

Dynamics of change of leaf attributes of Brussels sprouts in response to switches between high and low supply of nitrogen

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Abstract

In previous research nitrogen supply was shown to affect several components of foliar growth and development of Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* DC.). This paper extends the analysis of data on Brussels sprouts and focuses on the dynamics of change in components of leaf growth and nitrogen concentrations upon change in nitrogen supply. Four treatments were compared: a control without nitrogen limitation, a continuously nitrogen-deficient control, and two treatments with a switch from limiting supply to non-limiting supply or vice versa. Plant nitrogen concentrations changed first and fast upon change in soil nitrogen regime. Upon increase in supply rate, nitrogen concentration also increased in leaves that had completed their expansion. Changes in leaf growth started about 15 days after the switch in nitrogen regime. Leaves that were expanding at the switch responded by an increase in area when nitrogen supply became larger without a change in mass, i.e. specific leaf area increased. Areas of leaves as well as specific leaf area of expanding leaves decreased when the nitrogen supply became smaller. Control of leaf size during initiation and expansion is discussed.

Keywords: Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* DC.), leaf expansion, leaf size, nitrogen concentration

Introduction

The grower of a crop is faced with the challenge to match the nitrogen requirement and supply. From a theoretical point of view split applications represent an attractive strategy to achieve that objective since it enables the grower to tailor the rate of supply to the course of crop growth and to availability of nitrogen in the soil. However, dynamic strategies for nitrogen supply need to be based on thorough understanding of the reaction of the crop to temporal changes in availability of nitrogen, both in the soil and in the plant. Also insight needs to be gained in the plasticity of the crop response upon unintended temporary deficiency. Variable availability of nitrogen may also affect the pattern of plant growth and ultimately the level of production since

the plant has to 'take decisions' throughout the growing period on the initiation and growth rate of additional organs. Relevant questions are therefore under which conditions, to which extent and in which order processes of crop growth and development are altered by varying the availability of nitrogen to the plant.

This paper follows on a series of papers that examined the effects of amount and timing of nitrogen supply in potato (*Solanum tuberosum* L.) (Vos & Biemond, 1992; Biemond & Vos, 1992), Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* DC.) (Biemond *et al.*, 1995a,b), leek (*Allium porrum* L.) (Biemond, 1995a,b) and spinach (*Spinacia oleracea* L.) (Biemond, 1995c; Biemond *et al.*, 1996). Analyses particularly addressed the rates and duration of appearance and expansion of leaves, the patterns of distribution of dry matter and nitrogen, and the nitrate levels. The ultimate objectives of this approach were twofold: (i) to provide insight into nitrogen responses needed to design new dynamic systems of nitrogen supply and to understand the limitations of such systems, and (ii) to contribute to a more refined way of simulation of crop growth and development, accounting for responses to nitrogen.

The previous papers showed that nitrogen affects various characteristics of the foliage, among which leaf expansion rate and leaf size (area), number of leaves and nitrogen concentration are of primary importance. The relative distributions of dry matter and nitrogen among component above ground organs appeared to be fairly conservative, although nitrogen concentrations varied widely among nitrogen treatments.

The current paper attempts to analyse the dynamics of change, of leaf growth and nitrogen uptake and allocation in response to a switch in nitrogen supply from high to low doses or vice versa. To that end analyses are extended of an experiment on Brussels sprouts that was described in Biemond *et al.* (1995a,b). Four treatments will be compared: a control without nitrogen limitation, a continuously nitrogen-deficient control, and two treatments in which the nitrogen supply was switched from non-limiting to limiting supply or vice versa. The current analysis is expected to contribute to mechanistic modelling of nitrogen responses of vegetables.

Materials and methods

A detailed description of the pot experiment with Brussels sprouts is given by Biemond *et al.* (1995a; their Experiment 3). Briefly, the experiment was conducted in a greenhouse kept for 12 h during the day time at 18°C and for the rest of the diurnal period at 12°C. Pots of 20 litre were used containing sand free from organic matter. One seedling with five leaves present was planted per pot on April 14, 1992. Plant density was initially 4.5 m⁻²; it was decreased as plants grew and was 3.2 m⁻² from 70 DAP (days after planting) onwards. Four nitrogen treatments were applied (Figure 1), comprising (i) a nitrogen-limited control, (ii) a non-limited control, and two treatments in which switches were made from limiting supply to non-limiting supply or vice versa. All treatments received nutrient solution at nine dates throughout growth (Figure 1). The nitrogen-limited control received 5.6 g nitrogen per plant. This treatment will be named the 'LL-treatment' (for 'low-low' rates of supply ini-

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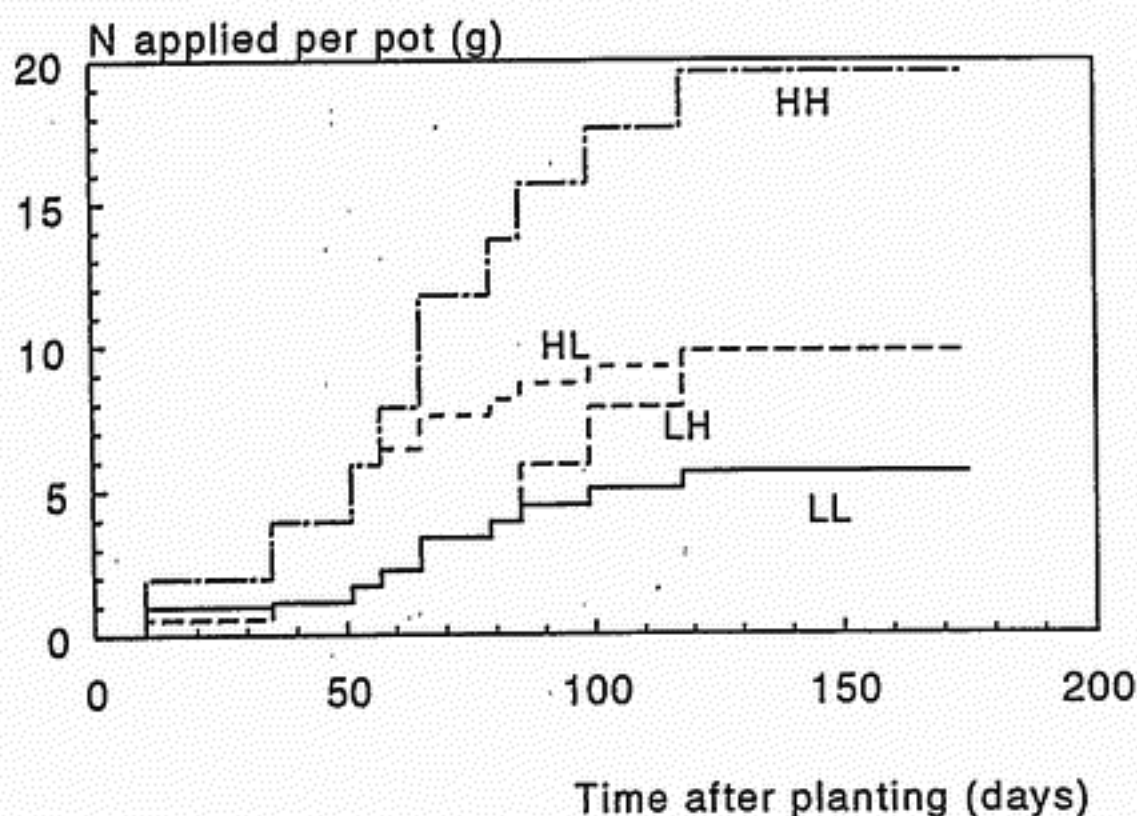


Figure 1. Cumulative amounts of nitrogen supplied per pot. HH is the non-limited control treatment; HL is the treatment with switch from high to low doses (at 57 DAP) and LH from low to high doses (at 85 DAP); LL is the nitrogen-limited control treatment. Low doses were 0.56 g nitrogen and high doses were 1.96 g per plant (double doses were given on 65 DAP).

tially as well as later during growth). The non-limited control (HH-treatment, *i.e.* 'high-high') received 19.6 g N per plant. Both the HL-treatment ('high-low') and the LH-treatment ('low-high') received 9.8 g nitrogen in total. Nitrogen supply to the HL-treatment was initially equal to the supply of the HH-treatment (*i.e.* doses of 1.96 g nitrogen); from 57 DAP onwards additions were equal to those of the LL-treatment (0.56 g per application, except for 65 DAP when two doses were combined into one application in all treatments). Nitrogen supply to the LH-treatment initially ran parallel to the supply of the LL-treatment (doses of 0.56 g nitrogen); at 85 DAP the switch was made to high doses as in the HH-treatment (doses of 1.96 g). Unintentionally, the first application in the LL-treatment amounted to 0.98 g instead of 0.56 g nitrogen (Figure 1 and Table 3 in Biemond *et al.*, 1995a). This mistake is suspected to have had an effect on the results. (In retrospect, the decision to combine two doses at 65 DAP unfortunately disturbed the regularity of the regimes of addition. Because we wanted to maintain fixed H and L doses and because we wanted the total amount added to HL and LH to be similar, it was not possible to make the L-H and H-L switch at the same point in time.)

Areas of leaves were measured per individual leaf number. To limit the number of samples to a manageable level, leaves from five successive nodes were bulked prior to determination of dry weight and nitrogen concentration (*i.e.* data per group of leaves and not per individual leaf number). Thus some degree of resolution of responses was lost. Growth analyses were done at 29, 48, 70, 90, 111, 132, 154 and 175 DAP.

Results

The first 15 leaves appeared at similar dates in all treatments (Figure 2). Compared to the non-limited control the LH-treatment and the nitrogen-limited control developed a lag in the number of appeared leaves between leaf numbers 15 and 25 (the period from *ca* 40 to 70 DAP). The change in nutritional regime at 85 DAP did not result in a faster rate of leaf appearance (RLA) in the LH-treatment; rather RLA continued at the same rate, whereas it gradually declined beyond 90 DAP in the nitrogen-limited control. The 'cross-over' in the curves of the LH-treatment and the nitrogen-limited control (Figure 2) occurred at *ca* 100 DAP, *i.e.* 15 days after the switch in nitrogen regime, when 45 leaves had appeared.

In the HL-treatment the change in nitrogen regime (at 57 DAP when about 32 leaves had appeared) did not result in a clearly lower RLA until about 85 DAP when 50 leaves had appeared. The HL- and LH-treatments happened to end up with the same final number of leaves, which number was intermediate between those of the nitrogen-limited control and the non-limited control.

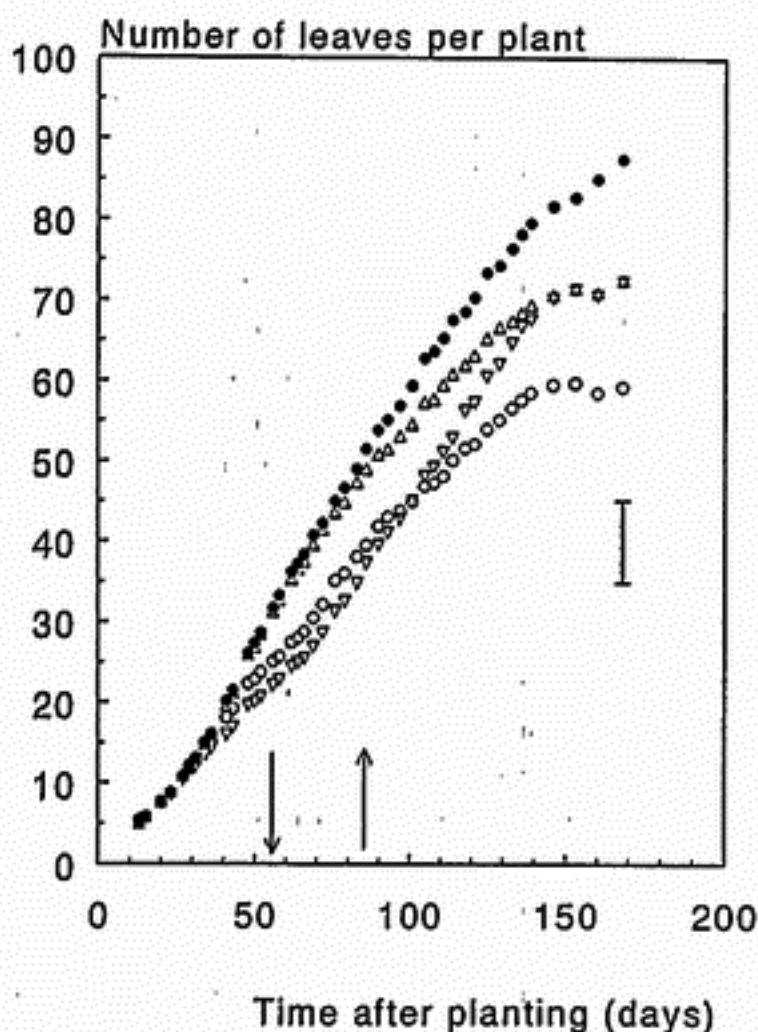


Figure 2, Change with time in the number of appeared leaves per plant in Brussels sprouts. Solid dots: HH-treatment (non-limited control); open circles: LL-treatment (nitrogen-limited control); triangles pointing up: HL-treatment; triangles pointing down LH-treatment. The vertical bar indicates the least significant difference (LSD) between treatment means at the last date of observation ($P=0.05$). The arrows indicate the time of switch in N doses for the HL and LH treatments, respectively.

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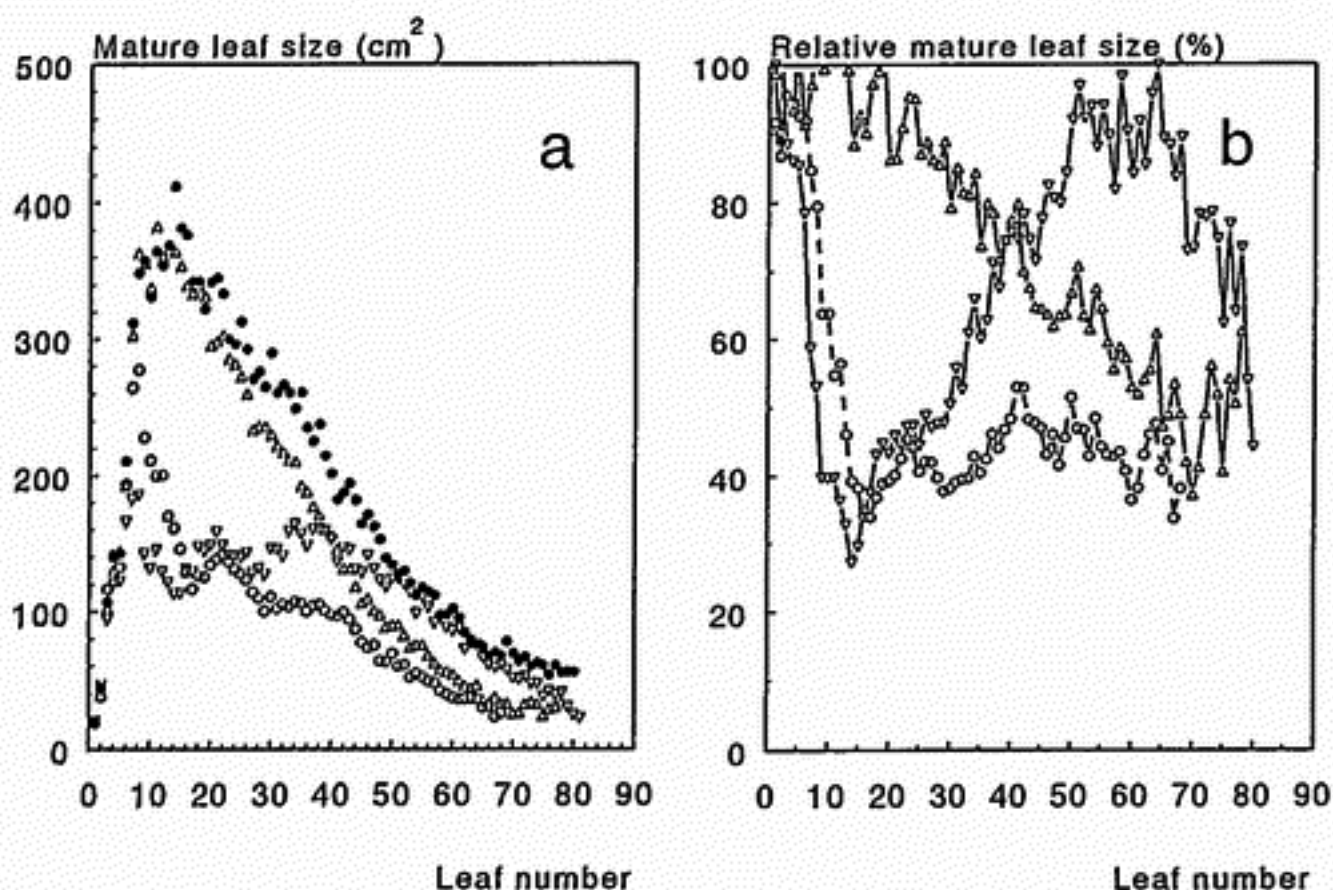


Figure 3. Effect of the nitrogen regime on sizes of mature leaves; (a): absolute values; (b) relative leaf areas, assigning 100 per cent to the leaf area of the non-limited control at each node. See legend of Figure 2 for explanation of symbols.

Mature leaf sizes responded to change in nutritional regime. Figure 3a shows the absolute leaf areas and Figure 3b the relative ones, assigning 100 per cent to the leaf area of the non-limited control at each node. The first eight leaves, approximately, grew to similar sizes, irrespective of nitrogen treatment. Up till approximately leaf 15, leaf size was greater in the nitrogen-limited control than in the LH-treatment (due to the mistake in the nitrogen regime?). Leaf sizes in the LH-treatment started to become clearly larger than those of the nitrogen-limited control above approximately leaf number 30. Leaf 30 of the LH-treatment appeared at *ca* 70 DAP and reached its final area at *ca* 108 DAP (data not shown, but see Figure 4 for leaf area growth of leaf numbers 26 and 34). Apparently, the change in nitrogen status affected the leaves that had appeared and were expanding at the moment of change. In the LH-treatment sizes of leaves with numbers higher than 45 to 50 became almost as large as comparable leaves of the non-limited control (relative leaf area varying around 90 per cent). Leaf numbers 45 to 50 appeared between 100 and 110 DAP in the LH-treatment, *i.e.* *ca* 15 days after the switch in nitrogen regime. Leaves of LH-plants beyond number 68 were again progressively smaller than those of the non-limited control.

In the HL-treatment leaf sizes were similar to the leaves of the non-limited control up to about leaf number 30 (relative leaf area of about 90 per cent or higher) and became progressively smaller than comparable leaves of the non-limited control for leaf numbers higher than about 30. Leaf number 30 of the HL-treatment appeared at

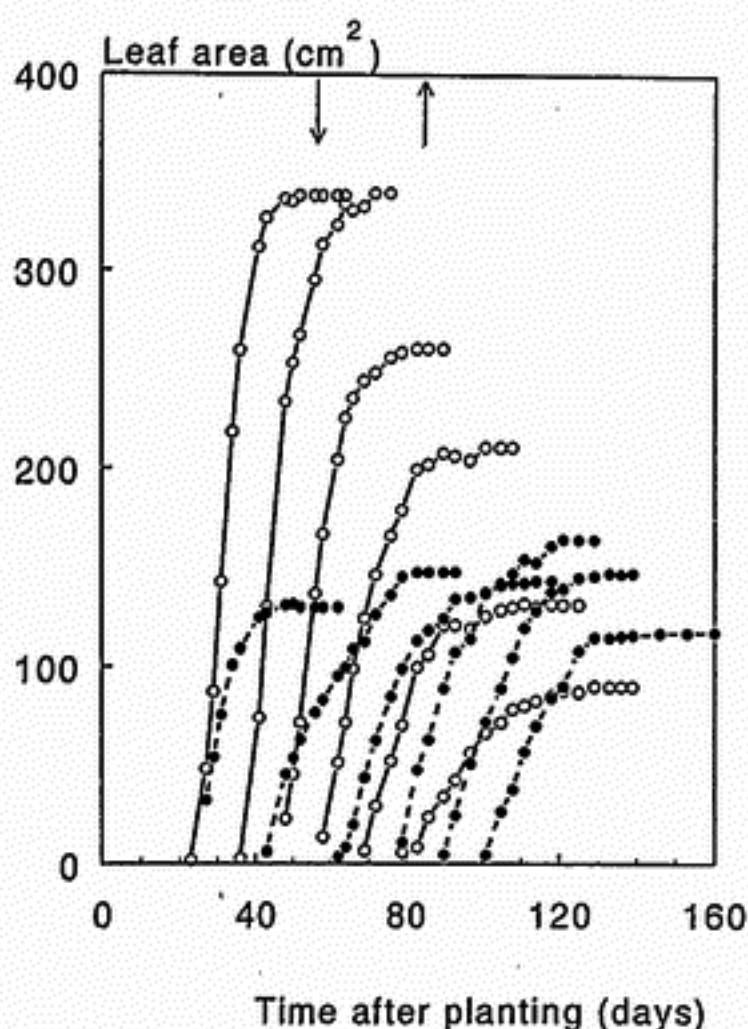


Figure 4. Change with time in area of selected leaf numbers. Open circles: HL-treatment; solid dots: LH-treatment. For both treatments the curves pertain to leaf numbers 10, 18, 26, 34, 42 and 50, respectively. Arrows at the top indicate the times of switch in N doses for the HL and LH treatment, respectively.

55 DAP and had reached its final area at *ca* 80 DAP (data not shown, but see Figure 4 for area growth curves of leaf numbers 26 and 34). The change in nitrogen regime which occurred during the period of growth of leaf 30 apparently already affected its final size. HL-leaves were smaller than LH-leaves beyond leaf number 45; HL-leaves with numbers higher than *ca* 65 (appearing after *ca* 125 DAP) had sizes close to those of comparable leaf numbers of the nitrogen-limited control.

Figure 4 shows the change with time in leaf area for selected leaf numbers, the first of those appeared before the switches in the nitrogen regimes and the last of those appeared at least two weeks after the switches. Figure 4 clearly illustrates that initially HL-leaves grew to larger sizes than LH-leaves and that the opposite was true later in the growing period. It was inferred from Figures 2 and 3 that the switches in nitrogen regimes affected the final size of leaves that were expanding at the moment of the switch. However, the change in leaf growth was not so instantaneous that a systematic point of inflection could be found in the growth curves of individual leaf numbers.

Figures 5 a-f illustrate the changes in leaf attributes in response to switch from a high rate of N supply to a low rate and vice versa. Data pertain to the *leaf blades* for

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groups of five leaf layers. Leaf group 16–20 appeared between 35 and 50 DAP in all treatments (Figure 2), *i.e.* these leaves had appeared and had reached their final sizes (Figure 4) before the switches in nitrogen regimes were made, but maximum weight was attained after the switches were made. Leaf group 36–40 appeared between *ca* 60 and 70 DAP in the HL-treatment and the non-limited control, *i.e.* immediately upon the switch from high to low nitrogen supply. For the nitrogen-limited control and the LH-treatment leaf group 36–40 appeared between *ca* 80 and 90 DAP, *i.e.* right at the switch from low N to high N supply. A switch from a low to a high rate of N supply led to the following responses:

- (i) The change with time in dry mass of neither of the two leaf groups was affected by a change in nutritional regime (Figures 5a and 5d). From about leaf number 50 onwards (Figure 3) were the leaf sizes similar in the LH-treatment and the non-limited control (leaf 50 appeared at 108 DAP, *i.e.* 23 days after the switch). In expanding leaves (group 36–40), the effect of the switch on leaf size apparently preceded an effect on leaf mass. In line with this, SLA of leaf group 36–40

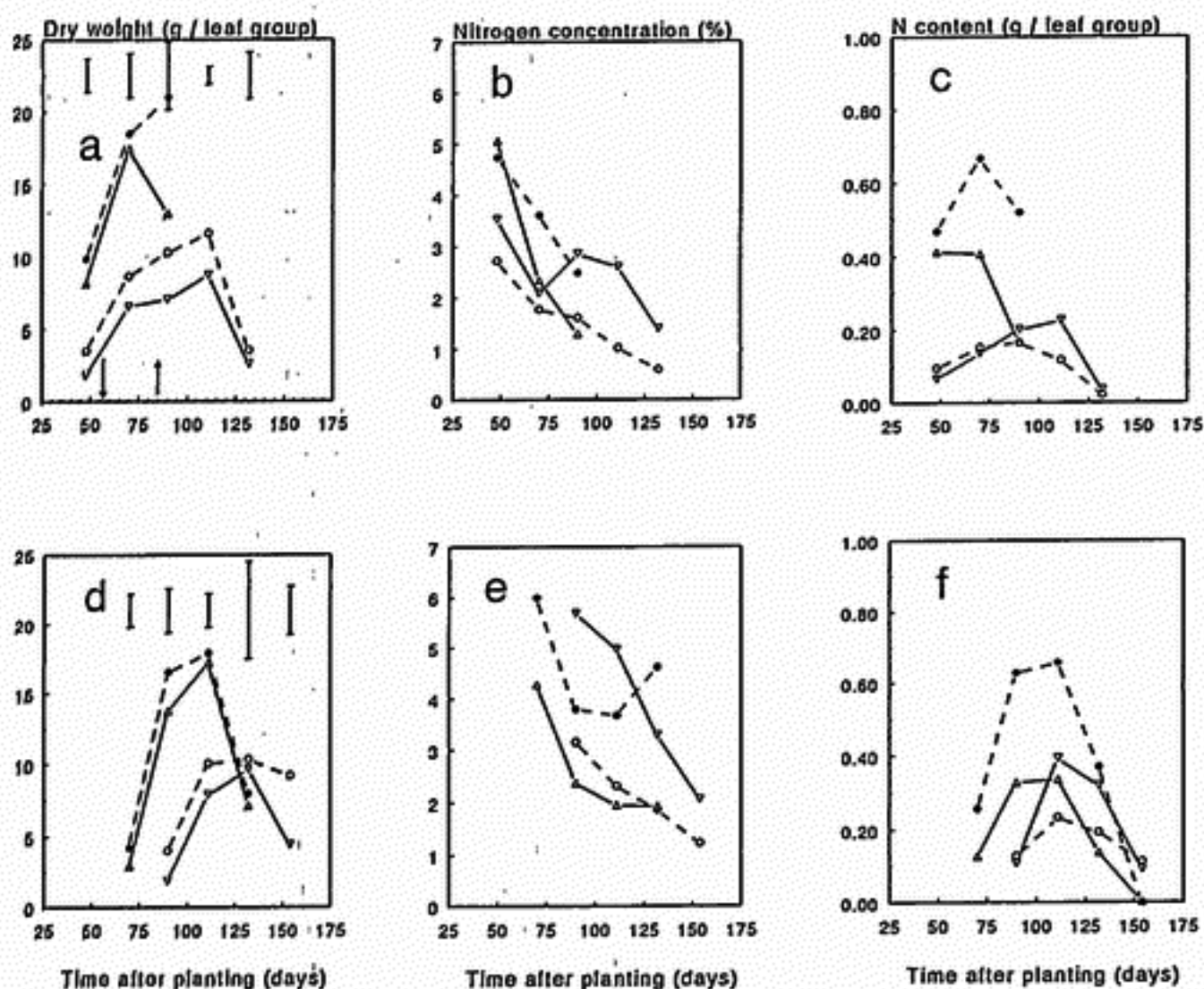


Figure 5. Changes with time in attributes of leaf blades: dry weight (a and d), nitrogen concentration in leaf dry matter (b and e) and amount of nitrogen in leaves (c and f) for leaf group 16–20 (bulked leaves; *n-c*) and for leaf group 36–40 (d–f). See legend of Figure 2 for explanation of symbols. Vertical bars represent LSDs between treatment means ($P=0.05$).

was significantly larger in the LH-treatment than in the nitrogen-limited control (see Figure 10 in Biemond *et al.*, 1995a).

- (ii) The trend of decline in nitrogen concentration with age was clearly temporarily reversed in leaf group 16–20 (*i.e.* after cessation of expansion of these leaves), bringing the values close to those for the non-limited control (Figure 5b); the response was very fast, since the third data point for the LH-treatment in Figure 5b was obtained five days after the switch in the nitrogen regime. For leaf group 36–40, the nitrogen concentration resembled that of the comparable leaves of the non-limited control (Figure 5e).

Switch from a high rate of nitrogen supply to a low rate of nitrogen supply led to the following responses:

- (i) The changes in leaf dry mass with time of the HL-treatment and the non-limited control were comparable. The last data points in the example in Figure 5a for leaf group 16–20 seem not too convincing in this respect, but for other sets of leaf groups not shown in Figure 5 the similarity was more obvious as is corroborated for leaf group 36–40 in Figure 5d. For leaf numbers higher than *ca* 40 to 45 the sizes in the HL-treatment became somewhat smaller than in the non-limited control (Figure 3). The switch from a high rate of nitrogen supply to a low rate had apparently no effect on dry matter accumulation of leaves at an advanced stage of expansion (leaf group 16–20). The same was true for leaves starting to expand (leaf group 36–40) at the time of switch. Leaf sizes, in contrast, were reduced in comparison to the non-limited control (Figure 3b). In line with this, SLA of leaf group 36–40 was significantly lower in the HL-treatment than in the non-limited control (see data on SLA from the current experiment in Figure 10 in Biemond *et al.*, 1995a).
- (ii) The nitrogen concentration in young HL-leaves was close to the initial values in the non-limited control (Figure 5b), but values dropped faster in time than in the non-limited control. Within two weeks upon change in nitrogen regime, nitrogen concentrations in expanded leaves (leaf group 16–20) and in expanding leaves (leaf group 36–40) attained levels which were close to those in comparable leaves of the nitrogen-limited control; the latter is particularly clear for leaf group 36–40 (Figures 5b and 5e).

Figures 5c and 5f show that the decline in nitrogen concentration that occurred early in the life of a leaf group did not necessarily imply export of nitrogen from the leaves. A faster rate of dry matter accumulation than of decline in nitrogen concentration led to a later point in time for maximum nitrogen content than for maximum nitrogen concentration.

Figures 6 a–d illustrate the consequences of the changes in leaf attributes for the whole plant. In the initial stages of growth, leaf area, dry weight and nitrogen uptake per plant and the average nitrogen concentration showed comparable changes with time for the nitrogen-limited control and the LH-treatment on the one hand and for the non-limited control and the HL-treatment on the other hand. The increase in total nitrogen uptake in the LH-treatment as compared to the nitrogen-limited control did not result in a comparable increase in total dry weight of the plant. Compared to the non-limited control the HL-treatment had a much lower nitrogen uptake (less than

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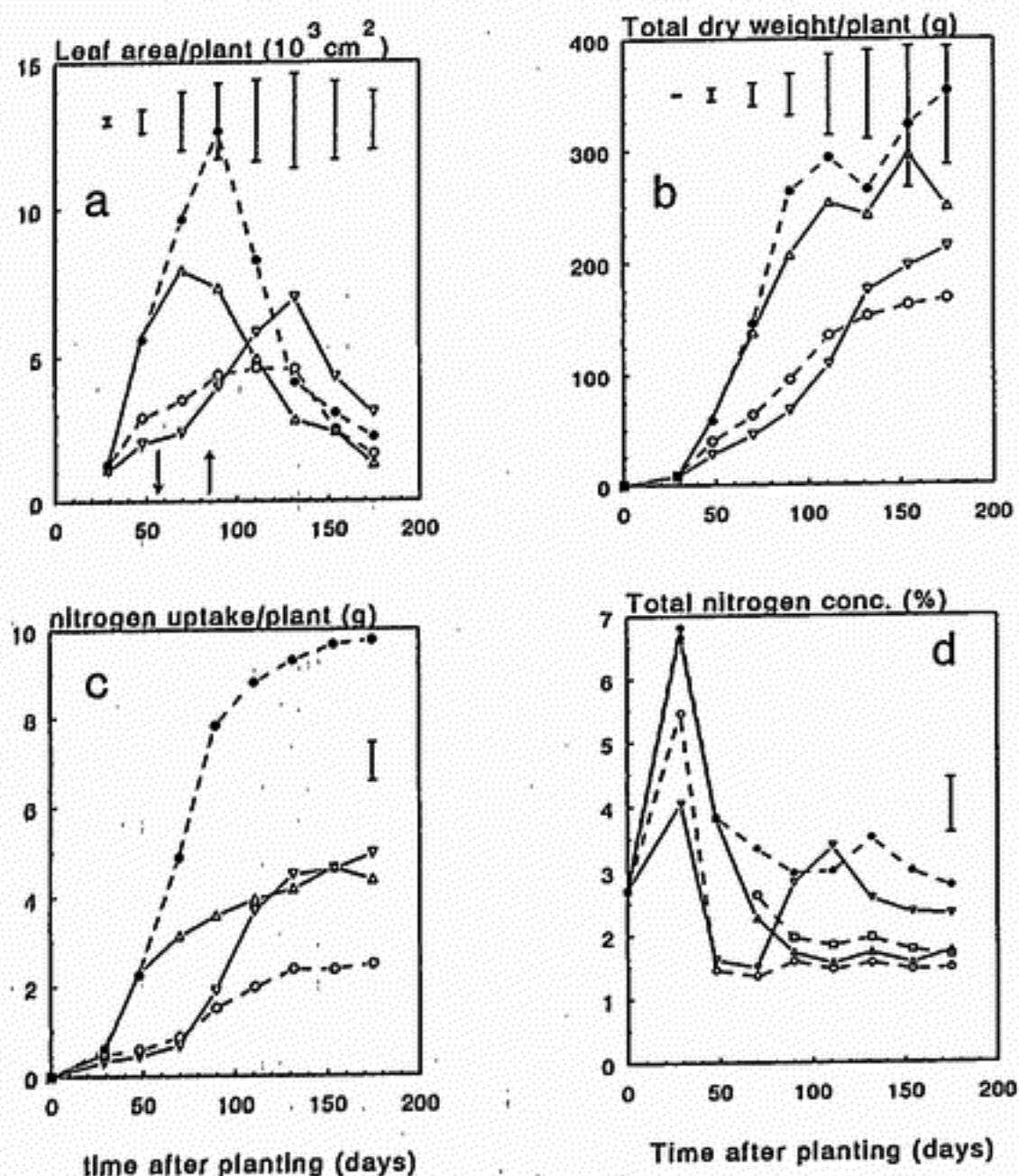


Figure 6, Changes with time in total leaf area per plant (a), total dry weight per plant (b), nitrogen uptake per plant (c) and average nitrogen concentration in plant dry matter (d). The dotted line with open squares represents the critical nitrogen concentration (Greenwood *et al.*, 1985; 1990) based on the dry weight of the non-limited control. See legend of Figure 2 for explanation of the other symbols. Vertical bars in graphs a and d represent LSDs between treatment means ($P=0.05$).

50%), but the total dry matter production was very much less affected. The average (overall) nitrogen concentration (Figure 6d) showed after the switch a quick increase in concentration in the LH-treatment, reaching values close to those of the non-limited control at 90 DAP, *i.e.* 5 days after the switch.

Inferences from Figure 6 are:

- (i) The nitrogen productivities (*i.e.* final amount of dry mass per unit nitrogen taken up) of the four treatments were largely different. For the nitrogen-limited control the value was 67 g dry matter (DM) produced per g nitrogen taken up; the values were 57, 43 and 36 g DM g⁻¹ N for the HL- and LH-treatments and the non-limited control, respectively (measurements at 175 DAP).

- (ii) Resumption of nitrogen supply following a period of deficiency boosted the uptake of nitrogen, followed much less or later by an increase in the rate of dry matter accumulation.

Discussion

The rate of nitrogen supply to the non-limited control was based on experiences in earlier experiments under comparable experimental conditions (Biemond *et al.*, 1995a,b). Still, this does not prove the absence of any nitrogen limitation in that treatment. Greenwood *et al.* (1985, 1990) developed the concept of minimum, critical and maximum concentrations of nitrogen in plant dry material. They found that the relation between crop dry mass per unit area and the critical nitrogen concentration (N_{crit} ; *i.e.* the concentration above which no further increase in growth rate occurs) could be described adequately with one single curve for C_3 crop species (Greenwood *et al.*, 1990). Justus *et al.* (1994) corroborated that curve for wheat. In Figure 6d a curve is included, showing N_{crit} as a function of time from 70 DAP onwards for the non-limited control (plant weights converted to weight per unit area at each point in time; plant density was last changed at 70 DAP). The concentrations of the non-limited control and of the LH-treatment were generally above N_{crit} and the concentrations of the nitrogen-limited control and HL-treatments lower than N_{crit} . Based on the earlier experiences and on the comparison with N_{crit} , it is fair to assume the absence of nitrogen limitation in the non-limited control.

Results showed that leaf properties differed in the time span that was required to produce responses to change in nitrogen regime. The effects are summarized in Table 1, distinguishing between groups of leaves of different growth stage at the times of the switch. Nitrogen concentrations responded fast (significant effects in the harvests after 5 days and 13 days in the LH- and HL-treatments, respectively). Interestingly, the allocation of dry matter to expanding leaves was not changed, but final leaf sizes were, resulting in change in SLA (Biemond *et al.*, 1995a). Changes in leaf growth started to show about 15 days after the switch in nitrogen regime. However, the leaves appearing about 25 days or 15 phyllochrons after the switch in the LH-treatment attained leaf attributes close to those of comparable leaves of the non-limited control. This suggests some slow operating mechanism of control of leaf size.

Caution is needed with regard to the interpretation of changes in RLA. Since a minimum length of 5 cm was necessary (Biemond *et al.*, 1995a) before a leaf was registered as having appeared, variations in expansion rate affect the moment of appearance. In other words: the observations on RLA do not closely reflect the events at the apex. About similar time of onset of changes in RLA and leaf growth can be taken to support a partial dependency of RLA on the rate of leaf expansion.

Control of number and sizes of leaves is exerted at several levels. Pieters and Van den Noort (1988) hypothesized that the (potential) size of a leaf is largely determined at the moment of its initiation on the apex. So ontogenetic change and growth conditions during leaf initiation would be the first level of control of leaf size. Since

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Table 1. Summary of responses to change in regime of nitrogen supply for different groups of leaves of Brussels sprouts.

Leaf stage at time of switch	LH-treatment (compared to the nitrogen-limited control)	HL-treatment (compared to the non- limited control)
Expansion completed	Temporary increase in N concentration	Faster decline in N concentration
Expanding	No change in dry weight increased N conc. increased maximum area, but area smaller than non-limited control Increased SLA	No change in dry weight faster decline in N conc smaller final size Decrease in SLA
Later appearing leaves	Attributes of leaves appearing after 25 d or 15 phyllochrons near those of non-limited control	Progressively lower values of all attributes

most of the cells of leaves are formed upon leaf appearance (Dale, 1982) there is apparently also control during the expansion phase. Leaf initiation on the apex occurs before the leaf appears. If the (potential) size of a leaf is largely determined at that stage, then leaves completing their expansion at any time during the growing season would reflect the conditions of earlier stages during the plant's life. How much earlier would depend on timing and pattern of production of primordia. In determinate plants such as cereals, leaf and floral primordia accumulate at the apex and are present in early growth phases. In potato the primordial stages of the inflorescence of the main stem are often present before or shortly after emergence (Kirk *et al.*, 1985; Firman *et al.*, 1991). In leek, leaf primordia do not accumulate on the apex; always there is only one primordium present on the apex (Hay & Brown, 1988). The authors are not aware of analyses at the level of the apex in Brussels sprouts, but the current data on comparatively slow change (15 phyllochrons) in relative leaf size upon change in nutritional regime suggests some form of long range control which could be located at the apex level. An alternative suggestion in the literature is that the time of appearance, growth and final size of leaf X+1 depend on those of leaf X. Such a mechanism would also result in a gradual change in leaf properties upon change in growing conditions.

The fact that the HL- and LH-treatments ended up with a similar number of appeared leaves can be the incidental result of more than one mechanism of control. It would have seemed possible for the LH-plants to produce as many leaves as the non-limited control, but this did not happen. Perhaps the reason is that relatively more plant nitrogen became tied up in other organs (sprouts) in the LH-treatment than in the non-limited control. That would also explain the declining relative area of the latest leaves that appeared in the LH-treatment.

Gradual adaptation to conditions is advantageous when conditions deteriorate:

plants in the HL-treatment still managed to produce a large total leaf area resulting in a comparatively large production. The total leaf area in LH-plants remained limited because the potentially large leaves remained actually only small, whereas later in the season the ontogenetically determined small potential size was a barrier precluding the development of a large leaf area per plant.

References

- Biemond, H., 1995a. 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., 1995b. 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., 1995c. Effects of nitrogen on accumulation and partitioning of dry matter and nitrogen of vegetables. 2. Leek. *Netherlands Journal of Agricultural Science* 43: 435-446.
- 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, 1995a. Effects of nitrogen on development and growth of the leaves of vegetables. 1. Appearance, expansion growth and life span of leaves of Brussels sprouts. *Netherlands Journal of Agricultural Science* 43:217-232.
- Biemond, H., J. Vos & P.C. Struik, 1995b. Effects of nitrogen on accumulation and partitioning of dry matter and nitrogen of vegetables. 1. Brussels sprouts. *Netherlands Journal of Agricultural Science* 43: 419-433.
- Biemond, H., J. Vos & P.C. Struik, 1996. Effects of nitrogen on accumulation and partitioning of dry matter and nitrogen of vegetables. 3. Spinach. *Netherlands Journal of Agricultural Science* (submitted).
- Dale, J.E., 1982 The growth of leaves. Studies in Biology No. 137. Edward Arnold Publishers Ltd, London, 60 pp.
- Firman, D.M., P.J. O'Brien & E.J. Allen, 1991. Leaf and flower initiation in potato (*Solanum tuberosum*) sprouts and stems in relation to number of nodes and tuber initiation. *Journal of Agricultural Science, Cambridge* 117:61-74.
- Greenwood, D., J.J. Neeteson & A. Draycott, 1985. Response of potatoes to N fertilizer: quantitative relations for growth. *Plant and Soil* 85:163-183.
- 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.
- 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.
- Justus, E., B. Mary, J-M. Beynard, J-M. Machet & L. Thelier-Huche, 1994. Determination of a critical nitrogen dilution curve for winter wheat crops. *Annals of Botany* 74:397-407.
- Kirk, W.W., H.V. Davies & B. Marshall, 1985. The effect of temperature on the initiation of leaf primordia in developing potato sprouts. *Journal of Experimental Botany* 336 (171):1634-1643.
- Pieters, G.A. & M.E. Van den Noort, 1988. Effect of irradiance and plant age on the dimensions of the growing shoot of poplar. *Physiologia Plantarum* 74:467-472.
- Vos, J. & H. Biemond, 1992. Effects of nitrogen on the development and growth of the potato plant. 1. Leaf appearance, expansion growth, life span of leaves and stem branching. *Annals of Botany* 70: 27-35.