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Full Length Article

Prediction of Nutrient Leaching from Culture of Containerized Buddhist Pine and Japanese Maple Seedlings Exposed to Extended Photoperiod

Kaiyuan Zhu[1], Huichun Liu[1*], Hongxu Wei[2*], Jianghua Zhou[1], Qingcheng Zou[1], Guangying Ma[1] and Jiaqiang Zhang[1]
[1]*Flower Research and Development Center, Zhejiang Academy of Agricultural Sciences, Hangzhou, China, 311202*
[2]*Northeast Institute of Geography and Agricultural Ecology, Chinese Academy of Sciences, Changchun, China, 130102*
[*]For correspondence: lhuichun@163.com; Weihongxu@iga.ac.cn

Abstract

Leachate nutrients from the nursery of containerized tree seedlings result in risks of inefficient fertilization through nutrient loss and contamination of ground water. In the present study, seedlings of Buddhist pine (*Podocarpus macrophyllus*) and Japanese maple (*Acer palmatum*) were cultured in containers of three differing sizes under contrasting photoperiods of naturally 11 h per day and an extended one of 20 h per day. Controlled release fertilizer (CRF, 14-14-14) was used to feed seedlings with sufficient nutrients for 5 months from June to November 2014. At the end of experiment, seedlings from all types of containers were measured for height and root collar diameter, whilst leachates were collected to determine nutrient concentrations of available phosphorus (P), potassium (K), ammonium and nitrate nitrogen (N) therein. Growth of both seedling species increased with the volume of container, but only growth of Japanese maple seedlings responded to photoperiod. In spite, there was greater amount of nutrients leached from containers planted with Buddhist pine than with Japanese maple; nearly no nutrient in the leachate affected by photoperiod. A negative correlation was found between concentrations of leachate nutrients and seedling height growth in all container sizes. In conclusion, nutrient leaching is a non-ignorable but predictable factor by seedling height growth during the intensive culture of slowly growing seedlings of ornamental trees under the extended photoperiod. © 2016 Friends Science Publishers

Keywords: Nutrient leaching; Kusamaki; Stocktype; Container size; Time of illumination

Introduction

Nutrients from leachates, such as nitrate and phosphate, during the fertilization to vegetative crops result in eutrophication of surface water and create a potential contamination to ground water. Although gross amount of fertilizers used for forest production of tree stocks may not be comparable as for agricultural and horticultural crops, however, the risk arisen from containerized tree stock culture cannot be overlooked (Juntunen and Rikala, 2001; Juntunen *et al.*, 2002, 2003; Dobrahner *et al.*, 2007; Gomez-Rey *et al.*, 2008). Thus, nursery bare-root stocks occupies a large proportion of tree seedling productions from the whole tree seedling culture system, wherein the proportion of containerized stocks, however is lower but growing dramatically at a considerable speed all over the world including Norway, Sweden, Finland, Canada (Juntunen *et al.*, 2002), USA (South *et al.*, 2005; Pinto *et al.*, 2011), and China (Xu *et al.*, 2013).

Series of manual practices during seedling culture promote nutrient utilization by seedlings, therefore nutrient wastage through leaching events could be alleviated through practical manipulations, such as fertilization method, type, and facility manipulations (Juntunen *et al.*, 2003; Park *et al.*, 2012). Additionally, the choice of container type, differed depending on size, is an operational practice for containerized tree stock culture (Faure-Lacroix *et al.*, 2013; Jelic *et al.*, 2014). There is a potentially positive relationship between container size and nutrient loss from leaching, hence smaller containers are believed to be more efficient to control leaching from containers planting tree seedlings (Murray *et al.*, 1996; Dumroese *et al.*, 2011). However, the trade-off does exist between container-type-related seedling growth and leachate nutrient loss, and end-of-season tree stock size is limited by small container volume (Pinto *et al.*, 2011; Dumroese *et al.*, 2011). Large container with a greater volume is advantageous for the culture of slowly growing tree species because a larger container usually resulted in a larger tree stock size at a given time (Faure-Lacroix *et al.*, 2013; Jelic *et al.*, 2014), hence efficiently increased was their growing rate. Most slowly growing tree species are of high value, which arouses an interest for stimulating growth rate of slowly growing tree species using larger containers with a greater volume but with the wish of less leaching nutrient loss.

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With the aim to control nutrient loss through leaching, fertilization using controlled release fertilizer (CRF) is an available method (Alva, 1992; Murray *et al.*, 1996; Hangs *et al.*, 2003; Klooster *et al.*, 2012). Additionally, production of large tree stock using large-volume containers has been proven to be highly efficient with CRF (Murray *et al.*, 1996; South *et al.*, 2005; Dumroese *et al.*, 2011). However, effects of CRF on seedling performance seemed to be derived mainly from nutrient availability, hence response of seedling growth replies on the interaction of tree species and CRF but not on single impact from CRF (Oliet *et al.*, 2004; Klooster *et al.*, 2012). Therefore, the question remains: how growth of slowly growing seedlings can be promoted with controlled nutrient leaching with CRF used in a large container.

Artificially prolonged photoperiod is a valid manipulation for accelerating tree seedling growth rate (Wheeler, 1979; Bongarten and Hanover, 1985; Macey and Arnott, 1985; Johnsen and Seiler, 1996; do Amaral *et al.*, 2006). The inherent affectivity works through the mechanism of a longer term of photosynthetic product synthesizing per day. A recently published study determined that an extended photoperiod could successfully promote seedling growth of Buddhist pine (*Podocarpus macrophyllus*), a slowly growing species mainly used as an ornamental tree (Wei *et al.*, 2013). Compared to the naturally regular illumination-time, the employment of the extended photoperiod has the reasonable potential to control nutrient loss in leachates because under an extended illumination root proliferation can be benefited from photosynthetic production and absorbed more nutrients detained within containers. On the basis of these proofs, it can be speculated that an extended photoperiod can help to counter nutrient loss through leaching due to more efficient nutrient uptake through roots (Zhang *et al.*, 2010). This can be supported by increased photosynthetic productions in a longer illumination. In contrast, this conjecture may also face an argue by disproving that larger container size also result in a larger root system (Murray *et al.*, 1996; Topic *et al.*, 2009), which has the full potential to protect supplied nutrients from leaching. This argument cannot be proved to be null on the basis of current evidence, because the general aim of performing an extended photoperiod is to promote tree growth. Hence, relevant studies have paid too much attention to determine tree-responses, such as morphology (Wheeler, 1979; Bongarten and Hanover, 1985), biomass accumulation (Macey and Arnott, 1985; Johnsen and Seiler, 1996), photosynthetic rate (do Amaral *et al.*, 2006), nutrient uptake and reserves (Wei *et al.*, 2013). As a result, information about leaching characteristics beneath containerized seedlings subjected to an extended photoperiod is quite limited.

Studies from scientific literature indicated that performance of large tree stock was tested on species mainly used for afforestation, such as birch (Aphalo and Rikala, 2003), pine (Sutherland and Day, 1988; South *et al.*, 2005; Pinto *et al.*, 2011), *Plinia* spp. (Danner *et al.*, 2007), eucalyptus (Close *et al.*, 2010), and koa (Dumroese *et al.*, 2011). The main concerns from these studies are generated about the cost during seedling culture (Bowden, 1993), performance of transplanted stocks (Aphalo and Rikala, 2003; South *et al.*, 2005; Pinto *et al.*, 2011), and the benefit for the whole silvi-cultural system from seed germination to transplanted seedling establishment (Sutherland and Day, 1988; Close *et al.*, 2010). For slowly growing tree species, quite little evidence can be found for the available approach to culture large ornamental tree stocks, although the benefit from a large stock production of landscape-used tree species is obviously greater than that of afforestation trees no matter considering commercial or ecological values. Therefore, in the present study, Buddhist pine and Japanese maple (*Acer palmatum*) seedlings were employed as plant materials due to their typically high-value for ornamentally landscape but apparently slowly growing rate in sub-tropical and tropical Asia. Additionally, containers with different sizes were used for seedling culture under the contrasting photoperiods of naturally regular and the extended ones. Our aim was to promote seedling growth of two tree species but with little loss of leaching nutrients. It was hypothesized that: (1) seedlings of both tree species grew faster in larger containers; (2) seedling growth would be promoted under the extended photoperiod; (3) nutrient loss through leaching could be controlled under an extended photoperiod; (4) leaching nutrients could be predicted by seedling morphologies. This study would have practical implications for producing high-quality ornamental tree stocks, and the results therein can supply experimental evidence to enhance seedling growth at a low cost of nutrient loss. Also, our study devotes to available solution for the risk of leaching nutrients originated from containerized tree seedling culture.

Materials and Methods

Seedling Material

Bare-root seedlings of Buddhist pine and Japanese maple were obtained from Chinese Tree Stock Market (30º09'N, 120º16'E), Xiaoshan District, Hangzhou City, Zhejiang Province, China, on 10 April 2014. For either of the two species, seedlings were cultured from the same seed provenance and chosen at the uniform size to eliminate the possible effect of provenance variation on results (Yao *et al.*, 2014). For Buddhist pine seedlings, initial height and root collar diameter (RCD) were measured to be 13.5 cm and 2.9 mm, respectively, while of Japanese maple seedlings, 30 cm and 3.5 mm, respectively. Seedlings were moisturized with soil blocks packaging around roots until transported to the experimental nursery of Flower Development and Research Center (30º19'N, 120º28'E), Zhejiang Academy of Agricultural Sciences, Xiaoshan District, Hangzhou City, Zhejiang Province, China.

Experiment Design

Due to different initial morphologies and growth rates between Buddhist pine and Japanese maple, the present experiment was conducted following a split-block design, wherein the 2 main blocks were separated into two groups being divided by tree species. Within each of the main blocks, the 2 sub-blocks were separated by photoperiod and three different container sizes involved in each of the sub-block. Main blocks were replicated for 6 times implemented along two adjacent nursery benches. Two seedlings bulked as one measuring unit; hence a total number of 72 seedlings (2 species × 2 photoperiods × 3 containers × 6 replicated blocks) were used.

Seedling Culture and Treatment

Seedlings arrived at the experimental nursery on 14 April 2014, where packaging soils around roots were washed away by soaking in running water. Seedlings free from root soils were cut for one third of total root length then planted into containers. Three container sizes of large, medium, and small volumes were used. Specific height and diameter of these containers are shown in Table 1. Peat (Klasmann-Deilmann®, Geeste, Germany) and perlite were mixed at a ratio of 3:1 (v/v) and filled into containers as substrates. When half of the container-volume was filled, CRF (14-14-14, micronutrients added, controlled release for 5 months) granules were placed on the surface of substrates. Seedlings were transplanted into containers to the depth at half of the container height, then pulled upwards to make the root collar emerged out of substrate. Fans and moist curtains were employed to keep the relative humidity of 70-90% and temperature in the green house at 22–26ºC during the night and 35–40ºC during the day time.

As described above, half of containerized seedlings were placed on one bench, leaving the other half on the other adjacent one. Between them, a plastic blackout curtain was hanged up from the ceiling downwards to the ground along the benches. At Xiaoshan locally natural photoperiod during the growing season was about 11 h per day, but in the extended photoperiod treatment prolonged to be 20 h per day by 125-W plant growing lamps (Oudi™, Huzhou, Zhejiang, China) and equipped 1.5 m over benches. Illuminations measured on top of apical tips of both species of seedlings were maintained to be 2000 lux (217-Lightmeter, GE®, CT, USA) during the lighting photoperiod. Photoperiod treatment was commenced at 17 June 2014 and ended at 25 November 2014 at termination of study.

Irrigation Regime and Leachate Determination

Trays were placed beneath containers to collect leachate water immediately followed by each of irrigations. Seedlings were irrigated using water kettles at the time of transplanting and on every first to third day depending on the daily temperature and container weight throughout the experiment. Daily irrigation resulted in approximately 1.1 L of water applied per seedling. This watering volume meets the locally practical protocol in Xiaoshan for seedling culture. Thus, different sizes of containers had different requirements for irrigation volumes, but this is one part of the treatment influences brought by container types.

Leachates were collected on the last three weeks of 11, 18, and 25 November 2014, before the experiment termination. These days for leachate sampling were selected because at this time CRF nutrients were about to be exhausted from containers but seedlings received effects from CRF treatments thoroughly. Leachates could not be collected for each size of containers because leaching water from some containers was too little to be collected under uniform watering volume as irrigation. Finally, a volume of 100 mL leachates was collected for each group of 6 containers of three sizes per time, which was subsequently moved to a bottle, sealed, and stored at 0–4ºC until determination for nutrient concentrations using the method of Wei *et al.* (2012). Briefly, ammonium- and nitrate-nitrogen (N) analyses were conducted using flow injection method (Lachat Instruments, Hach Co., Loveland, USA). Available phosphorus (P) and potassium (K) concentrations (mg L^{-1}) were determined by ICP-OES (Perkin Elmer Co., Waltham, USA). All determinations finished in 2 months after collection.

Seedling Harvest and Measurement

Seedlings were harvested at the same day with leachate collections on 11, 18 and 25 November 2014. Shoot height (cm) and RCD (mm) are easily measured shoot morphologies to predict seedling quality efficiently and used to test our third hypothesis. Hence, shoot height and RCD were measured for all seedlings immediately after each of the harvests. Thus, the time of seedling harvest fell into winter season locally in Xiaoshan and seedling growth was almost ceased. This phenomenon needs not to be considered for the necessity of measuring shoot morphologies repeatedly in winter season in our study, because this can fully satisfy the statistical requirement of regression analysis between data about morphology and leaching nutrients.

Statistical Analysis

Our dataset was established by two parts: seedling morphology and leaching nutrient concentrations. Therefore, different methods of data analysis were adapted for each of the results. For seedling height and RCD results, analysis was conducted separately without comparing between the two tree species because Japanese maple grew faster generally than Buddhist pine due to their natural species characteristics. For leachate nutrients, however, comparison between the main blocks of tree species was performed, while comparisons among container sizes were unavailable. Data from the three investigating days were analyzed separately.

Within each of the replicated blocks (n=6), seedling height and RCD were determined using the average of two measures, while leachate nutrients were determined using the average of three repeatedly technical replicates. Analysis of variance (ANOVA) based on the General Linear Model (GLM) procedure was performed to test effects of tree species (freedom degree, i=1), photoperiod (i=1), and container size (i=2) on height, RCD, and concentrations of ammonium-N, nitrate-N, available P, and available K by a split-block method using SAS (SAS Institute Inc., NC, USA). All factors were considered fixed. When treatment effect was detected to be significant, means were compared or ranked according to Turkey's studentized range test α=0.05 level.

Because leachate nutrients usually showed discrete distribution, ANOVA may be null sometimes for analyzing leachate data from fixed treatments (Dumroese *et al.,* 2011). Therefore, results of concentrations of ammonium-N, nitrate-N, available P and K were presented using Box-Whisker plots to suggest the potential traits of scattered values. Also for leachate data, principal component analysis (PCA) was performed using the FACTOR procedure of SAS to help analyzing variation of data. Ranking Spearman Regression was conducted between the first two PC (Factor 1 and 2) and the effect of species or photoperiod to indicate existed relationship, according which eigenvalues of Factor 1 and 2 could be related to each other to reveal the treatment effect on data variation. Pearson correlation was performed to detect the relationship between seedling shoot morphology and leachate nutrients, which could attribute to an available predictor of nutrient leaching through measuring growth performance.

Results

Seedling Growth

Shoot morphology response to photoperiod and container size differed between tree species (Table 2). Both treatments and their interaction significantly affected height and RCD of Japanese maple seedlings, but only container size showed significant effect on Buddhist pine. Results of Japanese maple on 25 November 2014 revealed that, only small size container under regular photoperiod resulted in small-height seedlings (Fig. 1A) and smaller RCD at any of photoperiods, while large container under the regular photoperiod resulted in highest RCD (Fig. 1B). Results of height and RCD in Japanese maple seedlings on 11 and 18 November followed the similar trends as for 25 November with a small difference in the same treatment for about 5% of change between dates.

Photoperiod resulted in increased seedling height for both species, but decreased RCD of Japanese maple at the same time (data not shown). Effect of container size on seedling height and RCD was significant, with values lower for small containers than large and medium containers (Table 3).

Nutrient Concentration in Leachates

Most nutrients collected from leachates did not respond to photoperiod except for available P determined on 18 November 2014 (Table 2), which was lower by 18% in the extended photoperiod (Extended, 35.96±35.88 mg L^{-1}; Regular, 43.80±42.92 mg L^{-1}; Mean±*SD*). There was a difference for each of the nutrient loss from leachates collected beneath containers for both tree species, and nutrient concentration was higher in leachates of Buddhist pine than Japanese on each of three investing dates (Fig. 2). Patterns of scattered plots revealed that difference between the two tree species was quite apparent for available P and ammonium N. For nitrate N on 18 November, difference between the two tree species disappeared temporarily due to more high-level percentiles of Japanese maple relative to Buddhist pine (Fig. 2D).

A PCA was conducted on data between tree species or photoperiods (Fig. 3). More than 70% of variation was accounted for by the first two PC (Factor 1 and 2) for most datasets. Therefore, results indicated that the first two PC were retained by the MINEIGEN criterion, except for the dataset of photoperiod treatment on 25 November, wherein approximately 70% of the variation was explained by Factor 1 alone hence only Factor 1 was retained (Fig. 3F).

Concentrations of available P, K, and ammonium N was distributed near the opposite ends of X- or Y-axis with higher absolute values for Buddhist pine than Japanese maple on 11 and 18 November 2014 (Table 5), when no correlation was detected between eigenvalues of Factor 1 or Factor 2 and nutrient concentrations for two species (Table 4). However, a significant correlation was found between eigenvalues of Factor 2 and nutrients on 25 November, and the absolute values of ammonium N and nitrate N were closer to zero of the Y-axis for Japanese maple relative to Buddhist pine, coincides to results of (Fig. 3C and D) indicating higher values for Buddhist pine.

Eigenvalues of Factor 1 were negatively correlated with nutrient concentrations under contrasting photoperiods on all three investigating dates (Table 4). Therefore, relative to the regular photoperiod, concentrations of available K and nitrate N tended to be declined and increased under the extended photoperiod on 11 November 2014 (Fig. 3D), because the relative vector directions increased and decreased following the intercepts on X-axis, respectively. However, these relationships turned to be inversed on 18 and 25 November (Fig. 3E and F). Additionally, eigenvalues of Factor 2 were positively correlated with nutrient concentrations on 18 November (Table 4), and concurred the relationship between nitrate N concentrations under contrasting photoperiods, because results under the extended photoperiod were closer to zero following the intercepts on Y-axis relative to regular photoperiod (Fig. 3E).

Table 1: Specific height and diameter of containers used in the study

Container type	Height (cm)	Top-diameter (cm)	Bottom-diameter (cm)
Large	16.0	21.0	14.5
Medium	15.5	18.0	12.5
Small	12.0	15.5	11.0

Table 2: *P* values from ANOVA analysis on effects of tree species, photoperiod, and container size on shoot morphologies of height and root collar diameter (RCD) and leachate nutrient concentrations of ammonium-nitrogen (N), nitrate-N, available phosphorus (P), and available potassium (K)

Source of variation	11 November		18 November		25 November	
Shoot morphology						
	Height	RCD	Height	RCD	Height	RCD
Buddhist pine						
Photoperiod	0.6753	0.4377	0.6652	0.6517	0.7916	0.3350
Container size	0.0003	0.0010	0.0004	0.0040	0.0005	0.0015
Photoperiod × Container size	0.7593	0.9991	0.6842	0.9517	0.7273	0.8180
Japanese maple						
Photoperiod	0.0472	0.0041	0.0658	0.0058	0.0343	0.0120
Container size	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Photoperiod × Container size	0.0166	0.0093	0.0320	0.0085	0.0133	0.0038
—Nutrient concentrations in leachates—						
Available P						
Photoperiod	0.4567		0.0404		0.4193	
Tree species	<0.0001		<0.0001		<0.0001	
Photoperiod × Tree species	0.2591		0.0822		0.5746	
Available K						
Photoperiod	0.9039		0.0957		0.8309	
Tree species	0.0088		0.0002		0.0036	
Photoperiod × Tree species	0.2096		0.5587		0.2912	
Ammonium N						
Photoperiod	0.4710		0.0789		0.0669	
Tree species	<0.0001		<0.0001		<0.0001	
Photoperiod × Tree species	0.6550		0.0646		0.1609	
Nitrate N						
Photoperiod	0.1530		0.4277		0.9898	
Tree species	0.0211		0.3739		0.0056	
Photoperiod × Tree species	0.4547		0.0992		0.0587	

Table 3: Seedling height and root collar diameter (RCD) measured for Buddhist pine (*Podocarpus macrophyllus*) seedlings cultured on 11, 18, and 25 November 2014, in three sizes (large, medium, and small) of containers

Date	Container size	Seedling height (cm)	RCD (mm)	Photoperiod	Seedling height (cm)	RCD (mm)
11 Nov	Large	27.92±4.78a	4.02±0.40x	Extended	25.36±4.89	3.65±0.56
	Medium	26.63±3.98a	3.86±0.43x	Regular	24.80±5.14	3.77±0.46
	Small	20.69±2.62b	3.27±0.34y			
18 Nov.	Large	27.81±4.23a	3.96±0.53x	Extended	25.42±4.14	3.73±0.58
	Medium	26.64±3.43a	3.98±0.49x	Regular	24.83±5.04	3.80±0.50
	Small	20.92±2.35b	3.35±0.30y			
25 Nov.	Large	28.15±4.82a	4.12±0.60x	Extended	25.50±4.80	3.96±0.68
	Medium	26.71±3.98a	4.13±0.49x	Regular	25.14±4.43	3.79±0.53
	Small	21.10±2.34b	3.38±0.27y			

Note: Different letters of a and b indicate significant difference for seedling height, while different letters of x and y indicate significant difference for RCD

Relationship between Seedling Morphology and Leaching Nutrients

Leachate nutrient concentrations of available P and nitrate N, and those of most available K and ammonium N were negatively correlated to height and RCD of seedlings planted in three sizes of containers (Table 5). Additionally, available P concentration was positively correlated with available K, ammonium N and nitrate N. Also positively correlated were nitrate-N and both available K and ammonium N.

Discussion

The results of present study showed that compared to small sized containerized seedlings, those transplanted

Table 4: Spearman correlation between principal component analysis (PCA) factors and nutrient concentrations of leaching solutions collected on 11, 18, and 25 November 2014, during the culture of different tree species subjected to contrasting photoperiods

PCA	11 November		18 November.		25 November	
	Species	Photoperiod	Species	Photoperiod	Species	Photoperiod
Factor 1	*R*=-0.14	*R*=-0.71	*R*=-0.24	*R*=-0.76	*R*=-0.14	*R*=-0.90
	P=0.74	*P*=0.04	*P*=0.57	*P*=0.03	*P*=0.73	*P*<0.01
Factor 2	*R*=0.14	*R*=0.33	*R*=0.10	*R*=0.93	*R*=0.71	-
	P=0.74	*P*=0.42	*P*=0.82	*P*<0.01	*P*=0.04	-

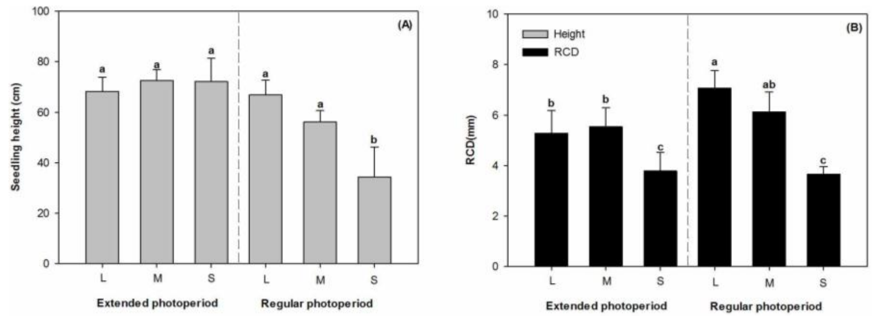

Fig. 1: Height (A) and root collar diameter (RCD) (B) of Japanese maple (*Acer palmatum*) seedlings cultured with three sizes (large, medium, and small) of containers subjected to contrasting photoperiods of regular- and extended-ones. Data were measured on 25 November 2014. Values are means±SD. Different letters indicate significant differences according to Turkey's Test at 0.05 level

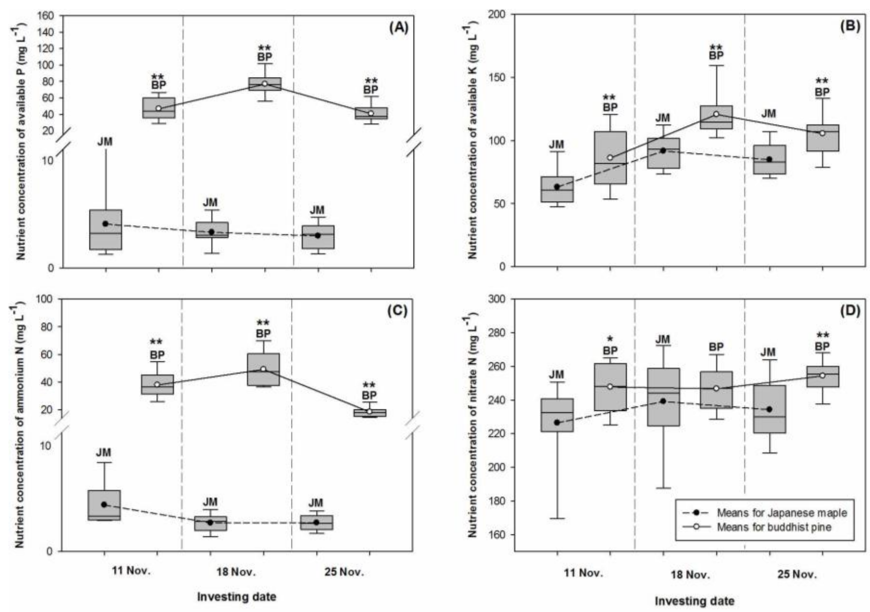

Fig. 2: Box-whisker plots with means of nutrient concentrations of phosphorus (P) (A), potassium (K) (B), ammonium nitrogen (N) (C), and nitrate N (D) in leaching solutions during the culture of seedlings of Japanese maple (JM) (*Acer palmatum*) and buddhist pine (*Podocarpus macrophyllus*) (BP) sampled on 11, 18, and 25 November 2014. Columns indicate ranges between one- and three-percentiles and bars indicate ranges between maximum and minimum observations. Transverse lines indicate half percentiles. Asterisks of "**" and"*" indicate significant difference between results of JM and BP at 0.05 and 0.01 levels, respectively, according to Turkey's Test at 0.05 level

in larger containers grew to be larger at the same time with faster growing speed. However, large and medium sizes of containers used in our study did not show significantly different effects on height and RCD for both species. The null effect of different sizes of containers on seedling morphology has also been found on koa (Dumroese *et al.*, 2011) and pine seedlings (Pinto *et al.*, 2011). Resource availabilities, such as nutrient and water, at a given space reserved within containers may suffer from some limits when their size increased to be some volume. However, it is reasonable to accept our first hypothesis.

Compared to Buddhist pine, Japanese maple seedlings showed better response to photoperiod (Table 2). This difference may be related to leaf-shapes, i.e. needles vs. broad attributing to specific leaf area (Nagel *et al.*, 1998). In contrast to our results of the null response of Buddhist pine seedlings to photoperiod, an earlier study suggested a stimulated response to height of juvenile Buddhist pine seedlings in response to extended photoperiod (Wei *et al.*, 2013). The most reasonable explanation of these varied responses can be concluded due to the difference of light-sensitivity driven by plant developmental stages. Wei *et al.* (2013) used Buddhist pine seedlings cultured from germination, thereafter initial and terminated height of

Table 5: Pearson correlations between nutrient concentrations of phosphorus (P), potassium (K), ammonium-nitrogen (N), and nitrate-N and height and root collar diameter (RCD) of seedlings of Japanese and buddhist pine cultured with three sizes (Large, medium, and small) of containers from 11 to 25 Nov. 2014

	P	K	Ammonium-N	Nitrate-N
P	-	-	-	-
	-	-	-	-
K	*R*=0.66	-	-	-
	P=0.02	-	-	-
Ammonium-N	*R*=0.98	*R*=0.56	-	-
	P<0.01	*P*=0.06	-	-
Nitrate-N	*R*=0.72	*R*=0.69	*R*=0.58	-
	P<0.01	*P*=0.01	*P*=0.05	-
—Height—				
Large	*R*=-0.97	*R*=-0.63	*R*=-0.96	*R*=-0.69
	P<0.01	*P*=0.03	*P*<0.01	*P*=0.01
Medium	*R*=-0.99	*R*=-0.64	*R*=-0.96	*R*=-0.76
	P=<0.01	*P*=0.03	*P*<0.01	*P*<0.01
Small	*R*=-0.89	*R*=-0.58	*R*=-0.81	*R*=-0.86
	P<0.01	*P*=0.04	*P*<0.01	*P*<0.01
—RCD—				
Large	*R*=-0.80	*R*=-0.52	*R*=-0.85	*R*=-0.38
	P<0.01	*P*=0.08	*P*<0.01	*P*=0.22
Medium	*R*=-0.94	*R*=-0.59	*R*=-0.96	*R*=-0.59
	P<0.01	*P*=0.04	*P*<0.01	*P*=0.04
Small	*R*=-0.92	*R*=-0.49	*R*=-0.90	*R*=-0.72
	P<0.01	*P*=0.10	*P*<0.01	*P*<0.01

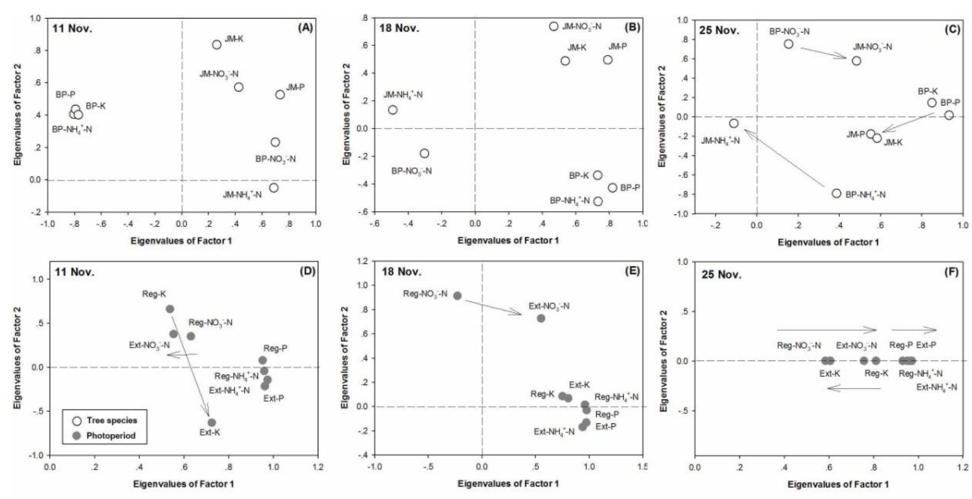

Fig. 3: Principle component analysis (PCA) analysis on nutrient concentrations of phosphorus (P), potassium (K), ammonium nitrogen (NH_4^+-N), and nitrate nitrogen (NO_3^--N) in leaching solutions during the culture of seedlings of Japanese maple (JM) (*Acer palmatum*) and buddhist pine (*Podocarpus macrophyllus*) (BP) subjected to contrasting photoperiods of regular- and extended-ones sampled on 11 (A, D), 18 (B, E), and 25 (C, F) November 2014. Scattered plots are located according to eigenvalues between Factor 1 (X-axis) and Factor 2 (Y-axis) from the first and the second PCA, respective. Arrows indicate vector directions between contrasting traits for one nutrient parameter

seedlings was about ~6 cm and ~14 cm, respectively. In our study, seedlings cultured for one growing season had initial and terminal height of ~14 cm and ~26 cm, respectively. Sysoeva *et al.* (2010) did a systematical review work on plant under continuous light, and suggested that plant response to photoperiod varied depending on different developmental stages. For tree cultivars, age-related variation of sensitivity to photoperiod has been well established in apple (Hauagge and Cummins, 1991) and pine (Wheeler, 1979). Probably, for juvenile Buddhist pine the first year germination was more sensitive to photoperiod than at mature stage. However, this conjecture needs future work of species-specific study to confirm.

Although Japanese maples seedlings showed better response of height growth to photoperiod (Fig. 1), their RCD declined under the extended photoperiod. This may be warning of declined seedling quality, because taller but slender seedlings induced by extended photoperiod would probably need more water and nutrient whilst transplanting. A typical parameter negatively quantifying seedling quality of sturdiness quotient (SQ) is calculated by height/diameter. Therefore, according to measures of morphology on end-of-nursery seedlings, those taller but slender stocks had higher SQ ratio. Although the high SQ is fairly accepted as positive predictor for seedling quality of hard wood species (Tsakaldimi *et al.*, 2013), opposite queries on the positive relationship between SQ and seedling quality have never stopped (Bayala *et al.*, 2009). Therefore, according to the calculation for SQ, quality of Japanese maples seedlings in our study tended to be declined by the extended photoperiod. The quality of these seedlings needs to be evaluated through continuously monitoring on transplanting performance after nursery culture in future work. Thus, our second hypothesis could be at least partly accepted due to response of Japanese maple.

A greater nutrient loss from containers with Buddhist pine seedlings than Japanese maple (Fig. 2) implicates that culture of the former would result in more nutrient loss than the later. This was also revealed by PCA wherein data of leachate nutrients were closer to zero of either X- or Y-axis for Japanese maple than Buddhist pine (Fig. 3). Regarding interspecies distinction, faster growth requires higher rate of nutrient flux (Boczulak *et al.*, 2014), the difference in our study may result from different growing rates between the two species according to their naturally relative growing rate (growth increment, Δ). Δ_{Height} and Δ_{RCD} were calculated to be 40 cm and 2.67 mm, respectively, for Japanese maple; and 14.65 cm, but only 1.28 mm, respectively for Buddhist pine seedlings. On the other hand, higher rate of root forage of a faster growing species could also attribute to less nutrient loss through leaching. After all, roots of Japanese maple seedlings presented a better morphology than those of Buddhist pine seedlings (data not shown).

Photoperiod did not show any significant effect on nutrient leaching at most investigating dates except for 18 November 2014 (Table 2), when the extended photoperiod resulted in lower available P loss from leaching. This was also confirmed by results of absolute values of available P between the two photoperiods from PCA (Fig. 3E; Table 4). This can be concluded as a general result, at least at the species specific level due to that on the same date no interactive effects of tree species and photoperiod were observed. Because leachates were collected by traits immediately after irrigation until the volume of 100 mL, therefore declined P loss may result from increased P uptake by seedlings of the two species under extended photoperiod. Wei *et al.* (2013) revealed that under extended photoperiod P uptake was fully promoted by needles, stem, and root of Buddhist pine seedlings. Accordingly, photoperiod was also found to affect P requirement for the maximum growth rate (Shatwell *et al.*, 2014). Evidence from *Eucalyptus globulus* revealed that requirement of P in the photosynthetic system was driven by the synthesis of phosphate and fructose 6-phosphate also accompanied by correlated changes of V_{cmax} (Warren, 2011). Therefore, all these series results together suggest the assumption that extended photoperiod controls nutrient leaching through promoting root-forage driven uptake can be reasonable for P availability. However, the third hypothesis needs more experimentation because most leachate nutrients did not respond to photoperiod except available P.

As mentioned earlier, a number of studies have monitored nutrient leaching from containers during tree seedling culture, but quite a few of them have predicted nutrient leaching through seedling morphology. After all, seedling shoot morphologies are easily measured and direct parameters revealing seedling growing dynamics, which has applicative practices for nursery seedling culture. According to our knowledge, at least three necessities can be put forward to perform this prediction. Firstly, this has practical implications to easily judge whether the current culture protocol results in risks for water contamination; Secondly, it is facilitate to timely know whether the fertilization regime is inefficient due to nutrient loss from leaching; Finally, it can tell whether it needs to care about nutrient leaching when using innovative methods for seedling culture, such as the manipulation of prolonged photoperiod performed in the present study. In the present study, we evaluated nutrient leaching through measuring morphologies, and found negative correlations between leachate nutrient concentrations and seedling height growth (Table 5). This indicated that acceleration of seedling height growth using large containers under the extended photoperiod could counter nutrient loss through leaching by promoting nutrient uptake efficiently. The results of insignificant correlations between leachate nutrient concentration and RCD for some large and small seedlings suggested that nutrients were not retained thoroughly for diameter growth. Positive correlations among leachate nutrient concentrations revealed their interplay during the uptake and loss within seedling containers. However, insignificant correlation between concentrations of available

K and ammonium N can probably be attributed to the competition of uptake between the two cations, which would occupy the transporter for each other within cell wall when being absorbed.

Conclusion

Results in the present study showed that container size can promote seedling growth significantly, but the effect of photoperiod was only effective to enhance growth for Japanese maple seedlings. Additionally, nutrient leaching also had a species-specific response but photoperiod did not have any effect on leaching nutrients. It was also found the negative relationship between leachate nutrients and height growth. Also, we put forward the utilization of predicting leaching nutrients with easily-measured shoot morphologies and results of the present study proved its validity.

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References

Alva, A.K., 1992. Differential leaching of nutrients from soluble vs controlled-release fertilizers. *Environ. Manage.*, 16: 769–776

Aphalo, P. and R. Rikala, 2003. Field performance of silver-birch planting-stock grown at different spacing and in containers of different volume. *New For.*, 25: 93–108

Bayala, J., M. Dianda, J. Wilson, S.J. Ouédraogo and K. Sanon, 2009. Predicting field performance of five irrigated tree species using seedling quality assessment in Burkina Faso, West Africa. *New For.*, 38: 309–322

Bongarten, B.C. and J.W. Hanover, 1985. Accelerating seedling growth through photoperiod extension for genetic testing: A case study with blue spruce (*Picea pungens*). *For. Sci.*, 31: 631–643

Bowden, R., 1993. Stock type selection in British Columbia. *In*: *Proceedings of the 1993 Forest Nursery Association of British Columbia Meeting*, pp: 17–20. Huber R. (ed.). Forest Nursery Association of British Columbia, USA

Boczulak, S.A., B.J. Hawkins and R. Roy, 2014. Temperature effects on nitrogen form uptake by seedling roots of three contrasting conifers. *Tree Physiol.*, 34: 513–523

Close, D.C., S. Paterson, R. Corkrey and C. McArthur, 2010. Influences of seedling size, container type and mammal browsing on the establishment of *Eucalyptus globulus* in plantation forestry. *New For.*, 39: 105–115

Do Amaral, J.A.T., A.B. Rena and J.F.T. do Amaral, 2006. Seasonal vegetative growth of the coffee plant and its relationship with the photoperiod, fructification, stomatic resistance and photosynthesis. *Pesq. Agric. Bras.*, 41: 377–384

Danner, M.A., I. Citadin, A.D. Fernande, A.P. Assmann, S.M. Mazaro and S.A.Z Sasso, 2007. Seedling development of *jabuticaba* fruit trees (*Plinia* sp.) in different substrates and sizes of containers. *Rev. Braz. Frut.*, 29: 179–182

Dobrahner, J., B. Lowery and J.G. Lyer, 2007. Slow-release fertilization reduces nitrate leaching in bareroot production of *Pinus strobus* seedlings. *Soil Sci.*, 172: 242–255

Dumroese, R.K., A.S. Davis and D.F. Jacobs, 2011. Nursery response of *Acacia koa* seedlings to container size, irrigation method, and fertilization rate. *J. Plant. Nutr.*, 34: 877–887

Faure-Lacroix, J., J.P. Tremblay, N. Thiffault and V. Roy, 2013. Stock type performance in addressing top-down and bottom-up factors for the restoration of indigenous trees. *For. Ecol. Manage.*, 307: 333–340

Gomez-Rey, M.X., M. Maderia and E. Vasconcelos, 2008. Effects of organic residue management and legume cover on growth of pine seedlings, nutrient leaching and soil properties. *Ann. For. Sci.*, 65: 807

Hauagge, R. and J.N. Cummins, 1991. Age, growing temperatures, and growth-retardants in fluence induction and length of dormancy in *Malus*. *J. Amer. Soc. Hortic. Sci.*, 116: 116–120

Hangs, R.D., J.D. Knight and K.C.J. Van Rees, 2003. Nitrogen accumulation by conifer seedlings and competitor species from ^{15}nitrogen-labeled controlled-release fertilizer. *Soil. Sci. Soc. Amer. J.*, 67: 300–308

Jelic, G., V. Topic, L. Butorac, Z. Durdevic, A. Jazbec and M. Orsanic, 2014. Container size and soil preparation effects on afforestation success of one year old stone pine (*Pinus pinea* L.) seedlings in Croatian Mediterranenan area. *Sumarski List*, 138: 463–475

Johnsen, K.H. and J.R. Seiler, 1996. Growth, shoot phenology and physiology of diverse seed sources of black spruce. 1. Seedling responses to varied atmosphere CO_2 concentrations and photoperiods. *Tree Physiol.*, 16: 367–373

Juntunen, M.L. and R. Rikala, 2001. Fertilization in Finnish forest nurseries from the standpoint of environmental impact. *New For.*, 21: 141–158

Juntunen, M.L., T. Hammar and R. Rikala, 2002. Leaching of nitrogen and phosphorus during production of forest seedlings in containers. *J. Environ. Qual.*, 31: 1868–1874

Juntunen, M.L., T. Hammar and R. Rikala, 2003. Nitrogen and phosphorus leaching and uptake by container birch seedlings (*Betula pendula* Roth) grown in three different fertilizations. *New For.*, 25: 133–147

Klooster, W.S., B.M. Cregg, R.T. Fernandez and P. Nzokou, 2012. Growth and physiology of deciduous shade trees in response to controlled-release fertilizer. *Sci. Hortic.*, 135: 71–79

Macey, D.E. and J.T. Arnott, 1985. Effect of supplemental light intensity on white spruce, Engelmann spruce, and mountain hemlock seedlings grown under an extended photoperiod. *Can. J. For. Res.*, 15: 295–300

Murray, C.L., G.P. Lumis and C. Chong, 1996. Fertilizer method and container size effects on shade trees grown in in-ground containers. *Can. J. Plant. Sci.*, 76: 507–513

Nagel, L.M., J.H. Bassman, G.E. Edwards, R. Robberecht and V.R. Franceshi, 1998. Leaf anatomical changes in *Populus trichocarpa*, *Quercus rubra*, *Pseudotsuga menziesii* and *Pinus ponderosa* exposed to enhanced ultraviolet-B radiation. *Physiol. Plant.*, 104: 385–396

Oliet, J., R. Planelles, M.L. Segura, F. Artero and D.F. Jacobs, 2004. Mineral nutrition and growth of containerized *Pinus halepensis* seedlings under controlled-release fertilizer. *Sci. Hortic.*, 103: 113–129

Pinto, J.R., J.D. Marshall, R.K. Dumroese, A.S. Davis and D.R. Cobos, 2011. Establishment and growth of container seedlings for reforestation: A function of stocktype and edaphic conditions. *For. Ecol. Manage.*, 261: 1876–1884

Park, B.B., M.S. Cho, S.W. Lee, R.D. Yanai and D.K. Lee, 2012. Minimizing nutrient leaching and improving nutrient use efficiency of *Liriodendron tulipifera* and *Larix leptolepis* in a container nursery system. *New For.*, 43: 57–68

Shatwell, T., J. Kohler and A. Nicklisch, 2014. Temperature and photoperiod interactions with phosphorus-limited growth and competition of two diatoms. *Plos. One.*, 9: e102367

Sutherland, D.C. and R.J. Day, 1988. Container volume affects survival and growth of white spruce, black spruce and jack pine seedlings: a literature review. *North. J. Appl. For.*, 5: 185–189

South, D.B., S.W. Harris, J.P. Barnett, M.J. Jainds and D.H. Gjerstad, 2005. Effect of container type and seedling size on survival and early height growth of *Pinus palustris* seedlings in Alabama, USA. *For. Ecol. Manage.*, 204: 385–398

Sysoeva, M.I., E.F. Markovskaya and T.G. Shibaeva, 2010. Plant under continuous light: A review. *Plant Stress*, 4: 5–17

Tsakaldimi, M., P. Ganatsas and D.F. Jacobs, 2013. Prediction of planted seedling survival of five Mediterranean species based on initial seedling morphology. *New For.*, 44: 327–339

Topic, V., L. Butorac, Z. Durdevic, B. Kekilic and G. Jelic, 2009. Effect of container type on growth and development of seedlings of common cypress (*Cupressus sempervirens* var. *pyramidalis* Nyman) in nurseries and forest cultures. *Sum. List*, 133: 121–134

Wheeler, N., 1979. Effect of continuous photoperiod on growth and development of lodgepole pine seedlings and grafts. *Can. J. For. Res.*, 9: 276–283

Warren, C.R., 2011. How does P affect photosynthesis and metabolite profiles of *Eucalyptus globulus*? *Tree Physiol.*, 31: 727–739

Wei, H.X., C.Y. Xu, B.J. Hawkins, L.Y. Ma and L.N. Jiang, 2012. Organic amendment and inorganic fertilization affect soil properties and quality of *Larix olgensis* bareroot stock. *New For.*, 43: 155–168

Wei, H.X., J. Ren J. and J.H. Zhou, 2013. Effect of exponential fertilization on growth and nutritional status in Buddhist pine (*Podocarpus macrophyllus* [Thunb.] D. Don) seedlings cultured in natural and prolonged photoperiods. *Soil Sci. Plant. Nutr.*, 59: 933–941

Xu, F., Y. Liu, G.L. Li, Q.Y. Sun, S. Zhang and W.W. Wang, 2013. Research advances of container seedling afforestation techniques in China. *World. For. Res.*, 26: 62–66

Yao, Z.M., C.Y. Xu, Y. Chai and H.X. Wei, 2014. Effect of light intensities on the photosynthetic characteristics of *Abies holophylla* seedlings from different provenances. *Ann. For. Res.*, 57: 181–196

Zhang, Y., Z.C. Zhou, X.H. Ma and G.Q. Jin, 2010. Foraging ability and growth performance of four subtropical tree species in response to heterogeneous nutrient environments. *J. For. Res.*, 15: 91–98

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