Seedling emergence and early growth of Chinese fir under different light levels and seed positions: implications for natural regeneration

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Abstract

Chinese fir, *Cunninghamia lanceolate* (Lamb.) Hook. (Taxodiaceae) is an evergreen conifer primarily distributed in southern China. Possibly due to low light and a thick litter layer, the species exhibits very poor natural regeneration. To improve the understanding of natural regeneration capacity of Chinese fir, in this study, we conducted a shade house experiment to determine the optimum light requirements and seed positions for seedling emergence and early growth. The experiment involved five light levels (100%, 60%, 40%, 15%, 5% of full sunlight) and four seed positions (1 cm beneath soil surface without litter, on soil surface without soil-seed contact, on soil surface and covered with litter, 1 cm beneath soil surface and covered with litter). Seedling emergence was highest at 5–15% sunlight, whereas seedling height, root length, root mass, stem mass, leaf mass, and total mass were highest at 60% sunlight. For each light level, seed position significantly affected emergence and growth. Above-litter position inhibited seedling emergence and survival, while below-litter position favored seedling emergence and early growth, particularly under high light. Based on these results, to enhance natural regeneration of Chinese fir, we recommend periodical thinning to increase light into understory after successful seedling emergence. We also recommend sowing seeds deeper into the litter to improve soil contact and moisture conditions.

Key words (5): Biomass allocation, Forest regeneration, Plantation, Seedling morphology, Silviculture

1. Introduction

Chinese fir, *Cunninghamia lanceolata* (Lamb.) Hook. Taxodiaceae) is an evergreen conifer with strong economic, environmental and social significance in southern China, with over 1000 years of cultivation in managed forests (Ma et al. 2007). Chinese fir trees are only found scattered in natural broad-leaved forests, and naturally occurring pure Chinese fir stands do not exist (Chen et al. 2000). Current pure Chinese fir plantations take up 17×10^6 ha worldwide, comprising 24% of forests in China and 6.1% of global forests, replacing numerous natural broadleaf and coniferous forests in China (FAO 2015; Yang et al. 2018). Unfortunately, the sustainability of these Chinese fir plantations is threatened by biodiversity reduction, production loss, soil degradation, and a lack of self-regeneration (Yang et al. 2004; Bi et al. 2007; Ma et al. 2007; Chen et al. 2014; Luo et al. 2014; Zhang et al. 2018). The lack of natural regeneration is of particular concern as forest management shifts focus from timber production to sustainability (i.e., maintaining ecological functions), and sustainable forestry relies on the ability of plant species to self-regenerate (Robert 2014; Bertacchi et al. 2016; Chazdon et al. 2016; Pirard et al. 2016; Liu et al. 2017). While understanding the factors that control tree regeneration is a major research priority for forest managers worldwide (Carnevale et al. 2002; Dupuy et al. 2008), surprisingly only a few studies have addressed the ecological factors that influence tree regeneration in Chinese fir plantations (e.g., Liu et al. 2017).

Seedling emergence and early growth are more sensitive than other developmental stages to environmental fluctuation, thus representing a major bottleneck to tree regeneration (Zhu et al. 2003; Walck et al. 2011). Environmental factors such as light, soil moisture, temperature, soil conditions, pH, and litter cover are known to

influence these two bottleneck developmental processes (Liu et al. 2011; Liu et al. 2017; Liu et al. 2018; Wu et al. 2017), although the degree of influence varies from region to region. In the subtropical ecosystems of southern China, for example, moisture and temperature are not limiting factors. Likewise, Fan et al. (2005) reported that lower pH (e.g., $\text{pH} < 3.5$) significantly inhibited seed germination, subsequent survival and growth, and pH 5.0 significantly stimulated seedling growth (Fan et al. 2005). The soil pH is more than 4.2 in the Chinese fir plantations, (Zhang et al. 2017), the effect of pH caused by acid rain on germination and seedling growth of Chinese fir is therefore small. At the same time, in coniferous plantations, high tree density blocks light from reaching the ground, and low litter decomposition rates can lead to heavy needle-layer accumulation (Cornett et al. 1998; Day et al. 2005; Wang et al. 2006; Zhu et al. 2014), especially for Chinese fir (Chen et al. 2014; Wang et al. 2007). Evidence from field observations suggests that Chinese fir can regenerate naturally along the edges of Chinese fir stands and in gaps of natural broad-leaved forests, but not in dense pure Chinese fir stands (Chen et al. 2000; Zhu et al. 2011). Therefore, low light penetration and thick litter layer are major limiting factors that influence seedling emergence and early growth of many coniferous species (Cornett et al., 1998; Day et al., 2005; Wang et al., 2006), including Chinese fir (Chen et al. 2014; Liu et al. 2017; Liu et al. 2018).

The influence of litter on seedling development may have a chemical or mechanical basis (Bosy and Reader 1995; Olson and Wallander 2002). Litter may exert an allelopathic effect on seeds, or else physically block access to nutrients, water and light (Hovstad and Ohlson 2008; Quddus et al. 2014). No research currently exists on how various light conditions affect these two bottleneck developmental processes in

Chinese fir, even though the species is light-demanding. This knowledge gap highlights the importance of this study because light requirements may differ in a species-specific manner between seed germination and subsequent seedling growth. For example, *Pinus strobus* L. exhibits significantly higher seedling emergence under low light (13% of full sunlight) than under moderate light (47% of full sunlight) (Parker et al. 2006), whereas the latter level favored seedling growth (Parker et al. 2004). *Pinus pinea* L. maintained high germination rate in dark conditions (Ganatsas and Tsakalidimi 2015), while *Pinus pinaster* experiences the highest germination percentage and aboveground dry weight at 25% and 100% light intensity, respectively (Ruano et al. 2009).

Because thick litter can act as a physical barrier, the seed's position relative to litter should be taken into consideration when investigating seedling emergence and growth (Zhang et al. 2012; Wellstein 2012). Indeed, thick Chinese fir litter was found to negatively affect seedling emergence and early growth (Liu et al. 2017). Generally, seeds are on the litter surface, between litter and soil, or buried in soil (Rotundo and Aguiar 2005; Wellstein 2012). Although germinating beneath a litter layer can be beneficial due to lower evaporation and attenuated temperature extremes (Jensen and Gutekunst 2003; Eckstein and Donath 2005; Rotundo and Aguiar 2005; Donath and Eckstein 2010), leaf litter can also physically prevent the upward growth of an emerging shoot, while blocking access to sufficient light. In contrast, although seedlings can absorb moisture and nutrients through direct soil contact with their primary root when they are on the top of litter, the above-litter position increase desiccation likelihood (Rotundo and Aguiar 2005; Donath and Eckstein 2010). The net benefits of litter with respect to seed position are

thus complex and could be species specific. To our knowledge, no studies have investigated the net benefits of litter with respect to seed position for Chinese fir.

In the present study, we investigated seedling emergence and early growth of Chinese fir in shade-house experiments with varying light levels and sowing positions. We specifically addressed the following questions. First, do seedling emergence and subsequent seedling growth have different light requirements? Second, does seed position affect seedling emergence and growth, and if so, how does this response change depending on different light levels? Our results should advance current understanding of the ecological factors that determine tree regeneration and thus inform the development of sustainable plantation management.

2. Methods

2.1. Seed collection and pretreatment

Chinese fir seeds were obtained from a plantation in Xinkou National Forest Farm (26°10' N, 117°27' E), Sanming City, Fujian Province, China. Seeds were collected from at least 10 individual trees during late November 2015, then manually cleaned, air-dried, sterilized, and stored at 4°C until needed for sowing. Sterilization involved soaking in a 0.5% K₂MnO₄ for 30 min and in 75% ethanol for 1 min. Seeds were subsequently soaked in distilled water for 24 h. Floating seeds were discarded, while seeds that sank immediately were considered viable. Only viable seeds with similar size and shape were used in this study.

2.2. Experimental design

The study tested five different light levels: 100% (control), 60%, 40%, 15%, and 5% of full sunlight. Each was created using houses covered with black nylon shade cloth

at increasing higher mesh gauges (the 100% sunlight control did not use shade cloth). Mesh gauges of 2, 3, 6, and 8 were used to intercept 40%, 60%, 85%, and 95% irradiance, resulting in conditions of 60%, 40%, 15%, and 5% sunlight, respectively. Houses were 2.0 m high, 6.0 m $\times$ 2.5 m in length and width, and were positioned parallel to the sun's daily motion to reduce spatial and temporal variation of solar radiation. Each of the five light levels contained four houses, one for each tested seed-sowing position relative to the 3-cm litter: (i) control: 1 cm beneath soil surface without litter; (ii) surface: on the soil surface without soil-seed contact; (iii) between: on soil surface and covered with litter; (iv) burial: 1 cm beneath soil surface and covered with litter. The experiment used plastic pots (18 cm $\times$ 20 cm), commercial sterilized potting soil, and Chinese fir litter from the same plantation as the seeds. Pots were spaces apart from each other to minimize any interplant competition. Each seed position had five replicates, and each pot sown 50 seeds.

To prepare the litter cover, leaves of Chinese fir were rinsed with distilled water to remove dust particles and air-dried under ambient temperature, then stored in paper bags until needed. A 3-cm thickness litter (i.e., 400 g litter m⁻²) was used to reflect the annual litter production naturally found in Chinese fir forests (Wang et al. 2007; Gao et al. 2015). Germination was defined as the first needle sprout becoming visible. The emergence experiment was terminated once no further germination occurred over two weeks.

In August 2016 (the experiment began in April 2016 and lasted for 4 moths), all seedlings were removed and carefully washed. Five seedlings per pot were randomly selected. Roots, stems, and leaves were separated for every plant. Longest root length and

shoot height (up to the apical meristem) were measured to characterize overall plant extension above- and below-ground. All biomass fractions were placed in paper bags, oven-dried at 75°C for 48 h, and weighed. We then calculated the emergence rate (number of germinated seeds/total number of seeds planted) and survival rate (number of living seedlings/number of germinated seeds). The following biomass fractions were also calculated: leaf mass fraction (leaf dry mass/total seedling dry mass); stem mass fraction (stem dry mass/total seedling dry mass); root mass fraction (root dry mass/total seedling dry mass); and root/shoot ratio (root dry mass/aboveground dry mass). Survival rates were not provided for full sunlight under “surface” treatment because seedlings experienced 100% mortality.

2.3. Statistical analysis

All statistical analyses were performed in SPSS version 24.0 (SPSS Inc., Chicago, IL, USA). Assumptions of normality were tested before analysis (one-sample Kolmogorov-Smirnov test, $p > 0.05$). Data are presented as means $\pm$ standard deviation (SD) for each treatment. The effects of light, seed position, and their interaction on seedling emergence, survival, root length, shoot height, root mass, stem mass, leaf mass, total mass, root-mass fraction, stem-mass fraction, leaf-mass fraction, and root/shoot ratio were examined with two-way ANOVA. In 100% sunlight, no seedlings survived under “surface” treatment, and less than 2 seedlings survived under “control”, “between” and “burial” treatments. Thus, with the exception of emergence and survival rate calculations, data analyses of 100% sunlight treatments excluded these sowing conditions. Post-hoc multiple comparisons of means were performed with Tukey’s tests. Significance was set at $p = 0.05$.

3. Results

3.1. Seedling emergence and survival

Light level, seed position and their interaction significantly affected emergence and survival rates ($P < 0.001$). Emergence and survival rates significantly decreased with increasing light levels, except between 5% and 15% sunlight (Fig. 1). Emergence and survival rates were significantly lower in “surface” treatment than in “between” and “burial” treatments (Fig. 1). Under 5%, 15%, and 40% sunlight, the “control” improved emergence and survival rates over the “surface” treatments. Under 5% sunlight only, “control” was also superior to “between” and “burial” treatments. However, emergence and survival rates dropped in “control” treatments compared with “between” and “burial” treatments under 60% and 100% sunlight (Fig. 1).

3.2. Root and stem growth

Light level, seed position and their interaction significantly affected root length and seedling height. Root length strongly increased with increasing light levels, except between 40% and 60% sunlight in “between” and “burial” treatments (Fig. 2A). Seedling height was significantly lower at 5% sunlight compared with 40% and 60%, regardless of seed position (Fig. 2B). Root length was significantly longer in “surface” than in “between” or “burial” treatments, except under 5% sunlight (Fig. 2A). Seedling height was shorter in “surface” treatment than in “between” and “burial” treatments, regardless of light level (Fig. 2B). Root length and seedling height were greater in “control” than in “between” and “burial” treatments under 15% sunlight, but no significant difference was observed under 40% and 60% sunlight (Fig. 2). Compared with “control”, root length and

seedling height were shorter in “surface” treatment under 40% and 60% sunlight, but not significantly different under 5% and 15% sunlight.

3.3. Biomass accumulation

Light level, seed position, and their interaction significantly affected biomass variables. In general, biomass exhibited a direct relationship with light (Fig. 3). Regardless of seed position, root mass significantly increased with increasing light penetration (60% > 40% > 15% > 5% sunlight) (Fig. 3A). Stem mass, leaf mass, and total mass were significantly higher at 60% and 40% sunlight compared with 15 % and 5% sunlight (Fig. 3B). Root mass was significantly higher in “surface” than in “between” and “burial” treatments, except under 5% and 15% sunlight (Fig. 3A). Stem mass, leaf mass, and total mass were significantly lower in “surface” than in “between” and “burial” treatments (Fig. 3B-D).

Under 15% sunlight, “control” increased root mass over “between” and “burial” treatments, but not under 5%, 40%, or 60% sunlight. “Control” also increased stem, leaf, and total mass over the other two treatments under 15% sunlight, but had the opposite effect under 60% sunlight (Fig. 3). The three seed positions did not differ in their effects on stem, leaf, and total mass under 5% and 40% sunlight.

3.4. Biomass allocation

As light increased, root mass fraction and root/shoot ratio increased, while leaf and stem mass fractions decreased (Fig. 4). Under “between” and “burial” treatments, root mass fraction, leaf mass fraction, and root/shoot ratio significantly differed across all light levels, except when comparing 60% and 40% sunlight. Stem mass fraction was higher under 5% and 15% sunlight than under 40% and 60% sunlight, regardless of seed

position. Root mass fraction and root/shoot ratio were significantly higher in “surface” than in “between” and “burial” treatments, regardless of light level (Fig. 4A). Finally, stem and leaf mass fractions were significantly lower in “surface” treatment than in “between” and “burial” treatments under all light levels except 5% sunlight (Fig. 3B-D).

4. Discussion

In this study, we demonstrated that light requirement differed between seedling emergence (including survival rate) and seedling growth. Consistent with other coniferous species, such as *Pinus strobus* and *P. koraiensis* (Parker et al. 2006; Zhang et al. 2014), seedling emergence and survival rate decreased with increasing light, irrespective of seed position. The highest seedling emergence and survival rate were observed at 5% and 15% sunlight, while the highest seedling growth (e.g., shoot or root morphology and biomass) occurred under 60% sunlight. Thus, while shading is critical to increasing germination and seedling survival of coniferous trees (Pons 1992), it has negative effect on subsequent seedlings growth. A trade-off between carbon allocation for development versus for storage and defense (Poorter and Kitajima 2007) could be responsible for this opposing effect of light, a phenomenon observed in other tree species (Parker et al. 2004; Ganatsas and Tsakalidimi 2015; Zhang et al. 2014). As herbivores and pathogens are the primary agents of seedling mortality (Myers and Kitajima 2007; Poorter and Kitajima 2007), Chinese fir may need to allocate more C for seedling defense and storage during initial growth stages, resulting in the overall highest survival rate under the lowest light levels (5% and 15%). However, as seedlings grow, higher light environment is necessary to support photosynthesis. In densely populated Chinese fir plantation, severe light competition occurred with neighboring plants forcing the

seedlings to shift their C allocation toward shoot elongation. As a result, seedling mortality may increase in low light environments because resources are diverted away from defense (Kurokawa et al. 2004) and storage (Myers and Kitajima 2007; Poorter and Kitajima 2007). Due to light requirement disparities between seedling emergence and subsequent growth, we recommend periodical thinning in Chinese fir plantations that will allow more light to reach the understory upon successful emergence.

We also demonstrated that seed position strongly affects seedling emergence and survival rate in Chinese fir. Seeds sown in direct contact with soil (between litter and soil or buried under soil) had higher emergence rates than seeds sown on the litter surface. This finding was consistent with research demonstrating *Bromus pictus* seeds sown within litter (no seed-soil contact) showed reduced emergence and growth (Rotundo and Aguiar 2005). Similarly, a study on three grassland species (*Pimpinella saxifraga*, *Leontodon autumnalis*, *Sanguisorba officinalis*) revealed that seedling emergence was significantly higher when seeds were sown below the litter than when they were sown on top of litter. Two factors may have contributed to lower seedling emergence and survival rate for seeds sown on top of litter. First, some of the seedlings may have suffered lethal desiccation during radicle establishment because thick leaf litter layer may act as a barrier towards the downward movement of plant roots. Consequently, seedling's primary root could not reach the soil and water rapidly enough. Second, seedling root growth itself may be significantly retarded by the thick litter layer (Green 1999), as opposed to when seeds germinated beneath the litter layer and in direct contact with the soil. Supporting these propositions was our observation that below-litter seeds had very straight roots, indicating unobstructed penetration through soil. In contrast, roots of above-litter seeds

were typically looped and curved, reflecting considerable obstruction before reaching the soil. Similar observations were also recorded for *Chrysophyllum* seedlings (Green 1999). Some data exist to suggest that the ideal position for seeds may be in direct soil contact beneath a litter layer, as this offers benefits such as attenuated temperature extremes and reduced water stress (Jensen and Gutekunst 2003; Eckstein and Donath 2005; Donath and Eckstein 2010).

In response to fluctuations in seed position, seedlings face a trade-off between aboveground growth (for light interception) and root growth (for nutrient and water acquisition) (Kostel-Hughes et al. 2005; Donath and Eckstein 2010). Our study provided evidence of biomass allocation shift in response to resource limitation (Poorter and Nagel 2000; Markesteijn and Poorter 2009). With decreasing light availability, Chinese fir seedlings allocated more resources to leaves at the expense of roots and stems, as indicated by higher leaf mass fraction and leaf/root ratio under low-light treatments (Fig. 4). Shoot development is thought to be an adaptive strategy for seedlings under low light environments (e.g., beneath the litter) (Peterson and Facelli 1992; Ellsworth et al. 2004; Kostel-Hughes et al. 2005), especially as root growth can be hampered by poor photosynthetic conditions that limit photosynthate availability. In contrast, under high-light environments, biomass allocation will shift to water and nutrient acquisition (Brown et al. 2014; Sevilano et al. 2016), as shown by increased root length, root/shoot ratio and root mass fraction (Fig. 4). We found evidence that when seeds were in the above-litter position, resource investment toward root elongation was crucial to overall seedling fitness (Green 1999; Wellstein 2012). Therefore, our results were consistent with the functional equilibrium model where plants allocate biomass depending on whether

above- or belowground regions have fewer resources (Poorter and Nagel 2000; Markesteijn and Poorter 2009). This plastic morphological response suggested that Chinese fir seedlings can use resource allocation to survival in both below- and above-litter positions, provided the litter is not overly thick. Similar observations were also found from studies of other tree species (e.g., *Fagus sylvatica* and *Quercus robur*; Sevillano et al. 2016).

5. Conclusions

Here we demonstrated that light requirements for Chinese fir shifted from 5–15% to 60% between seedlings emergence and growth. Although other natural environment factors may affect both of these processes, the shift in optimal light level across developmental stages partly explain the poor natural regeneration observed in Chinese fir plantations. In coniferous plantations, high tree density blocks light from reaching the ground (Cornett et al. 1998; Day et al. 2005; Wang et al. 2006), favoring seedling emergence but not subsequent growth. We also found that the optimum seed position for emergence and growth was beneath the litter, rather than above it, suggesting the importance of seed burial as a protective mechanism against temperature extremes and water stress. Notably, both conditions become more apparent under high light. The protective qualities of litter also benefit the development of silviculture practices (e.g., spatially- and temporally-regulated thinning) that generate favorable light levels for subsequent growth of Chinese fir seedlings. We also recommend sowing seeds deeper in the litter to improve soil contact and moisture conditions.

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Figure legends

Fig. 1 Emergence rate (A) and survival rate (B) across different light levels and seed positions in experimental shade houses. Bars are means $\pm$ SD. Different capital letters represent significant differences between light levels within the same seed position (Tukey's test at $P < 0.05$). Bars with different lowercase letters represent significant differences between seed positions within the same light level (Tukey's test at $P < 0.05$). Control: under 1 cm soil without litter, which was considered the control position; Surface: on top (no soil-seed contact); Between: on soil and covered with litter; Burial: under 1 cm soil and covered with litter.

Fig. 2 Root length (A) and seedling height (B) across different light levels and seed positions. Bars are means $\pm$ SD. Different capital letters represent significant differences between light levels within the same seed position (Tukey's test at $P < 0.05$). Bars with different lowercase letters represent significant differences between seed position within the same light level (Tukey's test at $P < 0.05$). Control: under 1 cm soil without litter, which was considered the control position; Surface: on top (no soil-seed contact); Between: on soil and covered with litter; Burial: under 1 cm soil and covered with litter.

Fig. 3 Root mass (A), stem mass (B), leaf mass (C), and total mass (D) across light levels and seed positions. Bars are means $\pm$ SD. Different capital letters represent significant differences between light levels within the same seed position (Tukey's test at $P < 0.05$). Different lowercase letters represent significant differences between seed positions within the same light level (Tukey's test at $P < 0.05$). Control: under 1 cm soil without litter, which was considered the control position; Surface: on top (no soil-seed contact); Between: on soil and covered with litter; Burial: under 1 cm soil and covered with litter.

Fig. 4 Root mass fraction (A), stem mass fraction (B), leaf mass fraction (C), and root/shoot ratio (D) across light levels and seed positions. Bars are means $\pm$ SD. Different capital letters represent significant differences between light levels within the same seed position (Tukey's test at $P < 0.05$). Different lowercase letters represent significant differences between seed positions within the same light level (Tukey's test at $P < 0.05$). Control: under 1 cm soil without litter, which was considered the control position; Surface: on top (no soil-seed contact); Between: on soil and covered with litter; Burial: under 1 cm soil and covered with litter.

Fig. 1

Fig. 2

Fig. 3

Fig. 4