Plant Cell Physiol. 37(8): 1083–1089 (1996)

Interaction between Nitrogen Deficit of a Plant and Nitrogen Content in the Old Leaves

Kiyomi Ono [1], Ichiro Terashima [2] and Akira Watanabe [1]

[1] *Department of Biological Sciences, Graduate School of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo, 113 Japan*
[2] *Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305 Japan*

We examined changes in nitrogen content of the first leaves in relation to growth and nitrogen status of sunflower (*Helianthus annuus* L.) plants that were raised hydroponically at two irradiance levels (high and low light, HL and LL) and at two nitrogen concentrations (high and low nitrogen, HN and LN). Initial increases in total dry mass and total nitrogen of the whole plant were faster in HL-plants than in LL-plants irrespective of nitrogen supply, but in LN-plants the increase in total nitrogen was soon blunted. When plants grown under the same irradiance were compared, nitrogen content of the first leaves (leaf N) decreased faster in LN-plants than in HN-plants, while for the plants grown at the same nitrogen concentrations, it decreased faster in HL-plants than in LL-plants. Since these changes in leaf N were not explained solely by the changes in plant dry mass or plant nitrogen, we introduced an index, 'nitrogen deficit (ND*)', to quantify nitrogen deficit of the whole plant. ND* was expressed as $ND^*(t)=[N_{max}-N(t)]\times DM(t)$, where N_{max} and N(t) were nitrogen contents in the young, expanding leaves that had just unfolded to expand, at the initial stage with sufficient nitrogen and at time t, respectively, and DM(t), plant dry mass at t. The decrease in leaf N was expressed as a liner function of ND* irrespective of the growth conditions, which indicates validity of this index. Limitation of the use of ND*, and mechanisms by which leaves sense nitrogen demand are also discussed.

Key words: Carbohydrates — Nitrogen — Senescence — Sink-source — Sunflower (*Helianthus annuus* L.).

Leaf senescence, which is usually assessed as the decrease in nitrogen and/or protein content of the leaves, is influenced by many environmental variables. In particular, light and nitrogen nutrition exert critical effects (Thomas and Stoddart 1980, Smart 1994).

Contents of nitrogen and/or protein in leaves decrease after their maturation (Thimann 1980, Thomas and Stoddart 1980, Gepstein 1988) and the decreasing rate is accelerated when nitrogen supply is restricted (Makino et al. 1983, Morris and Paulsen 1985). From such senescing leaves, nitrogen is retranslocated to the developing organs (Mae and Ohira 1981). In the plants with developing reproductive organs, remobilization of nitrogen from old leaves is shown to depend on the growth of the sink such as flowers and fruits (Wittenbach 1983, Khanna-Chopra and Reddy 1988, Guitman et al. 1991). For example, when sink capacity of soybean plants is suppressed by depodding, remobilization of nitrogen from old leaves is also suppressed (Wittenbach 1983, Crafts-Brandner and Egli 1987). These results indicate that the demand for nitrogen in the growing organ affects leaf senescence.

Light environment also influences leaf senescence. It is generally argued that the decrease in leaf nitrogen content is slower and leaf longevity is greater in shaded plants (Chabot and Hicks 1982, Kikuzawa 1989, Hidema et al. 1991, Dale and Causton 1992). However, there are studies which clearly showed that shading of leaves causes rapid decrease in their nitrogen contents (Hikosaka et al. 1993, 1994, Pons and Pearcy 1994). Hikosaka (1996) attributed this apparent contradiction to the differences in the manner of shading. When the whole plants are shaded, demand for nitrogen is lowered because growth is limited by the reduced energy input. On the other hand, when particular leaves of a plant are shaded but the remaining parts including growing organs are unshaded, demand for nitrogen in growing organs can be still large. When the plants suffer from nitrogen deficiency, and only particular leaves are shaded, nitrogen in such shaded leaves would be re-translocated to young organs.

From the above, it appears reasonable to infer that sink-source and/or demand-supply relationships of nitrogen regulate leaf senescence. Then, the effects on leaf senescence of both light environment and nitrogen nutrition can be explained in an unified way if their effects on the nitrogen status of the plant are properly assessed. However, as far as the plants in the vegetative phase are concerned, no studies examined leaf senescence in relation to the demand for nitrogen or nitrogen sink activity.

The purpose of this study is to examine the above hypothesis. We raised sunflower plants under different nitrogen and irradiance conditions and measured contents of nitrogen in the first leaves at various stages. We tried to relate

Abbreviations: leaf N, nitrogen content of the first leaves; ND* nitrogen deficit of the whole plant, expressed as the product of the nitrogen deficit in young, developing leaves and the plant dry mass; HL, high light; LL, low light; HN, high nitrogen; LN, low nitrogen.

the decrease in the leaf nitrogen content to various plant measures such as increases of plant dry mass and plant total nitrogen, and nitrogen deficit of the plants. The results showed that the decrease of nitrogen content in the first leaves was almost uniquely related to the development of the nitrogen deficit of the whole plant.

Materials and Methods

Growth conditions—Seeds of sunflower (*Helianthus annuus* L. cv. Russian Mammoth) were germinated in vermiculite and grown in an environment-controlled chamber (Koito KG-50HLA, Koito Industries, Ltd., Yokohama, Japan) at a PPFD of 450 μmol m^{-2} s^{-1} with a photoperiod of 13 h. Day/night temperatures were 23/20°C. When the first leaves were fully expanded (16 days after seeding), the plants were transferred to Wagner pots (12.5 cm diameter and 20 cm height) containing 1.5 liter hydroponic solution (one plant per pot). These plants were grown under either high light (450 μmol m^{-2} s^{-1}) or low light (150 μmol m^{-2} s^{-1}) at two different nitrogen levels. The hydroponic solution for the higher nutrient concentration (8 mM NO_3^-, pH 5.0) contained 2.7 mM KNO_3, 2.7 mM $Ca(NO_3)_2$, 1.0 mM $MgSO_4$, 0.89 mM NaH_2PO_4, 0.033 mM FeNa-EDTA, 6.7 μM $MnSO_4$, 0.67 μM $ZnSO_4$, 33 μM H_3BO_3, 0.33 μM Na_2MoO_4, 0.67 mM NaCl and 0.13 μM $CoSO_4$. For the low nutrient condition, concentrations of both KNO_3 and $Ca(NO_3)_2$ in the nutrient solution were reduced to 0.27 mM (0.8 mM NO_3^- in total) but KCl and $CaCl_2$ were added to keep concentrations of K^+ and Ca^{2+} constant. The replacement did not affect pH of the solution. The hydroponic solutions were continuously aerated. Nutrient solutions were renewed every four days.

Nitrogen and carbon content—All samples were harvested between 11:30 AM and 1:30 PM. For measurements of the nitrogen content, two or three discs of 1 cm diameter were punched out from each leaf and dried at 70°C for at least three days. Nitrogen and carbon contents of leaves were determined with an NC analyzer (NC-80, Sumitomo Chemical, Tokyo, Japan).

Carbohydrate content—Leaf discs were frozen in liquid nitrogen and stored at −30°C in a freezer until measurements. Carbohydrates were quantitated by the methods of Rufty and Huber (1983) and Rao et al. (1990). Samples were ground in liquid nitrogen with a mortar and pestle and suspended in 80% ethanol. The suspension was incubated ca. 80°C for 30 min and subsequently centrifuged for 10 min at 10,000 × *g*. The supernatant was transferred to a test tube. Procedures from suspension in 80% ethanol to transfer of supernatant were repeated twice or three times. Collected supernatant was evaporated to remove ethanol and redissolved in 0.5 ml of H_2O. To remove chlorophyll, 0.5 ml chloroform was added and the chloroform-water mixture was centrifuged for 10 min at 6,000 × *g*. The upper clear phase was used for glucose and sucrose analysis. Sucrose in the solution was hydrolyzed with invertase at 25°C for 60 min. Sucrose concentrations were determined from the difference between total glucose upon addition of invertase (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and free glucose. Glucose concentration was determined with a glucose assay kit (Glucose B-test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan). The residue after the 80% ethanol extraction was used for determination of starch content. It was suspended in 1.0 ml of 0.2 M KOH and placed in boiling water for 30 min. After cooling, 200 μl of 1.0 M acetic acid was added. Then dialyzed amyloglucosidase (from *Rhizopus mold*, Sigma, St. Louis, U.S.A.) solution (35 units ml^{-1} in 50 mM Na acetate buffer, pH 4.5) was added, and the mixture was incubated at 55°C for 30 min. After digestion of starch to glucose, the reaction mixture was centrifuged and glucose in the supernatant was analyzed using the glucose assay kit.

Statistics—The difference between or among regression lines was examined statistically by the method of Snedecor and Cochran (1967).

Results

Here, we refer to the growth conditions of the plants, high and low irradiance levels, and high and low nitrogen concentrations as HL and LL, and, HN and LN, respectively. The plants grown at high irradiance and high nitrogen concentration are, thus, expressed as HLHN-plants. Changes in dry mass of the whole plants are shown in Fig. 1A. Dry mass increased rapidly under HL-condition. Average dry mass of HLHN-plants was about 15 g on day 30 from the transfer, when the first leaves in HLHN-plants turned almost yellow. Compared with HLHN-plants, growth of the other plants was suppressed, and, at the time of yellowing of the first leaves, about 25, 35 and 40 days after transfer in HLLN-, LLLN-, and LLHN-plants, respectively, the plants were about 6 g in dry mass. Nitrogen content of the whole plants increased rapidly in HL-plants for a while but the increase in HLLN-plants was blunted around on day 15 from the transfer (Fig. 1B). For the LL-plants, increase in plant N was slower under the same nitrogen supply (Fig. 1B). The patterns of the increases in dry mass and nitrogen of the whole plants, therefore, differed considerably depending on growth irradiance and nitrogen nutrition.

Changes in nitrogen content in the first leaves (leaf N) were compared for different growth conditions. The first leaves of all these plants expanded under the identical HL-condition (see Materials and Methods). Thus, their nitrogen contents per unit leaf area were similar upon the transfer. Appreciable decreases in leaf N were observed after the 11th day from the transfer. Thus, the regression analyses were made on the data obtained on and after the 11th day after transfer (Fig. 2). Decreases in leaf N were faster in HL-plants than in LL-plants. Under the same growth light, leaf N decrease faster under the low nitrogen concentration.

We tried to relate the decreases in leaf N to the increases in dry mass or nitrogen content of the whole plants. If the decrease in leaf N was uniquely related to the increase in plant dry mass, leaf N plotted against plant dry mass would not be different between the conditions. However, difference between HN- and LN-plants was obvious (Fig. 3A). When changes in leaf N were plotted against the changes in plant nitrogen, distinct difference was also observed between HN- and LN-plants (Fig. 3B). The decreases in leaf N cannot be simply ascribed to the increases in plant dry mass or in plant nitrogen.

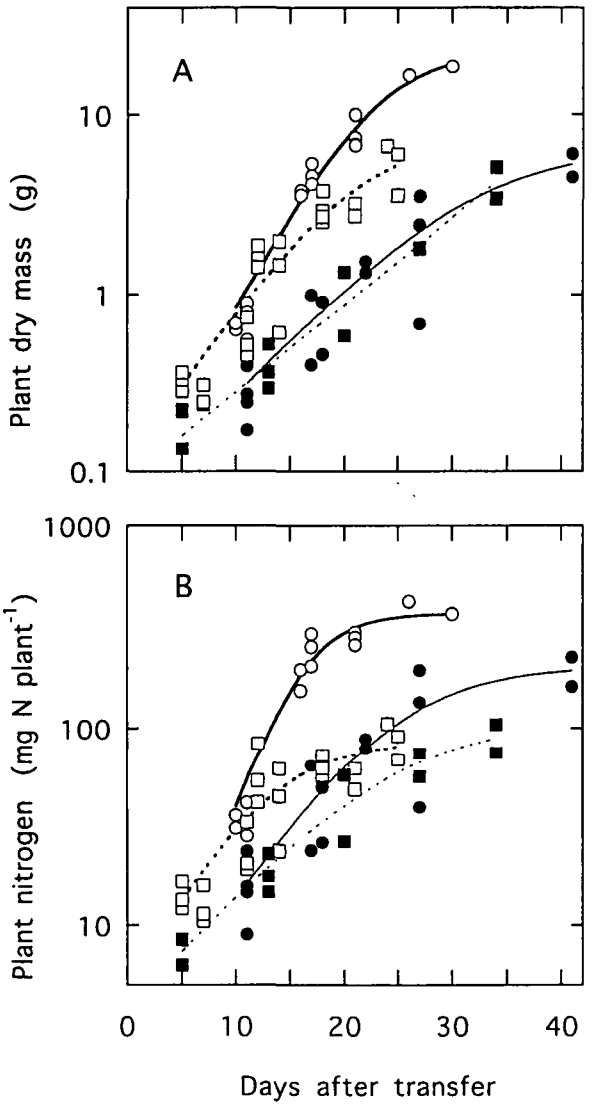

Fig. 1 Changes in plant dry mass (A) and total nitrogen of the whole plant (B). ○, HLHN; ●, LLHN; □, HLLN; ■, LLLN. Solid and dotted lines denote HN- and LN-plants. Thick and thin lines denote HL- and LL-plants. Each point shows the value of one plant. Logistic curves were fitted to the data.

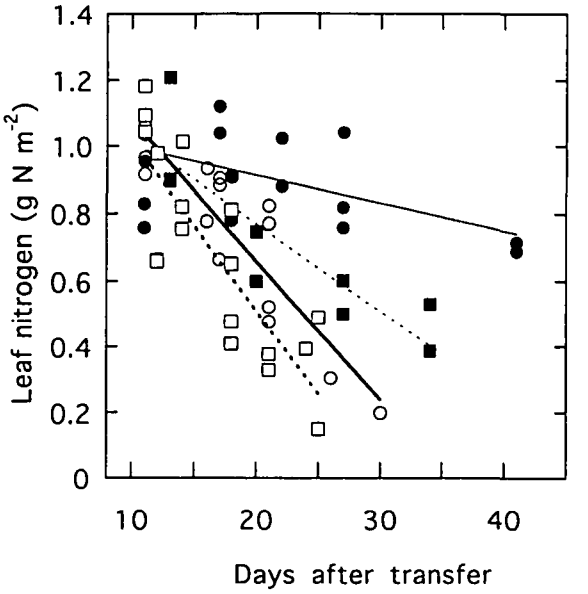

Fig. 2 Changes in nitrogen content per unit area of the first leaves (leaf N). Each datum point denotes one leaf. Symbols and lines are as in Fig. 1. Regression lines were fitted to the data obtained after the 11th day inclusive: for HLHN, $y=1.49-0.0418x$, $r^2=0.79$; LLHN, $y=1.08-0.00819x$, $r^2=0.28$; HLLN, $y=1.53-0.0508x$, $r^2=0.71$; LLLN, $y=1.29-0.0261x$, $r^2=0.76$. The difference between regression line for HLHN and for LLHN, HLLN and LLLN, HLHN and HLLN, and, LLHN and LLLN were significant at the significant level of $P<0.01$, $P<0.05$, $P<0.05$ and $P<0.05$, respectively (Snedecor and Cochran 1967).

As mentioned in Introduction, we hypothesized that nitrogen deficit of the plant causes the decrease in leaf N. To see if this is valid, we first examined the changes in nitrogen per dry mass. Figure 4 shows changes in nitrogen per dry mass in the whole plants (Fig. 4A) and that in the young parts of the plants (Fig. 4B). Nitrogen per dry mass in the whole plants grown under HLLN condition remained nearly constant up to the 10th day from the transfer, and then decreased markedly. The onset of appreciable decrease in HLHN-plants delayed for about 10 days from that of HLLN-plants. In LLHN-plants, the decrease was the smallest. Nitrogen per dry mass in young leaves, which had most recently unfolded to expand, also decreased in a way very similar to that of nitrogen per dry mass of the whole plant (Fig. 4B). The decrease in HLLN-plants occurred first. Subsequently, those of HLHN- and LLLN-plants decreased almost synchronously. In LLHN-plants, the decrease in nitrogen per dry mass in young leaves were small. For the analyses below, we use nitrogen per dry mass in young leaves as a qualitative measure of nitrogen deficit because nitrogen per dry mass for the whole plant would decrease even under the condition of sufficient nitrogen supply due to the relative increase of supporting tissues.

Quantitative value of nitrogen deficit (ND*) is expressed as:

$$ND^* = [N_{max} - N(t)] \times DM(t)$$

where, N_{max} is the maximum nitrogen per dry mass in the young, developing leaves, N(t) is the nitrogen per dry mass in the developing leaves at time t, and DM(t) is the plant dry mass at time t. N_{max} adopted here is 0.0717, which is the mean of the initial values of different growth conditions. We used this value for all the plants. The decreases in nitrogen with time are expressed as lines in Fig. 4B.

Nitrogen deficit thus expressed increased rapidly in HL-plants (Fig. 5) and the initial increase in HLLN-plants

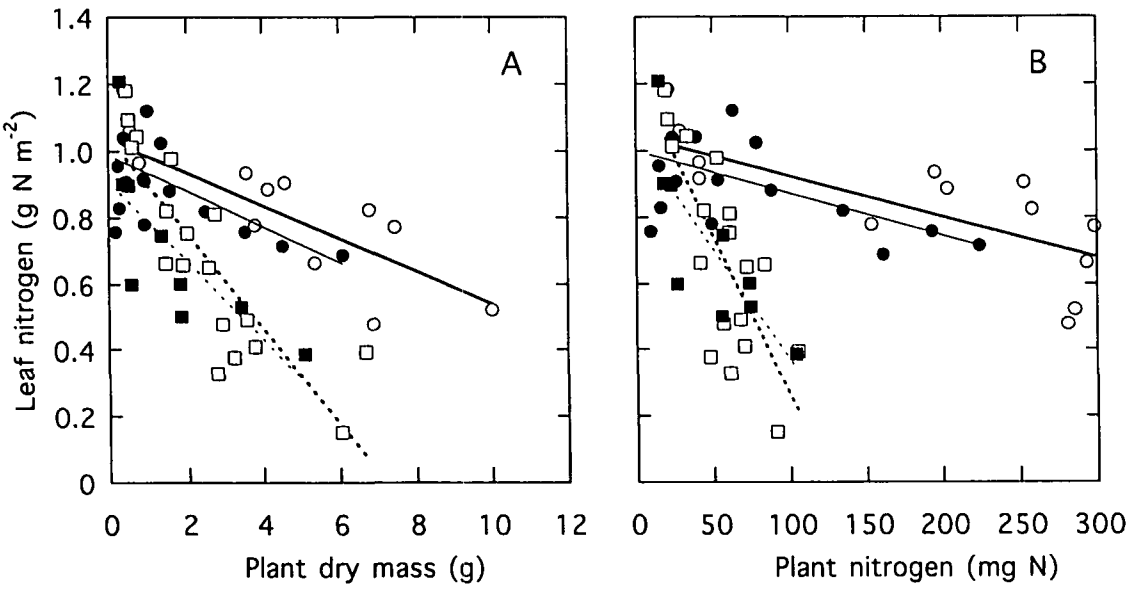

Fig. 3 Changes in nitrogen content per unit area of the first leaves (leaf N) versus dry mass of the plant (A) and total nitrogen of the plant (B). Symbols and lines are as in Fig. 1. For (A), regression lines are: HLHN, $y=1.034-0.0493x$, $r^2=0.65$; LLHN, $y=0.990-0.0538x$, $r^2=0.37$; HLLN, $y=1.045-0.1448x$, $r^2=0.71$; LLLN, $y=0.912-0.1197x$, $r^2=0.57$. For (B): HLHN, $1.046-0.001216x$, $r^2=0.51$; LLHN, $y=1.000-0.001266x$, $r^2=0.32$; HLLN, $y=1.219-0.00954x$, $r^2=0.58$; LLLN, $y=1.038-0.0007901x$, $r^2=0.65$. The difference among four lines were significant at $P<0.001$ and $P<0.001$, for (A) and (B), respectively (Snedecor and Cochran 1967).

occurred earlier than in HLHN-plants by about 3 days. In LL-plants, ND* increased much slower than in HL-plants. Increase in ND* was slowest in LLHN-plants. Trend of the increase in ND* among these plants was the same as that of the decreases in leaf N (compare Fig. 2, 5).

When leaf N was plotted against ND*, the regression lines were almost identical, independent of growth light and nutrient conditions (Fig. 6). As mentioned above, nitro-

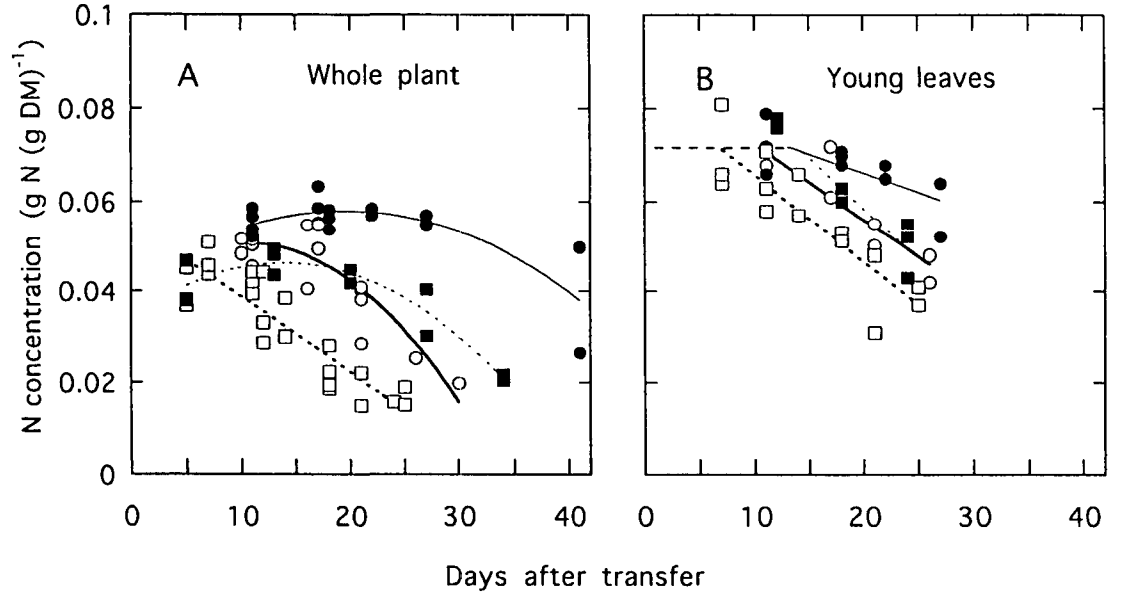

Fig. 4 Changes in nitrogen per dry mass of the whole plants (A) and those of the young developing leaves (B). Symbols and lines are as in Fig. 1. In (B), the horizontal line denotes the nitrogen concentration of 0.0717. Declines in nitrogen concentration were expressed by the linear regression lines: for HLHN, $y=0.0890-0.00165x$, $r^2=0.82$; LLHN, $y=0.0828-0.000829x$, $r^2=0.54$; HLLN, $y=0.0847-0.0019x$, $r^2=0.78$; LLLN, $y=0.103-0.00223x$, $r^2=0.91$.

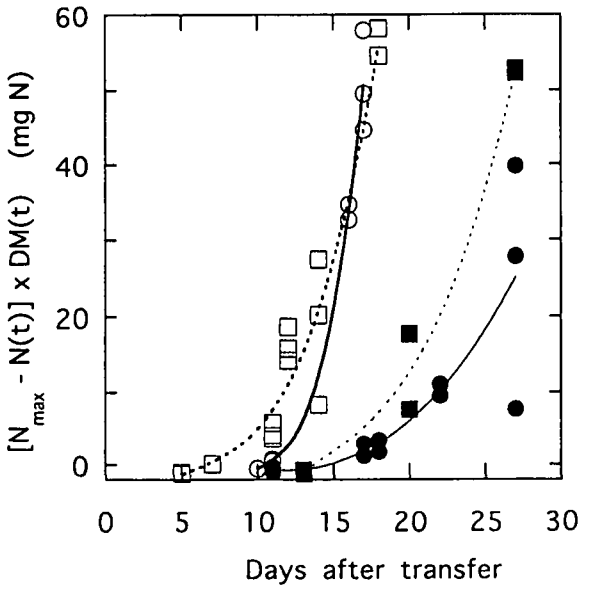

Fig. 5 Changes in nitrogen deficit (ND*) of the plants. Symbols and lines are as in Fig. 1.

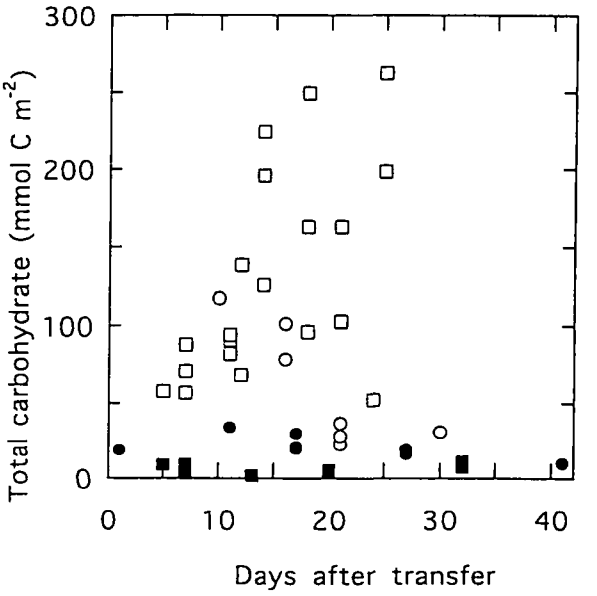

Fig. 7 Changes in content of carbohydrates (glucose+sucrose+starch) per unit are of the first leaves. Symbols are as in Fig. 1.

gen per dry mass of the whole plant would not be a good qualitative measure of nitrogen deficit. However, the similar tendency was also observed when N_{max} and N(t) in the above equation were replaced by the maximum nitrogen per dry mass and nitrogen per dry mass of the whole plants at time t (see Fig. 4A, data not shown), which would indicate the usefulness of the index.

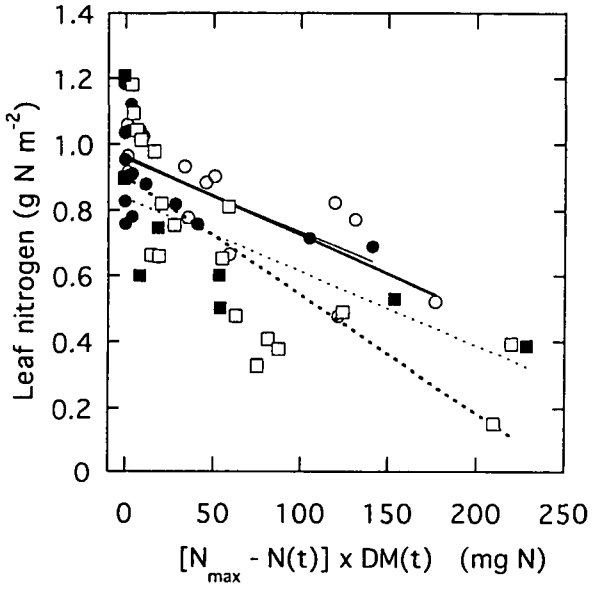

Fig. 6 Nitrogen content of the first leaves (leaf N) versus nitrogen deficit (ND*) of the plants. The data points below the ND* of 250 mg N are analyzed. Symbols and lines are as in Fig. 1. Regression lines are: HLHN, $y=0.964-0.00240x$, $r^2=0.62$; LLHN, $y=0.957-0.00222x$, $r^2=0.37$; HLLN, $y=0.902-0.00359x$, $r^2=0.62$; LLLN, $y=0.836-0.00225x$, $r^2=0.50$. The difference among four lines was not statistically significant ($P>0.05$) (Snedecor and Cochran 1967).

We also measured content of carbohydrate (glucose+sucrose+starch). Carbon is an important component of the plant as nitrogen is, and we expected that carbohydrate accumulated in leaves of plants under nitrogen deficiency. Contents of total carbohydrate in the first leaves were higher in HL-plants than in LL-plants (Fig. 7). Marked changes in these contents were not detected in the leaves of LL-plants. For the HL-plants, the carbohydrate levels in HLLN-plants were consistently greater than those in HLHN-plants.

Discussion

For the plants grown at the same nitrogen concentration, the decrease in leaf N started earlier and the rate of decrease was faster in HL-plants than in LL-plants (Fig. 2). Between the plants grown under the same irradiance level, leaf N decreased faster in LN-plants than in HN-plants (Fig. 2). These trends are consistent with the results of previous studies, although in these studies, the effects of growth irradiance (Hidema et al. 1991) and those of nitrogen supply (Makino et al. 1983, Morris and Paulsen 1985) were separately examined.

A novel point of the present study is that leaf N can almost uniquely be expressed as a function of ND* (Fig. 6). In other words, in spite of the fact that the onset of the development of ND* of the plants and the rate of its develop-

ment markedly differed depending on growth conditions (Fig. 5), we can predict the decrement in leaf N through analyzing the effects of growth conditions on ND* of the whole plants.

Growth irradiance appears to affect ND* and, whereby, leaf senescence, mainly through its effect on plant growth. In the present study, faster growth of HL-plants under relatively limited nitrogen supply resulted in the faster development of the ND* (Fig. 5). The difference in the rate of decrease in leaf N between HL- and LL-plants can thus be eventually attributed to their different growth rates. Anten and Werger (1996) investigated nitrogen allocation in large, dominant, and small, subordinate plants in a dense, monocultural stand of *Amaranthus dubius*. The nitrogen content of old leaves in the small plants that were heavily shaded by large plants decreased much slower than that in large plants. Since growth of small plants was light-limited and thereby sink for nitrogen was much smaller than that of large plants, this phenomenon may be explained by considering nitrogen deficit of the whole plant. As Hikosaka (1996) has pointed out, effects on leaf senescence of total shading to the whole plant differ from those of shading of a particular leaf, even though the irradiance level of the leaves under consideration is identical. This can also be explained by considering effects of these shadings on the plant nitrogen deficit. The former shading suppresses the development of nitrogen deficit, while the latter may not greatly affect the development of the nitrogen deficit of the plant.

On the other hand, lowered nitrogen supply raising the term ND* primarily through enhancing the term $N_{max} - N(t)$. In LN-plants, supply of nitrogen via roots was limited and the nitrogen deficit developed earlier (Fig. 4). This explains why the increase in ND* occurred earlier in LN-plants than in HN-plants (Fig. 5), in spite of the fact that increase in dry mass was faster in HN-plants (Fig. 1A). The faster increase in ND* in LN-plants would then lead to the earlier retranslocation of nitrogen from old leaves to the developing organs to compensate for the nitrogen shortage (Fig. 4B).

As detailed above, by considering the effects of these environmental variables on ND*, we can explain, to a considerable extent, the effects of growth irradiance and nitrogen nutrition on leaf senescence so far reported (eg. Makino et al. 1983, Morris and Paulsen 1985, Hidema et al. 1991, Hikosaka 1996, Anten and Werger 1996). Though light and nitrogen have been considered as environmental signals which directly affect leaf senescence (Thomas and Stoddart 1980, Smart 1994), leaf senescence should also be studied in relation to the nitrogen status of the whole plant.

Nitrogen deficit is a useful parameter, but there are cases in which it cannot explain the decrease in leaf N. For example, when the soybean plants with the first leaves shaded were compared with those with all leaves unshaded, the decrease in leaf N of the first leaves was faster in the shaded leaves (Pons and Pearcy 1994). Because these plants were grown under the same irradiance and the same nitrogen concentration, we can expect that they had almost the same ND*. In this case, however, source activities of the first leaves would be different between the conditions. Further study is needed to elucidate whether the leaves have mechanisms to monitor their current contribution as the source leaves.

For the changes of nitrogen in the first leaves to be dependent on the nitrogen deficit of the plants, the nitrogen status of the whole plants should be sensed by the first leaves. In this study, we measured carbohydrates to examine a possibility that the carbohydrate level may be engaged in the sensing mechanisms. Carbohydrates accumulate in the leaves of HL-plants (Fig. 7). This is probably due to both the limited nitrogen uptake and the considerable carbon fixation. Such accumulation of carbohydrates occurs when the content of carbon to that of nitrogen is unbalanced (Radin and Eidenbock 1986, Paul and Stitt 1993). Since the marked accumulation of the carbohydrate in HL-plants was observed where the rate of development of nitrogen deficit in these plants was greatest, the carbohydrates levels may affect leaf senescence. During leaf senescence, RuBP carboxylase decreased extensively and is often used as an index of leaf senescence. It has been proposed that metabolic events associated with the presence of high carbohydrate, repress expression of photosynthetic genes such as *rbcS* which codes for small subunit of RuBP carboxylase (Sheen 1990, Krapp et al. 1993). Since these results have been obtained with artificial systems such as maize protoplasts (Sheen 1990) and cold-girdled leaves (Krapp et al. 1993), further studies with more intact systems are needed to elucidate whether such repression really controls the resource utilization in the real plants.

The authors thank Drs. N. Adachi, N.P.R. Anten, K. Hikosaka, M. Ishibashi and K. Sonoike for valuable suggestions and discussions and Mr. A. Aoyama for technical assistance. This work was supported in part by the Sasakawa Scientific Research Grant from The Japan Science Society.

References

Anten, N.P.R. and Werger, M.J.A. (1996) Canopy structure and nitrogen distribution in dominant and subordinate plants in a dense stand of *Amaranthus dubius* L. with a size hierarchy of individuals. *Oecologia* 105: 30–37.

Chabot, B.F. and Hicks, D.J. (1982) The ecology of leaf life spans. *Annu. Rev. Ecol. Syst.* 13: 229–259.

Crafts-Brandner, S.J. and Egli, D.B. (1987) Sink removal and leaf senescence in soybean. *Plant Physiol.* 85: 662–666.

Dale, M.P. and Causton, D.R. (1992) The ecophysiology of *Veronica chamaedrys, V. montana and V. officinalis*. IV. Effects of shading on nutrient allocations.—a field experiment. *J. Ecol.* 80: 517–526.

Gepstein, S. (1988) Photosynthesis. *In* Senescence and Aging in Plants. Edited by Nooden, L.D. and Leopold, A.C. pp. 85–109. Academic Press, New York.

Guitman, M.R., Arnozis, A.A. and Barneix, A.J. (1991) Effect of source-sink relations and nitrogen nutrition on senescence and N remobilization in the flag leaf of wheat. *Physiol. Plant.* 82: 278–284.

Hidema, J., Makino, A., Mae, T. and Ojima, K. (1991) Photosynthetic characteristics of rice leaves aged under different irradiances from full expansion through senescence. *Plant Physiol.* 97: 1287–1293.

Hikosaka, K. (1996) Effects of leaf age, nitrogen nutrition and photon flux density on the organization of the photosynthetic apparatus in leaves of a vine (*Ipomoea tricolor* Cav.) grown horizontally to avoid mutual shading of leaves. *Planta* 198: 144–150.

Hikosaka, K., Okada, K., Terashima, I. and Katoh, S. (1993) Acclimation and senescence of leaves: their roles in canopy photosynthesis. *In* Photosynthetic Responses to the Environment. Edited by Yamamoto, H.Y. and Smith, C.M. pp. 1–13. American Society of Plant Physiologists, Lancaster.

Hikosaka, K., Terashima, I. and Katoh, S. (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.

Khanna-Chopra, R. and Reddy, P.V. (1988) Regulation of leaf senescence by reproductive sink intensity in cowpea (*Vigna unguiculata* L. Walp). *Ann. Bot.* 61: 655–658.

Kikuzawa, K. (1989) Ecology and evolution of phenological pattern, leaf longevity and leaf habit. *Evol. Trends Plants* 3: 105–110.

Krapp, A., Hofmann, B., Schafer, C. and Stitt, M. (1993) Regulation of the expression of rbcS and other photosynthetic genes by carbohydrates: a mechanism for the 'sink regulation' of photosynthesis? *Plant J.* 3: 817–828.

Mae, T. and Ohira, K. (1981) The remobilization of nitrogen related to leaf growth and senescence in rice plants (*Oryza sativa* L.). *Plant Cell Physiol.* 22: 1067–1074.

Makino, A., Mae, T. and Ohira, K. (1983) Photosynthesis and ribulose 1,5-bisphosphate carboxylase in rice leaves. *Plant Physiol.* 73: 1002–1007.

Morris, C.R. and Paulsen, G.M. (1985) Development of hard winter wheat after anthesis as affected by nitrogen nutrition. *Crop Sci.* 25: 1007–1010.

Paul, M.J. and Stitt, M. (1993) Effects of nitrogen and phosphorus deficiencies on levels of carbohydrates, respiratory enzymes and metabolites in seedlings of tobacco and their response to exogenous sucrose. *Plant Cell Environ.* 16: 1047–1057.

Pons, T.L. and Pearcy, R.W. (1994) Nitrogen reallocation and photosynthetic acclimation in response to partial shading in soybean plants. *Physiol. Plant.* 92: 636–644.

Radin, J.W. and Eidenbock, M.P. (1986) Carbon accumulation during photosynthesis in leaves of nitrogen- and phosphorus-stressed cotton. *Plant Physiol.* 82: 869–871.

Rao, I.M., Fredeen, A.L. and Terry, N. (1990) Leaf phosphate status, photosynthesis, and carbon partitioning in sugar beet. *Plant Physiol.* 92: 29–36.

Rufty, T.W. and Huber, S.C. (1983) Changes in starch formation and activities of sucrose phosphate synthase and cytoplasmic fructose-1,6-bisphosphatase in response to source-sink alterations. *Plant Physiol.* 72: 474–480.

Sheen, J. (1990) Metabolic repression of transcription in higher plants. *Plant Cell* 2: 1027–1038.

Smart, C.M. (1994) Gene expression during leaf senescence. *New Phytol.* 126: 419–448.

Snedecor, G.W. and Cochran, W.G. (1967) Statistical Methods (6th ed.), Iowa State Univ. Press, Ames.

Thimann, K.V. (1980) The senescence of leaves. *In* Senescence in Plants. Edited by Thimann, K.V. pp. 85–115. CRC Press, Boca Raton, FL.

Thomas, H. and Stoddart, J.L. (1980) Leaf senescence. *Annu. Rev. Plant Physiol.* 31: 83–111.

Wittenbach, V. (1983) Effect of pod removal on leaf photosynthesis and soluble protein composition of field grown soybeans. *Plant Physiol.* 73: 121–124.

(Received June 17, 1996; Accepted August 30, 1996)