Jour. Korean For. Soc. Vol. 95, No. 5, pp. 614~620 (2006)

JOURNAL OF KOREAN FOREST SOCIETY

Changes in Chlorophyll Contents and Photosynthetic Characteristics of Hardwood Species According to Artificial Shade Treatment

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Abstract : To study the chlorophyll contents and photosynthetic characteristics of 4 tree species of deciduous hardwoods; *Betula platyphylla* var. *japonica*, *Zelkova serrata*, *Acer mono* and *Prunus sargentii* were treated in 3 stages of shading; the full sun treatment, the medium shade treatment with 30% of transmittance comparing to full sun, the intense shade treatment with 8% of transmittance and their changes in chlorophyll contents and photosynthetic characteristics were examined and analyzed. Most hardwoods showed differences in the total chlorophyll contents in the order of May < September < July, however, that in *Prunus sargentii* increased progressively along with the lapse of time. Concerning the degree of shading, total chlorophyll contents increased in proportion to the level of shading. *Betula platyphylla* var. *japonica* and *Prunus sargentii* showed more than 2-3 times difference between the full sun treatment and the intense shade treatment. The changes in photosynthetic characteristics, the range of the light saturation point of the trees was 1,000~1,100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in May, before the shading was applied, and the intensity was shown in the order of *Betula platyphylla* var. *japonica* > *Zelkova serrata* > *Acer mono* > *Prunus sargentii.* The photosynthetic rate was 6.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$~27.1 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$ in the order of *Betula platyphylla* var. *japonica* > *Prunus sargentii* > *Acer mono* > *Zelkova serrata* that there were differences between species. Concerning the changes in light saturation point in each growth period after shading treatment, the light saturation point in the full sun treatment was found in the range of 560~1,100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and the level of intensity was shown in the order of May > July > September. The light saturation point decreased as the level of shading intensified and the level of changes in light compensation point in the full sun treatment for *Betula platyphylla* var. *japonica* and *Prunus sargentii* was shown in the range of 2.9~27.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in the order of May > July > September, however, for *Zelkova serrata* and *Acer mono* was shown in the range of 3.9~11.7 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$ in the order of July > May > September that there were differences between species.

Key words : Betula platyphylla var. japonica, Zelkova serrata, Acer mono, Prunus sargentii, *Chlorophyll contents, Photosynthetic characteristics*

Introduction

The forest is an organism that reacts to continuous and interactive factors of the environment and clarifying the relationship with the condition of the forest. The inorganic environmental factors is a concrete way to know the growth and development of trees constituting the forest, the enhancement of productivity of the forest, and so forth. Also, the forest forms a unique environment with the crown of forest trees such as the light within the forest, temperature and humidity that strongly influences the type and appearance rate of understory vegetation, the growth and survival of young trees (Choi, 2001; Lee *et al*, 1997; Lee and Woo, 2000; Kramer and Kozlowski, 1979). Especially, the light gives impact on various inorganic environment along with water within the forest ecosystem and it provides direct and indirect causes inducing the loss of moisture contained in the tree by accelerating the transpiration of leaves. The studies to find out the physiological and ecological impact of light have been made through many researchers in a long term in advanced countries and many results have been reported. The physiological and ecological studies related

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with moisture and light have been progressed by a few researchers that this were considered as an important task that needs more research efforts (Alberte *et al*, 1977; Ashton and Berlyn, 1994; Bahari *et al*, 1985; Boardman, 1977; Castrillo, 1983; Kramer, 1983; Lee *et al*, 2004, Sim and Han, 2003). Therefore, there are problems in applying the results of studies made in overseas directly to our forest where the composition of trees and ecological environment of forest are different while there was the need for study results by the tree species unique to Korea. This study analyzed the impact of artificial shade treatment on the chlorophyll contents and photosynthesis by seasonal changes with the seedlings of 4 hardwood species in order to find out the physiological characteristics of each species including light requirements and shade tolerance. The purpose of this was to provide basic data required for developing the landscaping and tree, urban forest and forest managing technologies to improve the shade tolerance of trees.

Material and Methods

1. Materials

The subjects of this study were the major species of hardwood trees such as *Betula platyphylla* var. *japonica*, *Zelkova serrata*, *Acer mono* and *Prunus sargentii.* Approximately 2-year-old seedlings were planted repeatedly at intervals of 15 cm×15 cm in a seedbed ground at Chungnam National University. After taking their roots for a certain period, the seedlings underwent artificial shade treatment with the shade levels divided into 3 levels, using a glare screen to provide the degrees of shade as shown in Figure 1. To determine the light quantity being transmitted under the shade screen at each time zone per day on the basis of sunny days in May, July and September was measured repeatedly in the vertical direction to toward the sun. The light quantity at midday in each month showed some variation in each month. The maximum quantity of light was about 1,600~1,700 $\mu E \cdot m^{-2} \cdot s^{-1}$ in May, 1,700~1,900 $\mu E \cdot m^{-2} \cdot s^{-1}$ in July and about 1,900~2,100 $\mu E \cdot m^{-2} \cdot s^{-1}$ in September. As for changes during the course of the day, the light quantity from morning till midday showed similar changes in each month only to show a sudden decrease as the sun went down after 2 p.m. With the control (A) being assigned a total light treatment values of 100%, the relative light quantity in each artificial shade treatment control(B) was 30% for the medium shade treatment, and the intense shade treatment with 8% for the C treatment control, respectively. Thus, the treatment controls showed wide variation compared with the total light treatment as the intensity of shade increased.

2. Analysis of chlorophyll contents

To study the artificial shade treatments on the chlorophyll contents of 4 tree species of deciduous hardwoods were treated in 3 levels of shadings. Tree seedlings were randomly selected for three periods; May which was before shade treatment, July when the leaves were

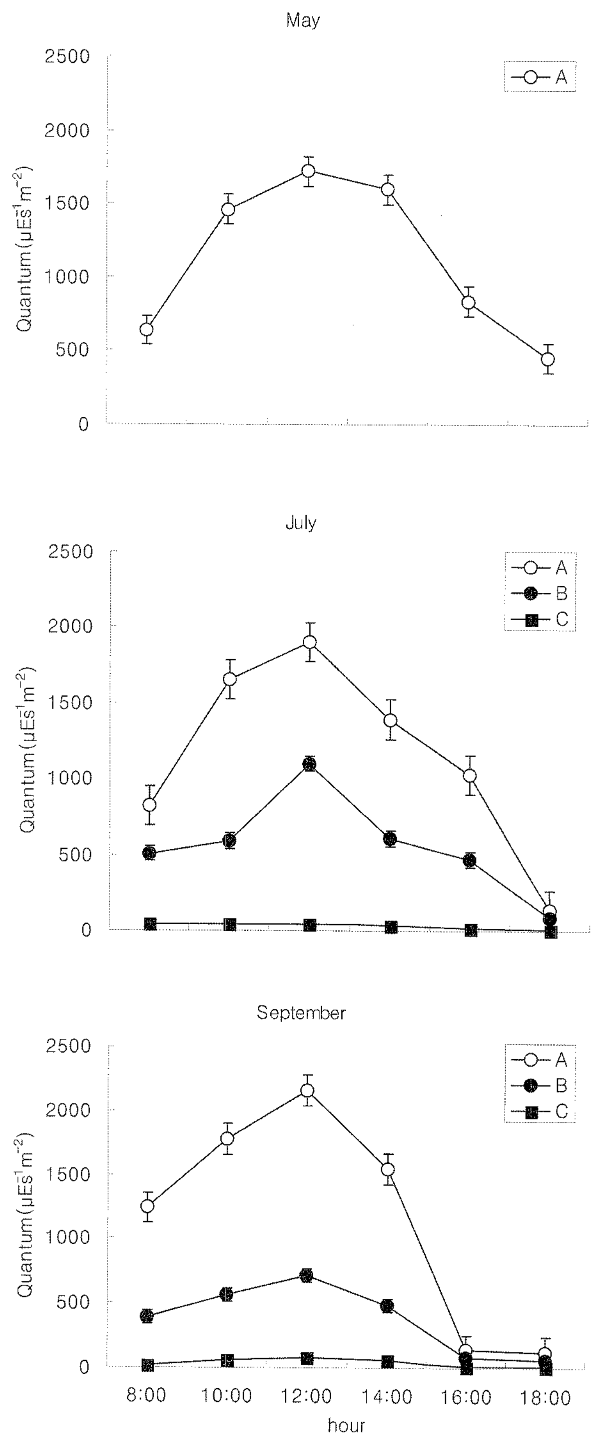

Figure 1. Seasonal and diurnal changes of light intensity in three treatment levels of artificial shading in sunny days showing the light intensity of A(θ) curves. *Light transmittances; A : 100%, B : 30%, C : 8%

maturing during the shade treatment and September when the tree's physiological metabolic activities slowed down, and the chlorophyll contents were analyzed 3 times for each seedling, total 9 times, in order to analyze the seasonal changes of chlorophyll contents according to the level of shade treatment.

For the extraction of chlorophyll contents, the method using Dimethylsulfoxide (DMSO) conforming to Hiscox and Israelstam (1978) method was applied, and DMSO 10 ml was placed into a capped test tube (15 mm× 12.5 cm), 0.1g of leaf in the central region was precisely weighed with a electric balance capable of measuring down to the level of 0.1 mg and inserted into the test tube prepared and immediately placed into an electric water bath heated to 65 ± 1°C for approximately 6-7 hours to measure the chlorophyll content.

After a fixed period time, the light absorption was measured from the extract inside the test tube, the leaf tissue of which turned light brown, in the wavelength of 66 nm and 645 nm using spectrophotometer and obtained chlorophyll contents a and b using the expression of Arnon (1949) and Mackinney (1941).

Chlorophyll a ($mg \cdot g^{-1} \cdot freshwt$) =
$(0.0127 \times OD663 - 0.00269 \times OD645) \times f$
Chlorophyll b ($mg \cdot g^{-1} \cdot freshwt$) =
$(0.0229 \times OD645 - 0.00468 \times OD663) \times f$
*OD : Optical Density

In this expression, OD663 and OD645 represent the light absorption at 663 and 645 nm respectively, and f is the function related to the fresh weight and the dilution factor according to DMSO contents. The measured contents was converted into the chlorophyll contents per dry weight by measuring the moisture contents of the leaves.

3. Net photosynthesis measurement

To analyze the photosynthetic characteristics of the experimental trees in relation to shade treatment, the time-serial changes such as photosynthetic rate, light saturation point and light compensation point were examined and compared by each species and the level of shade treatment using portable photosynthesis measuring device in three periods; May which was before shade treatment, mid July when the leaves are maturing during the shade treatment, and early September when the growth of the tree slows down.

Each examination avoided rain, the day before and after rain and chose clear days to reduce the impact different to each other according to the changes of moisture conditions and eliminate the impact by high intensity of light.

Light-saturated net photosynthesis (An) was measured on fully expanded, mature leaf number 4 counted from each shoot apex on every individual in the treatments. Net photosynthesis was measured with a broad-leaf cuvette of the Li-cor 6400 Potable Photosynthesis System (Li-cor Inc., USA), the leaf was sealed and CO_2 concentration was allowed to be maintained at ambient levels. Air flow through the analyzer was adjusted to maintain leaf cuvette relative humidity near ambiance level (70 ± 10%) during measurement. The average cuvette temperature was maintained at 25°C. For photosynthesis capacity, this study measured the light-photosynthesis curve by adjusting the light intensity from 0 to 2,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with a photosynthesis analyzer (Choi, 2001; Kwon *et al*, 2006).

Net Photosynthesis was calculated as :

$$A_n = A_n = \frac{u_e(c_e - c_c)}{100s} - c_c E$$

A_n; Net Photosynthesis ($\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$), u_e; mole flow rate of air entering the leaf chamber ($\mu mol \cdot s^{-1}$), c_e; mole fraction of CO_2 in the leaf chamber ($\mu mol \cdot CO_2 \cdot mol^{-1}$ air), c_c; mole fraction of CO_2 entering in the leaf chamber ($\mu mol \cdot CO_2 \cdot mol^{-1}$ air), s; leaf area (cm^2), E; transpiration ($mmol \cdot H_2O\ m^{-2}s^{-1}$)

This study also performed a regression analysis and obtained photosynthesis curves using SigmaPlot(SPSS Inc.) to estimate precise light-photosynthesis curves and light compensation points.

$$y = ax / (b + x)$$

A light compensation point was obtained when y = 0 in the equation above.

Results and Discussion

1. Analysis of changes in chlorophyll contents

Among tree species, most hardwoods showed differences in chlorophyll contents within the range of 2.70~4.50 mg/g < 3.62~5.91 mg/g < 2.93~7.34 mg/g in the order of May < September < July however *Prunus sargentii* showed steady increase in proportion to the progress of the season. By the level of shade treatment (Figure 2), most species showed the increase of chlorophyll contents in proportion to the intensity of shade treatment. The chlorophyll contents in *Betula platyphylla* var. *japonica* and *Prunus sargentii* were 2.72~5.91 mg/g in full sun treatment, 6.56~9.24 mg/g in medium shade treatment with 30% of transmittance and 8.25~12.10 mg/g in intense shade treatment with 8% of transmittance that there was approximately 2~3 times much difference according to the intensity of treatments. Although there are some exceptions, *Zelkova serrata* and *Acer mono* showed the increase of chlorophyll contents in proportion to the intensity of shade treatment.

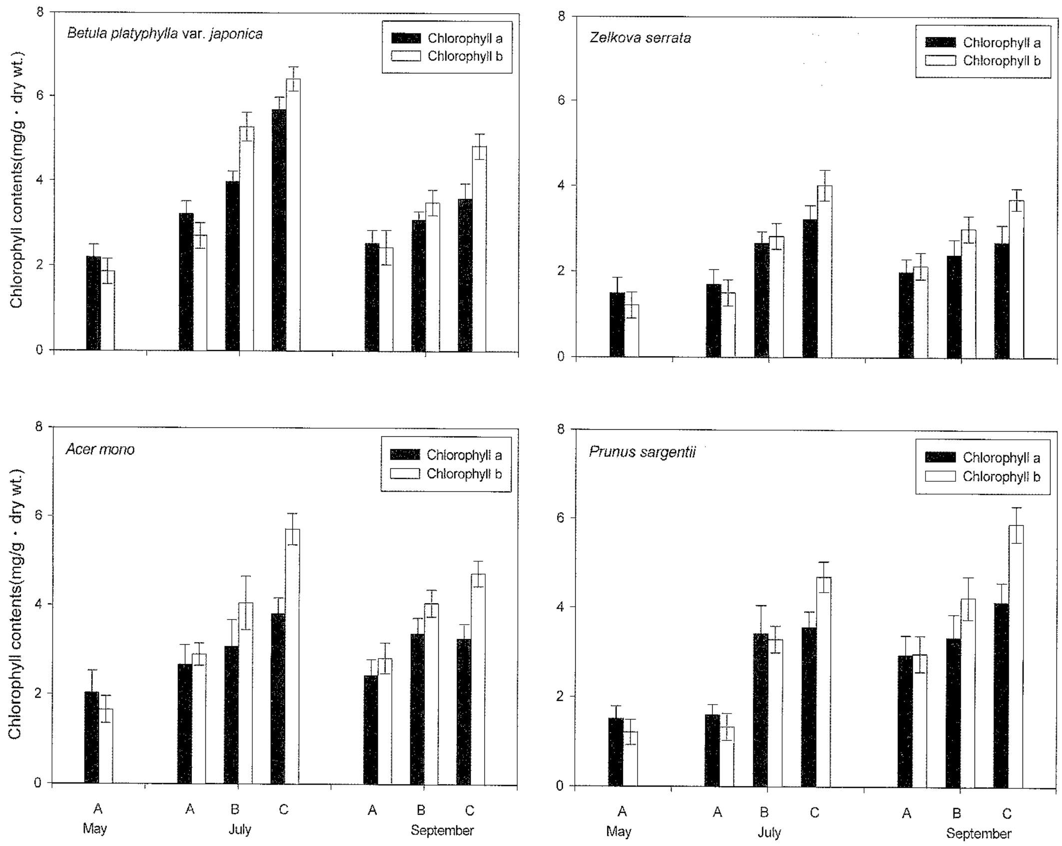

Figure 2. The effect of shade in temporal changes of chlorophyll contents of four broad leaved tree species subjected to three levels of artificial shade treatment.
***Light transmittances; A : 100%, B : 60%, C : 8%**

Especially, the chlorophyll contents a and b showed differences according to the level of shading and season within the range of 1.49~5.68 mg/g and 1.21~7.29 mg/g in overall, and both increased as the intensity of shade treatment increased and the chlorophyll contents a and b in the medium shade treatment with more or less than 30% of transmittance and in the intense shade treatment in July increased twice as much compared to the full sun (Choi, 2001; Castrillo, 1983).

The chlorophyll contents b according to artificial shade treatment increased relatively larger than a, and the overall chlorophyll contents a/b decreased in proportion to the intensity of the shade treatment. Also, as the season passed, the chlorophyll contents b increased relatively larger than a in most species in each level of shade treatment in July, and the chlorophyll contents a/b in July and September was lower than those in May (Choe and Lee, 1995; Kim, 2003; Kramer and Kozlowski, 1979).

2. Photosynthetic characteristics

To study the photosynthetic characteristics of 4 tree species of deciduous hardwoods; *Betula platyphylla* var. *japonica*, *Zelkova serrata*, *Acer mono* and *Prunus sargentii* were treated in 3 levels of shading treatment and photosynthetic characteristics were analyzed (Table 1).

The differences in photosynthetic characteristics between treatments in September were small and the light saturation point reduced as the intensity of shading increased.

Changes in light saturation point of *Acer mono* in July and September in each treatment was found within the range of 11.7~2.9 $\mu mol{\cdot}CO_2{\cdot}m^{-2}{\cdot}s^{-1}$ that the full sun treatment and the intense shade treatment showed twice to 3 times much differences, the light compensation point and the light saturation point largely decreased as the intensity of shading increased as compared with the figures in May that they tended to decrease as the intensity of shading increased. The light compensation point of *Prunus sargentii* in July was 10.1±1.5 $\mu mol{\cdot}CO_2{\cdot}m^{-2}{\cdot}s^{-1}$, 9.0±1.0 $\mu mol{\cdot}CO_2{\cdot}m^{-2}{\cdot}s^{-1}$ and 6.0±0.8 $\mu mol{\cdot}CO_2{\cdot}m^{-2}{\cdot}s^{-1}$ respectively that the full sun and the normal shading treatment with 30% of transmittance showed similar light compensation points while it relatively decreased in the intense shading treatment with less than 8% of transmittance and the light saturation point also decreased with the intensity of shading increased. Such outcomes show that the difference of luminosity of strong ray brings impact on the physiological conditions of the tree and it has

Table 1. Seasonal changes of photosynthetic rate on the light saturation points of the four deciduous tree species subjected to artificial shade treatments. (unit : $\mu mol{\cdot}CO_2{\cdot}m^{-2}{\cdot}s^{-1}$)

Species	Season	Shading level*		
		A	B	C
Betula platyphylla var. *japonica*	May	27.1±1.0		
	July	17.5±1.0	13.9±1.2	5.9±0.9
	September	2.9±1.1	2.6±0.7	2.4±0.8
Zelkova serrata	May	6.4±1.5		
	July	8.0±1.9	7.0±1.5	6.1±0.5
	September	3.9±0.8	3.6±0.9	3.4±0.7
Acer mono	May	8.5±1.1		
	July	11.7±1.5	9.0±1.1	4.7±0.8
	September	7.9±2.0	6.5±1.5	2.9±0.5
Prunus sargentii	May	17.5±2.0		
	July	10.1±1.5	9.0±1.0	6.0±0.8
	September	7.1±1.4	6.0±0.9	5.0±0.9

*Light transmittances ; A : 100%, B : 30%, C : 8%

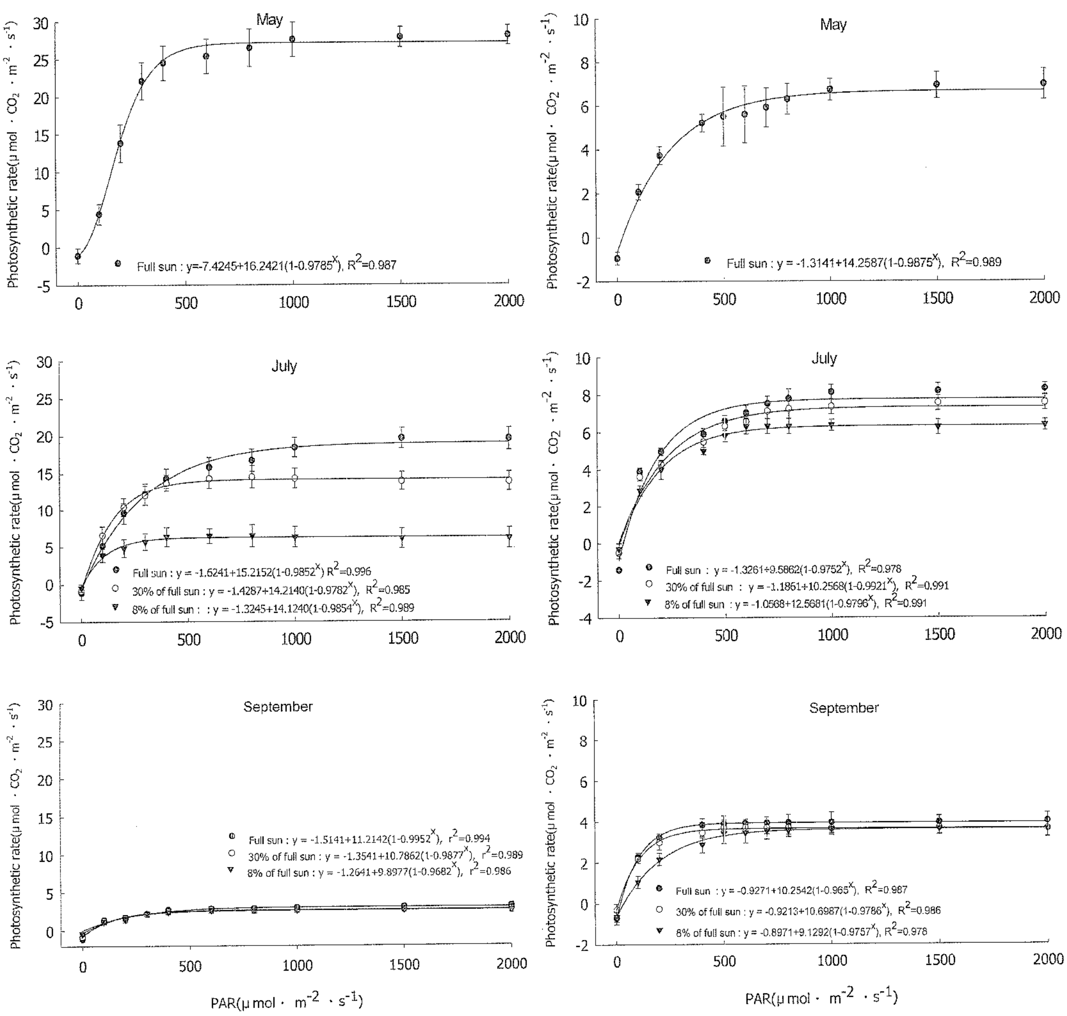

Figure 3. Effect of artificial shading treatment on the photosynthetic rate of *Betula platyphylla* var. *japonica* (left) and *Zelkova serrata* (right) seedlings subjected to artificial shade treatments. *Light transmittances; ●: 100%, ○: 60%, △: 8%

critical influence upon the photosynthesis and respiration using the light efficiency (Choi, 2001; Kim, 2003; Kramer and Kozoloski, 1979; Han and Sim, 1989; Sim and Han, 2003).

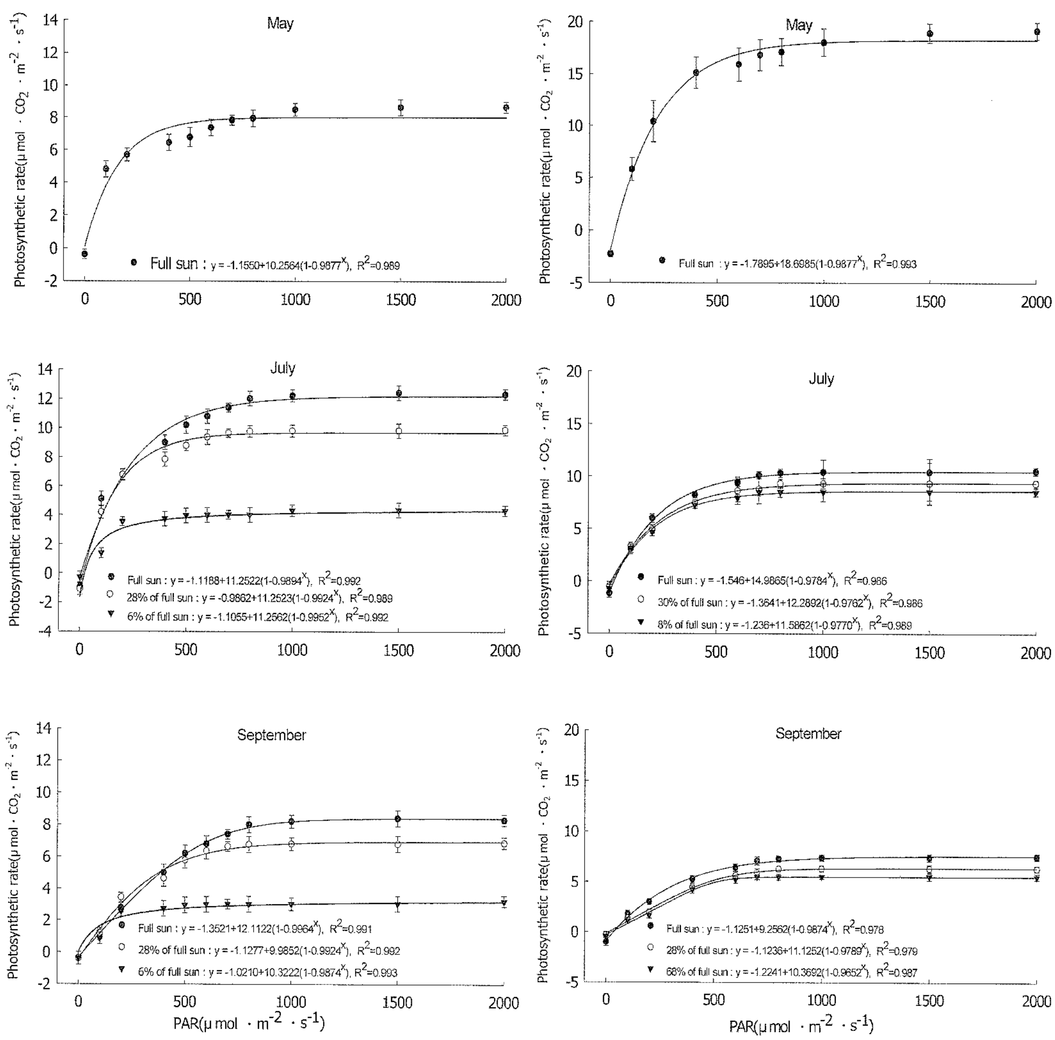

Figure 4. Effect of artificial shading treatment on the photosynthetic rate of *Acer mono* (left) and *Prunus sargentii* (right) seedlings subjected to artificial shade treatments.
***Light transmittances; ●: 100%, ○: 60%, ▽: 8%**

The light saturation point of the trees test in May, before shading were applied, was 1,000~1,100 μmolm^{-2}s^{-1}, and by species, *Betula platyphylla* var. *japonica* showed 1,100 μmol·m^{-2}·s^{-1}, *Zelkova serrata* showed 1,000 μmol·m^{-2}·s^{-1}, *Acer mono* showed 1,100 μmolm^{-2}s^{-1} and *Prunus sargentii* showed 1,000 μmol·m^{-2}·s^{-1}.

The intensity of the photosynthetic rate was shown in the order of *Betula platyphylla* var. *japonica* > *Prunus sargentii* > *Acer mono* > *Zelkova serrata* in the range of 6.4~27.1 μmol·m^{-2}·s^{-1} that there were differences between species (Figure 3, 4).

Changes in light saturation point in each growth period after shading in the full sun treatment for *Betula platyphylla* var. *japonica*, *Zelkova serrata*, *Acer mono* and *Prunus sargentii* were found within the range of 560~1,100 μmol·m^{-2}·s^{-1} in the order of May > July > September, and the light saturation point decreased as the strength of shading increased as compared with the full sun treatment; 500~800 μmol·m^{-2}·s^{-1} for the medium shade treatment with more or less than 30% of transmittance and 470~710 μmol·m^{-2}·s^{-1} for the intense shade treatment with less than 8% of transmittance.

The light compensation point in the full sun treatment for *Betula platyphylla* var. *japonica* and *Prunus sargentii* was shown in the range of 2.9~27.1 μmol·m^{-2}·s^{-1} in the order of May > July > September, however, for *Zelkova serrata* and *Acer mono*, it was found in the range of 3.9~11.7 μmol·m^{-2}·s^{-1} in the order of July > May > September.

The photosynthesis mechanism appear differently between species and depending on the environment and the age of the same species however the bio-association and each region of individual organism and plant growing in an environment with different luminosity showed different photosynthetic reaction according to the luminosity, the leaves that grew with weak sun ray had more chlorophyll contents per weight and the photosynthetic characteristics also changed accordingly. Such outcomes imply that the photosynthesis and respiration of tree are interactively influenced by various external conditions and the physiological conditions of the tree itself however the influence by the difference of luminosity brings decisive impact on photosynthesis and respiration and the light compensation point and the light saturation point show differences (Boardman, 1977; Freedman, 1989; Kim, 2003).

Concerning the photosynthetic characteristics of target tree in each shading treatment, the light compensation point of *Betula platyphylla* var. *japonica* in July was shown as 17.5±1.0 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$, 13.9±1.2 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$ and 5.9±0.9 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$ in the full sun treatment, the medium shade treatment with more or less than 30% of transmittance and the intense shade treatment with less than 8% of transmittance, respectively that it showed about 3 times much difference. The light compensation point of *Zelkova serrata* in July was 8.0±1.9 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$, 7.0±1.5 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$ and 6.1±0.5 $\mu mol \cdot CO_2 \cdot m^{-2} \cdot s^{-1}$ in respective treatments that the differences between treatment were not as much however the light compensation point decreased as the intensity of shading increased and the light saturation point was 1,000 $\mu mo \cdot m^{-2} \cdot s^{-1}$, 780 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 650 $\mu mol \cdot m^{-2} \cdot s^{-1}$ that it also tended to decrease as the intensity of shading increased.

Acknowledgments

This paper was supported by a grant from Wonkwang University in 2005

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(Received Spetember 4, 2006; Accepted October 12, 2006)