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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

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Abstract Effects of leaf age, nitrogen nutrition and photon flux density (PFD) on the distribution of nitrogen among leaves were investigated in a vine, *Ipomoea tricolor* Cav., which had been grown horizontally so as to avoid mutual shading of leaves. The nitrogen content was highest in newly developed young leaves and decreased with age of leaves in plants grown at low nitrate concentrations and with all leaves exposed to full sunlight. Thus, a distinct gradient of leaf nitrogen content was formed along the gradient of leaf age. However, no gradient of leaf nitrogen content was formed in plants grown at a high nitrate concentration. Effects of PFD on the distribution of nitrogen were examined by shading leaves in a manner that simulated changes in the light gradient of an erect herbaceous canopy (i.e., where old leaves were placed under increasingly darker conditions with growth of the canopy). This canopy-type shading steepened the gradient of leaf nitrogen content in plants grown at a low nitrogen supply, and created a gradient in plants grown at high concentrations of nitrate. The steeper the gradient of PFD, the larger the gradient of leaf nitrogen that was formed. When the gradient of shading was inverted, that is, younger leaves were subjected to increasingly heavier shade, while keeping the oldest leaves exposed to full sunlight, an inverted gradient of leaf nitrogen content was formed at high nitrate concentrations. The gradient of leaf nitrogen content generated either by advance of leaf age at low nitrogen availability, or by canopy-type shading, was comparable to those reported for the canopies of erect herbaceous plants. It is concluded that both leaf age and PFD have potential to cause the non-uniform distribution of leaf nitrogen. It is also shown that the contribution of leaf age increases with the decrease in nitrogen nutrition level.

Key words Distribution of leaf nitrogen content
Leaf age · Nitrogen availability · Photon flux density
Vine (*Ipomoea tricolor*)

Introduction

In a leaf canopy, photon flux density (PFD) is highest at the top of the canopy and decreases with depth (Monsi und Saeki 1953). There is also a vertical gradient of leaf nitrogen content determined on the basis of leaf area (N_L) in leaf canopies (DeJong and Doyle 1985; Hirose and Werger 1987a, b; Werger and Hirose 1991). Since leaves which have high nitrogen contents can realize high photosynthetic rates at high PFD, it is advantageous for nitrogen-abundant leaves to be at the top of the canopy (Field 1983). On the other hand, investment of great amounts of nitrogen into leaves at lower positions would be inefficient because the maximum photosynthetic rate cannot be attained in the shade (Mooney and Gulmon 1979). In addition, an increase in leaf nitrogen content is generally accompanied by enhanced respiratory activity, which would lead to a decreased gain or even a net loss of carbon from shade leaves (Hirose and Werger 1987a). Several studies based on the cost-benefit hypothesis (Mooney and Gulmon 1979) have shown that the gradient of N_L gives rise to a higher carbon gain of a whole canopy than would one where nitrogen is uniformly distributed among leaves (Field 1983; Hirose and Werger 1987b; Pons et al. 1989; Schieving et al. 1992). The vertical gradient of N_L is, therefore, regarded as an adaptive response of leaves to the light gradient inside a canopy.

There are two factors which could contribute to formation of the gradient of N_L in a canopy. Mooney et al. (1981) indicated that, in old-field plants, N_L decreased with leaf age even when aged leaves had not been heavily shaded. They suggested that the decrease in N_L with

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advancing age of a leaf is a genetically programmed process. Another factor to be considered is the light environment of leaves. Hirose et al. (1988) found that the vertical gradient of N_L was steeper in a denser stand of *Lysimachia vulgaris* L. than a thinner one and concluded that the light gradient within the leaf canopy, which is strongly affected by the stand density, plays an important regulatory role in the formation of the gradient of N_L. A dominant effect of PFD on the distribution of nitrogen was also suggested by experiments with the canopy of *Carex acutiformis* Ehrh. These plants form a tussock, with the oldest parts of their long erect leaves receiving the highest PFD at the top of the canopy. N_L increased significantly with plant height even though the tissue age was older at the top of the canopy (Hirose et al. 1989; Schieving et al. 1992). Evans (1989), who grew cucumber plants with leaves shaded to various extents, showed that the nitrogen content of individual leaves varied depending on PFD rather than on leaf age. More recently, Ackerly (1992) reported that, irrespective of leaf age, N_L was correlated with the light environments of leaves in field-grown tropical vines of *Syngonium podophyllum* Schott. These studies indicate that PFD is a prevailing factor determining the distribution of nitrogen in a canopy. However, a question still remains as to whether or not leaf age affects the distribution of N_L among the leaves of an actual canopy. If so, it is also important to clarify how leaf age and PFD interact with each other in the formation of a vertical gradient of N_L.

In the present study, attempts were made to evaluate effects of leaf age and PFD on the distribution of nitrogen among leaves separately. Generally, it is difficult to distinguish between the effects of these two factors because older leaves are more or less shaded by upper young leaves. Therefore, we used a vine, *Ipomoea tricolor* Cav., that had been grown horizontally to avoid mutual shading of leaves. Because all leaves of the vine had developed under uniform light conditions, we were able to evaluate the effect of leaf age on distribution of N_L separately from that of PFD. Then, light environments of individual leaves were varied, simulating the light conditions inside a canopy. This enabled us to investigate the effect of PFD on the nitrogen content of leaves of different ages. We also examined the influence of nitrogen nutrition on the distribution of N_L. Preliminary accounts of a part of this study have appeared elsewhere (Hikosaka et al. 1992, 1993).

Materials and methods

Seeds of *Ipomoea tricolor* Cav. cv. Heavenly Blue (morning glory) from Takii Syubyo (Kyoto, Japan) were germinated in vermiculite. When cotyledons had expanded, seedlings were transferred to Wagner pots of 12.5 cm diameter and 20 cm height (one plant per pot), which contained 1.5 l hydroponic solution. The solution was continuously aerated. The standard hydroponic solution (12 m mol NO_3^- l^{-1}) contained 4 m mol KNO_3 l^{-1}, 4 m mol $Ca(NO_3)_2$ l^{-1}, 1.5 m mol $MgSO_4$ l^{-1}, 1.33 m mol NaH_2PO_4 l^{-1}, 0.05 m mol FeNa-EDTA l^{-1}, 10 µmol $MnSO_4$ l^{-1}, 1 µmol $ZnSO_4$ l^{-1}, 1 µmol $CuSO_4$ l^{-1}, 50 µmol H_3BO_3 l^{-1}, 0.5 µmol Na_2MoO_4 l^{-1}, 0.1 m mol NaCl l^{-1} and 0.2 µmol $CoSO_4$ l^{-1} (Hewitt and Smith 1975). When nitrate concentration was reduced, KCl and $CaCl_2$ were added to keep concentrations of K^+ and Ca^{2+} constant. Concentrations of all the elements other than NO_3^- and Cl^- were 10%, 30%, 60% and 100% of those of the standard solution in 0.04, 0.12, 0.24 and 1.2 m mol NO_3^- l^{-1} solutions, respectively. In order to make up the hydroponic solution, the concentrated nutrient solutions were added to tap water. The same volumes of the concentrated nutrient solutions were added to the hydroponic solution in the pot every 4 days and the whole hydroponic solution was renewed every 12 days. In the second shading experiment, plants were grown in sand in the same pots and 50 ml of the standard solution (12 m mol NO_3^- l^{-1}) was added to each pot every 2 days.

Vines were grown on wire nets and leaves were fixed to the net with vinyl-coated wire to avoid mutual shading. Where indicated, leaves were differently shaded with wire boxes (11 × 11 × 5 cm height) with all the sides except the bottom covered with one to six layers of shade cloth. Plants were grown in a greenhouse. On clear sunny days, the maximum PFD was about 1200 $\mu mol\ m^{-2}\ s^{-1}$ at noon. In the experiments shown in Fig. 1, day length was adjusted by supplementary light of 240 $\mu mol\ m^{-2}\ s^{-1}$ from 1000-W halide-lamps (Neo halide lamp, Toshiba, Tokyo, Japan) from 0500 to 0600 hours and from 1900 to 2000 hours. All lateral buds were removed before they reached 1 cm in length.

For measurement of nitrogen content, three discs of 1 cm diameter were punched out from each leaf and dried at 70° C for at least 3 days. Leaf nitrogen content was measured with a NC analyzer (NC-80, Sumitomo Chemical, Tokyo, Japan), in which NO_x from the combusted sample was converted to N_2, and then the amount of N_2 was measured with gas chromatography. Leaf area was determined with an area meter (Hayashi Denkoh, Tokyo, Japan).

The gradient of N_L was evaluated in terms of K_a, a coefficient of leaf nitrogen allocation defined by Hirose and Werger (1987b) for canopies of *Solidago altissima*,

$$N = N_0 \exp(-K_a\ F/F_t) \qquad (1)$$

where F and F_t denote leaf area cumulated from the youngest leaf to the leaf under consideration and total cumulative leaf area, and N and N_0 are N_L at F and $F = 0$, respectively.

Results

Effects of leaf age on distribution of leaf nitrogen

First, experiments were carried out to investigate whether the distribution of N_L is regulated by leaf age alone through growing plants with all the leaves uniformly exposed to sunlight. Effects of nitrogen supply were also examined by growing plants at 0.04, 0.24, 1.2 and 12 m mol NO_3^- l^{-1}. None of the leaves were wilted during the experiments. Figure 1 shows the N_L values of plants grown at different nitrate concentrations. Because the number of leaves developed per plant varied considerably depending upon nitrogen supply, the distributions of N_L values were compared in terms of F/F_t. The data points at or near $F/F_t = 0$ and 1 indicate the N_L values of the youngest and oldest leaves, respectively. Figure 1 a–d shows the data collected from plants grown for four different periods. It is seen that, in plants grown at low nitrate concentrations, N_L decreased with increase of F/F_t or with time elapsed after transplanting. Consequently, a decreasing gradient of N_L from the top to the base of the vine was formed. The gradient became progressively steeper with plant age. Because all the

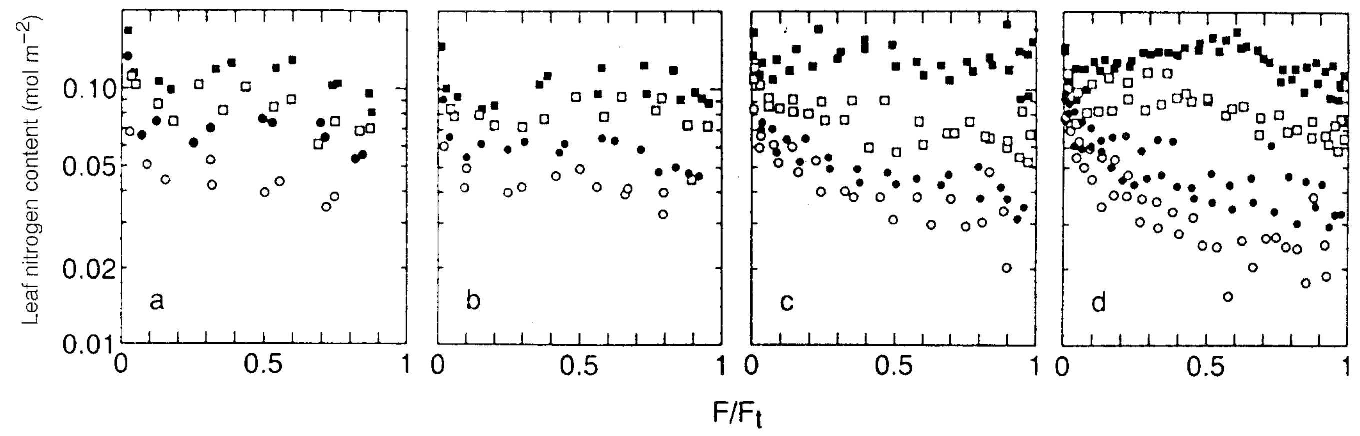

Fig. 1a–d Time-dependent changes in distribution of nitrogen among leaves of plants grown at different concentrations of nitrate, 0.04 (○), 0.24 (●), 1.2 (□) and 12 m mol l^{-1} (■). Leaves were harvested **a** 22 d, **b** 29 d, **c** 43 d and **d** 58 d after transplanting. At each nitrate level, leaves were harvested from two plants and analysed for nitrogen. Each data point indicates a single leaf

leaves had developed under uniform light conditions, the gradient of N_L generated along the vine is ascribed to the gradient of leaf age. The data also show that the distribution of nitrogen in leaves is strongly affected by nitrogen availability. A large gradient of N_L was formed only under nitrogen-limited conditions. When plants had been grown at higher concentrations of nitrate, the gradient of N_L became less marked and nitrogen contents of all the leaves remained at a high and constant level throughout the experiment.

K_a values were estimated as a measure of the steepness of gradients of N_L, by regression of the data points using the least squares method, even though some data obtained from plants grown at low nitrate concentrations often deviated considerably from straight lines. Figure 2 shows the K_a values thus determined as a function of time after transplanting. Relatively large K_a values were obtained for young plants (22 d) which had small numbers of leaves. Except for this, there was a clear trend demonstrating that the K_a value increased with time in plants grown at low nitrate concentrations. However, K_a remained at low levels in plants grown at 12 m mol NO_3^- l^{-1}.

It should be mentioned that, even when nitrogen is supplied at a constant rate, the internal nitrogen level of a whole plant grown under nitrogen-limited conditions would decrease with advancing age of the plant because the biomass of the plant increases more or less exponentially. In fact, mean N_L value (N_p) of plants grown at 0.04 and 0.24 m mol nitrate l^{-1} decreased from 45 and 68 mmol m^{-2} at 22 d to 33 and 45 mmol m^{-2} at 58 d, respectively. Thus, K_a should be related to the internal nitrogen level of plants or N_p rather than to the concentration of nitrate added. Figure 3 shows that K_a decreases with increasing value of N_p. The K_a values of plants at three different stages of growth are indicated by different symbols. Regression of the data points from each age-group of plants yielded a straight line with negative

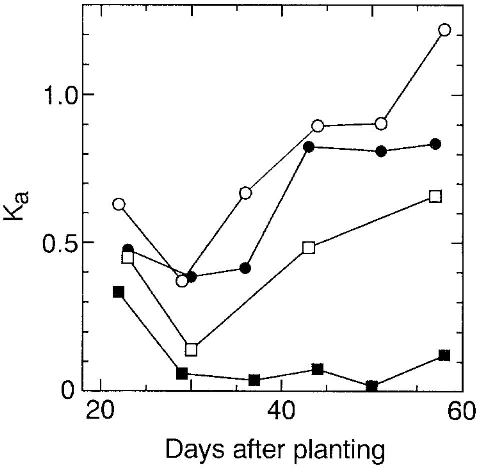

Fig. 2 Changes in K_a with growth of plants at different nitrate concentrations. The K_a values were estimated from the data in Fig. 1 and other data not presented. Symbols are as in Fig. 1

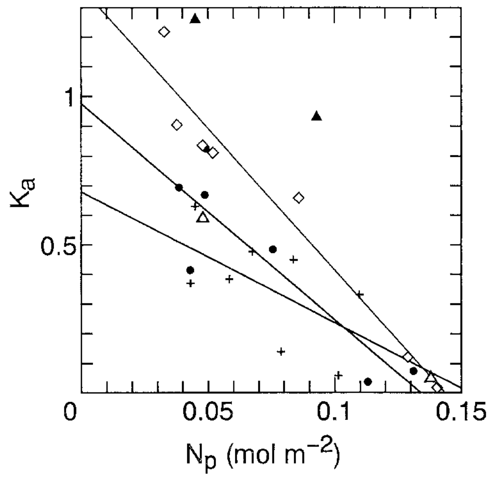

Fig. 3 K_a values as a function of N_p. The data points were collected from the experiment shown in Fig. 2 and divided into three groups depending upon growth periods. +, 22–30 d; ●, 36–44 d; ◇, 50–58 d after transplanting. The regression lines are $K_a = 0.68–4.42\ N_p$, $r^2 = 0.35$ (not significant) for 22–30 d; $K_a = 0.98–7.28\ N_p$, $r^2 = 0.77$ ($P < 0.01$) for 36–44 d; $K_a = 1.37–9.52\ N_p$, $r^2 = 0.95$ ($P < 0.01$) for 50–58 d. The slopes of regression lines are statistically different from each other ($P < 0.001$). For comparison, the K_a values obtained from plants grown under "canopy-type shading" condition (▲) and the non-shaded control (△) are shown

Table 1 Time schedules of shading treatments. 100% indicates full sunlight. Experiment I, "canopy-type shading": leaves younger than 7th leaves were not shaded. In experiment II, canopy-type shading simulating light regimes inside stands of three different plant densities: only the attenuation pattern of low density shading is shown. Intermediate and high density shading were carried out with the same time schedule but PFD was more strongly reduced (see text). Experiment III, "inverse canopy-type shading": figures in parentheses are the attenuation pattern for control experiments (canopy-type shading). Days are counted from the day of transplanting of seedlings to the pots.

Experiment	Days after transplanting	Irradiances (%)						
		Leaf order 1	2	3	4	5	6	$7\leq$
I	21	35	100	100				
	25	35	35	100	100			
	29	14	35	35	100	100		
	31	14	14	35	35	100	100	
	33	3.7	14	14	35	35	100	100
	35	3.7	3.7	14	14	35	35	100
II	14	50	100	100				
(low density)	16	50	50	100	100			
	19	35	50	50	100	100		
	21	35	35	50	50	100	100	
III	16	100	100	35				
		(35	100	100)				
	19	100	100	35	35			
		(35	35	100	100)			
	24	100	100	35	35	14	14	14
		(14	14	35	35	100	100	100)

slope. This indicates that K_a increases with the increasing degree of nitrogen deficiency at any stage of plant growth. Note that the slope of the regression lines significantly increased with plant age. Thus, at any N_p value, older plants gave larger K_a values than did younger plants. It is concluded, therefore, that the gradient of N_L is determined by both plant age and degree of nitrogen deficiency of plants.

Effects of PFD on distribution of leaf nitrogen

For investigation of the effects of light environment, plants were grown under full sunlight for 20 days and then shade treatments were initiated, simulating light conditions inside the canopy of an erect herbaceous plant. The time schedule of the shade treatment is shown in Table 1. The principle is that a leaf was one step more heavily shaded when two newer leaves than the leaf under consideration had unfolded. Thus, while newly unfolded leaves were always exposed to full sunlight, older leaves were placed under increasingly shaded conditions. Plants were grown at two different nitrate concentrations (0.12 and 12 m mol l^{-1}) and leaves were harvested at day 43–47 after transplanting. The N_L values determined are plotted against F/F_t in Fig. 4. Note that the canopy-type shading resulted in large declines in N_L of older leaves and consequently an significant gradient of N_L was formed in plants grown at 12 m mol nitrate l^{-1}, which otherwise showed a high and constant level of N_L. The gradient of N_L in plants grown at 0.12 m mol nitrate l^{-1} became more marked under the shading treatment. The K_a values determined for plants subjected to the shading treatment are shown in Table 2. For comparison, the K_a values obtained under canopy-type shading conditions are shown in Fig. 3. They were higher than those estimated for unshaded plants of the similar ages and of comparable N_p values.

The gradient of PFD inside a canopy varies depending upon stand density. The second shading experiment was carried out to examine how distribution of nitrogen is affected by the steepness of the light gradient. Three groups of plants grown under full sunlight for 14 days were differently shaded. The pattern of stepwise shading for the first group is given in Table 1. Two other groups of plants were shaded under the same time schedule but

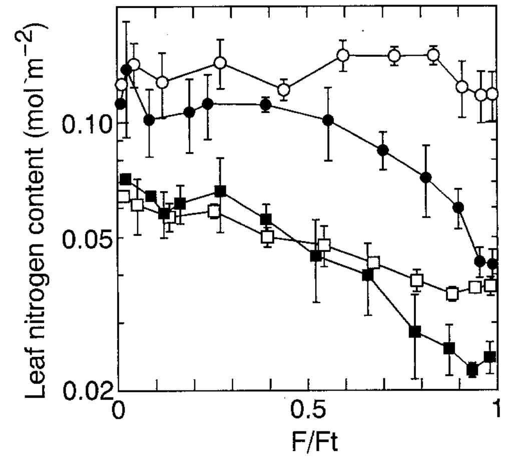

Fig. 4 Effects of canopy-type shading on the distribution of nitrogen in leaves. Each *data point* and *bar* indicate mean and SD ($n = 3$ leaves of 3 plants), respectively. *Squares* and *circles*, plants grown at 0.12 and 12 m mol nitrate l^{-1}, respectively. *Open symbols*, plants grown without any shading treatment; *closed symbols*, plants grown under the canopy-type shading conditions. For the time schedule for shading, see Table 1

Table 2 Effects of the canopy-type shading on K_a, N_0 and N_p. K_a and N_0 are estimated from regression between log N_L and F/F_t for all the leaves of three plants ($n = 30$–32). For each N_p, mean ±SD ($n = 3$) is shown

Treatment	K_a	N_0	r^2	N_p
		(mol m^{-2})		(mol m^{-2})
12 m mol NO_3^- l^{-1}				
No shading	0.05^a	0.135	0.01^{ns}	0.138±0.001
canopy-type shading	0.93^b	0.134	0.62**	0.093±0.016
0.12 m mol NO_3^- l^{-1}				
No shading	0.59^c	0.064	0.83**	0.048±0.001
canopy-type shading	1.26^d	0.080	0.87**	0.045±0.002

For K_a values (regression coefficient): different superscripts indicate the statistically different values ($P < 0.001$)
ns, correlation coefficient not significantly different from zero; ** $P < 0.01$

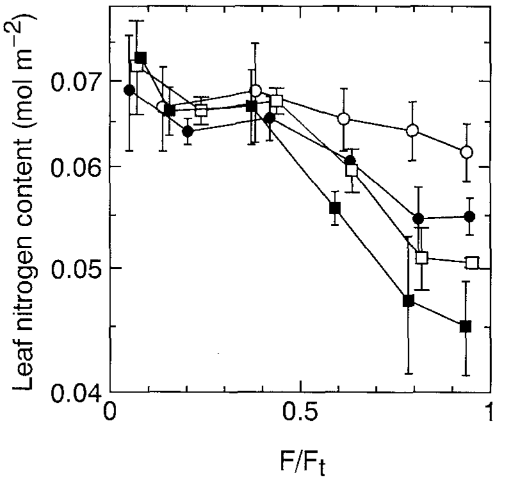

Fig. 5 Effects of canopy-type shading with different attenuation patterns on the distribution of nitrogen in leaves. Plants were grown in sand and 50 ml of standard solution (12 m mol nitrate l^{-1}) was added every 2 days. ○, no shading treatment; ●, "low density shading"; □ , "intermediate density shading"; ■, "high density shading". Each *data point* and *bar* indicate mean and SD ($n = 3$), respectively. For the time schedule for shading, see Table 1

PFD was more steeply reduced; 100%, 35%, and then 14%, for the second and 100%, 20% and then 3.7% for the third group. For convenience, the treatments applied to the first, second and third group are called low, intermediate and high density shadings, respectively. Leaves were harvested 24–26 days after transplanting for analysis of N_L. Figure 5 shows that, whereas the 4th–6th leaves which had not or had only briefly, been shaded retained high levels of N_L, the 1st–3rd leaves lost larger amounts of nitrogen depending on duration and degree of the shading. Thus, the gradient of N_L was formed in response to the gradient of PFD applied to the leaves.

In the third experiment, leaves were shaded in a manner opposite to the canopy-type shading. The 1st and 2nd leaves were unshaded throughout the experiment, whereas growth irradiance of the 3rd and 4th, and the 5th and 6th leaves were reduced to 35% and 14% of full sunlight, respectively (see Table 1). The inverse canopy-type shading resulted in a non-uniform distribution of nitrogen in leaves of plants grown at the high nitrogen level (Fig. 6). The gradient of N_L formed was opposite to that generated by the canopy-type shading, but in both cases N_L decreased as growth irradiance was reduced. These results indicate that PFD is an important factor regulating the distribution of N_L.

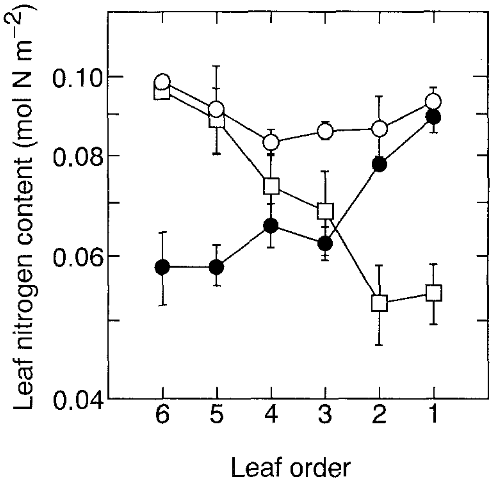

Fig. 6 Effects of inverse canopy-type shading on the distribution of nitrogen in leaves. Leaf order denotes the order of leaves on a vine counted from the base. Each *data point* and *bar* indicate mean and SD ($n = 3$), respectively. Plants were grown hydroponically in the standard solution (12 m mol NO_3^- l^{-1}) and leaves were harvested 32 d after transplanting. ○, plants grown without any shading treatment; □, the canopy-type shading; ●, inverse canopy-type shading. Data from the 7th and younger leaves are omitted because they were not yet fully expanded at harvest

Discussion

Effects of PFD and leaf age on the gradient of N_L within a plant

In the first part of this study the effects of leaf age on the nitrogen abundance of leaves were investigated. In canopies of erect herbaceous plants, there are a decreasing gradient of PFD and an increasing gradient of leaf age from top to bottom. The use of vines grown horizontally allowed us to evaluate the effect of leaf age on the distribution of nitrogen among leaves separately from that of PFD. The effects of nitrogen nutrition on the distribution of nitrogen were also examined. Leaf senescence, usually assessed as loss of chlorophyll or protein, is known to be accelerated by nitrogen deficiency (Thomas and Staddart 1980; Makino et al. 1984). However, effects of nitrogen availability on the distribution pattern of nitrogen among leaves of a whole plant have not yet been analyzed in detail.

When *I. tricolor* was grown at high nitrogen level, the N_L values were constant and independent of leaf age. By contrast, distinct gradients of N_L were created along the vine of plants grown at low nitrogen concentrations because newly developed leaves always contained high levels of nitrogen but N_L decreased with advancing age of leaves (Fig. 1). The K_a values of plants grown at low nitrate concentrations increased with advance of plant age (Fig. 2). These results suggest that, when the supply of nitrogen is limited, the gradient of N_L is formed by translocating nitrogen from old leaves to developing young leaves. Mooney et al. (1981) have suggested that the gradient of N_L is formed by leaf aging in old-field plants. We stress that the gradient of N_L is created along that of leaf age only when nitrogen is limited.

The present study also shows that PFD strongly affects the distribution of nitrogen among leaves. A distinct gradient of N_L was formed by the canopy-type shading in plants grown at a sufficiently high nitrogen level (Fig. 4, Table 2). The steeper the gradient of PFD, the larger the gradient of N_L that was formed (Fig. 5). Moreover, the gradient of N_L was inverted by shading leaves in a manner opposite to the canopy-type shading: the older leaves which had received a higher PFD contained larger amounts of nitrogen than leaves kept under darker conditions (Fig. 6). These results indicate that light environment is an important factor in regulating the distribution of nitrogen. Our results are consistent with previous studies which indicated that PFD plays a dominant role in the formation of gradient of N_L in natural and artificial canopies (Ackerly 1992; DeJong and Doyle 1985; Hirose et al. 1988, 1989; Schieving et al. 1992; Werger and Hirose 1991; but see Mooney et al. 1981).

Hirose and co-workers have reported K_a values obtained in stands of several species: *Solidago altissima*, a clonal plant, 0.80 (Hirose and Werger 1987b); *Lysimachia vulgaris*, an annual, 0.48 and 1.20 in thin and dense stands, respectively (Hirose et al. 1988); *Carex acutiformis*, a tussock grass, 0.12–0.70 (Hirose et al. 1989). Although these species have different life forms, they showed comparable values of K_a. This has been regarded as evidence that PFD is the most important factor defining the gradient of N_L. Interestingly, the K_a values in the plants grown without shading under low nitrogen availability are comparable to the reported values (Fig. 2, Table 2). Thus, it is suggested that leaf age also has a potential to generate a significant gradient of N_L.

However, a question remains as to how leaf age and PFD interact with each other in formation of a gradient of N_L. Of particular interest in this context is the observation that, when grown under the same shading conditions, the K_a values were always significantly greater in plants grown at lower nitrogen levels than in those grown at higher nitrogen levels (Table 2, Hikosaka et al. 1993). We suggest that, at low nitrogen availability, in addition to PFD, leaf age significantly contributes to the formation of gradient of N_L. Hirose et al. (1988), who found that the gradient of N_L was steeper in a denser stand of *Lysimachia vulgaris* than in a thinner one, concluded that N_L is controlled by the light microenvironment of leaves. Their conclusion is consistent with our results shown in Fig. 5. However, the nitrogen availability of individual plants should be lower in the dense stand than in the thinner one because of competition among plants for the nutrient. Therefore, the steeper gradient of N_L in the dense stand may partly be attributed to low nitrogen availability and the leaf age effect.

Concluding this section, our results indicate that each of leaf age and PFD alone has a potential to generate a gradient of N_L as large as those existing in natural canopies. Nitrogen availability is also an important factor affecting the distribution of nitrogen among leaves and both of leaf age and PFD contribute to formation of the gradient of N_L under nitrogen-limiting conditions. Thus, the present study provides useful information for investigation of mechanisms underlying non-uniform distribution of N_L in natural canopies of herbaceous plants.

Effects of the gradient of PFD and leaf age on canopy/plant photosynthesis

As stated in the introduction, past studies have shown that vertical gradients of N_L contribute to a high photosynthetic gain of the whole canopy (Field 1983; Hirose and Werger 1987b; Pons et al. 1989; Werger and Hirose 1991). Photosynthetic production in the canopy of *Solidago altissima* with a significant gradient of N_L was estimated to be 21% greater than a canopy in which the same total amount of nitrogen is uniformly distributed among leaves (Hirose and Werger 1987b). Similar results have been reported for stands of *Lysimachia vulgaris* (Pons et al. 1989) and *Carex acutiformis* (Schieving et al. 1992). On the other hand, Field (1983) showed theoretically that, if all leaves receive the same PFD, the carbon gain of a whole plant is maximized when nitrogen is distributed uniformly among leaves. It is remarkable, therefore, that a significant gradient of N_L was formed in *I. tricolor* with leaves developed under uniform light conditions (Fig. 1, Table 2). It should be noted that the magnitude of the decrement of whole plant photosynthesis due to non-uniform distribution of N_L under unshaded conditions strongly depends on the nature of the relationship between daily photosynthesis and N_L. If daily photosynthesis of leaves is almost linear to N_L, the decrease in daily photosynthetic production of a whole plant with all leaves exposed to full sunlight due to non-uniform distribution of N_L is small. In contrast, if the dependency of daily photosynthesis on N_L is saturating, non-uniform distribution of nitrogen gives rise to a lower carbon gain than does uniform distribution of nitrogen. This is because, when the relationship between daily photosynthesis and N_L is curved, the sum of daily photosynthesis of two leaves with the different N_L is smaller than that of two leaves with the same N_L that is equal to the mean of the N_L values of the above two leaves. In view of a non-linear relationship between photosynthetic capacity and N_L in several plants (Evans

1983; Terashima and Evans 1988; Connor et al. 1993), it is probable that the daily photosynthesis-N_L relationship could be fairly curved. Thus, an important question, whether there is a limit to the ability of plants to acclimate to growth irradiance, remains to be answered. Experiments in this line are in progress.

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