leaf insertion number as affected by nitrogen supply for Experiment 1. (□) N(4.5/3); (○) N(4.5/9); (■) N(13.5/3); (●) N(13.5/9).

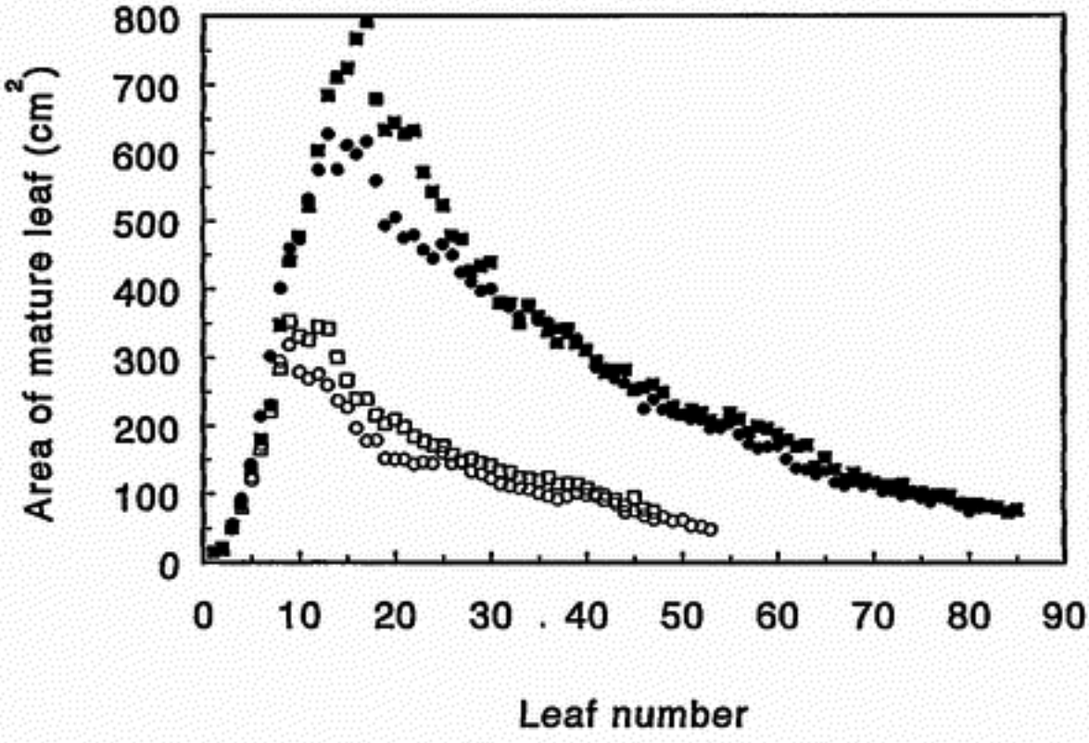

Figure 4. Mature leaf area of leaves of Experiment 1 in relation to leaf insertion number and nitrogen treatment. (□) N(4.5/3); (○) N(4.5/9); (■) N(13.5/3); (●) N(13.5/9).

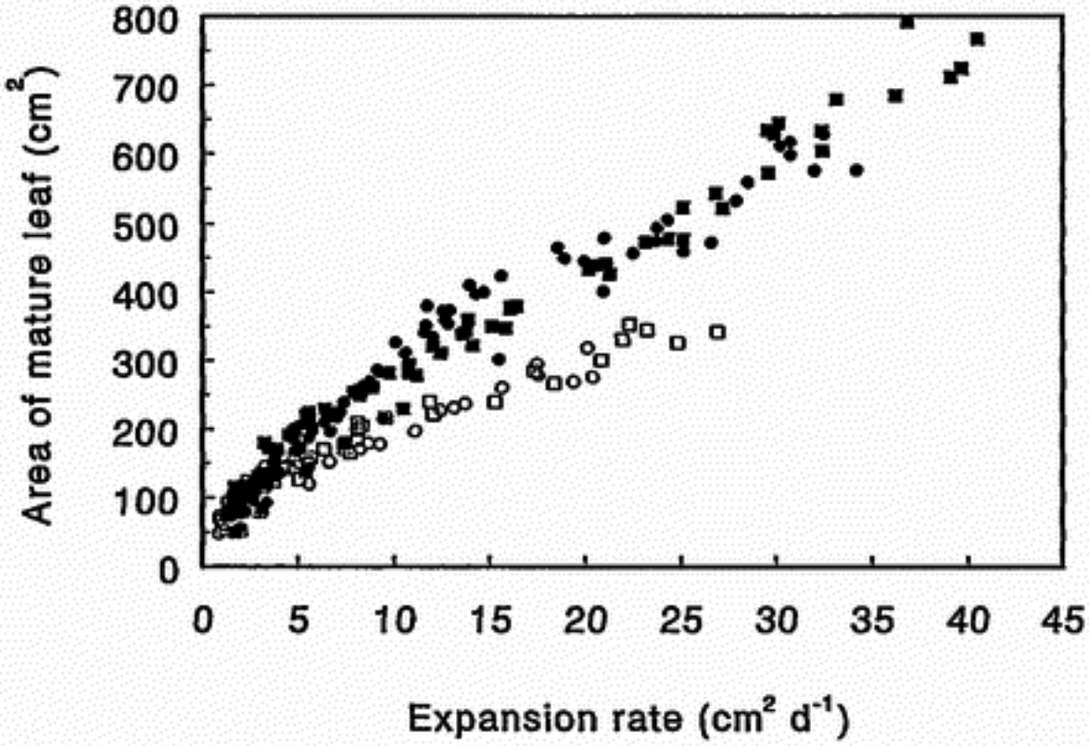

Figure 5. The relation between mature leaf size and leaf expansion rate, irrespective of leaf position (Experiment 1). (□) N(4.5/3); (○) N(4.5/9); (■) N(13.5/3); (●) N(13.5/9).

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 6: Experiment 1). The increase was faster for N(4.5/3) and N(4.5/9) than for

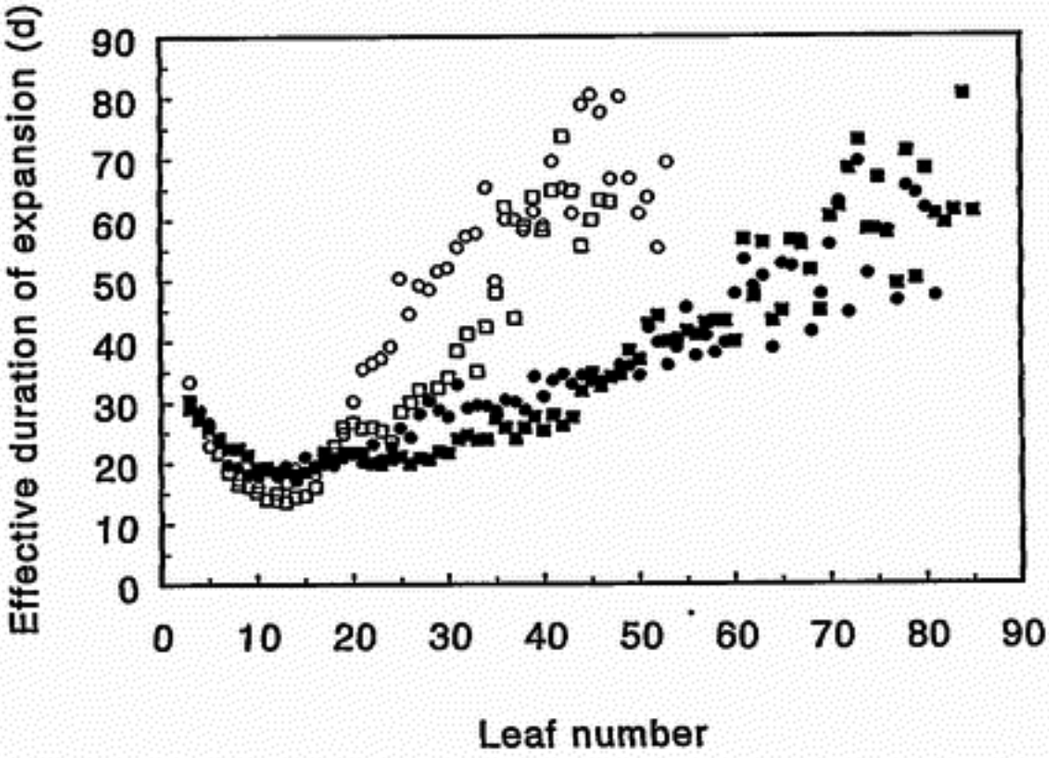

Figure 6. The effective duration of expansion as a function of leaf position and nitrogen treatment (Experiment 1). (□) N(4.5/3); (○) N(4.5/9); (■) N(13.5/3); (●) N(13.5/9).

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.

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 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 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

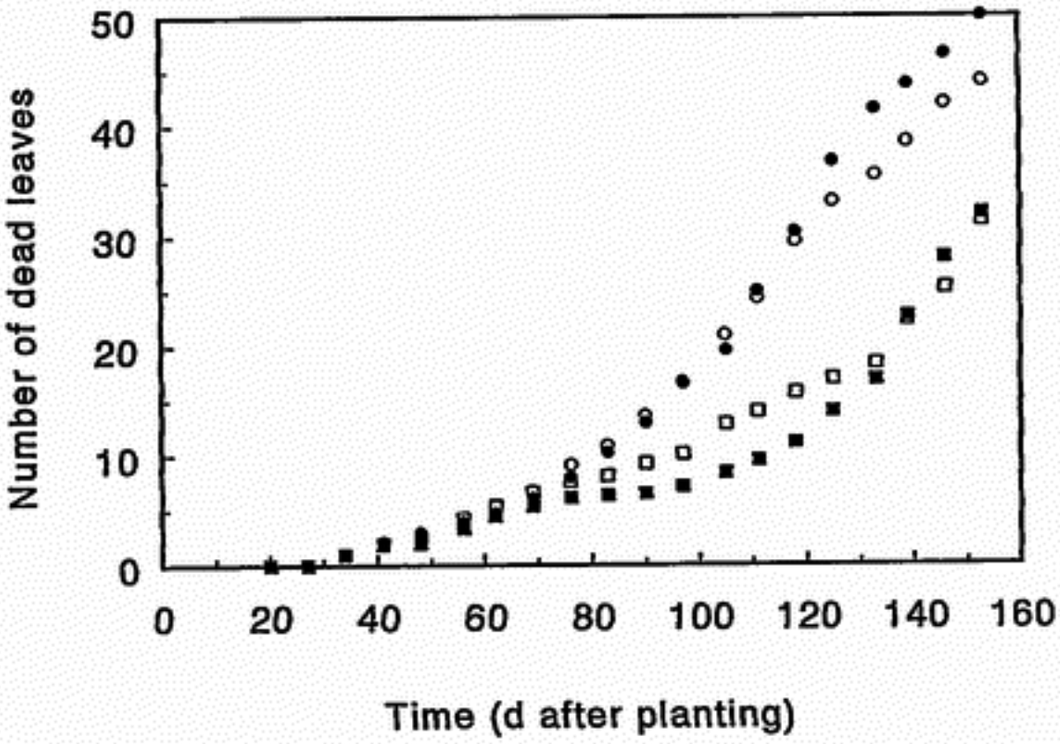

Figure 7. The number of dead leaves as a function of time and nitrogen treatment for Experiment 3. (□) N(5.6); (○) N(9.8;E); (■) N(9.8;L); (●) N(19.6).

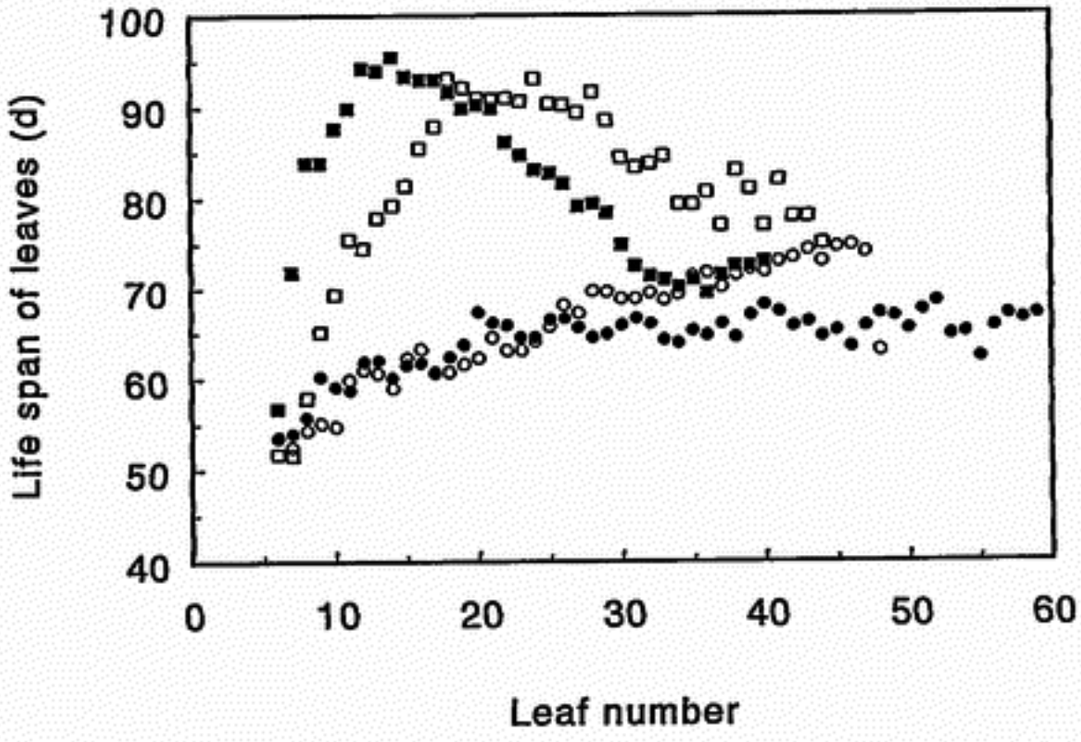

Figure 8. The life span of leaves of Experiment 3 as a function of leaf insertion number and nitrogen treatment. (□) N(5.6); (○) N(9.8;E); (■) N(9.8;L); (●) N(19.6).

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 maxi-

mum 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.

Specific leaf area

Specific leaf area (SLA) was calculated over all green leaves of a plant at each (intermediate) harvest. In Experiment 3 (Figure 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 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

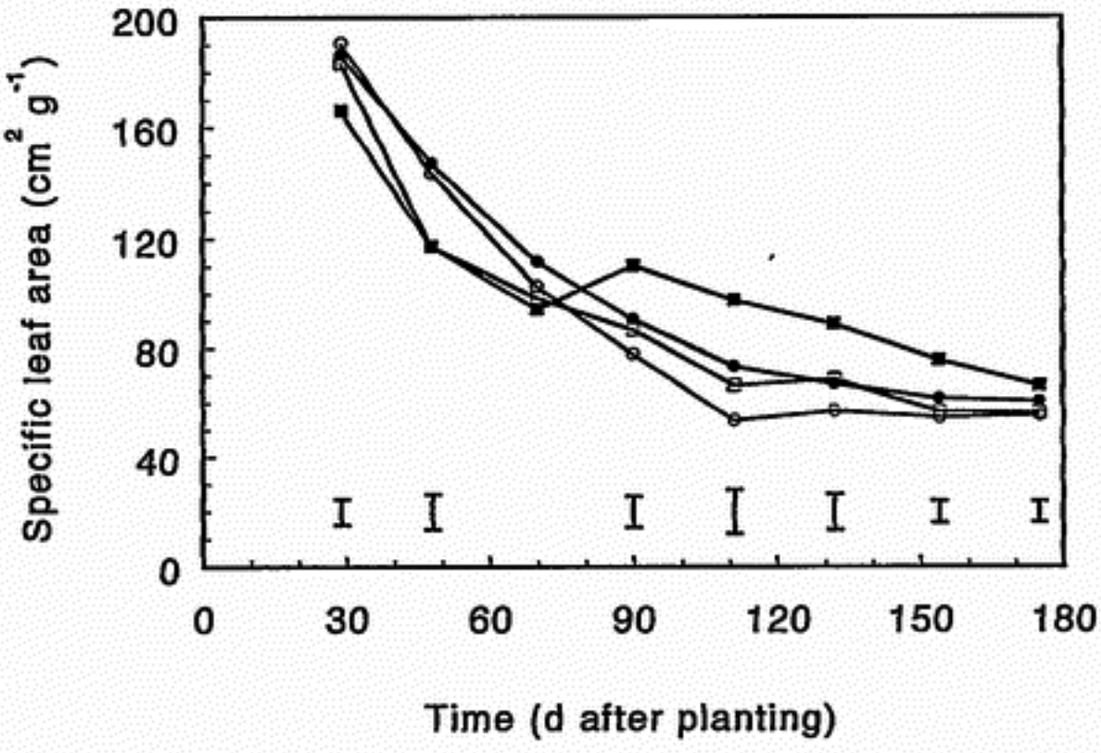

Figure 9. Specific leaf area over all leaves of a plant for Experiment 3 as a function of time and nitrogen treatment. The vertical bars represent LSD values (P=0.05). (□) N(5.6); (○) N(9.8;E); (■) N(9.8;L); (●) N(19.6).

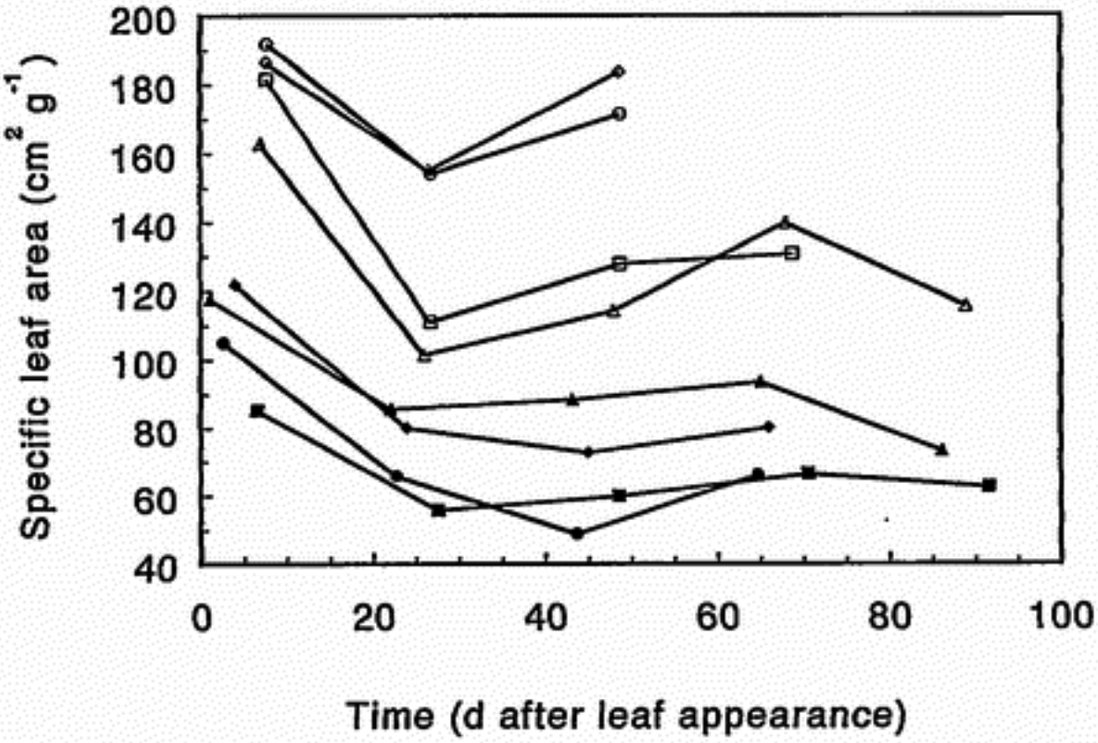

Figure 10. Specific leaf area of two groups of five leaves against days after leaf appearance (Experiment 3). Leaf blades 6–10: (open markers; (□) N(5.6); (○) N(9.8;E); (△) N(9.8;L); (◇) N(19.6)); leaf blades 36–40: (closed markers; (■) N(5.6); (●) N(9.8;E); (▲) N(9.8;L); (◆) N(19.6)).

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.

Total leaf area per plant

Figure 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),

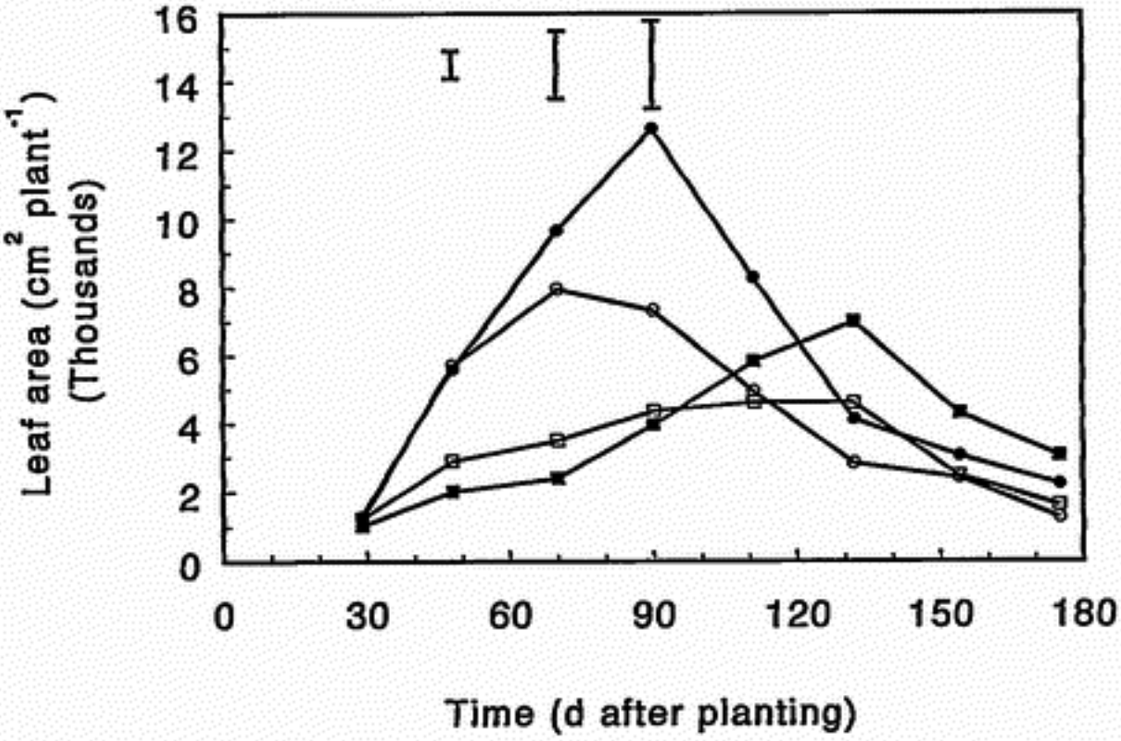

Figure 11. Total leaf area per plant for Experiment 3 as a function of time and nitrogen treatment. The vertical bars represent LSD values (P=0.05). (□) N(5.6); (○) N(9.8;E); (■) N(9.8;L); (●) N(19.6).

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.

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 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).

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