**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 (Pₐ), stomatal conductance (gₛ), transpiration rate (E), total aboveground and root dry weight (DW), maximum net photosynthetic rate (Aₘₐₓ), and maximum rate of electron transport at saturating irradiance (Jₘₐₓ) 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 (Aₘₐₓ and Jₘₐₓ). 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 (PEP) 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 ≈46 mol·m⁻²·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 (Sapindus saponaria), 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 ≈5 m, with a crown circumference of ≈4.5 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., 2017a). 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 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...
(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) 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 μmol·m⁻²·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/m² 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 (Zhen-gling, 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 CO₂ 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 μmol·m⁻²·s⁻¹ (Li et al., 2017) and an ambient CO₂ concentration of 400 μmol·m⁻³. The flow rate was 500 μmol·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 Pn/E ratio according to Colla et al. (2012). The stomatal limitation value (Lₜ) was calculated as 1 – C_i/C_a (where C_i is the intercellular CO₂ concentration and C_a is the concentration of CO₂ 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: RWC = [(FW – DW)/(SM – DW)] × 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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**Fig. 1.** The growth situation of different planting densities of tung tree in the same field. (A) Mature trees spaced at 6 × 6 m have some fruit without shading. (B and C) Mature trees spaced at 4 × 5 m with dead lower branches.

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

| Treatment symbol | Light transmission (%) | DLI in July (mol·m⁻²·d⁻¹) | DLI August (mol·m⁻²·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                      |

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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 acetic and 15 mL pure aceton. After 24 h in the dark, the leaves changed to white at 4 °C refrigeration. Absorbance was recorded using a spectrophotometer (SPECCORD 50 PLUS; Jena, Germany) at an absorbance of 663 nm and 465 nm; there were three replicates for each treatment. Chlorophyll content was calculated using the following equation: Total chlorophyll (mg/100 g FW) = \((20.2 \times \text{OD}_{663}) + (8.02 \times \text{OD}_{465})\), 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, 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, 0.2 mL 40 mM PEP, 0.3 mL 1 mM ethylenediaminetetraacetic acid (EDTA), 0.3 mL 2 mM NADH, 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 (=105 U). The solution was mixed for 10 min at 28 °C, after which 200 µL PEPC extraction solution was added. After extraction, OD\(_{440}\) 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\(^{-1}\)). The internal oxygen concentration inside the chamber was maintained at 400 µmol·mol\(^{-1}\). To generate P\(_c\)/P\(_c\) 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\(_c\)/P\(_c\) 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\(_c\). 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.

\[ A_{max}(\text{apparent quantum yield}) = \frac{\sqrt{\beta + \gamma}}{\gamma} \cdot R_d \]
where \(\alpha\) is QY, \(\beta\) and \(\gamma\) are coefficients, \(I\) is PAR, \(R_d\) is the rate of dark respiration, \(I_{sat}\) is saturation light intensity (LSL), and \(A_{max}\) is the maximum photosynthetic rate. For the fitting of the A/Ci curves, the program Farquhar von Caemmerer Berry (Duursma, 2015) was used, which is based on the equations of Farquhar et al. (1980). The rate of Rubisco carboxylation (V\(_{\text{cmax}}\)), CO2 compensation point (CCP) and \(J_{\text{max}}\) 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 paraphin section technique, we studied leaf anatomic structure. Leaf samples of paraphin 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 hr 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 \(P \leq 0.05\).

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. L50 and L20 were 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

| Time  | Treatment* | 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    | 7.6 ± 0.25     | 7.66 ± 6.3          | 26.31 ± 3.95                 | 6.15 ± 0.61                | 16.81 ± 1.20          | 2.62 ± 0.37          |
|       | L20        | 22.5 ± 3.4    | 6.6 ± 0.23     | 138 ± 8.6           | 1250 ± 1.3                   | 4.83 ± 0.42               | 13.12 ± 0.31          | 2.01 ± 0.21          |
|       | L50        | 25.7 ± 4.6    | 7.3 ± 0.4      | 207.5 ± 2.64        | 2075 ± 2.64                  | 3.56 ± 0.39               | 11.80 ± 0.87          | 1.23 ± 0.19          |
|       | L20        | 21.0 ± 2.9    | 7.3 ± 0.4      | 753 ± 4.3           | 733 ± 1.54                   | 1.58 ± 0.17               | 6.64 ± 0.56           | 0.80 ± 0.11          |
| August| L100       | 35.3 ± 4.6    | 9.9 ± 0.52     | 232.6 ± 15.3        | 92 ± 0.58                    | 28.31 ± 1.96              | 68.55 ± 3.65          | 13.73 ± 1.68         |
|       | L75        | 39.8 ± 5.1    | 7.9 ± 0.39     | 192.1 ± 1.11        | 48.12 ± 3.4                  | 11.01 ± 0.86              | 35.47 ± 1.88          | 4.12 ± 0.64          |
|       | L50        | 52.6 ± 5.9    | 7.1 ± 0.31     | 245.9 ± 10.1        | 45.42 ± 2.52                 | 10.68 ± 0.88              | 34.02 ± 3.51          | 4.09 ± 0.71          |
|       | L20        | 75.3 ± 3.8    | 7.9 ± 0.3      | 126.8 ± 10.4        | 116.8 ± 0.40                  | 8.07 ± 0.71               | 1.34 ± 0.20           | 0.80 ± 0.11          |

*1L100, 100% light intensity; L75, 75% light intensity; L50, 50% light intensity; L20, 20% light intensity. Data represent the mean ± s (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).
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. Pn, gs, and E in all treatments decreased as light intensity decreased and as the treatment duration continued (Fig. 2A–C). Compared with the control, Pn 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 Pn between July and August in all treatments, except for the L50 treatment (Fig. 2A). The influence of light intensity on gs and E in all treatments was similar to that of Pn. Compared with the control, gs 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 in July decreased gradually, whereas in August, C first decreased and then increased (Fig. 2D). C in July was similar between the L100 and L75 treatments, whereas C in the L50 and L20 treatments decreased slightly. C 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 with July, WUE increased significantly over time in August (P < 0.05), except for the L20 treatment (Fig. 3A). As light intensity decreased, Ls increased by 9.56%, 11.11%, and 20.57% (P < 0.05) in July. Ls also increased by 16.21%, 18.92%, and 22.97% (P < 0.05) in August (Fig. 3B). However, no significant differences were detected in Ls 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 between the L100 and L75 treatments, and between the L50 and L20 treatments, and were greater in July compared with August.

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 treatments 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 \(P_e–P_{\text{PFD}}\) response curves showed the typical characteristics of photosynthesis of plants acclimatized under different light intensities: a linear increase at low PPDF and saturation at high PPDF in all treatments (Fig. 6). Photosynthetic capacity varied with light intensity. The leaves in the control treatment exhibited \(A_{\text{max}}\) at 22.5 \(\mu\text{mol CO}_2/\text{m}^2/\text{s}\) in July. Compared with the control, \(A_{\text{max}}\) 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, \(A_{\text{max}}\) in the L100 treatment in August increased 16.89%. \(A_{\text{max}}\) 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 \(A_{\text{max}}\). 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 39.7%, 46.01%, and 46.72% \((P < 0.05)\), respectively. In August, LSP, LCP, and \(R_d\) in the L20 treatment decreased by 64.7%, 47.46%, and 46.72% \((P < 0.05)\), 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 PPDF of 300 \(\mu\text{mol m}^{-2}\text{s}^{-1}\) was reached, and then increased linearly until saturated in all treatments (Fig. 7). In August, \(g_s\) 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 PPDF 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 \(P_e–C_i\) curves of tung tree leaves showed distinct differences among different light treatments, particularly in August (Fig. 8). As the \(\text{CO}_2\) concentration increased from 0 to 400 \(\mu\text{mol CO}_2/\text{mol,}\ \text{Pn}\) increased in all treatments. As the \(\text{CO}_2\) concentration continued to increase, the slope of the response curve gradually became steady (Fig. 8). At low light intensities, \(A_{\text{max}}, V_{\text{max}}\), and \(I_{\text{max}}\) were less (Table 5). Compared with the control, \(V_{\text{max}}\) in the L75, L50, and L20 treatments decreased by 25.36%, 65.49%,
and 80.26% \( (P < 0.05) \) in August, respectively, and \( J_{\text{max}} \) 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 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.

### Table 4. Apparent quantum yield (AQY), maximum net photosynthetic rate (\( A_{\text{max}} \)), 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 | AQY \((\mu \text{mol CO}_2/\text{m}^2/\text{s})\) | \( A_{\text{max}} \) \((\mu \text{mol CO}_2/\text{m}^2/\text{s})\) | LSP \(\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\) | LCP \(\text{mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}\) | \( R_d \) \((\mu \text{mol CO}_2/\text{m}^2/\text{s})\) |
|-------|-----------|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| July  | L100      | 0.055 ± 0.010 Ac                 | 22.5 ± 2.56 Ba  | 1563 ± 78 Ba     | 47.5 ± 2.2 Aa    | 1.65 ± 0.18 Aa  |
|       | L75       | 0.064 ± 0.012 Ab                 | 18.8 ± 1.25 Ab  | 975 ± 62 Ab      | 22.3 ± 1.6 Bb    | 1.37 ± 0.16 Ab  |
|       | L50       | 0.071 ± 0.009 Aa                 | 15.0 ± 1.46 Ac  | 683 ± 51 Ac      | 16.7 ± 1.1 Ac    | 1.08 ± 0.11 Ac  |
|       | L20       | 0.079 ± 0.013 Aa                 | 10.3 ± 1.08 Ad  | 652 ± 22 Ac      | 7.6 ± 1.3 Ad     | 0.91 ± 0.09 Ac  |
| August| L100      | 0.057 ± 0.010 Ac                 | 26.3 ± 2.76 Aa  | 1752 ± 85 Aa     | 42.6 ± 2.8 Bb    | 1.67 ± 0.15 Aa  |
|       | L75       | 0.069 ± 0.011 Ab                 | 16.7 ± 1.55 Bb  | 903 ± 53 Ab      | 26.7 ± 1.7 Ab    | 1.29 ± 0.12 Aa  |
|       | L50       | 0.075 ± 0.012 Aab                | 12.7 ± 1.06 Bbc | 658 ± 44 Ac      | 10.9 ± 1.5 Bc    | 0.97 ± 0.14 Ac  |
|       | L20       | 0.082 ± 0.009 Aa                 | 9.9 ± 0.86 Ac   | 617 ± 37 Ac      | 6.3 ± 0.7 Acd    | 0.90 ± 0.08 Ac  |

* L100, 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 different months. Lowercase letters indicate significant differences among light treatments in the same month \((P \leq 0.05)\, \text{Duncan’s multiple range test}\).
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 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 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 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
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; Lichtenhalter 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 $P_e$ 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 $P_e$ was related to nonstomatal limitations (Brodribb, 1996). When $g_S$ and $C_i$ decrease simultaneously, the decline in $P_e$ 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 $P_{e}$, $g_S$, and $E$ significantly in tung tree seedlings. $C_i$ in the L75 and L50 treatments was related to a decrease in $g_S$ and $P_e$; 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 indicated 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 $P_e$ 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 $P_e$–PPFD and $P_e$–$C_i$ 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, A$_{QY}$, $A_{max}$, 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 $R_A$ in the L75, L50, and L20 treatments, indicating that physiological activity may have declined (Talts et al., 2004). Furthermore, $A_{max}$, $V_{max}$, and $I_{max}$ 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, $P_e$, $g_S$, $E$, Rubisco and PEPC assimilatory enzyme activities, $V_{max}$ 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$46 mol·m$^{-2}$·d$^{-1}$ (or 1400 $\mu$mol·m$^{-2}$·s$^{-1}$ PPFD at noon) on sunny days in summer are recommended to ensure proper tung tree growth and yield.

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