Effects of Slow-Release Fertilizer on Growth, Photosynthetic Physiology, and Nutrient Accumulation of Chinese Fir Container Seedlings: Postprint

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Date: 2022-07-05T00:00:00+00:00

Abstract

To explore the response characteristics of Chinese fir container seedling growth, photosynthetic traits, and nutrient accumulation to different slow-release fertilizer application rates, this study investigated the effects of six slow-release fertilizer treatments (0, 200, 400, 800, 1,000, and 1,200 $g \cdot m^{-3}$) on Chinese fir seedling growth, photosynthetic pigment content, chlorophyll fluorescence characteristics, and nutrient content. A comprehensive evaluation of growth and physiological indices was conducted using the subordinate function method to screen for optimal fertilization levels for Chinese fir container seedling growth and provide a reference for efficient cultivation of high-quality Chinese fir seedlings. The results showed that: (1) Compared with the control, slow-release fertilizer application promoted Chinese fir seedling height, ground diameter growth, and total plant biomass accumulation to varying degrees. (2) Slow-release fertilizer treatments significantly increased chlorophyll and carotenoid contents in Chinese fir leaves, and enhanced maximum fluorescence (Fm), variable fluorescence (Fv), PS II maximum photochemical efficiency (Fv/Fm), PS II potential photochemical efficiency (Fv/Fo), and actual quantum yield (QY) values. (3) Slow-release fertilizer treatments promoted nutrient accumulation in Chinese fir seedlings to varying degrees, with the most significant changes observed in manganese, iron, and zinc accumulation. (4) Subordinate function analysis revealed that the subordinate value was highest at a slow-release fertilizer application rate of 1,000 $g \cdot m^{-3}$, indicating the best comprehensive growth status under this treatment. In summary, a slow-release fertilizer application rate of 1,000 $g \cdot m^{-3}$ is optimal for cultivating robust Chinese fir seedlings. Under this treatment, the accumulation of elements closely related to photosynthesis within the plant was promoted, leaf photosynthetic pigment content increased, leaf PS II photochem-

ical efficiency and electron transport rate improved, thereby enhancing leaf light energy capture and utilization efficiency, ultimately improving seedling growth.

Full Text

Preamble

Effects of Slow-Release Fertilizer on Growth, Photosynthetic Physiology, and Nutrient Accumulation in Container Seedlings of *Cunninghamia lanceolata*

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Abstract: This study investigated the responses of growth, photosynthetic characteristics, and nutrient accumulation in *Cunninghamia lanceolata* container seedlings to different slow-release fertilizer application rates. Six fertilizer treatments (0, 200, 400, 800, 1,000, and 1,200 $g \cdot m^{-3}$) were applied to examine their effects on seedling growth, photosynthetic pigment content, chlorophyll fluorescence parameters, and nutrient content. A subordinate function method was employed for comprehensive evaluation of growth and physiological indices to identify optimal fertilizer levels for *C. lanceolata* container seedlings and provide references for efficient cultivation of high-quality seedlings. The results showed that: (1) Compared with the control, slow-release fertilizer application promoted seedling height, ground diameter growth, and total biomass accumulation to varying degrees. (2) Fertilizer treatments significantly increased leaf chlorophyll and carotenoid contents, as well as maximum fluorescence (Fm), variable fluorescence (Fv), PSII maximum photochemical efficiency (Fv/Fm), PSII potential photochemical efficiency (Fv/Fo), and actual quantum yield (QY). (3) Fertilizer treatments promoted nutrient accumulation in seedlings, with Mn, Fe, and Zn showing the most significant changes. (4) Subordinate function analysis revealed the highest membership value at 1,000 $g \cdot m^{-3}$ fertilizer application, indicating the best overall seedling growth under this treatment. In conclusion, 1,000 $g \cdot m^{-3}$ is the optimal slow-release fertilizer rate for *C. lanceolata* seedling cultivation. This treatment improves seedling growth by promoting accumulation of photosynthesis-related elements, increasing leaf photosynthetic pigment content, enhancing PSII photochemical efficiency and electron transport rate, and ultimately improving light energy capture and utilization efficiency.

Keywords: seedling cultivation, slow-release fertilizer, *Cunninghamia lanceo-*

lata, chlorophyll fluorescence parameters, container seedlings, seedling quality

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Cunninghamia lanceolata is an important fast-growing timber species in southern China. According to the ninth national forest inventory, Chinese fir plantations rank first among major artificial forest tree species in both area and volume, playing a crucial role in ensuring national ecological and timber security. With expanding plantation area, market demand for high-quality Chinese fir seedlings has increased annually. Traditional seedling cultivation primarily relies on bare-root seedlings in field nurseries, which suffer from low outplanting rates, root damage during lifting, limited planting seasons, and requirements for crop rotation, significantly increasing nursery costs and reducing afforestation survival rates. Container seedlings effectively overcome these limitations and have become an important alternative for Chinese fir propagation. However, the limited growth space and relatively low nutrient availability in container substrates cannot meet the nutrient demands of rapidly growing seedlings, making fertilization a critical measure for ensuring high-quality growth. Although fertigation is commonly used in container seedling production, this conventional approach often leads to waste of water and fertilizer, reduces nutrient use efficiency, increases production costs, and may cause environmental pollution. Therefore, research on fertilization techniques for Chinese fir container seedlings is essential for improving seedling quality, enhancing stress resistance, and optimizing afforestation outcomes.

As a novel fertilizer type, slow-release fertilizer offers high nutrient use efficiency, minimal volatilization and leaching, and long-lasting effects, with increasingly widespread application in seedling cultivation. Studies on pecan container seedlings demonstrated that 3 $kg \cdot m^{-3}$ slow-release fertilizer effectively promoted growth and root development by improving plant N, P, and K nutrition. Research on *Quercus aliena* container seedlings indicated that 0.95 $g \cdot L^{-1}$ slow-release fertilizer optimally promoted seedling height, ground diameter, biomass, and nutrient accumulation. Similarly, optimal growth of *Aquilaria sinensis* container seedlings occurred at 2.5 $kg \cdot m^{-3}$, with significantly higher seedling height, ground diameter, and biomass compared to other treatments. In *Pistacia chinensis*, low fertilizer rates favored root growth, while increased rates significantly improved stem and leaf growth, reaching maximum values at 1.6 $kg \cdot m^{-3}$. These findings demonstrate substantial variation in optimal slow-release fertilizer rates among different species due to biological differences, highlighting the theoretical and practical importance of investigating fertilizer responses in Chinese fir container seedlings.

Previous research on Chinese fir fertilization has primarily focused on conventional fertilization, formula fertilization, and exponential fertilization, with relatively few studies on slow-release fertilizer effects and incomplete understanding of growth response mechanisms. Meanwhile, promotion of superior Chinese fir varieties such as the elite clone 'Yang-061' has significantly improved genetic quality and economic benefits, yet corresponding seedling cultivation techniques remain underdeveloped. Superior varieties exhibit different nutrient requirements due to their distinct biological characteristics and demand higher cultivation standards than traditional techniques can provide. Therefore, research on slow-release fertilizer application techniques tailored to superior Chinese fir materials is crucial for achieving efficient cultivation. This study used the elite clone 'Yang-061' to investigate effects of different slow-release fertilizer rates on growth, biomass accumulation, photosynthetic pigment synthesis, chlorophyll fluorescence parameters, and nutrient content of container seedlings in light substrate. Using subordinate function analysis for comprehensive evaluation, we addressed: (1) How do Chinese fir seedling growth, photosynthesis, and nutrient accumulation respond to different fertilizer rates? (2) What is the optimal fertilizer rate for 'Yang-061', and how does it improve seedling growth? The findings provide theoretical basis and technical support for efficient cultivation of high-quality Chinese fir seedlings.

1.1 Experimental Site

The experiment was conducted in a field greenhouse at Jinshan Campus, Fujian Agriculture and Forestry University (119°13 E, 26°05 N). The site is at 10 m altitude with a subtropical monsoon climate, mean annual temperature of 25°C, annual sunshine of 1,700–1,980 h, annual precipitation of 900–2,100 mm, and a frost-free period of 326 days.

1.2 Experimental Materials

Cutting shoots were obtained from Fujian Yangkou State-owned Forest Farm, using uniform one-year-old shoots of 'Yang-061'at (10$±0.4)cmlength.Thelightsubstrate(peatsoil, perlite, andChi
2 : 2(V : V : V)ratio.ThechemicalpropertiesofthemixedsubstratearesshowninTable1.Theslow−
releasefertilizer(Apex, USA)contained180g · kg^{-1}$ total N, 60 $g \cdot kg^{-1}$ total P, and 120 $g \cdot kg^{-1}$ total K.

1.3 Experimental Design

In mid-April 2019, different amounts of slow-release fertilizer were added to the light substrate (peat:perlite:bark = 1:2:2 V:V:V). Six treatments were established: CK (0 $g \cdot m^{-3}$), T1 (200 $g \cdot m^{-3}$), T2 (400 $g \cdot m^{-3}$), T3 (800 $g \cdot m^{-3}$), T4 (1,000 $g \cdot m^{-3}$), and T5 (1,200 $g \cdot m^{-3}$). The substrate and fertilizer were thoroughly mixed and placed in non-woven fabric bags (6 cm diameter × 10 cm height). Cuttings were inserted into the bags, with one seedling per bag and 300 seedlings per treatment. The experiment used a randomized block design with three replicates of 90 seedlings each (30 seedlings per replicate), totaling 540 seedlings across six treatments. Normal water and weed management were maintained during cultivation. The experiment concluded in mid-November 2020.

1.4 Measurement Methods

1.4.1 Growth Indices In mid-November 2020, seedling height and ground diameter were measured using vernier calipers and rulers. Three standard seedlings were selected per replicate based on mean values. These were separated into roots, stems, and leaves, killed at 105°C for 2 h, then oven-dried at 75°C to constant weight. Seedling quality index was calculated following Zhu et al. (2018):

$$\text{Seedling quality index} = \frac{\text{Total seedling dry mass}}{\left(\frac{\text{Height}}{\text{Ground diameter}} + \frac{\text{Aboveground dry mass}}{\text{Belowground dry mass}}\right)}$$

1.4.2 Photosynthetic Pigment Content Healthy mature leaves from the first whorl of upper branches were selected for photosynthetic pigment determination using the ethanol-acetone extraction method (Lin et al., 2016). After extraction, 200 L of extract was placed in a microplate and absorbance measured at 470, 645, and 663 nm (A_{470}, A_{645}, A_{663}) with three replicates per treatment. Pigment contents were calculated as:

$$\text{Chlorophyll a (Ca)} = (12.7 \times A_{663} - 2.69 \times A_{645}) \times \frac{\text{Extract volume} \times \text{Dilution factor}}{\text{Sample mass}}$$

$$\text{Chlorophyll b (Cb)} = (22.88 \times A_{645} - 4.76 \times A_{663}) \times \frac{\text{Extract volume} \times \text{Dilution factor}}{\text{Sample mass}}$$

$$\text{Total chlorophyll} = (20.29 \times A_{645} + 8.04 \times A_{663}) \times \frac{\text{Extract volume} \times \text{Dilution factor}}{\text{Sample mass}}$$

$$\text{Carotenoid content} = \frac{1000 \times A_{470} - 3.27 \times \text{Ca} - 104 \times \text{Cb}}{229}$$

1.4.3 Chlorophyll Fluorescence Parameters A PAM-2500 portable chlorophyll fluorometer (Walz, Germany) was used to measure chlorophyll fluorescence parameters on healthy mature leaves from the first whorl of upper branches, following Tao et al. (2011). Parameters were calculated according to Baker (2008).

1.4.4 Nutrient Content Determination Dried root, stem, and leaf samples were ground and sieved through a 0.149 mm mesh. Samples were digested using microwave high-pressure digestion, and element contents (Mg, P, K, Ca, Mn, Fe, Zn) were determined by inductively coupled plasma mass spectrometry (ICP-MS).

1.5 Data Analysis

Data were processed using Excel 2019. The subordinate function method was used for comprehensive evaluation of seedling growth traits. For traits positively correlated with growth, the subordinate function value was calculated as (X - X)/(X - X); for negatively correlated traits, the value was 1 - (X - X)/(X - X), where X is the measured value, X is the maximum value, and X is the minimum value (Dai et al., 2021). SPSS 26.0 was used for one-way ANOVA, with LSD tests for mean comparisons. Figures were created using Origin 8.5.

Results

2.1.1 Effects on Seedling Height and Ground Diameter

All fertilizer treatments promoted seedling height and ground diameter growth compared to CK (Figure 1 [Figure 1: see original paper]). Seedling height increased by 26.94-73.83% (Figure 1A) and ground diameter by 8.79-17.79% (Figure 1B), with maximum values observed in the T4 treatment.

2.1.2 Effects on Biomass Accumulation

Biomass in roots, stems, leaves, and total plant generally increased with fertilizer application, though stem and total biomass showed initial increases followed by decreases (Table 2). Root biomass was higher than CK only in T4 and T5 treatments. Compared to CK, stem, leaf, and total biomass increased by 12.23-122.28%, 5.07-108.57%, and 1.27-85.00%, respectively (except T1 leaf biomass), with maximum values in T4.

2.1.3 Effects on Seedling Quality Index

Fertilizer application significantly affected seedling quality index ($P<0.05$) (Figure 2 [Figure 2: see original paper]). The index showed a decreasing then increasing trend with fertilizer rate, following the order: T5 > T4 > T3 > CK > T2 > T1. T3, T4, and T5 treatments increased the index by 9.25%, 44.34%, and 60.08% compared to CK, respectively.

2.2 Effects on Photosynthetic Pigment Content

Fertilizer application significantly increased chlorophyll a, chlorophyll b, carotenoid, and total chlorophyll contents ($P<0.05$) (Table 3). Maximum chlorophyll a, b, and total contents occurred in T5, while maximum carotenoid content was in T4. The chlorophyll a/b ratio showed an initial increase then decrease with fertilizer rate, but differences among treatments were not significant ($P>0.05$).

2.3 Effects on Chlorophyll Fluorescence Parameters

Initial fluorescence (Fo) and non-photochemical quenching (NPQ) generally decreased with increasing fertilizer rate (Table 4). Compared to CK, Fo decreased by 17.38-23.73% and NPQ by 13.28-53.63%. Maximum fluorescence (Fm), variable fluorescence (Fv), PSII potential photochemical efficiency (Fv/Fo), and

PSII maximum photochemical efficiency (Fv/Fm) increased with fertilizer application, peaking in T4. T4 showed significant differences from CK in Fo, NPQ, Fm, Fv, and Fv/Fo, while effects on Fv/Fm, actual quantum yield (QY), and photochemical quenching coefficient (Qp) were not significant among treatments ($P>0.05$).

2.4 Effects on Nutrient Content

Except for Ca in the low-rate T1 treatment, all fertilizer treatments promoted element accumulation in 'Yang-061' seedlings (Figure 3 [Figure 3: see original paper]). Compared to CK, P, K, and Mg contents increased by 29.50–103.65%, 22.92–80.06%, and 14.52–74.07%, respectively, with maxima in T4. Ca accumulation showed an initial decrease then increase, peaking in T5. Among micronutrients, Mn, Fe, and Zn contents reached maxima in T4, increasing by 157.14%, 216.39%, and 238.08% compared to CK.

2.5 Comprehensive Evaluation Using Subordinate Function Method

Seedling growth is a comprehensive trait that cannot be accurately assessed by single indicators. Therefore, we used the subordinate function method to comprehensively evaluate growth, photosynthetic pigment content, and chlorophyll fluorescence parameters. The comprehensive evaluation index followed the order: T4 > T5 > T3 > T2 > T1 > CK (Table 5).

Discussion

Fertilization is a critical component of seedling cultivation. Rational fertilization reduces nutrient loss, improves fertilizer use efficiency, enhances seedling quality, and lowers production costs. Slow-release fertilizer, with its long-lasting and stable nutrient release, can synchronize nutrient supply with plant demand, substantially improving nutrient use efficiency. Numerous studies have reported on container seedling cultivation using slow-release fertilizer in light substrates. Research on species including pecan, *Quercus aliena*, *Aquilaria sinensis*, *Pistacia chinensis*, *Schima superba*, and *Cyclobalanopsis gilva* demonstrated that 0.95–3.50 $kg \cdot m^{-3}$ slow-release fertilizer effectively promoted container seedling growth, indicating substantial interspecific variation in optimal fertilizer rates, possibly related to fertilizer type and substrate composition. This study compared 0.2–1.2 $kg \cdot m^{-3}$ slow-release fertilizer effects on the elite Chinese fir clone 'Yang-061' , finding that fertilizer application significantly promoted seedling height, ground diameter, and biomass accumulation, but growth indicators declined at 1.2 $kg \cdot m^{-3}$. This pattern aligns with previous container seedling studies, indicating that growth promotion occurs within an optimal fertilizer range, while excessive rates inhibit growth.

Seedling quality index is an important metric for assessing seedling quality. In this study, slow-release fertilizer improved Chinese fir seedling quality, but significant differences were only observed between T5 and CK, similar to findings by Li et al. (2020a). Two possible explanations exist: First, the non-woven fabric bags induced "air pruning" when roots filled the container, limiting further root growth. Due to small container volume, root systems of all treatments eventually filled the bags, minimizing differences in root biomass and potentially confounding the seedling quality index. Second, the small-volume bags may have allowed nutrient and water exchange with air and nutrient leaching by rainfall, potentially affecting results. Specific mechanisms require further investigation.

Photosynthesis provides the primary energy source for plant growth and represents the material basis for biomass production. Chlorophyll is the main photosynthetic pigment, playing a key role in light capture, transfer, and conversion, making photosynthetic capacity closely related to pigment content. Fertilization enhances photosynthetic capacity largely by increasing leaf pigment content. This study found that slow-release fertilizer significantly increased chlorophyll and carotenoid contents with increasing application rate, consistent with Li et al. (2020b), indicating that fertilization enhances photosynthetic capacity by increasing pigment content. The chlorophyll a/b ratio, often used with pigment content to characterize light use efficiency, increased with fertilization (though not significantly), suggesting that slow-release fertilizer enhances light absorption efficiency, directing more light energy to photosynthesis and promoting growth, consistent with our biomass results.

Chlorophyll fluorescence parameters characterize light energy absorption, transfer, dissipation, and allocation in photosystems, commonly used to study light use capacity under stress conditions. Compared to CK, fertilizer application significantly reduced initial fluorescence (Fo), indicating less thylakoid membrane damage and better maintenance of PSII reaction center activity. This may result from improved leaf nutrition preventing reactive oxygen accumulation and reducing damage to photosynthetic structures under nutrient deficiency stress. Maximum fluorescence (Fm), variable fluorescence (Fv), PSII maximum photochemical efficiency (Fv/Fm), and PSII potential photochemical efficiency (Fv/Fo) increased then decreased with fertilizer rate, peaking in T4. These increases enhance the rate and efficiency of light-to-chemical energy conversion, providing more energy for carbon assimilation and improving light use efficiency. Similar results have been reported for *Heritiera littoralis* and switchgrass, indicating that improved light use efficiency and enhanced carbon assimilation represent the photosynthetic physiological basis for growth promotion by slow-release fertilizer. Additionally, Fv/Fm values typically range 0.80–0.85, with stress causing reductions. The control value was significantly below 0.80, indicating nutrient stress and photoinhibition. Non-photochemical quenching (NPQ) is a photoprotective mechanism dissipating excess light energy as heat. Except for T5, all fertilizer treatments showed significantly lower NPQ than CK, suggesting that appropriate fertilization reduces heat dissipation and directs more absorbed light to carbon assimilation, while nutrient stress and excessive fertilization increase heat dissipation to protect photosynthetic apparatus, reducing light energy available for carbon assimilation. This may partially explain the growth decline observed in T5.

Fertilization also promoted nutrient element accumulation. Compared to CK, slow-release fertilizer increased nutrient contents, particularly micronutrients, though excessive fertilizer caused declines while remaining above CK levels. This pattern aligns with studies on *Larix olgensis* and precious tree species, possibly because excessive fertilizer concentrations cause ion toxicity, inhibiting nutrient absorption. Notably, under appropriate fertilization, contents of photosynthesis-related elements (Mg, K, P, Fe, Zn) were significantly higher than in the control. These elements are essential components of photosynthetic pigments or participate in key photosynthetic processes, and their enhancement improves light capture and conversion capacity, thereby increasing photosynthesis and promoting growth. This aligns with our chlorophyll fluorescence results, indicating that appropriate fertilization improves light use efficiency by enhancing nutrient status, particularly photosynthesis-related elements.

Comprehensive evaluation using the subordinate function method identified 1,000 $g \cdot m^{-3}$ as the optimal slow-release fertilizer rate for 'Yang-061' seedlings. This treatment enhanced growth by increasing leaf photosynthetic pigment content, improving light capture and absorption, promoting accumulation of photosynthesis-related elements, increasing chlorophyll fluorescence parameters (Fm, Fv, Fv/Fm, Fv/Fo), enhancing light use efficiency, and ultimately promoting photosynthetic carbon assimilation and biomass accumulation.

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Note: Figure translations are in progress. See original paper for figures.