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RESEARCH ARTICLE

Optimum nitrogen fertilization of *Calophyllum inophyllum* seedlings under greenhouse conditions

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Abstract A greenhouse pot experiment was conducted to study the effects of nitrogen fertilization on *Calophyllum inophyllum* seedlings grown with 0, 50, 100, 150, 200, 300, 400 and 600 mg N per seedling according to exponential functions. Seedling height, root collar diameter, leaf area and total biomass increased with increasing fertilization from 0 to 200 mg N per seedling and decreased with further increase in fertilization from 300 to 600 mg N per seedling. The net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate of *C. inophyllum* seedlings showed a unimodal parabolic trend, with peak values of 7.29 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 0.071 $mol \cdot m^{-2} \cdot s^{-1}$, 220 $\mu mol \cdot mol^{-1}$ and 1.34 $mmol \cdot m^{-2} \cdot s^{-1}$, respectively, when the rate of fertilization was 200 mg N per seedling. Photosynthetic gas exchange parameters were significantly different among nitrogen treatments. Based on the critical values of leaf N and P concentration and N/P ratio, the optimum amount of nitrogen of *C. inophyllum* seedlings was 200–400 mg per seedling for leaf N and P concentration, and 100–400 mg per seedling for N/P ratio. It was concluded that 200–400 mg N per seedling was the most suitable nitrogen range for *C. inophyllum* seedlings.

Keywords *Calophyllum inophyllum*, growth, nitrogen fertilization, nutrient status, photosynthesis

1 Introduction

Nitrogen is a key factor affecting seedling growth and development[1–3], and is important in regulating plant growth and metabolism. Suitable nitrogen fertilization can lead to high-quality seedling production[4,5].Therefore, the amount and effectiveness of nitrogen fertilizer has been a strong research focus. Currently in seedling production, nitrogen use efficiency is low because of inappropriate fertilization methods. Studies have shown that 32%–85% of nitrogen fertilizer cannot be absorbed or used by forest seedlings in the nurseries[6].

During the seedling growth, fertilization methods have usually involved supplying fertilizers in equal doses. This method results in a surplus of nutrients at the beginning followed by deficiency later in seedling growth[7]. Since the 1980s, researchers have discovered that exponential fertilization can actively enhances nutrient utilization efficiency[8], including black spruce (*Picea mariana*) [9–11], white spruce (*Picea glauca*)[12], Norway spruce (*Picea abies*)[13] and birch (*Betula alnoides*)[14].

Calophyllum inophyllum is a salt-tolerant and wind-resistant evergreen species distributed in the tropical and subtropical regions of Asia, Oceania and South America[15]. In China, it is mainly found in Hainan Island[16]. *C. inophyllum* has a high value for development and utilization because of its ecological, medicinal and seed oil functions. Researchers have extensively investigated the biological characteristics[17], ecological functions[18,19], medicinal efficacy[20,21] and economic values[22,23] of *C. inophyllum*. However, little is known about its nutrient requirements, which potentially creates inefficiencies in its silviculture.

The objective of this study was to determine the optimum nitrogen fertilization for *C. inophyllum* seedlings grown under greenhouse conditions. Responses of seedlings to nitrogen fertilizer treatments, including growth characteristics, photosynthetic gas exchange parameters and nutrient status, were examined. Optimum fertilization was determined by the critical value method to provide a theoretical basis for fertilization practice and cultivation technology for this species.

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2 Materials and methods

2.1 Plant materials and growth condition

Healthy one-month old seedlings of *C. inophyllum* about 6.5 cm in height were grown in a greenhouse at the Research Institute of Tropical Forestry, Guangzhou, China. The seedlings were cultured in 12 cm × 15 cm round, tapered plastic pots filled with peat, vermiculite and pearlite (3:2:2 v/v). Each pot was irrigated to 75%–80% of field capacity determined gravimetrically at transplanting[24]. Plant Prod (Plant Products Co. Ltd, Brampton, Ontario, Canada) soluble fertilizer ($N:P_2O_5:K_2O$, 20:10:20) was applied twice per week. Plastic bags were placed inside the pots to prevent drainage of water and nutrients. The average daily temperature in the greenhouse ranged from 23 to 38°C and night temperature from 14 to 22°C. Relative humidity ranged from 34% to 78%. To reduce the edge effects, the positions of the posts were rotated every two weeks. Fungal diseases were prevented by injecting a 0.2% solution of carbendazim into the growth medium.

2.2 Experimental design and fertilizer treatments

The fertilizer was dissolved in distilled water and the same volume carefully applied to seedlings using a syringe. To ensure similar moisture conditions of the seedlings, each seedling pot was weighed and an appropriate amount of water added.

Eight nutrient treatments (N1 to N8 = 0, 50, 100, 150, 200, 300, 400 and 600 mg N per seedling) were applied to seedlings with an exponentially increasing amount applied from June to December 2010 (Table 1). Each treatment had four replicates; each replicate consisted of 12 seedlings, totalling 384 seedlings across all treatments. Fertilizer treatments commenced two weeks after transplanting and were continued for 24 weeks.

The fertilizer treatments followed exponential functions to match nutrient supply with seedling growth according to Salifu and Timmer[11].

$$N_t = N_s(e^{rt-1}) - N_{(t-1)} \quad (1)$$

where N_t is the amount of N to be added at time t for a given relative additional rate r, N_s is the initial quantity of N at the commencement of the treatment, and $N_{(t-1)}$ is the cumulative amount of N added. r was calculated using the method described by Dumroese et al.[25].

$$N_T = N_s(e^{rt-1}) \quad (2)$$

where N_T is the desired amount to be added over the number of fertilizer applications (t = 12). N_s was predetermined from an additional 60 seedlings at 12.1 mg N per seedling.

2.3 Measurements

Root collar diameter and height of all seedlings were measured at the end of the experiment. Total leaf area of four seedlings randomly selected from each treatment was determined using a hand-held laser leaf area meter (CI-203, CID Bio-Science, Inc., Camas, WA, USA), and net photosynthesis,transpiration rate, stomatal conductance and intercellular CO_2 concentration were also measured using a portable photosynthesis measurement system (Licor-6400, Li-cor Biosciences, Lincoln, NE, USA). Four seedlings of each treatment were harvested and separated into leaves, stems and roots, and then oven-dried at 65°C for 48 h to estimate biomass production. The dried

Table 1 Program for exponential nitrogen fertilization of *Calophyllum inophyllum* seedlings

Week	Nitrogen fertilizer per seedling/mg							
	N1	N2	N3	N4	N5	N6	N7	N8
0	0	2.6	4.0	4.9	5.7	6.8	7.6	8.9
2	0	2.8	4.6	5.7	6.7	8.2	9.5	11.3
4	0	3.0	5.0	6.6	7.9	10.0	11.8	14.6
6	0	3.6	5.7	7.6	9.3	12.2	14.7	18.8
8	0	3.6	6.4	8.8	11.0	14.9	18.3	24.2
10	0	3.9	7.2	10.3	13.0	18.1	22.8	31.1
12	0	4.2	8.1	11.9	15.4	22.1	28.4	39.9
14	0	4.5	9.2	13.8	18.2	26.9	35.3	51.3
16	0	4.9	10.3	15.9	21.6	32.8	44.0	66.0
18	0	5.3	11.6	18.4	25.5	39.9	54.7	84.8
20	0	5.8	13.1	21.4	30.1	48.7	68.2	109.0
22	0	6.2	14.8	24.7	35.6	59.3	84.9	140.1
Total	0	50.0	100.0	150.0	200.0	300.0	400.0	600.0

seedling samples were finely milled using a H_2SO_4 and K_2SO_4-$CuSO_4$ mixture catalyst to determine total N by the diffusion method, and were wet-digested using HNO_3-$HClO_4$ mixture solution to analyze P by the molybdenum blue method and K by atomic absorption.

2.4 Data analysis

After checking for normality and homoscedasticity, the effects of nutrient application on seedling height, root collar diameter, leaf area and biomass were assessed using a one-way analysis of variance by SPSS 16.0 (IBM, Armonk, NY, USA). Significant differences among treatment means were further assessed using Duncan's multiple range tests at the 5% level. Effects of nitrogen fertilization on photosynthetic gas exchange parameters and leaf nutrient status were plotted using Excel 2003 (Microsoft Corp. Redmond, WA, USA). Regression equations between nutrient concentration or ratio and total biomass of the seedlings were fitted and then diagnostic equations were accepted that were statistically significant. The critical value and the optimum concentration range were calculated using the nutrient concentration which corresponded to 90% maximum biomass from the equation[26,27].

3 Results

3.1 Root collar diameter, height and leaf area

The root collar diameter, height and leaf area at the end of experiment of *C. inophyllum* seedlings are shown in Table 2. The values recorded for these growth parameters increased with an increase in nitrogen fertilizer peaking at N4 to N5 (150–200 mg N per seedling) and then decreased with further increase in the nitrogen fertilizer levels.

3.2 Biomass

Total biomass of *C. inophyllum* seedlings increased with increasing nitrogen fertilizer from N1 to N5, and decreased with further increase in the nitrogen fertilization from N6 to N8 (Fig. 1). The greatest biomass production was observed in N5 with 10.4 g per seedling compared to the 5.5 g per seedling in N1.

3.3 Photosynthetic gas exchange parameters

The net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate of *C. inophyllum* seedlings showed a unimodal parabolic trend. The peak values recorded for 200 mg N per seedling were 7.29 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 0.071 $mol \cdot m^{-2} \cdot s^{-1}$, 220 $\mu mol \cdot mol^{-1}$ and 1.34 $mmol \cdot m^{-2} \cdot s^{-1}$ (Fig. 2).

3.4 Leaf nutrient status

As the amount of nitrogen supplied increased, leaf nitrogen concentration increased from 6.28 $mg \cdot g^{-1}$ in N1 to 19.4 $mg \cdot g^{-1}$ in N8 (Table 3). Leaf nitrogen content increased with increasing nitrogen fertilization from N1 to N5, followed by a slight decline in N6, then increased again from N7 to N8. In contrast, leaf biomass of the seedlings increased with increasing nitrogen fertilizer from N1 to N5 then declined from N6 to N8.

3.5 The optimal nitrogen determined by the critical value

There were parabolic relationships between biomass and leaf N and P concentration, and N/P ratio as illustrated by the scatter plots (Fig. 3).

There were significant differences ($P < 0.01$) between leaf N and P concentrations, and N/P ratio (Table 4). The parabolic relationships between leaf K concentration, N/K and P/K ratio and seedling biomass were not significantly different.

The critical values of the leaf N and P concentrations, and N/P ratio of *C. inophyllum* seedlings were 9.49 $mg \cdot g^{-1}$, 0.48 $mg \cdot g^{-1}$ and 17.2, respectively, and their optimum concentration ranges were 9.49–17.04 $mg \cdot g^{-1}$, 0.48–0.73 $mg \cdot g^{-1}$ and 17.2–23.7, respectively. There-

Table 2 Effects of nitrogen fertilizer treatments on growth of *Calophyllum inophyllum* seedlings

Nitrogen treatment	Nitrogen fertilizer per seedling/mg	Seedling height/cm	Root collar diameter/cm	Leaf area/cm^2
N1	0	13.9 ± 0.80^{d}	0.52 ± 0.01^{f}	127 ± 11.3^{g}
N2	50	17.2 ± 2.15^{c}	0.55 ± 0.03^{de}	193 ± 19.0^{f}
N3	100	18.7 ± 0.84^{bc}	0.56 ± 0.06^{cde}	242 ± 17.8^{de}
N4	150	21.8 ± 2.14^{a}	0.63 ± 0.01^{ab}	312 ± 15.1^{ab}
N5	200	22.1 ± 1.51^{a}	0.65 ± 0.01^{a}	327 ± 15.0^{a}
N6	300	21.0 ± 0.22^{ab}	0.61 ± 0.01^{abc}	289 ± 6.4^{bc}
N7	400	20.4 ± 0.47^{ab}	0.60 ± 0.02^{bcd}	266 ± 13.0^{cd}
N8	600	19.6 ± 2.28^{abc}	0.59 ± 0.01^{bcd}	234 ± 21.8^{e}

Note: Each value is the mean of four replicates±SE. For each column, different letters denote significant differences among the treatments according to Duncan's Multiple Range Test ($P < 0.05$)

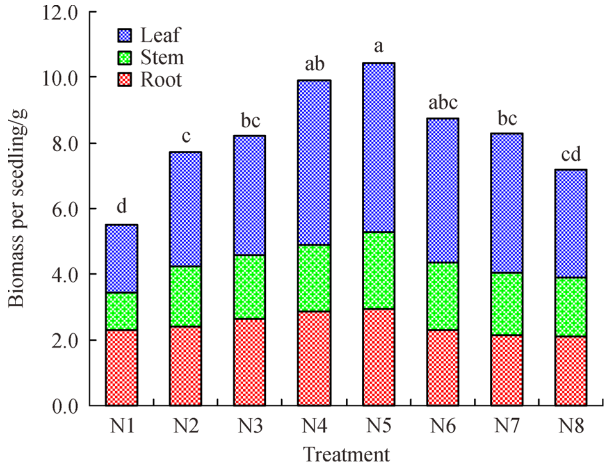

Fig. 1 Effects of nitrogen fertilizer treatments on biomass of *Calophyllum inophyllum* seedlings. Different letters above the columns indicate significant differences between treatments according to Duncan's Multiple Range Test ($P < 0.05$). Data are means ± SE (n = 4).

fore, given these optimum concentration ranges, the optimum amount of nitrogen for seedlings for these three measures were 200–400 mg per seedling, 200–400 mg per seedling and 100–400 mg per seedling, respectively.

4 Discussion

Numerous research results have shown that nitrogen influences the growth of young trees[3,5,28]. In our study, we observed that seedling height, root collar diameter, leaf area and biomass of *C. inophyllum* seedlings were enhanced by nitrogen fertilization. Dosage of 200 mg N per seedling appears to be the critical point for optimum growth of the seedlings. When the application was more than 200 mg N per seedling, excessive nitrogen reduced carbon assimilation and RuBP carboxylase activity, resulting in decreased photosynthesis and increased seedling respiration. In addition, photosynthesis was weakened while respiration of seedlings was strengthened. The growth of young leaves and seedlings was slow, thus the appropriate amount of nitrogen fertilizer can promote seedling growth and development. Our results are in accord with those reported by other researchers[29–31].

The peak values of net photosynethetic rate, stomatal conductance, intercellular CO_2 concentration and transpiration rate of *C. inophyllum* seedlings were obtained with fertilization of 200 mg N per seedling (N5). Nitrogen fertilizer treatments had significant effects on the photosynthetic gas exchange parameters (Fig. 2), which suggests that an appropriate amount of nitrogen can enhance the

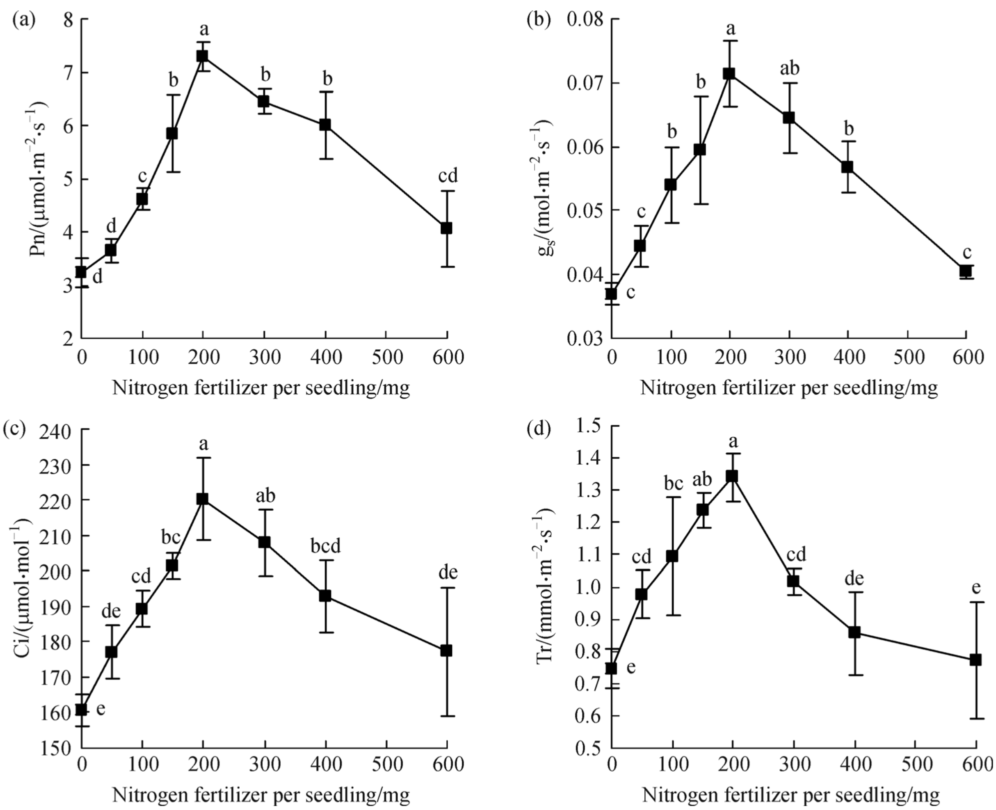

Fig. 2 Effects of nitrogen fertilizer treatments on photosynthetic gas exchange parameters of *Calophyllum inophyllum* seedlings. (a) Net photosynthetic rate; (b) stomatal conductance; (c) intercellular CO_2 concentration; (d) transpiration rate. Different letters indicate significant differences between treatments according to Duncan's Multiple Range Test ($P < 0.05$). Data are means±SE (n = 4).

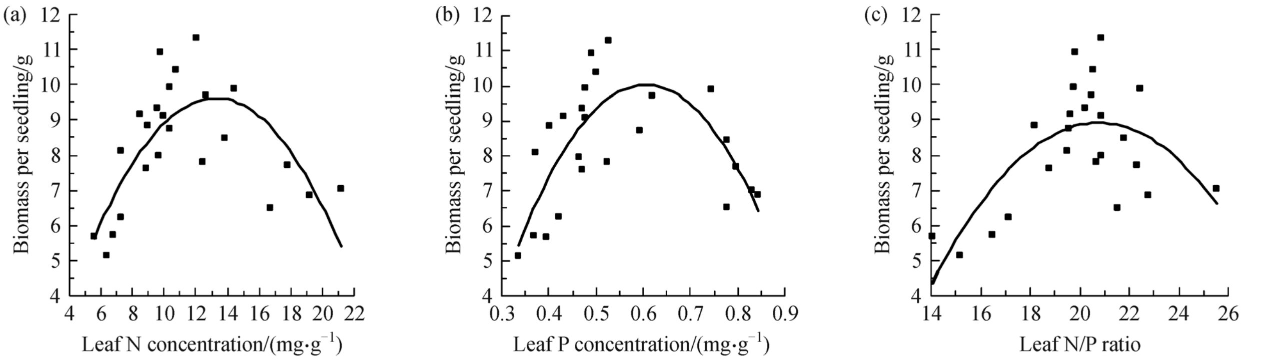

Fig. 3 The quadratic relationship between leaf N and P concentrations, N/P ratio and biomass of *Calophyllum inophyllum* seedlings. (a) The relationship between leaf N and biomass; (b) the relationship between leaf P and biomass; (c) the relationship between leaf N/P ration and biomass.

Table 3 Effects of nitrogen fertilizer treatments on leaf nutrient situation of *Calophyllum inophyllum* seedlings

Nitrogen treatment	Nitrogen fertilizer per seedling/mg	Leaf N concentration/$(mg \cdot g^{-1})$	Leaf biomass per seedling/g	Leaf N content per seedling/mg
N1	0	6.3 ± 0.37^{f}	2.06 ± 0.08^{c}	12.9 ± 0.95^{d}
N2	50	7.8 ± 0.57^{ef}	3.48 ± 0.22^{b}	27.3 ± 3.16^{c}
N3	100	9.0 ± 0.36^{e}	3.66 ± 0.46^{b}	32.8 ± 3.44^{c}
N4	150	9.8 ± 0.12^{de}	5.02 ± 0.26^{a}	49.0 ± 2.73^{b}
N5	200	11.1 ± 0.51^{cd}	5.16 ± 0.29^{a}	57.4 ± 5.90^{ab}
N6	300	11.8 ± 0.72^{c}	4.38 ± 0.22^{ab}	51.7 ± 3.35^{ab}
N7	400	15.0 ± 0.89^{b}	4.23 ± 0.58^{ab}	62.9 ± 6.47^{a}
N8	600	19.4 ± 0.97^{a}	3.30 ± 0.32^{b}	63.5 ± 3.02^{a}

Note: Each value is the mean of four replicates±SE. For each column, different letters denote significant differences between the treatments according to Duncan's Multiple Range Test ($P < 0.05$).

Table 4 Regression equations for nutrient concentration or ratio versus biomass of *Calophyllum inophyllum* seedlings

Dependent variable	Independent variable	Regressive equation	*R* value	Significant
(*Y*) Biomass	*X*1(N)	$Y = -0.0675X1^2 + 1.791X1 - 2.248$	0.735	$P < 0.01$
	*X*2(P)	$Y = -63.02X2^2 + 76.33X2 - 13.08$	0.732	$P < 0.01$
	*X*3(K)	$Y = -0.0505X3^2 + 1.274X3 + 0.8612$	0.346	$P > 0.05$
	*X*4(N/P)	$Y = -0.1009X4^2 + 4.191X4 - 34.59$	0.687	$P < 0.01$
	*X*5(N/K)	$Y = -1.319X5^2 + 9.141X5 + 0.4887$	0.623	$P > 0.05$
	*X*6(P/K)	$Y = 2139X6^2 - 51.49X6 + 5.565$	0.549	$P > 0.05$

photosynthetic capacity of *C. inophyllum* seedlings. Brown et al. concluded that the photosynthetic rates increased linearly with nitrogen addition to a maximum at 21 $mg \cdot g^{-1}$ and declined at higher rates of nitrogen addition when the seedlings of the evergreen conifers *Picea sitchensis, Thuja plicata* and *Tsuga heterophylla* were fertillized with nitrogen using exponentially increasing rates[32]. Nakaji et al. found the net photosynthetic rate of *Pinus densiflora* was reduced by the high nitrogen treatments in the first half of the growing season, and suggested that the reduced photosynthesis under high nitrogen dosage was mainly due to the decline of both CO_2 fixation and photosystem II activity in the chloroplasts[33].

Most studies have used exponential fertilization to indicate the relationship between nitrogen concentration and supply. Almost all these studies considered that seedling biomass and nitrogen content had an increase-steady-decline trend, with nitrogen concentration rising continuously as nitrogen supply increased[34]. We came to the same conclusion from the findings presented here (Fig. 3). Salifu and Jacobs studied the exponential fertilization in *P. mariana* and observed the optimum nitrogen range was 30–64 mg N per seedling[35]. These authors also found the most appropriate amount of nitrogen for *Quercus rubra* was 25–100 mg N per seedling. Wei et al. reported that 24.3–33.7 mg N per seedling resulted in the greatest biomass and highest nitrogen efficiency in *Larix olgensis*[30]. Chen et al. determined that the suitable nitrogen range for *B. alnoides*was 200–400 mg N per seedling using exponential fertilization[14]. In our research, leaf biomass reached its peak at N5 treatment (200 mg N per seedling), with the leaf nutrient level being satisfactory for seedling growth. From N6 to N8, leaf biomass of *C. inophyllum* seedlings decreased while the leaf nitrogen concentration increased, indicating that leaf nutrient status was in the luxury consumption under these conditions. This luxury nutrient consumption by seedlings and the concentration effect resulting from reduction in the leaf biomass may cause a continuing increase of leaf nitrogen concentration. Therefore, we conclude that 200–400 mg N per seedling is the most appropriate nitrogen range for *C. inophyllum* seedlings. All results from this and other studies indicate that there are marked differences for nutrient requirements between different tree species[13,36].

The optimum nitrogen range in our study was determined by the early growth performance of newly-transplanted seedlings. However, it should be noted that there are likely to be differences between greenhouse and field environments[37]. Further study in the field is thus warranted to provide a sound theoretical basis for fertilization of *C. inophyllum*.

5 Conclusions

Nitrogen fertilization had significant effects on the height, root collar diameter, leaf area, total biomass, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration. Transpiration rate of *C. inophyllum* seedlings, and the peak values of the eight measures were obtained with fertilization of 200 mg N per seedling. It was concluded that 200–400 mg N per seedling was the most suitable nitrogen fertilization range for *C. inophyllum* seedlings.

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Compliance with ethics guidelines Wentao Zou, Ruifeng Jia, Jinchang Yang, Rongsheng Li, and Guangtian Yin declare that they have no conflicts of interest or financial conflicts to disclose.

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References

1. Burdett A. Physiological processes in plantation establishment and the development of specifications for forest planting stock. *Canadian Journal of Forest Research*, 1990, **20**(4): 415–427
2. Nambiar E K S, Sands R. Competition for water and nutrients in forests. *Canadian Journal of Forest Research* , 1993, **23**(10): 1955–1968
3. Stumpf B, Yan F, Honermeier B. Nitrogen fertilization and maturity influence the phenolic concentration of wheat grain (*Triticum aestivum*). *Journal of Plant Nutrition and Soil Science*, 2015, **178** (1): 118–125
4. Imo M, Timmer V R. Nitrogen uptake of mesquite seedlings at conventional and exponential fertilization schedules. *Soil Science Society of America Journal*, 1992, **56**(3): 927–934
5. Paul Jackson D, Kasten Dumroese R, Barnett J P. Nursery response of container *Pinus palustris* seedlings to nitrogen supply and subsequent effects on outplanting performance. *Forest Ecology and Management*, 2012, **265**(1): 1–12
6. Juntunen M L, Hammar T, Rikala R. Leaching of nitrogen and phosphorus during production of forest seedlings in containers. *Journal of Environmental Quality*, 2002, **31**(6): 1868–1874
7. Imo M, Timmer V R. Growth, nutrient allocation and water relations of mesquite seedlings at differing fertilization schedules. *Forest Ecology and Management*, 1992, **55**(1–4): 279–294
8. Ingestad T, Lund A B. Theory and techniques for steady state mineral nutrition and growth of plants. *Scandinavian Journal of Forest Research*, 1986, **1**(1–4): 439–453
9. Timmer V R, Armstrong G, Miller B. Steady-state nutrient preconditioning and early outplanting performance of containerized black spruce seedlings. *Canadian Journal of Forest Research*, 1991, **21**(5): 585–594
10. Quoreshi A M, Timmer V R. Early outplanting performance of nutrient-loaded containerized black spruce seedlings inoculated with *Laccaria bicolor*: a bioassay study. *Canadian Journal of Forest Research*, 2000, **30**(5): 744–752
11. Salifu K F, Timmer V R. Optimizing nitrogen loading of *Picea mariana* seedlings during nursery culture. *Canadian Journal of Forest Research*, 2003, **33**(7): 1287–1294
12. McAlister J A, Timmer V R. Nutrient enrichment of white spruce seedlings during nursery culture and initial plantation establishment. *Tree Physiology*, 1998, **18**(3): 195–202
13. Rytter L, Ericsson T, Rytter R. Effects of demand-driven fertilization on nutrient use, root: plant ratio and field performance of *Betula pendula* and *Picea abies*. *Scandinavian Journal of Forest Research*, 2003, **18**(5): 401–415
14. Chen L, Zeng J, Xu D P, Zhao Z G, Guo J J, Lin K Q, Sha E. Effects of exponential nitrogen loading on growth and foliar nutrient status of *Betula alnoides* seedlings. *Sceintia Silvae Sinicae*, 2010, **46**(5): 35–40
15. Dweck A C, Meadows T. Tamanu (*Calophyllum inophyllum*) – the African, Asian, Polynesian and Pacific Panacea. *International Journal of Cosmetic Science*, 2002, **24**(6): 341–348
16. Wu D. Plant Directory in the coastal island in Hainan and Guangdong province. Beijing: *Science Press*, 1994.
17. Ji X, Zeng D, Huang S. *Calophyllum inophyllum*, a tree species for landscape in south subtropical zone. *Chinese Journal of Tropical Agriculture*, 2004, **24**(6): 44–45 (in Chinese)
18. Pradhan L, Pattnaik R, Sahoo A. *Calophyllum inophyllum*—an

economic tree in coastal tract of Orissa. *Environment and Ecology*, 1998, **16**(2): 476–477

19. Yang X, Fang J. Analysis of the development and application value of *Calophyllum inophyllum* L. *Chinese Wild Plant Resources*, 2001, **20**(6): 33–35 (in Chinese)
20. Ito C, Itoigawa M, Miyamoto Y, Rao K S, Takayasu J, Okuda Y, Mukainaka T, Tokuda H, Nishino H, Furukawa H. A new biflavonoid from *Calophyllum panciflorum* with antitumor-promoting activity. *Journal of Natural Products*, 1999, **62**(12): 1668–1671
21. Pawar K D, Joshi S P, Bhide S R, Thengane S R. Pattern of anti-HIV dipyranocoumarin expression in callus cultures of *Calophyllum inophyllum* Linn. *Journal of Biotechnology*, 2007, **130**(4): 346–353
22. Friday J, Okano D. *Calophyllum inophyllum* (kamani). *Species Profiles for Pacific Island Agroforestry*, 2006, **2**(1): 1–17
23. Su Y, He L. Study on the components of fat acids in the oil from seeds of *Calophyllum inophyllum* L. *Chinese Journal of Analysis Laboratory*, 2007, **26**(6): 62–64 (in Chinese)
24. Timmer V R, Armstrong G. Growth and nutrition of containerized *Pinus resinosa* seedlings at varying moisture regimes. *New Forests*, 1989, **3**(2): 171–180
25. Dumroese R K, Page-Dumroese D S, Salifu K F, Jacobs D F. Exponential fertilization of *Pinus monticola* seedlings: nutrient uptake efficiency, leaching fractions, and early outplanting performance. *Canadian Journal of Forest Research*, 2005, **35**(12): 2961–2967
26. Shedley E, Dell B, Grove T. Diagnosis of nitrogen deficiency and toxicity of *Eucalyptus globulus* seedlings by foliar analysis. *Plant and Soil*, 1995, **177**(2): 183–189
27. Huang Y Z, Feng Z W, Li Z X, Li X D, Yang B Q. Diagnosis of foliar nutrients (N, P, K, Ca, Mg, B) of young *Eucalyptus urophylla* trees. *Acta Ecologica Sinica*, 2002, **22**(8): 1254–1259 (in Chinese)
28. Li Y, Liu X, Ma J, Shi G, Yang S. Effects of topdressing nitrogen on growth and nutrient status in leaves of *Phoebe chekiangensis* container seedlings. *Journal of Nanjing Forestry University*, 2016, **40**(1): 33–38
29. Wu F Z, Bao W K, Li F L, Wu N. Effects of drought stress and N supply on the growth, biomass partitioning and water-use efficiency of *Sophora davidii* seedlings. *Environmental and Experimental Botany*, 2008, **63**(1–3): 248–255 (in Chinese)
30. Wei H X, Xu C Y, Ma L Y, Jiang L N, Jiang C J, Liu F S, Zhang Q C. Nutrient uptake of *Larix olgensis* seedlings in response to different exponential regimes. *Acta Ecologica Sinica*, 2010, **30**(3): 0685–0690 (in Chinese)
31. Zhang D, Chen Z X, Huang S N. Effects of fertilization on growth of *Archidendron clypearia* seedlings. *Forest Research*, 2015, **28**(6): 906–909 (in Chinese)
32. Brown K R, Thompson W A, Camm E L, Guy R D, Hawkins B J. Effects of N addition rates on the productivity of *Picea sitchensis, Thuja plicata*,and *Tsuga heterophylla* seedlings. *Trees*, 1996, **10**(3): 189–197
33. Nakaji T, Takenaga S, Kuroha M, Iuta T. Photosynthetic response of *Pinus densiflora* seedlings to high nitrogen load. *Environmental Sciences*, 2002, **9**(4): 269–282
34. Timmer V R. Exponential nutrient loading: a new fertilization technique to improve seedling performance on competitive sites. *New Forests*, 1997, **13**(1): 279–299
35. Salifu K F, Jacobs D F. Characterizing fertility targets and multi-element interactions in nursery culture of *Quercus rubra* seedlings. *Annals of Forest Science*, 2006, **63**(3): 231–237
36. Cortina J, Vilagrosa A, Trubat R. The role of nutrients for improving seedling quality in drylands. *New Forests*, 2013, **44**(5): 719–732
37. Schott K M, Snively A E K, Landhäusser S M, Pinno B D. Nutrient loaded seedlings reduce the need for field fertilization and vegetation management on boreal forest reclamation sites. *New Forests*, 2016, **47**(3): 1–18