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Seasonal changes in the photosynthetic capacity of cones on a larch (*Larix kaempferi*) canopy

W.-J. WANG[*], Y. WATANABE[**], I. ENDO[***], S. KITAOKA[***], and T. KOIKE[***,+]

Key Laboratory of Forest Plant Ecology, Northeast Forestry University, Harbin 150040, P. R. China [*]
Hokkaido University Forests, FSC, Sapporo 060-0809, Japan [**]
Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan [***]

Abstract

The seasonal changes of photosynthesis of cones of Japanese larch (*Larix kaempferi* Carr.) trees showed that gross photosynthetic rate of young cones (P_G) was 2–3 μmol m^{-2} s^{-1} at surface area unit and P_G/R_D (dark respiration of cones) peaked about 0.7 in the same period, indicating that 70 % of respiratory CO_2 was re-fixed. With maturation, P_G and P_G/R_D sharply decreased. Chlorophyll content in cones was 3–20 % of that in leaves, which made it a limiting factor for photosynthesis and its content was closely correlated with photosynthetic capacity. Although sunken and linearly arranged stomatal organs were found on the scale of young cones, differently from the significant regulation of leaf photosynthesis, these stomata tended to be non-functional since CO_2 is not limiting factor for cone photosynthesis. Thus photosynthesis of larch cones is an additional contribution to their development.

Additional key words: chlorophyll; re-fix ratio; respiration; seasonal changes; stomata.

Introduction

Japanese larch (*Larix kaempferi*) produces large amount of cones in masting year, which need large supply of photosynthates. Although young larch cones are green, little information is available on the significance of their chlorophyll (Chl) content for cone development (Wang *et al*. 2001a,b). If this green matter could fix CO_2, how large is its capacity at different stages of cone development and how much of the respiratory CO_2 could be re-fixed? A seasonal and annual study on cone gas exchange traits could answer these questions.

Furthermore, the two substrates for photosynthesis are the photons captured by Chl and the CO_2 supplied *via* stomata diffusion from atmosphere or respiratory internal cycling. These substrates could limit photosynthetic capacity. In healthy leaves, photon capture by Chl seldom affects photosynthesis (Larcher 2003) and significant stomatal regulation could be manifested by the close correlation between stomatal conductance (g_s) and photosynthetic rate (Farquhar and Sharkey 1982, Wang *et al.* 2001b, 2003). However, cone structure and function are different from that of leaves. For example, although stomata exist on non-photosynthetic organs, their amount is small compared with that on leaves and also there are no reports on larch species (Blanke 1993, Peschel *et al.* 2003, Wang 2005). Chl in cone scale gradually degrades with development, while Chl in leaves is stable during the same period. Moreover, respiratory activity of cones is much higher than that of leaves (Kozlowski and Pallardy 1997, Wang 2005). It is expected that these differences may influence the relations between Chl, g_s, and gross photosynthetic rate (P_G).

For understanding the photosynthetic traits of Japanese larch cones, we determined seasonal changes in P_G measured *in situ*, Chl content, anatomical structure of cone scale, and g_s.

Materials and methods

Study site and plants: This study was carried out in a plantation of Japanese larch (*Larix kaempferi*, 49-y-old in 2004) at the Sapporo Experiment Forest (43°44'N, 141°31'E) in Northern Japan. The altitude is *ca*. 60 m

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[+]Corresponding author; fax: (+81)-11-706-3450, e-mail: tkoike@exfor.agr.hokudai.ac.jp
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a.s.l., the average tree height is 18–20 m, and the soil is brown forest soil mixed with volcanic ash. A 15-m step was erected for gas exchange measurements.

Gas exchange: Dark respiration rate (R_D) and P_G of cones were measured by a *LI-6400* portable photosynthesis system *in situ* using a conifer chamber. After at least 5-min balance of intact cones in chamber, P_G was recorded at full sunlight, and R_D was measured after covering the chamber by aluminium foil. Five cones were measured in each time. P_G was computed as the difference between photosynthetic rate at saturation irradiance and R_D. Under full sunlight, the role of photorespiration is important to avoid photoinhibition (Kozaki and Takeba 1996). Further study will be needed to access the role of photorespiration in cone development in the field. Data were recalculated according to the surface area of the larch cone. This area was calculated by the formula of a cone-shape as follows:

$$A = \pi a\,(a + \sqrt{a^2 + b^2}) \quad (1),$$

where A is the surface area of cones, a is half of the cone width, and b is height of cones. g_s of cones was measured simultaneously with P_G and recalculation was done according to Eq. 1.

Chl content of cone scale and needles was measured by DMSO method as described by Barnes *et al.* (1992) and Shinano *et al.* (1996).

Anatomical observations: To check the cone scale structure and stomatal aperture, the transverse slices of the cone scale were prepared. The transverse slices were frozen in a drop of distilled water and sectioned with a sliding microtome. The cone scale structure on the transverse sections (about 30 μm thick) was tested by a conventional light microscope (*Axioskop2 Plus*, *Zeiss*, Germany).

Results

Seasonal changes in photosynthesis: Young cones had a fairly high resulting gas exchange rate, even near 0 μmol m^{-2} s^{-1} in saturation irradiance. With cone development, photosynthesis became less sensitive to increasing PPFD (photosynthetic photon flux density) (Fig. 1). P_G peaked 2–3 μmol m^{-2} s^{-1} in young cones, while it decreased sharply with cone maturation. It differed in 2001 and 2004, and showed about 0.1–0.5 μmol m^{-2} s^{-1} in September and completely no photosynthesis in October (Fig. 2*A*). Peak of ratio of P_G and R_D was about 0.7 in young cones; and then it sharply decreased to zero in October.

Chl content in cone scale peaked at 113 g kg^{-1}(FM) in young cone, which was 20 % of that in needles. It gradually decreased in July and August, was only 20 g kg^{-1}(FM) in September (7 % of that in needles), and almost no Chl was found in October (Fig. 2*B*).

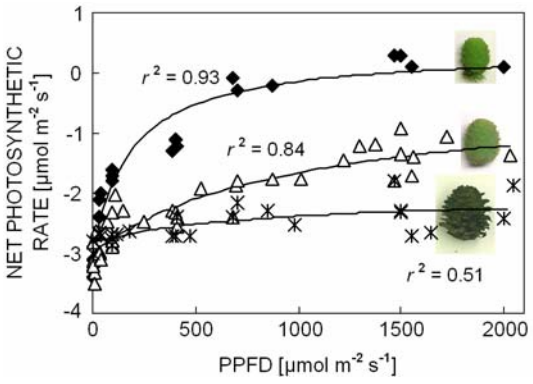

Fig. 1. Irradiance response curves of cone net photosynthetic rate at different phases of development: ♦ green, 15 June; Δ brown green, 20 July; ⁎ mature, 20 September.

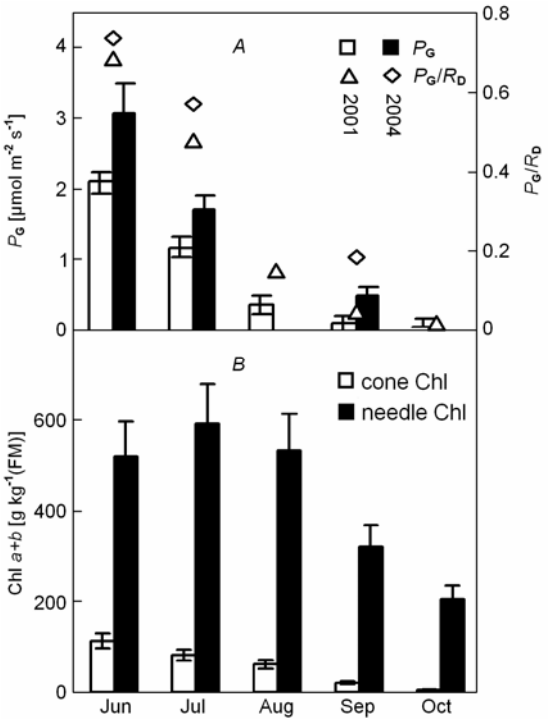

Fig. 2. *A*: Seasonal changes in gross photosynthetic rate (P_G) and ratio between P_G and dark cone respiration (R_D) measured in 2001 and 2004. *B*: Changes of chlorophyll content in cones and leaves during development of larch cones.

Anatomical structure: "Stomata-like" apparatus was observed on cone scale. Moreover, these sunken stomata were linearly arranged and trichomes were frequently observed (Fig. 3*A*). Typical stomata on cone scale included two guard cells and four subsidiary cells (Fig. 3*B*). In the cross section of stomata, two guard cells

were clearly observed and chloroplasts were also observed in other cells (Fig. 3*C*). However, when the cone became mature, chloroplasts disappeared and stomata were only hardly recognised (Fig. 3*D*).

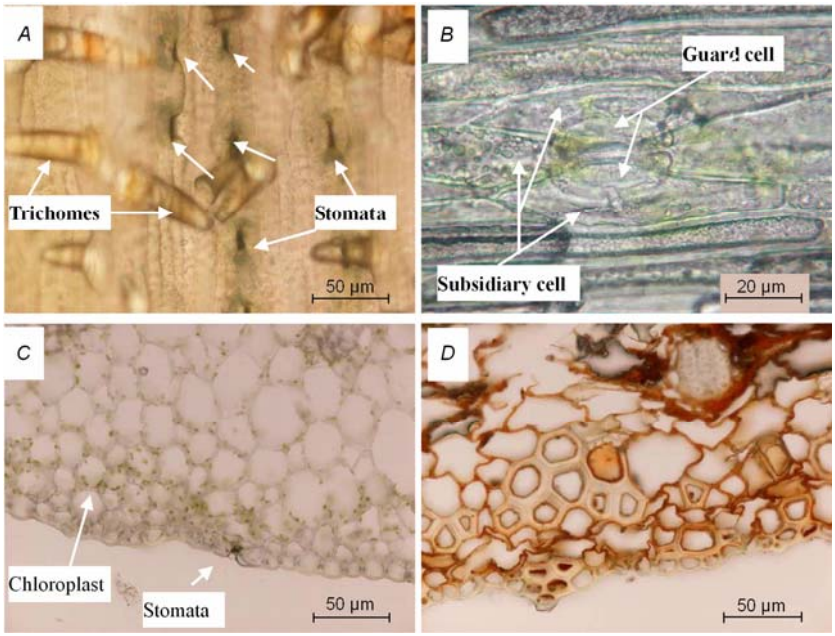

Fig. 3. The structure of stomata on cone scale. *A*: Sunken stomata linearly distributed on the scale of young cone (June 2004). *B*: Guard cells and subsidiary cells of the stoma. *C*: Cross section of stomata, most chloroplasts only distributed in surface layer of cone scale. *D*: Chloroplasts disappeared and stomata were also difficult to recognize when cone was mature (September 2004).

Relations between g_s, Chl content, and P_G: Chl content was closely correlated with photosynthetic capacity ($y = 0.023\ x - 0.302$, $r^2 = 0.90$, $p<0.0001$), while no correlation was found between g_s and P_G ($y = 2.532\ x + 1.001$, $r^2 = 0.10$, $p>0.01$).

Discussion

Contribution of cone photosynthesis and CO_2 re-fixation during its development: Many reproductive organs are photosynthetically active. Ogawa *et al.* (1988) found that cones of hinoki (*Chamaecyparis obtusa*) could re-fix 55–57 % of their daily respiratory CO_2, while fruits of *Cinnamomum camphora* could re-fix 17–51 % over the growth period (Ogawa and Takano 1997). Some evergreen pine cones could re-fix 50–85 % (Linder and Troeng 1981, Aschan and Pfanz 2003), olive fruits 40–80 % (Proietti *et al.* 1999), and orchid fruits 10–60 % (Zotz *et al.* 2003). Our studies showed that maximum P_G was 2–3 μmol $m^{-2}\ s^{-1}$, which took *ca*. 70 % of respiratory CO_2, while in mature cones, no photosynthesis was observed. This value is within the range of previous reports. Therefore, the green of cone scale made the cone a better user of respiratory CO_2 during the cone development (Aschan and Pfanz 2003).

Chl content determines photosynthetic capacity: Although Chl is not a limiting factor in most of the healthy leaves in nature (Larcher 2003), the Chl in young cone scale is only 20 % of that in leaves, which significantly limits the photosynthetic capacity of cones (Fig. 2). Trichomes and compact and waxy surface of cone scale may also decline the penetration of photons into the internal space. Thus, the photosynthesis of cone scale is limited by the light reactions of the photosynthetic process *via* Chl deficiency.

Marginal function of stomata in photosynthesis: The stomatal regulation of leaf photosynthesis is due to the shortage of substrate CO_2 *via* stomata diffusion when the conductance of stomata is too small (Jones 1992). It can be manifested by the close correlation between g_s and photosynthesis (Farquhar and Sharkey 1982, Wang *et al.* 2001b, 2003). However, no such correlation was found in cone photosynthesis. Hence, stomata of cone can not regulate cone photosynthesis. The presence of almost no positive net photosynthetic rate (Fig. 1) manifests that the maximum role of cone photosynthesis is to internally recycle the respiratory CO_2. Thus, there is no need for income of atmospheric CO_2 *via* stomatal diffusion. Therefore, CO_2 as a substrate for cone photosynthesis is not a limiting factor for cone photosynthesis, reflecting the absence of stomatal regulation in cone photosynthesis.

In conclusion, the green of cone scale could recycle as much as 70 % of total respiratory CO_2, which might contribute photosynthates for the cone development. Chl as a photon receptor strongly limited cone photosynthesis, while CO_2, as another substrate for photosynthesis, did not limit photosynthesis. This made the stomata non-functional in regulating photosynthesis of cones.

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