Growth, Physiological, and Biochemical Responses of Tung Tree (*Vernicia fordii*) Seedlings to Different Light Intensities

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Abstract. As a result of its high photosynthetic efficiency, the tung tree (*Vernicia fordii*) is a fast-growing heliophile, yielding fruit within 3 years. In addition, tung oil extracted from the fruit seeds is an environmentally friendly paint used widely in China. However, mutual shading inside a tung tree canopy leads to a low yield of fruit because of weak or dead lower branches. In this project, a pot experiment was conducted to understand the growth, physiological, anatomical structure, and biochemical responses of tung trees under various shading levels. Tung tree seedlings were subjected to different light intensities—100% sunlight (no cover), L100; 75% sunlight (25% shading), L75; 50% sunlight (50% shading), L50; and 20% sunlight (80% shading), L20—from June to August. Results indicate that the L75 treatment reduced significantly the net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), total aboveground and root dry weight (DW), maximum net photosynthetic rate (A_{max}), and maximum rate of electron transport at saturating irradiance (J_{max}) compared with the control, although plant height and leaf area (LA) were not reduced. Lower light intensities (L50 and L20) and longer duration of treatment led to greater reduction in growth, leaf thickness, and photosynthetic potential (A_{max} and J_{max}). Chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll content were increased in the L50 and L20 treatments compared with L100 and L75. There was no significant reduction in the enzyme activities of ribulose-1,5-bisphosphate carboxylase (Rubisco) and phosphoenolpyruvate (PEPC) of the seedlings using the L75 treatment; however, lower light intensities (L50 and L20) and longer duration of shade treatment resulted in a significant reduction in enzyme activity. In summary, the results suggest that tung trees have greater photosynthetic activity under high light intensity. Shading, even at 20%, especially for the longer term, reduced photosynthetic efficiency and growth. To prevent growth reduction, tung trees should be grown under full sun with a daily light integral (DLI) of $\approx 46 \text{ mol} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$, and mutual shading should be avoided by proper spacing and pruning.

Tung tree is a member of the Euphorbiaceae family, a native tree species that has been cultivated for more than 1000 years in China. Along with the oil-tea tree (*Camellia oleifera*), walnut (*Juglans regia*), and tallow tree (*Sapium sebiferum*), tung tree is one of the four major woody oil trees in China. Tung

trees have been cultivated in central and southern areas of China (lat. 22°15'–34°30'N; long. 99°41'–122°07'E) and are usually cultivated in mountainous and hilly areas at less than 1000 m above sea level. Mature tung trees can reach $\approx 5 \text{ m}$, with a crown circumference of $\approx 4.5 \text{ m}$ (Tan et al.,

2011). Tung oil, which is extracted from tung seeds, exhibits traits that are highly valued in many industries (Park et al., 2008; Pfister et al., 2008), including rapid drying, chemical resistance, adhesiveness, and sleekness. These properties make tung oil a valuable drying ingredient in paints, varnishes, and other coatings and finishes (Cao and Shockey, 2012; Li et al., 2017b). With recent human population increases, tung trees have become a valuable biofuel species, with the potential to help resolve energy shortage problems (Tan et al., 2011). Furthermore, tung trees grow quickly, yielding fruit within 3 years as a result of their high photosynthetic efficiency (Li et al., 2017a).

The primary problem in tung tree production is the weak or dead lower branches of mature tung trees caused by heavy shade of the upper branches, resulting in a lower yield. For example, tung trees in a shady, sloped planting site had a 58.4% yield reduction with smaller fruit than those grown in a sunny planting site (Li and Zhu, 2014). In addition, most of the lower branches of mature tung trees appear to be dead, which seriously affects the healthy development of the tung tree industry in China (Fig. 1). To date, there have not been any studies on the effects of growth and photosynthesis of tung trees under different light intensities. Currently, the lowest DLI for suitable for tung tree growth is not known, especially in southern China, which has frequent cloudy and rainy days. By studying the growth and physiological responses of tung tree seedlings to different light intensities, the minimum DLI requirement can be determined for the growth of tung trees, which provides a theoretic basis and technical support for the determination of a suitable planting density of tung trees.

The productivity of plants depends on soil, LA index and efficiency of light conversion, and CO₂ absorption (Lone and Khan, 2007). Light is a major environmental factor that affects leaf photosynthesis, traits, and plant growth, and determines the geographic distribution of plants (Kim et al., 2011). Plants experiencing shade stress often exhibit serious dysfunctions in terms of appearance and physiology, including reduced photosynthetic potential, stomatal density, and damage to various cellular structures (Holland and Richardson, 2009; Kim et al., 2011; Nobel et al., 1993; Tsukaya, 2005). Reduced photosynthesis may be the result of either stomatal closure restricting the availability of CO₂ for carboxylation or nonstomatal inhibition caused by abiotic stress on the photosynthetic apparatus (Chen et al., 2009; Colla et al., 2012b; Rivelli et al., 2002). However, plants adopt different strategies to adapt to shade stress. For example, to absorb sufficient light energy, LA and plant height increase under shade conditions, increasing light-harvesting capabilities in a light-limited environment (Huang et al., 2016; Johnston and Onwueme, 1998; Khan et al., 2000). Similarly, superoxide dismutase and peroxidase in leaves increase in response to shade stress (Ou et al., 2015). Many authors

Fig. 1. The growth situation of different planting densities of tung tree in the same field. (A) Mature tree spaced at 6×6 m have some fruit without shading. (B and C) Mature trees spaced at 4×5 m with dead lower branches.

(Morandi et al., 2011; Zibordi et al., 2009) have reported that shade causes a decrease in the net C exchange rates in young apple canopies. The decrease in fruit growth rate in young apple trees is mainly the result of a reduction in import through the phloem rather than a direct effect of shading on fruit sink strength. Iqbal et al. (2012) hypothesized that the photosynthetic potential of leaves that were lower on the plant axis was less than that of the upper leaves in the plant canopy. Previous studies have shown that light-use efficiency and decreased photosynthetic activity from the apex of the plant to the lower axis (Khan and Lone, 2005; Lone et al., 2008). Lugassi-Ben-Hamo et al. (2010) reported that shade treatments may have an adverse effect on plant growth or flower yield and quality in *Lisianthus*. Several studies have shown that shade tolerance is associated with a wide range of traits, including pigment biosynthesis, photosynthesis, and morphological and physiological traits (Huang et al., 2016; Khan et al., 2000; Kim et al., 2011).

In our study, we investigated the changes in growth, chlorophyll content, relative water content, photosynthesis, and enzyme activity under different light intensities. The aim of our study was to understand the acclimation mechanism under lower light conditions and to determine the optimal DLI for tung tree seedlings. From this DLI, we can determine the proper planting density and cultivation technique, such as pruning, to prevent mutual shading and ensure sufficient light for tung tree growth and high yield.

Materials and Methods

Plant material and growth conditions. Tung tree seedlings were grown in plastic containers (diameter, 23 cm; height, 24 cm)

Table 1. Treatment description and daily light integral (DLI) on sunny days.

Treatment symbol	Light transmission (%)	DLI in July ($\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)	DLI August ($\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$)
L100	100 (control)	48.69	46.26
L75	75	35.30	34.70
L50	50	24.21	23.13
L20	20	9.26	9.25

containing potting soil (mixed with peat, perlite, and vermiculite):sand (1:1, v/v) under outdoor conditions at Central South University of Forestry and Technology, Changsha, China. During the experimental period, the average air temperature was 30.2°C , the average relative humidity was 65.6%, and the maximum photosynthetic active radiation (PAR) was $1,860 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at noon without shade. When the experiment was started in June, the seedlings were ≈ 20 cm high and had formed three to five true leaves. In addition, all plants had similar growth (plant height, leaf number, number of nodes, etc.). The planting density was 10 plants/ m^2 with 15 cm between rows and 10 cm between plants.

Treatment. Different light intensities were achieved by covering the plants over a frame (height, 1.5 m) with black shade nets (Zhengling, Chang Sha, China) with 100%, 75%, 50%, and 20% light intensities (L100, L75, L50, and L20, respectively) (Table 1). The light intensity levels (treatments) were created by using different layers of shade net, and the light levels at noon were measured using a PAR sensor (6400XT portable photosynthesis system; LI-COR Biosciences, Lincoln, NE). The light intensities on sunny days were measured hourly in July and August for the L100, L75, L50, and L20 treatments, and DLIs were calculated (Table 1). A total of 72 seedlings were used in the experiment. Plants were divided randomly into four groups, and each treatment group consisted of 18 plants.

After 1 and 2 months (July and August), treatment under different light intensities, growth, chlorophyll content, gas exchange, and physiological responses were measured. Immediately after the measurements of gas exchange, the leaves were cut, weighed, wrapped in tinfoil, frozen in liquid N, and stored at -80°C until analysis. The collected leaf sections were used for enzyme activity measurements. During the experimental period, all treatments were watered (1000 mL/plant) twice and fertilized once per week with

500 mL Hoagland solution (Hoagland and Arnon, 1950).

Measurements of growth parameters. Using grid paper, LA was measured in six replications per treatment. After the gas exchange measurements, nine plants were selected randomly to measure plant height and stem diameter using tape and Vernier calipers. Total aboveground fresh weight (FW) and root FW (roots washed before weighing them) were measured using an analytical balance (precision, 0.1 mg). To determine the total aboveground dry weight (DW) and total root DW, plant tissues were dried in an oven at 105°C for 30 min and then at 65°C until constant weight was attained.

Measurements of photosynthetic characteristics. Photosynthetic gas exchange was measured on the fifth leaf (the same in light and CO_2 response measurements) using the previously mentioned LI-COR portable photosynthesis system between 9:00 AM and 11:00 AM during each measurement period (one leaf per plant; six plants per replicate). Light was provided by light-emitting diodes emitting in the blue and red light source with a constant saturating light intensity of $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Li et al., 2017) and an ambient CO_2 concentration of $400 \mu\text{mol}\cdot\text{mol}^{-1}$. The flow rate was $500 \mu\text{mol}\cdot\text{s}^{-1}$, the temperature of the leaf cuvette was 30°C , and the relative humidity was 70%. Water-use efficiency (WUE) was calculated as the P_n/E ratio according to Colla et al. (2012). The stomatal limitation value (L_s) was calculated as $1 - C_i/C_a$ (where C_i is the intercellular CO_2 concentration and C_a is the concentration of CO_2 in the atmosphere) according to Farquhar and Sharkey (1982).

Leaf relative water content (RWC) and chlorophyll content. RWC was determined according to FW, saturated mass (SM), and DW of the leaves. The equation is as follows: $\text{RWC} = [(FW - DW)/(SM - DW)] \times 100\%$. We used an analytical balance (precision, 0.1 mg) to measure the FW, SM, and DW of the leaves. The SM was obtained after 24 h of

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leaf immersion in distilled water in the dark. The DW was obtained after drying the leaves in an oven for 48 h at 65 °C. Total chlorophyll content was determined according to Zhang (1986). Fresh leaves (0.2 g) were homogenized in 50-mL scale test tubes containing 15 mL pure ethanol acetone and 15 mL pure acetone. After 24 h in the dark, the leaves changed to white at 4 °C refrigeration. Absorbance was recorded using a spectrophotometer (SPECORD 50 PLUS; Jena, Germany) at an absorbance of 663 nm and 645 nm; there were three replicates for each treatment. Chlorophyll content was calculated using the following equation: Total chlorophyll (mg/100 g FW) = (20.2 × OD₆₄₅) + (8.02 × OD₆₆₃), where OD is optical density (Arnon, 1949).

Rubisco and PEPC assimilatory enzyme extraction and assays. Fresh leaves (0.5 g) were ground with 3 mL ice-cold 100 mM Tris-HCl buffer (pH 8.2) containing 5% glycerin, 1 mM ethylenediaminetetraacetic acid (EDTA), 7 mM mercaptoethanol, and 1% PVP. The homogenates were centrifuged at 4 °C for 20 min at 15,000 g_n and the supernatant was stored at 4 °C for enzyme activity assay. The carbon assimilatory enzyme, Rubisco, was assayed according to Siegel and Lane (1975). The reaction mixture included 1 mL 100 mM Tris-HCl buffer (pH 8.0), 0.3 mL 100 mM MgCl₂, 0.3 mL 1 mM EDTA, 0.3 mL 50 mM ATP, 0.3 mL 50 mM DTT, 0.3 mL 2 mM NADH, 0.1 mL 200 mM NaHCO₃, 0.8 mL distilled water, 0.1 mL 9 mM RuBP, and 3-phosphoglycerate kinase/3-phosphoglycerate dehydrogenase (15 U/15 U). After mixing the solution for 10 min at 30 °C, the reaction began with the addition of 0.1 mL of the Rubisco extraction solution. For each sample, OD₃₄₀ values were obtained. Enzymatic activity was calculated according to the following equation: Enzymatic activity (μmol CO₂/g/min) = (ΔOD × V)/(2d × ζ × Δt × M), where ΔOD is the change in OD value, V is the reaction volume, d is the optical path of a cuvette, Δt is the reaction time, M is the amount of enzyme, and ζ is the extinction coefficient for 1 μM NADH at 340 nm. PEPC was assayed according to Shi et al. (1979). The reaction mixture comprised 1 mL 100 mM Tris-HCl buffer (pH 9.2), 1 mL enzyme extraction solution, 0.1 mL 10 mM MgCl₂, 0.1 mL 10 mM NaHCO₃, 0.2 mL 40 mM PEP, 0.3 mL 1

mg·mL⁻¹ NADH (pH 8.9), and 0.3 mL excess malic acid dehydrogenase (≈10.5 U). The solution was mixed for 10 min at 28 °C, after which 200 μL PEPC extraction solution was added. After extraction, OD₃₄₀ was obtained and enzymatic activity was calculated, as described earlier.

Light and CO₂ response measurements. Variable photosynthetic photon flux density (PPFD) levels were projected from an internal blue and red light source, providing a range of PPFD levels (2400, 2100, 1800, 1500, 1200, 900, 600, 300, 200, 150, 100, 75, 50, 25, and 0 μmol·m⁻²·s⁻¹), and the CO₂ concentration inside the chamber was maintained at 400 μmol·mol⁻¹. To generate P_n/C_i (CO₂ assimilation rate vs. intercellular CO₂ concentration) response curves, the external CO₂ concentration was manipulated from 50 to 1500 μmol·mol⁻¹, and PPFD was set to 1500 μmol·m⁻²·s⁻¹. The initial point in each P_n/C_i curve was 400 μmol·mol⁻¹. CO₂ concentration was then lowered to 300, 200, 150, 100, 70, and 50 μmol·mol⁻¹, and then raised to ambient concentration and allowed to stabilize at the original values of P_n. The CO₂ level was then raised to 600, 800, 1000, 1200, and 1500 μmol·mol⁻¹ using an automatic measurement program. The leaf temperature and air humidity were set to 30 ± 1 °C and 70%, respectively.

A_{max}, apparent quantum yield (AQY), light saturation point (LSP), and dark respiration rate (R_d) were calculated from P_n in response to various PPFDs by nonlinear regression curve fitting to an exponential model (Ye, 2007; Ye and Yu, 2008):

$$A_n(I) = \alpha \frac{1 - \beta I}{1 + \gamma I} I - R_d$$

$$I_{\text{sat}} = \frac{\sqrt{(\beta + \gamma)/\beta} - 1}{\gamma}$$

$$A_{\text{max}} = \alpha \left(\frac{\sqrt{\beta + \gamma} - \sqrt{\beta}}{\gamma} \right)^2 - R_d$$

where α is AQY, β and γ are coefficients, I is PAR, R_d is the rate of dark respiration, I_{sat} is saturation light intensity (LSP), and A_{max} is the maximum photosynthetic rate. For the fitting of the A/C_i curves, the program Farquhar von Caemmerer Berry (Duursma,

2015) was used, which is based on the equations of Farquhar et al. (1980). The maximum rate of Rubisco carboxylation (V_{cmax}), CO₂ compensation point (CCP) and J_{max} at a standard temperature of 25 °C were calculated with a model of the temperature dependence of the photosynthetic parameters.

Leaf anatomic structure. Using optical microscopy and paraffin section technology, we studied leaf anatomic structure. Leaf samples of paraffin sectioning were fixed in Carnoy's solution (acetic acid:ethanol ratio, 1:3) for 24 h and subsequently maintained in 70% alcohol. The leaves were softened for 5 h in 8 mol·L⁻¹ NaOH before observation. The leaves were then washed thoroughly with distilled water. The softened style was torn open along the leaves and stained for 5 h with 0.5% water-soluble aniline blue (prepared in 0.15 N dipotassium hydrogen phosphate buffer). The transverse leaf sections were observed microscopically (Leica GRE), and the palisade tissue, sponge tissue, and total thickness of leaves were measured.

Statistical analysis. All data were collected in triplicate and the results presented are means. A two-way analysis of variance was used to test the differences between the months (July or August) and light treatments. SPSS software (version 17.0) was used with Duncan's multiple range test when the main effect was significant at P ≤ 0.05

Results

Growth characteristics. Plant height, stem diameter, and average LA were affected significantly by light intensity (Table 2). Plant height was highest in the L50 treatment for both July and August, whereas no differences were observed for the L100, L75, and L20 treatments in July, and for the L100 and L20 treatments in August. Lower light intensities reduced stem diameter in July. Stem diameter in the L75, L50, and L20 treatments decreased by 20.54%, 28.86%, and 42.69% (P < 0.05), respectively, compared with the control in August. LA in July was greater in the L75 and L50 treatments than in the control and the L20 treatment. LA in August was greatest in the control and the L50 treatment and lowest in the L20 treatment. Total aboveground FW and DW, and total

Table 2. Plant height, stem diameter, leaf area, total aboveground fresh weight, total aboveground dry weight, total root fresh weight, and total root dry weight of tung tree seedlings grown under different light intensities.

Time	Treatment ^a	Plant ht (cm)	Stem diam (mm)	Leaf area (cm ²)	Total aboveground fresh wt (g)	Total aboveground dry wt (g)	Total root fresh wt (g)	Total root dry wt (g)
July	L100	20.5 ± 2.3 Bb	7.02 ± 0.28 Ba	76.6 ± 6.3 Bb	26.31 ± 3.95 Ba	6.15 ± 0.61 Ba	16.81 ± 1.20 Ba	2.62 ± 0.37 Ba
	L75	22.3 ± 3.5 Bb	6.87 ± 0.26 Ba	138.0 ± 8.6 Ba	24.94 ± 2.77 Bab	4.83 ± 0.42 Bb	13.12 ± 1.91 Bb	2.01 ± 0.24 Bb
	L50	25.7 ± 4.6 Ba	6.60 ± 0.23 Ab	138.3 ± 9.4 Ba	20.75 ± 2.64 Bb	3.56 ± 0.39 Bc	11.80 ± 0.87 Bc	1.23 ± 0.19 Bc
	L20	21.0 ± 2.9 Bb	5.72 ± 0.27 Ac	75.3 ± 4.3 Bb	7.33 ± 1.54 Bc	1.58 ± 0.17 Bd	6.64 ± 0.56 Ad	0.80 ± 0.11 Bd
August	L100	35.3 ± 4.6 Ac	9.98 ± 0.52 Aa	232.6 ± 15.3 Aa	92.65 ± 5.08 Aa	28.31 ± 1.96 Aa	68.53 ± 3.65 Aa	13.73 ± 1.68 Aa
	L75	39.8 ± 5.1 Ab	7.93 ± 0.39 Ab	192.1 ± 11.1 Ab	48.12 ± 3.40 Ab	11.01 ± 0.86 Ab	35.47 ± 1.88 Ab	4.12 ± 0.64 Ab
	L50	52.6 ± 5.9 Aa	7.10 ± 0.31 Abc	245.9 ± 10.1 Aa	45.42 ± 2.52 Ac	10.68 ± 0.88 Ab	34.02 ± 3.51 Ab	4.09 ± 0.71 Ab
	L20	36.5 ± 3.8 Ac	5.94 ± 0.25 Ac	112.6 ± 8.8 Ac	12.50 ± 1.31 Ad	3.68 ± 0.40 Ac	8.07 ± 0.71 Ac	1.34 ± 0.20 Ac

^aL100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity.

Data represent the mean ± SE (n = 3). Different capital letters indicate significant differences between July and August. Lowercase letters indicate significant differences among different light treatments in the same month (P ≤ 0.05, Duncan's multiple range test).

Fig. 2. Effect of different light levels on assimilation rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate in tung tree seedling leaves. L100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity. Data represent the mean $\pm$ SE ($n = 3$). Different capital letters indicate significant differences between July and August. Lowercase letters indicate significant differences among different light treatments in the same month ($P \leq 0.05$, Duncan's multiple range test).

root FW and DW in July decreased with light intensity. The reduction magnitude in FW and DW in August resulting from low intensity was greater in August than in July, indicating that a long duration of shading stress reduces seedling growth further.

Leaf gas exchange. P_n , g_s , and E in all treatments decreased as light intensity decreased and as the treatment duration continued (Fig. 2A–C). Compared with the control, P_n decreased by 39.24%, 64.45%, and 72.05%, respectively, in the L75, L50, and L20 treatments in August ($P < 0.05$). No differences were detected in P_n between July and August in all treatments, except for the L50 treatment (Fig. 2A). The influence of light intensity on g_s and E in all treatments was similar to that of P_n . Compared with the control, g_s decreased by 55.21%, 73.30%, and 78.68% ($P < 0.05$), respectively (Fig. 2B); and E decreased by 41.89%, 63.14%, and 71.33% ($P < 0.05$), respectively, in the L75, L50, and L20 treatments in August (Fig. 2C). As light intensity decreased, C_i in July decreased gradually, whereas in August, C_i first decreased and then increased (Fig. 2D). C_i in July was similar between the L100 and L75 treatments, whereas C_i in the L50 and L20 treatments decreased slightly. C_i in August was similar among the L75, L50, and L20 treatments, and was 8.60%, 13.98%, and 11.69% less than the control.

WUE was unaffected by the light intensity treatment, except the L20 treatment in July, which had the greatest value (3.8). Compared

Fig. 3. Effect of different light levels on (A) water use efficiency and the (B) stomatal limitation value in tung tree seedling leaves. L100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity. Data represent the mean $\pm$ SE ($n = 3$). Different capital letters indicate significant differences between July and August. Lowercase letters indicate significant differences among different light treatments in the same month ($P \leq 0.05$, Duncan's multiple range test).

with July, WUE increased significantly over time in August ($P < 0.05$), except for the L20 treatment (Fig. 3A). As light intensity decreased, L_s increased by 9.56%, 11.11%, and 20.57% ($P < 0.05$) in July. L_s also increased by 16.21%, 18.92%, and 22.97% ($P < 0.05$) in August (Fig. 3B). However, no significant differences were detected in L_s in all treatments between July and August.

Leaf RWC. As light intensity decreased, RWC values increased gradually in July and August compared with the control. There were no significant differences in RWC in July among the L100, L75, L50, and L20 treatments; however, RWC values increased in August by 8.56%, 14.45%, and 26.75% ($P <$

0.05), respectively, in the L75, L50, and L20 treatments compared with the control (Fig. 4).

Chlorophyll content. Chl a, Chl b, and total chlorophyll contents in the L50 and L20 treatments were greater than those in the L100 and L75 treatment in tung tree leaves in both July and August (Table 3). There were no differences in Chl a, Chl b, and total chlorophyll contents between the L100 and L75 treatments, and between the L50 and L20 treatments, and were greater in July compared with August.

Rubisco and PEPC assimilatory enzymes. The low-light intensity treatment decreased the activity of both Rubisco and PEPC

enzymes, and this effect became more pronounced over time (Fig. 5). Compared with the control, in July, Rubisco activity decreased in the L50 and L20 treatments by 41.94% and 66.66% ($P < 0.05$), respectively.

In August, Rubisco activity decreased by 62.16% and 78.38% ($P < 0.05$) in these treatments, respectively (Fig. 5A). PEPC activity was also reduced significantly by the low-light intensity treatments, except for

the L75 treatment in July (Fig. 5B). In July, PEPC activity in the L50 and L20 treatments was reduced by 50.59% and 65.88% ($P < 0.05$), respectively. In August, PEPC activity in the L75, L50, and L20 treatments was reduced by 26.09%, 66.30%, and 81.52% ($P < 0.05$), respectively.

Photosynthetic characteristics. The P_n -PPFD response curves showed the typical characteristics of photosynthesis of plants acclimatized under different light intensities: a linear increase at low PPFD and saturation at high PPFD in all treatments (Fig. 6). Photosynthetic capacity varied with light intensity. The leaves in the control treatment exhibited A_{max} at $22.5 \mu\text{mol CO}_2/\text{m}^2/\text{s}$ in July. Compared with the control, A_{max} in the L75, L50, and L20 treatments was reduced by 16.44%, 33.33%, and 54.22% ($P < 0.05$), respectively (Table 4). Compared with the L100 in July, A_{max} in the L100 treatment in August increased 16.89%. A_{max} in the L75, L50, and L20 treatments was reduced by 36.50%, 51.71%, and 62.36% ($P < 0.05$), respectively, compared with the L100 treatment in August. The changes in LSP, light compensation point (LCP), and R_d in the L75, L50, and L20 treatments were similar to those in A_{max} . LSP, LCP, and R_d were reduced significantly as light intensity decreased and as treatment duration continued ($P < 0.05$) (Table 4). In July, LSP, LCP, and R_d in the L20 treatment decreased by 58.29%, 84.00%, and 44.85% ($P < 0.05$), respectively. In August, LSP, LCP, and R_d in the L20 treatment decreased by 64.78%, 85.21%, and 46.11% ($P < 0.05$), respectively. However, AQY increased gradually as light intensity decreased and as treatment duration continued, and AQY increased by 21.05%, 31.58%, and 43.86% ($P < 0.05$), respectively.

In July, g_s increased slowly until a PPFD of $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was reached, and then increased linearly until saturated in all treatments (Fig. 7). In August, increases in g_s in both the L100 and L75 treatments were similar to those in July. However, in both the L50 and L20 treatments, the increase in g_s was slow and did not change during the whole PPFD range (Fig. 7). The leaves in the L50 and L20 treatments had a significantly less g_s compared with those in the L100 and L75 treatments in August (Fig. 7). The g_s values in the L100 and L75 treatments were greater than those in the same treatments in July, whereas g_s values in the L50 and L20 treatments were less than those in the same treatments in July (Fig. 7).

The P_n - C_i curves of tung tree leaves showed distinctly differences among different light treatments, particularly in August (Fig. 8). As the CO_2 concentration increased from 0 to $400 \mu\text{mol CO}_2/\text{mol}$, P_n increased in all treatments. As the CO_2 concentration continued to increase, the slope of the response curve gradually became steady (Fig. 8). At low light intensities, A_{max} , V_{cmax} , and J_{max} were less (Table 5). Compared with the control, V_{cmax} in the L75, L50, and L20 treatments decreased by 25.36%, 65.49%,

Fig. 4. Effect of different light levels on relative leaf water content of tung tree seedling leaves. L100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity. Data represent the mean $\pm$ SE ($n = 3$). Different capital letters indicate significant differences between July and August. Lowercase letters indicate significant differences among different light treatments within the same month at $P \leq 0.05$ according to Duncan's multiple range test.

Table 3. Chlorophyll a (Chl a), chlorophyll b (Chl b), and total chlorophyll content of tung tree seedlings grown under different light treatments.

Time	Treatment ^a	Chl a (mg·g ⁻¹)	Chl b (mg·g ⁻¹)	Total chlorophyll content (mg·g ⁻¹)
July	L100	0.86 $\pm$ 0.09 Bb	0.22 $\pm$ 0.01 Bb	1.08 $\pm$ 0.10 Bb
	L75	0.93 $\pm$ 0.08 Bb	0.29 $\pm$ 0.02 Bb	1.22 $\pm$ 0.12 Bb
	L50	1.23 $\pm$ 0.07 Ba	0.39 $\pm$ 0.02 Ba	1.62 $\pm$ 0.09 Ba
	L20	1.35 $\pm$ 0.11 Ba	0.51 $\pm$ 0.01 Ba	1.86 $\pm$ 0.11 Ba
August	L100	1.21 $\pm$ 0.09 Ab	0.34 $\pm$ 0.11 Ab	1.55 $\pm$ 0.15 Ab
	L75	1.48 $\pm$ 0.04 Ab	0.53 $\pm$ 0.01 Ab	2.01 $\pm$ 0.05 Ab
	L50	2.03 $\pm$ 0.17 Aa	0.89 $\pm$ 0.18 Aa	2.92 $\pm$ 0.20 Aa
	L20	2.07 $\pm$ 0.13 Aa	0.95 $\pm$ 0.13 Aa	3.02 $\pm$ 0.19 Aa

^aL100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity. Data represent the mean $\pm$ SE ($n = 3$). Different capital letters indicate significant differences between July and August. Lowercase letters indicate significant differences among different light treatments in the same month ($P \leq 0.05$, Duncan's multiple range test).

Fig. 5. Effect of different light levels on (A) ribulose-1,5-bisphosphate carboxylase (RuBP) and (B) phosphoenolpyruvate (PEPC) activity in tung tree seedling leaves. L100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity. Data represent the mean $\pm$ SE ($n = 3$). Different capital letters indicate significant differences between July and August. Lowercase letters indicate significant differences among different light treatments in the same month ($P \leq 0.05$, Duncan's multiple range test).

Fig. 6. Net CO₂ photosynthetic rate in response to photosynthetic photon flux density in tung tree seedling leaves under different light treatments. L100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity. Data represent the mean $\pm$ SE (n = 3).

Table 4. Apparent quantum yield (AQY), maximum net photosynthetic rate (A_{max}), light saturation point (LSP), light compensation point (LCP), and dark respiration rate (R_d) of tung tree seedlings grown under different light treatments.

Time	Treatment ^a	AQY	A_{max} (μmol CO ₂ /m ² /s)	LSP (μmol·m ⁻² ·s ⁻¹)	LCP (μmol·m ⁻² ·s ⁻¹)	R_d (μmol CO ₂ /m ² /s)
July	L100	0.055 $\pm$ 0.010 Ac	22.5 $\pm$ 2.56 Ba	1563 $\pm$ 78 Ba	47.5 $\pm$ 2.2 Aa	1.65 $\pm$ 0.18 Aa
	L75	0.064 $\pm$ 0.012 Ab	18.8 $\pm$ 1.25 Ab	975 $\pm$ 62 Ab	22.3 $\pm$ 1.6 Bb	1.37 $\pm$ 0.16 Ab
	L50	0.071 $\pm$ 0.009 Aa	15.0 $\pm$ 1.46 Ac	683 $\pm$ 51 Ac	16.7 $\pm$ 1.1 Ac	1.08 $\pm$ 0.11 Ac
	L20	0.079 $\pm$ 0.013 Aa	10.3 $\pm$ 1.08 Ad	652 $\pm$ 22 Ac	7.6 $\pm$ 1.3 Ad	0.91 $\pm$ 0.09 Acd
August	L100	0.057 $\pm$ 0.010 Ac	26.3 $\pm$ 2.76 Aa	1752 $\pm$ 85 Aa	42.6 $\pm$ 2.8 Ba	1.67 $\pm$ 0.15 Aa
	L75	0.069 $\pm$ 0.011 Ab	16.7 $\pm$ 1.55 Bb	903 $\pm$ 53 Ab	26.7 $\pm$ 1.7 Ab	1.29 $\pm$ 0.12 Ab
	L50	0.075 $\pm$ 0.012 Aab	12.7 $\pm$ 1.06 Bbc	658 $\pm$ 44 Ac	10.9 $\pm$ 1.5 Bc	0.97 $\pm$ 0.14 Ac
	L20	0.082 $\pm$ 0.009 Aa	9.9 $\pm$ 0.86 Ac	617 $\pm$ 37 Ac	6.3 $\pm$ 0.7 Acd	0.90 $\pm$ 0.08 Ac

^aL100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity.

Data represent the mean $\pm$ SE (n = 3). Different capital letters indicate significant differences between different months. Lowercase letters indicate significant differences among light treatments in the same month ($P \leq 0.05$, Duncan's multiple range test).

Fig. 7. Stomatal conductance in response to photosynthetic photon flux density (PPFD) in tung tree seedling leaves grown under different light levels. L100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity. Data represent the mean $\pm$ SE (n = 3).

and 80.26% ($P < 0.05$) in August, respectively, and J_{max} in the L75, L50, and L20 treatments decreased by 15.16%, 38.30%, and 56.56% ($P < 0.05$), respectively. CCP increased in all treatments, except for the L50 treatment in August (Table 5). Compared with the control, CCP in the L50 and L20 treatments increased by 17.95% and 27.35%, respectively, in July; and CCP in the L75, L50, and L20 treatments increased by 29.11%, 20.89%, and 32.36%, respectively, in August (Table 5).

Leaf anatomic structure. Palisade tissue thickness, sponge tissue thickness, and leaf thickness were affected significantly by light intensity, especially in August with the prolongation of shade time (Table 6). Palisade tissue thickness, sponge tissue thickness, and leaf thickness were greatest in the L75 treatment in July only, but palisade tissue thickness, sponge tissue thickness, and leaf thickness decreased by 45.63%, 36.48%, and 35.88% ($P < 0.05$), respectively, compared with the control in August. Lower light

intensities reduced leaf thickness in both July and August except for the L75 treatment in July. Compared with the control, leaf thickness in the L50 and L20 treatments decreased by 20.28%, and 26.17% ($P < 0.05$), respectively, in July; and leaf thickness in the L50 and L20 treatments decreased by 44.17% and 46.90% ($P < 0.05$), respectively, in August (Table 6, Fig. 9). The reduction magnitude of palisade tissue thickness, sponge tissue thickness, and leaf thickness was greater in August than in July as a result of low light intensity,

Fig. 8. Net photosynthetic rate in response to CO₂ concentration in tung tree seedling leaves under different light levels. L100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity. Data represent the mean $\pm$ SE (n = 3).

Table 5. Maximum rate of carboxylation (V_{cmax}), maximum rate of electron transport at saturating irradiance (J_{max}), and CO₂ compensation point (CCP) of tung tree seedlings grown under different light treatments.

Time	Treatment ^a	V_{cmax} ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	J_{max} ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
July	L100	73.5 $\pm$ 3.8 Aa	112.6 $\pm$ 6.2 Ba	46.8 $\pm$ 5.2 Bb
	L75	62.5 $\pm$ 2.5 Ab	98.3 $\pm$ 5.8 Ab	50.8 $\pm$ 6.1 Bb
	L50	45.8 $\pm$ 4.6 Ac	85.1 $\pm$ 4.6 Ac	55.2 $\pm$ 6.8 Ba
	L20	27.7 $\pm$ 2.5 Ad	73.3 $\pm$ 7.5 Ad	59.6 $\pm$ 7.9 Ba
August	L100	76.5 $\pm$ 5.7 Aa	122.7 $\pm$ 9.1 Aa	58.4 $\pm$ 6.7 Ac
	L75	57.1 $\pm$ 5.4 Bb	104.1 $\pm$ 5.0 Ab	75.4 $\pm$ 5.4 Aa
	L50	26.4 $\pm$ 3.6 Bc	75.7 $\pm$ 4.3 Bc	70.6 $\pm$ 7.1 Ab
	L20	15.1 $\pm$ 3.2 Bd	53.3 $\pm$ 4.9 Bd	77.3 $\pm$ 6.1 Aa

^aL100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity. Data represent the mean $\pm$ SE of three replicates. Different capital letters indicate significant differences between different months. Lowercase letters indicate significant differences among light treatments in the same month ($P \leq 0.05$, Duncan's multiple range test).

Table 6. Palisade tissue thickness, sponge tissue thickness, and leaf thickness of tung tree seedlings grown under different light intensities.

Time	Treatment ^a	Palisade tissue thickness/ μm	Sponge tissue thickness/ μm	Leaf thickness/ μm
July	L100	70.83 $\pm$ 3.22 Bb	80.33 $\pm$ 5.02 Bab	196.76 $\pm$ 3.62 Bab
	L75	77.81 $\pm$ 1.36 Aa	86.49 $\pm$ 2.17 Aa	200.13 $\pm$ 1.84 Aa
	L50	56.00 $\pm$ 3.99 Ac	71.41 $\pm$ 4.78 Ab	156.86 $\pm$ 4.53 Ac
	L20	44.61 $\pm$ 3.55 Ad	73.47 $\pm$ 4.91 Ab	145.26 $\pm$ 10.04 Ad
August	L100	75.43 $\pm$ 2.54 Aa	93.25 $\pm$ 7.71 Aa	205.57 $\pm$ 13.28 Aa
	L75	41.01 $\pm$ 0.90 Bb	59.24 $\pm$ 2.37 Bb	131.81 $\pm$ 2.26 Bb
	L50	36.76 $\pm$ 1.63 Bc	52.95 $\pm$ 0.45 Bb	114.77 $\pm$ 1.32 Bc
	L20	30.11 $\pm$ 1.44 Bd	54.26 $\pm$ 2.87 Bb	109.16 $\pm$ 2.76 Bc

^aL100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity. Data represent the mean $\pm$ SE (n = 3). Different capital letters indicate significant differences between July and August. Lowercase letters indicate significant differences among different light treatments in the same month ($P \leq 0.05$, Duncan's multiple range test).

indicating that a long duration of shading stress reduced leaf thickness further.

Discussion

Tolerance to shade or acclimation to shade varies largely with species. When the light intensity decreases to a threshold, plants start to show decreased photosynthetic rate, elevated WUE (Hu et al., 2015), decreased antioxidant enzyme activity (Ou et al., 2015), and damaged PSI and PSII functions (Chen et al., 2016). Apparently, the threshold for this light intensity for tung trees is relatively high. In other words, tung trees have low

tolerance to shade. The differences in growth, physiological, and biochemical responses of tung trees between July and August indicate that the longer exposure to shade stress led to more severe damage of the photosynthetic apparatus. These results explain the reason for dead and weak lower branches in a tung tree forest: heavy shading by upper branches (Fig. 1).

In our study, lower light treatments reduced stem diameter and biomass significantly at the beginning of the treatment, whereas plant height increased under lower light intensity conditions, which is common as a result of the changes in light quality

under the canopy. Furthermore, LA increased in the L75 and L50 treatments, except for the L50 treatment in August (Table 2). A similar result was found in four ramie (*Boehmeria nivea*) cultivars in which plant height and LA increased under shade treatment (Huang et al., 2016). This result may also be due to photosynthate allocation patterns favoring shoot elongation and LA, thus increasing light-harvesting capabilities in a light-limited environment (Huang et al., 2016; Johnston and Onwueme, 1998; Khan et al., 2000). Small stem diameter and low biomass may be attributed to shade-reduced g_s and inhibition of P_n under stress conditions (Huang et al., 2016; Schwarz et al., 2001). In July, palisade tissue thickness, sponge tissue thickness, and leaf thickness in the L75 treatment were greater than that of the control L100 treatment, but were significantly less than the control in August. These results indicate that a slight shading treatment of tung tree seedlings could increase leaf thickness, and slight shading is good for healthy growth of tung tree seedlings. However, with the prolongation of shading time, palisade tissue thickness, sponge tissue thickness, and leaf thickness decreased under all shading treatments in August. Therefore, long-term shading leads to a decrease in leaf thickness in the tung tree.

Water is an important factor in the growth and development of plants (Blakey and Bower, 2009; Bower, 1985; Bower and Cutting, 1988). RWC started to increase in the L75, L50, and L20 treatments, but there were no statistical differences in July, whereas RWC increased significantly in the L50 and L20 treatments in August compared with the control. Compared with all treatments in July, the RWC in August increased significantly in the L20 S3 treatments, whereas no differences were observed for the L100, L75, and L50 treatments. These results indicate that lower light intensity treatments improved RWC and reduced dry matter accumulation in tung tree seedlings. Heavy shading increased chlorophyll

Fig. 9. Anatomic structure of tung tree seedling leaves under different light levels. (A) One hundred percent light intensity (L100) in July. (B) Seventy-five percent light intensity (L75) in July. (C) Fifty percent light intensity (L50) in July. (D) Twenty percent light intensity (L20) in July. (E) One hundred percent light intensity (L100) in August. (F) Seventy-five percent light intensity (L75) in August. (G) Fifty percent light intensity (L50) in August. (H) Twenty percent light intensity (L20) in August. Bar = 50 μm .

contents in tung trees, possible because the chloroplasts of shade leaves have higher and broader thylakoid stacks and invest primarily in the development of the pigment antenna (Kim et al., 2011; Lichtenthaler et al., 2007; Sarijeva et al., 2007). Our results agree with those found in blueberry (*Vaccinium corymbosum* cv. Bluecrop), in which shade increased chlorophyll content (Kim et al., 2011).

The decrease in P_n response to shade, was related to a g_s decrease; in contrast, C_i increased or remained unchanged in shade conditions, suggesting that the decrease in P_n was related to nonstomatal limitations (Brodribb, 1996). When g_s and C_i decrease simultaneously, the decline in P_n is caused mainly by stomatal limitation. If g_s decreases while C_i increases, then photosynthesis is limited mainly by nonstomatal factors (Farquhar and Sharkey, 1982). Our investigation indicated that shade decreased P_n , g_s , and E significantly in tung tree seedlings. C_i in the L75 and L50 treatments was related to a decrease in g_s and P_n ; this result indicates that photosynthesis in the L75 and L50 treatments was limited mainly by stomatal limitation. However, C_i in the L20 treatment remained unchanged in July, and increased in August, compared with the control. This result indicates that the decrease in photosynthesis in the L20 treatment was caused by nonstomatal factors, which may represent a reduction in photosynthetic enzymatic activity. Interestingly, the activity of both Rubisco and PEPC enzymes was reduced in the L50 and L20 treatments, which strongly suggests that the decrease in photosynthesis in L20 was caused by nonstomatal factors. In addition, WUE was calculated as the ratio of P_n to E in

our study. We found that WUE increased in shade treatments because of the rapid decrease in E (Colla et al., 2010).

The P_n -PPFD and P_n - C_i response curves can help to explain the adaptability of plants to changes in two important factors: PAR and CO_2 concentration (Chen et al., 2012; Huang et al., 2015). In our study, AQY, A_{max} , LSP, and LCP in tung tree seedlings were found to decrease with an increase in shade levels, suggesting that light utilization efficiency was reduced dramatically in tung tree seedlings (Chen et al., 2005; Sui et al., 2012; Yan et al., 2013). In addition, there was a significant reduction of R_d in the L75, L50, and L20 treatments, indicating that physiological activity may have declined (Talts et al., 2004). Furthermore, A_{max} , V_{cmax} , and J_{max} were found to decrease as the shade level increased, mainly because photosynthetic enzymatic activity was reduced in shade treatments. Therefore, a greater amount of shade may have reduced the efficiency of CO_2 assimilation in tung tree seedlings (Huang et al., 2015). In summary, our experiments examined the effects of different light intensity on growth and photosynthetic characteristics of tung tree. We also determined photosynthetic physiological indexes in July and long-term stress in August. Compared with July, with lower light intensity, P_n , g_s , E , Rubisco and PEPC assimilatory enzyme activities, V_{cmax} of the seedlings decreased in August. This shows that the long time in low light intensity can cause fluctuations in tung tree physiological metabolism, which leads to a decrease in photosynthetic enzyme activity, and eventually leads to poor growth of tung trees.

Conclusion

Based on the growth, physiological, and biochemical responses to different light intensity treatments, we conclude that tung trees are considerably heliophilic and shading caused a reduction in photosynthetic efficiency, enzymatic activity, and gas exchange rate, and consequently reduced seedling growth. The L75 treatment reduced net photosynthesis by 40% and biomass by 62% over the 2 months. The longer period of shading led to more reduction of growth and more severe damage to the photosynthetic apparatus, as reflected by the lower enzymatic activity and photosynthetic characteristics. Thus, shade should be avoided by all means and a DLI of $\approx 46 \text{ mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ (or $1400 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD at noon) on sunny days in summer are recommended to ensure proper tung tree growth and yield.

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