

well understood (see Booi-James et al 2002). Suggestions for its function include a possible role in regulating D1 turnover (see Baena-Gonzalez et al 1999) as well as a possible role in signal transduction. The results presented here suggest an additional possibility, i.e., a possible function of sustained high D1/PSII core protein phosphorylation in photoprotection in overwintering leaves. One may speculate that sustained high PSII phosphorylation could be involved in converting PSII cores to dissipating centers where zeaxanthin may aid in thermal dissipation. Such a conversion of PSII cores to photochemically inactive, dissipating centers may serve not only to counteract singlet oxygen formation in these cores but also to prevent charge separation and a transfer of high energy electrons to oxygen, leading to superoxide formation in the chloroplast. Sustained D1 phosphorylation, F_v/F_m depression, and Z+A retention were observed irrespective of the type of stress that caused photoinhibition and is thus a characteristic feature of photoinhibited leaves of evergreens in general. Being more prominent in evergreens with their high stress resistance, photoinhibition may thus be one of the many “talents” of evergreens.

ACKNOWLEDGMENTS

We are indebted to Drs. Autar K. Mattoo and Anna Sokolenko for making antibodies against the unphosphorylated form of D1 and TLP40, respectively, available to us. Technical assistance of Lisa Schaffer and David Mellman is also appreciated. This work has been supported by grants of the United States Department of Agriculture (Award No. 00-35100-9564), the National Science Foundation (Award No. IBN-9974620), and the Andrew W. Mellon Foundation (Award No. 20200747).

REFERENCES

- Adams, W. W. III, Demmig-Adams, B., Rosenstiel, T. N., Brightwell, A. K. & Ebbert, V. (2002) *Plant Biol.* 4: 545–557.
- Adams, W. W. III, Zarter, C. R., Ebbert, V. & Demmig-Adams, B. (2004) *BioScience* 54: 41–49.
- Baena-Gonzalez, E., Barbato, R. & Aro, E.-M. (1999) *Planta* 208: 196–204.
- Booi-James, I. S., Swegle, W. M., Edelman, M. & Mattoo, A. K. (2002) *Plant Physiol.* 130: 2069–2075.
- Demmig-Adams, B. & Adams, W. W. III (2002) *Science* 298: 2149–2153.
- Demmig-Adams, B. & Adams, W. W. III (2003) In: *Encyclopedia of Applied Plant Science: Environmental Regulation of Growth and Development* (B. Thomas, D. Murphy & B. Murray, Eds.) pp. 707–714. Academic Press, London, UK.
- Ebbert, V., Demmig-Adams, B., Adams, W. W. III, Mueh, K. E. & Staehelin, L. A. (2001) *Photosynth. Res.* 67: 63–78.
- Öquist, G. & Huner, N. P. A. (2003) *Ann. Rev. Plant Biol.* 54: 329–355.
- Rintamäki, E., Salonen, M., Suoranta, U.-M., Carlberg, I., Andersson, B. & Aro, E.-M. (1997) *J. Biol. Chem.* 272: 30476–30482.
- Vener, A. V., Rokka, A., Fulgosi, H., Andersson, B. & Herrmann, R. G. (1999) *Biochemistry* 38: 14955–14965.

OPTIMAL LEAF NITROGEN CONTENT OF AN EVERGREEN UNDERSTOREY PLANT IN A TEMPERATE CLIMATE

Onno Muller^{1,2}, Kouki Hikosaka², Niels P. R. Anten¹, Tadaki Hirose², Marinus J. A. Werger¹. ¹Dept. of Plant Ecology Utrecht University P.O.Box 80084. 3508 TB Utrecht, The Netherlands. ²Graduate School of Life Sciences, Tohoku University, Aoba, Sendai, 980-8570, Japan

Keywords: optimal leaf nitrogen, carbon gain, NUE, photosynthetic acclimation, *Aucuba japonica*

INTRODUCTION

In a temperate climate, evergreen species in the understorey are exposed to large seasonal changes in light and temperature. Photosynthetic activity reacts directly to changes in temperature and light. The light utilization (LU) function of the photosynthetic apparatus (electron transport proteins, ATP-synthesis and carbon reduction cycle) is more sensitive to temperature than to light while the reverse holds for the light harvesting function (LH, chlorophyll-protein complexes). Acclimation of the photosynthetic apparatus to changes in either light or temperature by which a balance between the amount of LU and LH components are maintained are well documented (e.g., Berry & Björkman 1980, Björkman 1981, Evans & Seemann 1989). But acclimation to simultaneous changes in light and temperature has been less studied (e.g., Huner et al 1998), and not at all under natural conditions.

Photosynthesis also depends strongly on the amount of leaf nitrogen, the main component of the photosynthetic enzymes. Hirose (1984) proposed the optimal leaf nitrogen content as the nitrogen content that maximizes the carbon gain per unit leaf nitrogen. This hypothesis has been used to predict leaf nitrogen contents under different light conditions (e.g., Anten et al 1996, Hikosaka & Terashima 1995, Hirose & Werger 1987). However evergreen understorey species in temperate forests experience large seasonal differences in temperature and light. Do such species show seasonal changes in nitrogen contents in accordance with the optimization theory? Does temperature or light have the strongest effect on photosynthetic acclimation under natural conditions in a temperate climate?

MATERIALS AND METHODS

For this study we used *Aucuba japonica* Thunb. a common evergreen shrub with wide distribution on the main island of Japan. Its leaf longevity is 2-3 years and new leaves are formed in a single flush in spring (Yamamura 1986). We studied plants growing at three sites with different light regimes: understorey of temperate forest, understorey of evergreen forest, and in a gap of 20 × 20 m in a mixed forest. All sites are located in the Botanical garden of Tohoku University within 150 meters of each other. Every month, photosynthesis (LI6400, li-Cor, Lincoln, NE) was measured at light intensities of 0, 10, 25, 50 and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and at average temperature of two weeks before measurements. The leaves used were fully exposed to the local light climate. The PFD and temperature in each site were determined every 15 minutes. After photosynthesis measurements

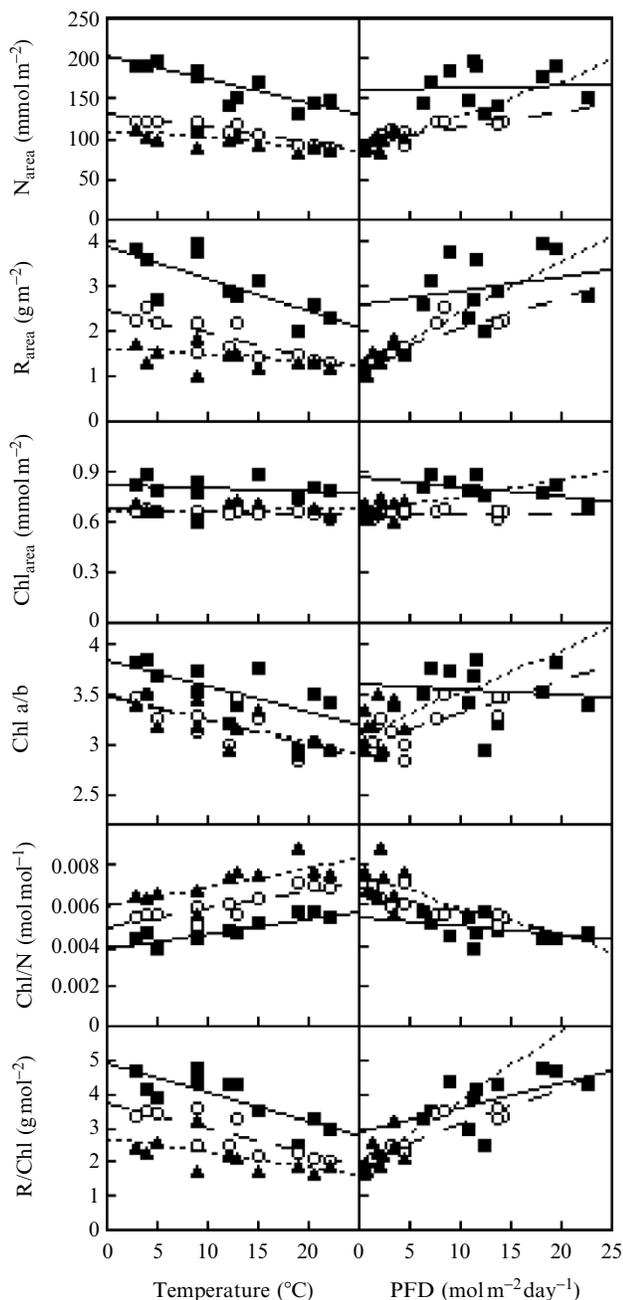


Figure 1: Photosynthetic components in relation to temperature and PFD: leaf nitrogen content per area (N_{area}), Rubisco content per area (R_{area}), Chlorophyll content per area (Chl_{area}), Chlorophyll a/b ratio (Chl a/b), Chlorophyll/Nitrogen ratio (Chl/N) and Rubisco/Chlorophyll ratio (R/Chl). Linear regression lines are for different sites; GAP (closed squares, solid line) DEC (open circles, broken line), and EVER (closed triangles, dotted line).

the leaves were taken to the laboratory for determination of leaf nitrogen content (N_i), Chlorophyll, Chlorophyll a/b ratio and rubisco (Muller et al 2005).

Model. The carbon exchange rate (CER) was calculated assuming a photosynthesis light response curve expressed as a non-rectangular hyperbola:

$$\text{CER} = ([I\phi + P_{\text{max}} - \{(\phi I + P_{\text{max}})^2 - 4\phi IqP_{\text{max}}\}^{1/2}]/2q) - R \quad (1)$$

Where I = photon flux density ($\text{mol m}^{-2} \text{s}^{-1}$), P_{max} = gross photosynthesis rate under saturating PFD ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), ϕ = quantum

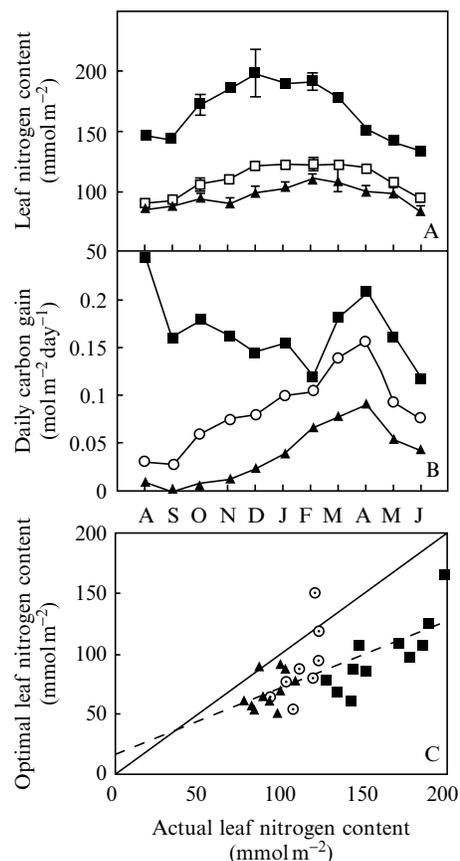


Figure 2: Monthly changes from August 2001 to July 2002 in leaf nitrogen content (A) in average daily carbon gain (B) and the relationship between actual and optimal leaf nitrogen content ($y = 0.56x + 15.18$ $r^2 = 0.48$) (C) for *A. japonica* growing in the GAP (closed squares), under DECiduous canopy (open circles) and EVERgreen canopy (closed triangles). Values in (A) are 3–6 leaves \pm S.E.

yield ($\mu\text{mol CO}_2 \mu\text{mol}^{-1}$), (q = convexity (dimensionless) and R = respiration ($\mu\text{mol CO}_2 \text{ m}^{-1}$).

The convexity factor, respiration and quantum yield were set to the average measured values of 0.9, $0.2 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ and $0.04 \mu\text{mol CO}_2 \mu\text{mol}^{-1}$, respectively.

P_{max} measurements for each month were fitted to N_i using a curvilinear relationship. Carbon gain for a 2-week period was then calculated with Eq. 1 using measured PFD values. Optimal N_i values for maximum carbon gain were determined by means of iteration.

The x-intercept of the N_i -Rubisco relation (29.8 mmol m^{-2}) did not change during the year and was used for the x-intercept of the curvilinear N_i - P_{max} relationship.

RESULTS AND DISCUSSION

Photosynthetic Acclimation to PFD and Temperature. Most of the components of the photosynthetic apparatus were strongly related with both PFD and temperature (Fig. 1). Low temperature and high PFD increased the amount of LU components. The photosynthetic apparatus acclimated to the light regime in which the leaves developed. But in developed leaves, in spite of large seasonal variation in PFD, acclimation occurred primarily in response to temperature and secondarily to PFD. This was shown by multiple

regression analysis in Muller et al (2005) but can also be seen in Fig. 1.

Carbon Gain, NUE and Optimal Leaf Nitrogen Content. The benefit of seasonal acclimation to temperature and PFD at the sites is analyzed in terms of carbon gain and carbon gain per unit nitrogen. The leaf nitrogen content showed significant seasonal changes (Fig. 2A) and was strongly correlated to temperature and light (Fig. 1). Carbon gain was highest in April for plants under deciduous and evergreen canopies (Fig. 2A). In the gap the carbon gain was highest in August with a second maximum in April. Under the deciduous and evergreen canopies, carbon gain was highest at low temperatures and relatively bright conditions in winter. In the gap most of the carbon was gained in the summer and at the end of winter.

The NUE can be inferred from Fig. 2A and B and was highest in summer in the gap. NUE from December through June was equal in the gap and under the deciduous canopy, with a peak in April. NUE under the evergreen canopy also peaked in April but was lower throughout.

Optimal nitrogen content was closely related to the actual leaf nitrogen content (Fig. 2C), though the actual leaf nitrogen content was higher than the optimal leaf nitrogen content.

In conclusion actual nitrogen content is strongly correlated with calculated optimal nitrogen content, and the actual is always higher than the optimal content. Actual nitrogen content changes seasonally and is highest in winter. The higher leaf nitrogen content in winter increases photosynthesis both per unit leaf area and per unit nitrogen.

ACKNOWLEDGMENTS

We would like to thank R.Oguchi and Y.Onoda for help during the study, the Botanical Garden of Tohoku University for allowing use of their forests. Financial support came from the Dutch Schure-Beijrinck-Popping-fund, VSB-fund and Funke-fund, and the AIEJ Short-term student exchange promotion scholarship, the MEXT scholarship and in part by the Ministry of Education of Japan.

REFERENCES

- Anten, N. P. R., Hernandez, R. & Medina, E. (1996) *Funct. Ecol.* 10: 491–500.
- Berry, J. & Björkman, O. (1980) Photosynthetic response and adaptation to temperature in higher plants. *Annu. Rev. Plant Physiol.* 31: 491–543.
- Björkman, O. (1981) In: *Encyclopedia of Plant Physiology*, new series, Vol. 12A (O. L. Lange, P. S. Nobel, C. B. Osmond & H. Ziegler, Eds.) pp. 57–109. Springer-Verlag, Berlin.
- Evans, J. R. & Seemann, J. R. (1989) In: *Photosynthesis* (W. R. Briggs, Ed.) pp. 183–205. Liss, New York.
- Hikosaka, K. & Terashima, I. (1995) *Plant Cell Environ.* 18: 605–618.
- Hirose, T. (1984) *Ann. Bot. (Lond.)* 54: 695–704.
- Hirose, T. & Werger, M. J. A. (1987) *Physiol. Plant* 70: 215–222.
- Huner, N. P. A., Oquist, G. & Sarhan, F. (1998) *Trends Plant Sci.* 3: 224–230.
- Muller, O., Hikosaka, K. & Hirose, T. (2005) *Oecologia* (in press).
- Yamamura, Y. (1986) *Botanical Magazine-Tokyo* 99: 323–332.

OJIP CHLOROPHYLL A FLUORESCENCE MEASUREMENT TO ASSESS THE EFFECT OF PESTICIDE-COPPER MIXTURES ON DUCKWEED PHOTOSYNTHESIS

Philippe Eullaffroy, Cécile Frankart, Sylvie Biagianti. Laboratoire d'Eco-Toxicologie. Unité de Recherches "Vignes et Vins de Champagne" (UPRES EA 2069). Université de Reims Champagne-Ardenne, 51687 Reims 02 – France

Keywords: Chlorophyll fluorescence, Pesticides, Duckweed, Copper

INTRODUCTION

The substantial use of copper sulfate during the past hundred years as a fungicide increased the input of copper into the aquatic environment through surface water runoff. Since this heavy metal is often in contact with other pesticides, we investigated the effects of three herbicides (diuron, paraquat, flazasulfuron) and copper alone and in combination on the photosynthetic apparatus of an aquatic plant, *Lemna minor*. These effects were determined by means of the Chl fluorescence transient O-J-I-P. Beside the results we demonstrated that Chl fluorescence analysis from polyphasic kinetics can be used as a useful physiological tool to assess early change in photosynthetic performance of *Lemna minor* in response to contaminant mixture pollutions.

MATERIALS AND METHODS

Plant material. *Lemna minor* was cultured (21 °C, 100 $\mu\text{E m}^{-2} \text{s}^{-1}$) in a mineral medium (pH 6.5) (Chollet 1993).

Chlorophyll fluorescence. Transients were measured by a PEA fluorometer (Hansatech, England). The following equations were used to assess the photosynthetic performance of duckweed: the maximum efficiency of PSII photochemistry in the dark-adapted state, $F_V/F_M = (F_M - F_O)/F_M$; $V_j = (F_{2ms} - F_O)/(F_M - F_O)$, and the fraction of inhibited centres, $FIC = (V_{j\text{contaminated}} - V_{j\text{control}})/(1 - V_{j\text{control}})$. The F_V/F_M and V_j parameters were calculated according to Genty (Genty et al 1989) and Appenroth (Appenroth et al 2001), respectively.

Pesticide applications. Plants were exposed 48 h to copper at concentrations of 5, 50, 100, 300 $\mu\text{g l}^{-1}$ of Cu^{2+} and to diuron, paraquat and flazasulfuron at concentrations of 5, 10, 20 and 100 $\mu\text{g l}^{-1}$. The concentration of copper was fixed at 50 $\mu\text{g l}^{-1}$ when combined with other pesticides.

Combination of copper and diuron, paraquat or flazasulfuron. Possible interaction between two chemicals were estimated using Abbott's formula (Gisi 1996). A ratio of inhibition (RI) was then calculated. A RI > 1 indicating potential synergism; RI = 1, a simple additivity, and RI < 1, an antagonism between the two chemicals.

RESULTS AND DISCUSSION

Copper decreased the fluorescence intensity at the F_M level without significant change (curve not shown) of the curve showing a very slight decrease of F_V/F_M (3 to 5%) (Fig. 1). Copper also induced an increase by 25% of the inhibited centres (Fig. 2). These effects were attributed to an inhibition of the acceptor (quinones) and donor side (OEC) of the PSII, respectively (Barón et al 1995). Diuron induced a