

## Combined pre-hardening and fall fertilization facilitates N storage and field performance of *Pinus tabulaeformis* seedlings

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Exponential fertilization during the pre-hardening stage and fall fertilization during the hardening stage have each been used independently to nutrient load seedlings. However, nursery and field responses of seedlings to the combination of exponential fertilization and fall fertilization have received little attention. Chinese pine (*Pinus tabulaeformis* Car.) container seedlings were exponentially fertilized with accumulated totals of 40, 80 or 120 mg N per seedling during pre-hardening, and fall-fertilized with 0, 12, 24 or 48 mg N per seedling, and were subsequently outplanted and followed for two growing seasons. Interactions of exponential and fall fertilization had significant effects on plant N content in the nursery and first-year height after outplanting. Fall fertilization promoted additional nutrient loading during hardening for the 40-80 mg N per seedling pre-hardening regimes. The highest exponential fertilization rate enhanced N concentration in foliage and roots compared to the other two rates. Maximum diameter was observed in the lowest exponential fertilization rate at the second year after outplanting. Fall fertilization enhanced foliar N concentration. Supplemental 12 and 24 mg N per seedling during fall were more effective in increasing height increment at the second year after outplanting. Our results indicate that pre-hardening fertilization is a useful tool to nutrient load Chinese pine in the nursery and facilitate outplanting performance in the field. In combination, fall fertilization has potential to further augment this response, although further research is needed to precisely match rates of pre-hardening and fall fertilization to optimize seedling performance.

**Keywords:** Exponential Fertilization, Fall Fertilization, Nutrient-loading, Field Performance, Chinese Pine

### Introduction

More nitrogen (N) is retranslocated from perennial tissues to new apical growth tissues in seedlings with rich N reserves, especially when seedlings are outplanted in poor fertility soil (Salifu & Timmer 2003a, Oliet et al. 2009, Villar-Salvador et al. 2012). Thus, additional N fertilization during nursery culture may provide an effective solution for enhancing field performance and has received worldwide attention (Timmer 1996, Andivia et al. 2011, Villar-Salvador et

al. 2012, Oliet et al. 2013).

Exponential fertilization during the pre-hardening stage and fall fertilization during the hardening stage are two approaches to facilitate N storage in seedlings (Timmer 1996, Sung et al. 1997, Dumroese 2003). Exponential fertilization reflects application of fertilizer at exponential rates corresponding closer to the desired relative growth rate of plants during their exponential phase of growth (Timmer 1996, Birge et al. 2006). It synchronizes nutrient supply with

crop demand, referred to as steady-state nutrition (Imo & Timmer 1992), and has proven to facilitate nutrient storage in a variety of tree species (Timmer & Armstrong 1987, Miller & Timmer 1994, Quoreshi & Timmer 2000, Salifu & Timmer 2001, 2003b, Qu et al. 2003). Previous studies comparing one exponential fertilization rate with one or more conventional fertilization rates, have effectively demonstrated that greater plant N reserves induced by exponential fertilization have the potential to promote seedling field performance over conventional fertilization (Dumroese et al. 2005, Close et al. 2005, Way et al. 2007). When outplanted onto a field site, how seedlings respond to varying exponential fertilization rates in the nursery has been studied on several species, including western hemlock (*Tsuga heterophylla* (Raf.) Sarg. – Hawkins et al. 2005), northern red oak (*Quercus rubra* L.) and white oak (*Q. alba* L. – Salifu et al. 2009). However, less attention has been given to pines (*Pinus* spp.), which are among the most widely distributed and planted species worldwide.

Fall fertilization is another approach to facilitate nutrient loading. Fall fertilization could counter nutrient dilution due to biomass accumulation and promote storage of nutrient reserves in seedlings (Van Den

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Driessche 1985). Therefore, it has been implemented in a variety of tree species such as loblolly pine (*Pinus taeda* L. – Sung et al. 1997), slash pine (*Pinus elliottii* var. *elliottii* Engelm. – Irwin et al. 1998), red pine (*Pinus resinosa* Ait. – Islam et al. 2009), Norway spruce (*P. abies* (L.) Karsten – Rikala et al. 2004, Heiskanen et al. 2009), black spruce (*Picea mariana* Milli.B.S.P. – Boivin et al. 2004), Lutz spruce (*Picea x lutzii* Littl. – Jonsdottir et al. 2013), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco – Van Den Driessche 1985, Margolis & Waring 1986a, Margolis & Waring 1986b), holm oak (*Q. ilex* spp. *ballota* Desf. – Oliet et al. 2011, Andivia et al. 2011), Olga Bay larch (*Larix olgensis* Henry – Li et al. 2012, Zhu et al. 2013).

In addition, the efficacy of fall fertilization on seedling nutrient reserves is dependent on pre-hardening nutrient status (Boivin et al. 2004, Li et al. 2014). If seedlings are provided with high rates of fertilizer and thus have substantial amounts of stored nutrients during the pre-hardening period, then fall fertilization has little additional impact on nutrient reserves (Boivin et al. 2004). For example, in the deciduous Chinese cork oak (*Quercus variabilis* Blume), root N concentration was affected by the interaction of pre-hardening and fall fertilization (Li et al. 2014). Due to variable strategies of nursery fertilization among tree species (Valdecantos et al. 2006), little information is available on how the combination of pre-hardening and fall fertilization impacts nutrient storage of *Pinus* spp. seedlings.

Stored nutrients in nursery seedlings are often correlated with early seedling survival after outplanting (Van Den Driessche 1985, Villar-Salvador et al. 2012), and nutrient differences among fall fertilization treatments are influenced by pre-hardening fertilization (Boivin et al. 2004, Li et al. 2014). Earlier papers have primarily dealt with the relationship between field performance and pre-hardening or fall fertilization independently (Qoreshi & Timmer 2000, Rikala et al. 2004, Hawkins et al. 2005, Close et al. 2005, Dumroese et al. 2005, Oliet et al. 2011, 2013), but expanded study aimed at understanding how their interactive effects impact nursery and field performance are needed, especially for species of widely planted genera such as *Pinus*.

Chinese pine (*Pinus tabulaeformis* Carr.) is a native evergreen coniferous species and the most widely planted species in northern China. Because of its resistance to drought and low soil fertility, it is planted across a wide range of site types and is often exposed to environmental stresses (Xu et al. 1981, Wu & Feng 1994). Nursery fertilization has been shown to promote Chinese pine seedling nutrient reserves (Huang et al. 2004, Wang 2014), suggesting potential to increase stress resistance and improve outplanting performance (Villar-Salvador et al. 2012, Oliet et al. 2013). Thus, our objectives were to: (1) examine the

combined effects of these two nutrient loading approaches on Chinese pine seedling nutrient storage in the nursery; and (2) assess how field performance responds to the application of combined exponential and fall fertilizations in the nursery. We focused on N because this nutrient is often the most limiting factor for seedling growth in peat-growing media (Folk et al. 1992), is required in the largest amount for intensive nursery culture of conifers (Salifu & Timmer 2003b), and is highly correlated with field performance after outplanting (Villar-Salvador et al. 2012, Oliet et al. 2013). We hypothesized that: (1) pre-hardening and fall fertilization would each promote nutrient reserves in Chinese pine seedling yet vary in their effectiveness; (2) pre-hardening and fall fertilization would be more advantageous in combination than individually; and (3) outplanting performance would be strongly correlated with seedling growth and nutrient storage from the nursery phase.

## Materials and methods

### Nursery fertilization treatments and outplanting trial

The experiment was conducted in a greenhouse at the Chinese Academy of Forestry Sciences in Beijing (40° 40' N, 116° 14' E). Our experiment investigated the main and interacting effects of three rates of pre-hardening N fertilization and four rates of N fertilization during hardening (fall fertilization). On 21 February 2010, seeds from a regional seed orchard (National Seed Orchard for Chinese Pine, Qigou Forest Farm, 41° 00' N, 118° 27' E, 526 m a.s.l.) were sown into plastic containers (8 cm diameter × 8 cm deep, 187 ml capacity) filled with a 3:1 (v:v) peat:vermiculite medium. Thirty containers were randomly assigned to a tray (53 cm long × 44.5 cm wide), providing a density of 127 seedlings m<sup>-2</sup>.

Three pre-hardening fertilization rates based on another *Pinus* species study (Miller & Timmer 1994), starting 1 week after germination and continuing for 25 weeks, were tested: (i) 40 mg N per seedling; (ii) 80 mg N per seedling; and (iii) 120 mg N per seedling following exponential (E) functions (McAlister & Timmer 1998, Dumroese et al. 2005). A total of 48 trays were arranged in a completely randomized design with sixteen trays for each rate.

In this study, the average initial N content per seed (N<sub>s</sub>) was determined to be 1.58 mg N. The total number of fertilizations (t) was 25. N was supplied as urea (Xilong Chemical Co., China), a N source commonly used in Chinese nurseries. Elemental P and K were supplied as KH<sub>2</sub>PO<sub>4</sub> (Guangdong Guanghua Sci-Tech Co. Ltd., China). On 16 August the pre-hardening fertilization was terminated, each seedling received total amounts of 26.2 mg P and 32.9 mg K which were evenly split and applied weekly in addition to the N fertilizer (1 March-16 August, 2010). The desired amounts of N, P

and K were dissolved in distilled water and added manually to each seedling once per week with a hand-sprayer. No micronutrients were applied during the experiment. Seedlings were rinsed after each application to avoid foliar fertilizer burn. Additional irrigation was provided as necessary, about two times each week. The trays on raised benches were rotated every week to minimize edge effect.

Fall fertilization started two weeks after the pre-hardening fertilization ended when most seedlings had already formed the terminal buds, and lasted for four weeks (30 August-20 September, 2010). The doses of N were evenly split and applied weekly as four fall fertilization rates: (i) control (no additional fertilization); (ii) 12 mg N per seedling; (iii) 24 mg N per seedling; and (iv) 48 mg N per seedling. Fall fertilization was applied within each of the three pre-hardening regimes (40, 80, 120 mg N per seedling) as a factorial 3×4 design that was randomly arranged with four replications per treatments combination. The desired amount of N was supplied as urea; 20 ml of appropriate fertigation solution was added by hand to each seedling. On 24 October, all seedlings were moved outdoors to hasten hardening. After 28 November, seedlings were stored under snow cover over winter.

Temperature was measured with a JL-18 Series thermometer (Huayan Instrument and Equipment Co., Shanghai, China) at 10-min intervals. From sowing throughout the end of the pre-hardening fertilization, the daily average for day:night temperature in the greenhouse was 25:18 °C, respectively. During the fall N application period and until the seedlings were moved outdoors, greenhouse temperatures averaged 19:14 °C (day:night). Once outside, seedlings were exposed to temperatures averaging 13:9 °C (day: night).

On 1 May 2011, seedlings from the twelve fertility treatments were shipped and outplanted in a previously cultivated land at the Beijing Forestry University Northern Experimental Base at Pingquan, Hebei province (41° 13' N, 118° 40' E). The soil was ploughed before outplanting. Weeds were removed by hand. The site has an average elevation of 765 m a.s.l. with a small slope (< 2%). The depth of soil varied between 45 and 60 cm. The surface soil (0-20 cm) is 73% sand, 11% silt, and 16% clay (a sandy clay loam) with a pH of 6.2 and soil organic carbon of 0.7%. Average total N, available P, and available K were 628.7, 139.5, and 113.5 mg kg<sup>-1</sup>, respectively. The soil was considered to have moderate fertility according to the macronutrient classification criteria (Bao 2000). The area has a temperate continental monsoon climate, characterized by dry winter and spring seasons. During 2011 and 2012, mean annual air temperature was 7.4 °C and 6.8 °C, respectively. Annual rainfall was 486 mm in 2011 and 626 mm in 2012.

The field experiment was arranged as a

**Tab. 1** - P-values derived from ANOVA of the main effects pre-hardening fertilization (PF), fall fertilization (FF) and of their interaction effect (PF×FF) on Chinese pine seedling biomass, N concentration and N content prior to planting.

| Source  | df | Biomass |       |       |       | N concentration |       |        |        | N content |       |       |        |
|---------|----|---------|-------|-------|-------|-----------------|-------|--------|--------|-----------|-------|-------|--------|
|         |    | Foliage | Stem  | Root  | Plant | Foliage         | Stem  | Root   | Plant  | Foliage   | Stem  | Root  | Plant  |
| PF      | 2  | 0.829   | 0.417 | 0.360 | 0.273 | <0.001          | 0.191 | <0.001 | <0.001 | 0.070     | 0.076 | 0.061 | <0.001 |
| FF      | 3  | 0.152   | 0.784 | 0.892 | 0.624 | 0.001           | 0.434 | 0.120  | 0.867  | 0.373     | 0.493 | 0.248 | 0.137  |
| PF × FF | 6  | 0.407   | 0.205 | 0.113 | 0.346 | 0.147           | 0.219 | 0.832  | 0.919  | 0.520     | 0.375 | 0.083 | 0.010  |

randomized complete block experimental design with four replicates. Each block measured 14×16.5 m<sup>2</sup> and was separated from adjacent blocks by 1 m buffers. Fifteen randomly selected seedlings from a tray per treatment were planted in single parallel rows within each block, resulting in a total of 720 seedlings planted. Seedlings were planted in 0.4×0.4×0.4 m manually dug planting holes with a 1×1.5 m<sup>2</sup> spacing.

**Harvesting and measurements**

Seedlings were sampled prior to planting to evaluate biomass allocation and N storage responses to nursery fertilization. Thirty-two seedlings per nursery fertilization treatment (eight seedlings per replicated tray) were randomly chosen. After carefully washing, seedlings were separated into needles, stems and roots. Organs of seedlings were oven-dried at 65 °C for 48 h to determine biomass. Organ N concentration was determined from a composite sample of eight seedlings for each treatment from a tray using semi-micro Kjeldahl distillation (Bremner & Mulvaney 1982).

Immediately after planting on 2 May 2011, seedling height and diameter were measured (T<sub>1</sub>). When seedling growth ceased in late October of 2011 (T<sub>2</sub>) and 2012 (T<sub>3</sub>), survival, height and diameter were measured. Survival was calculated as the number of saplings of the original fifteen remaining alive for each nursery-treatment replicate

at the time of each annual measurement. Diameter was measured at ground rate on each tree stem, and total seedling height was measured from the root collar to the tip of the terminal bud. Net increment of height in cm (or diameter in mm) was defined as the difference observed between T<sub>2</sub> and T<sub>1</sub>, and between T<sub>3</sub> and T<sub>2</sub>.

**Statistical analyses**

Effects of pre-hardening fertilization (3 rates), fall fertilization (4 rates), and their interactions were assessed using a two-way analysis of variance (ANOVA) applied on a completely randomized design at the nursery experiment and on a randomized complete block design in the outplanting trial. When ANOVA results showed a significant effect, post-hoc Duncan’s test was carried out for multiple comparisons among treatments (α=0.05). Statistical analyses were performed using SPSS® 16.0 (Chicago, Illinois, USA). The “explore” function of SPSS was used to examine data prior to ANOVA, and survival was arcsine-transformed to fulfill normality and variance homogeneity requirements.

**Results**

**Nursery phase**

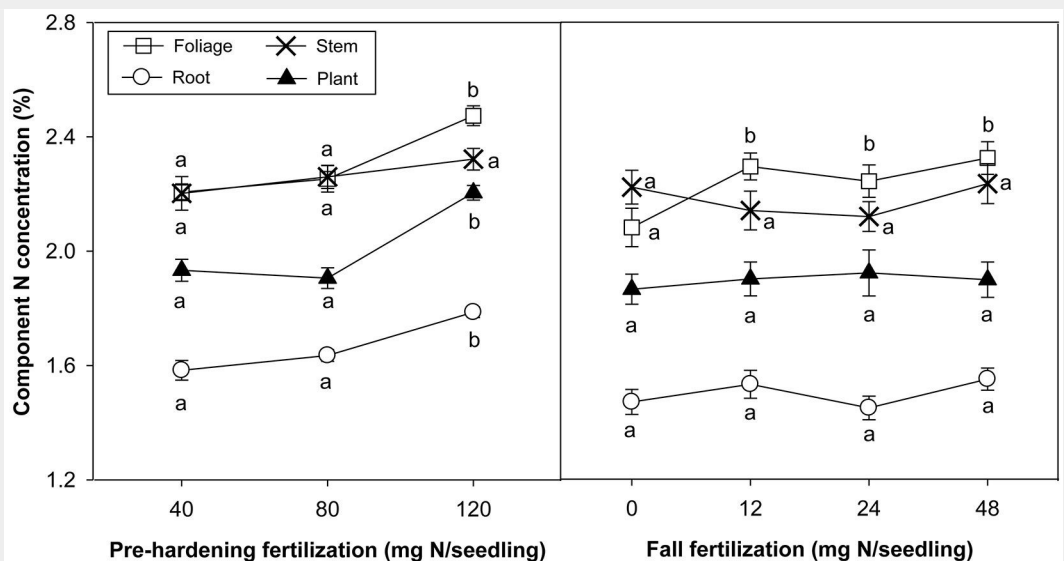
Fertilization factors and its interaction did not significantly affect biomass of specific organs or the whole plant (Tab. 1). The foliage, stem, and root biomass of seedlings

averaged 0.33 ± 0.01, 0.16 ± 0.001, and 0.40 ± 0.01 g, respectively. N concentration in foliage, root and whole plant was higher at 120 than at 40 and 80 mg N per seedling of pre-hardening fertilization. Fall fertilization enhanced foliar N concentration relative to the control (Tab. 1, Fig. 1). Organ N content did not differ among pre-hardening fertilization or fall fertilization, although pre-hardening fertilization marginally affected N content of three organs (Tab. 1). Overall mean of N content was 7.7 ± 0.16, 3.5 ± 0.08, and 6.7 ± 0.13 mg in foliage, stem and root, respectively. The N content of whole plants, however, was influenced by the interaction of pre-hardening fertilization and fall fertilization (Tab. 1, Fig. 2). At the lowest pre-hardening fertilization regime (40 mg N per seedling), 24 mg N per seedling of fall fertilization significantly increased the N content. At 80 mg N per seedling of pre-hardening fertilization regime, 12 mg N per seedling of fall fertilization yielded a similar effect.

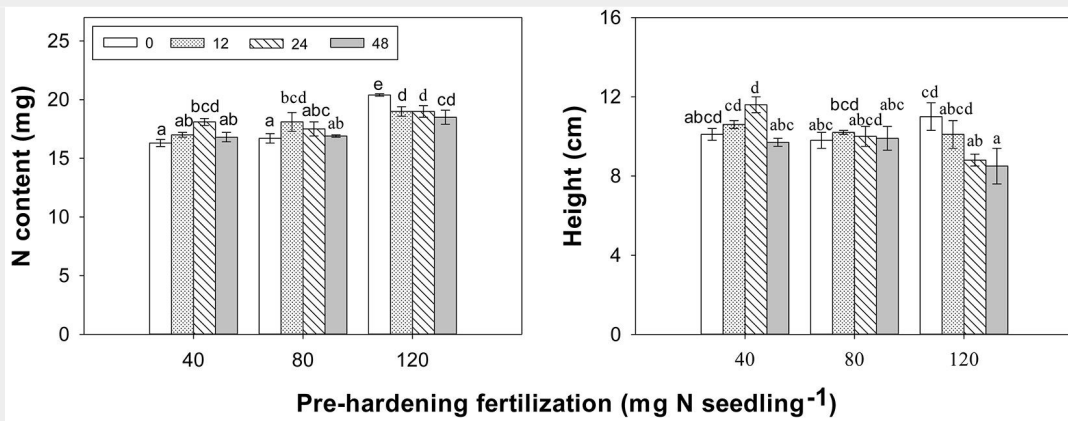
**Field performance**

For all field attributes, the interaction of pre-hardening fertilization and fall fertilization only had a significant effect on total height at the first growing season after outplanting (Tab. 2). The combination of pre-hardening at 40 mg N per seedling and 24 mg N per seedling of fall fertilization (24 mg) yielded the maximum height (Fig. 2). Within the highest pre-hardening fertiliza-

**Fig. 1** - Chinese pine seedling organ N concentration (means ± SE) in relation to pre-hardening and fall fertilization prior to planting. No significant interaction between pre-hardening and fall fertilization occurred in N concentration. Bars marked with different letters significantly differed for each organ and the whole plant after Duncan’s test (α = 0.05).







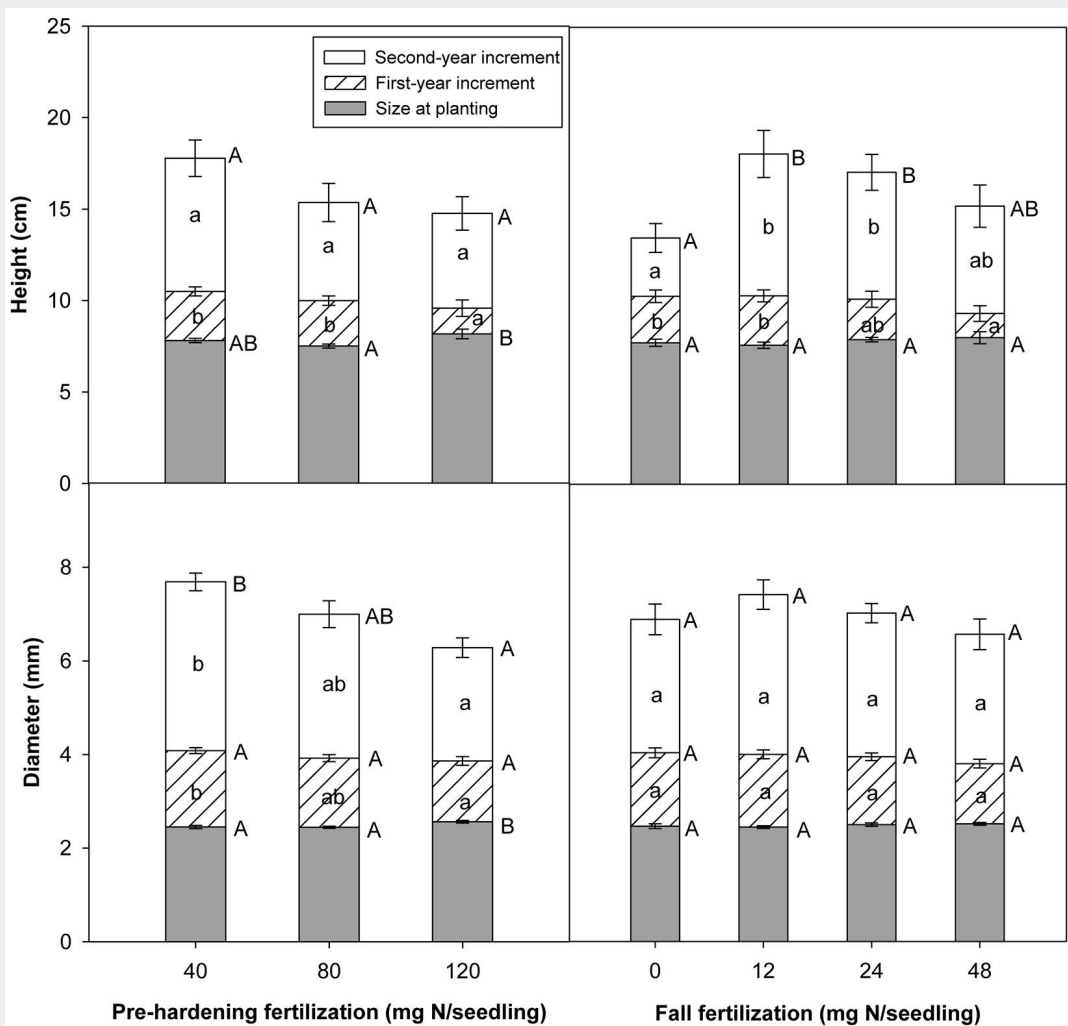
**Fig. 2** - Effect of combination treatments between pre-hardening fertilization and fall fertilization (0, 12, 24, 48) on Chinese pine seedling N content (mg N/seedling) prior to planting and total height (cm) at the end of first growing season (2011) after planting. Means ± SE marked with different letters significantly differed after Duncan's test ( $\alpha = 0.05$ ).

**Tab. 2** - P-values derived from ANOVA of main effects pre-hardening fertilization (PF), fall fertilization (FF) and of their interaction effect (PF×FF) on Chinese pine seedlings survival, total height, diameter and their increments prior to planting (Initial), at the end of first (2011) and second (2012) growing seasons after planting.

| Source | Height  |       |       | Height increment |       | Diameter |       |       | Diameter increment |       | Survival |       |
|--------|---------|-------|-------|------------------|-------|----------|-------|-------|--------------------|-------|----------|-------|
|        | Initial | 2011  | 2012  | 2011             | 2012  | Initial  | 2011  | 2012  | 2011               | 2012  | 2011     | 2012  |
| PF     | 0.028   | 0.054 | 0.079 | 0.005            | 0.231 | 0.002    | 0.088 | 0.004 | 0.022              | 0.005 | 0.161    | 0.373 |
| FF     | 0.458   | 0.087 | 0.031 | 0.021            | 0.029 | 0.303    | 0.201 | 0.309 | 0.138              | 0.366 | 0.458    | 0.793 |
| PF×FF  | 0.780   | 0.026 | 0.682 | 0.077            | 0.699 | 0.094    | 0.677 | 0.786 | 0.937              | 0.834 | 0.847    | 0.598 |

tion regime, 24 and 48 mg N per seedling of fall fertilization decreased height compared to the control.

Pre-hardening fertilization had no significant effect on survival either during the first or second growing season (Tab. 2). Survival of 40, 80 and 120 mg N per seedling of pre-hardening fertilization was 90%, 95%, and 89% in the first growing season, and was 79%, 79%, and 70% in the second growing season, respectively. Pre-planting height was superior for the highest pre-hardening fertilization rate. However, first-



**Fig. 3** - Chinese pine seedlings total height, diameter and their increments in relation to pre-hardening and fall fertilization at planting (Initial), at the end of first and second growing seasons after planting. Error bars represent SE of increments at the end of first and second growing seasons, and of size prior to planting. Bars marked with different capital and lower case letters significantly differed for total height (diameter) and its increment according to Duncan's test ( $\alpha = 0.05$ ), respectively. Comparison letters for first-year height are not presented owing to an occurring interaction.

year height increment of later rate was minimum. There was no significant difference in second-year height among pre-hardening rates. The dominance of total height in the highest exponential fertilization vanished at end of the second growing season (Tab. 2, Fig. 3). Both first-year and second-year diameter increments were superior in the 40 mg N per seedling of pre-hardening fertilization treatment, with the greatest diameter occurring in the lowest pre-hardening fertilization rate at the end of second growing season (Tab. 2, Fig. 3).

Fall fertilization also did not affect survival of Chinese pine seedlings. Survival of the control, 12, 24 and 48 mg N per seedling of fall fertilization was 92%, 93%, 93%, and 87% in the first growing season, and was 81%, 74%, 77%, and 73% in the second growing season, respectively. Pre-planting height and diameter were similar between fall-unfertilized and fertilized seedlings. Compared to the control, significant height increments in 12 and 24 mg N per seedling of fall fertilization treatments were not observed until the second growing season.

## Discussion

### *Effect of pre-hardening fertilization on nursery and field performance*

Under luxury N consumption, seedling dry mass remains unchanged but tissue N content continuously increases (Timmer 1996, McAlister & Timmer 1998, Salifu & Timmer 2003a, Birge et al. 2006). As defined by the conceptual model (Timmer 1996), absence of effects of exponential fertilization on biomass indicated that 40 mg N already reflected luxury consumption for the studied seedlings. This result is similar to our previous study on Chinese pine seedlings (Wang et al. 2015). Deficiency, luxury consumption, and toxicity have been determined in species of *Quercus* and *Picea* by evaluating the nursery response to more than six exponential fertilization rates (Salifu & Timmer 2003b, Birge et al. 2006, Salifu & Jacobs 2006). For red pine, Timmer & Armstrong (1987) proposed that 9.75 mg N yielded luxury consumption. In another study of red pine, however, 25 mg N was not designated as luxury consumption because a significant increase in biomass was still detected at 75 mg N (Miller & Timmer 1994). These conflicting results for *Pinus* spp. suggest that a wider range of nutrient availability should be tested to more accurately quantify N luxury consumption under varying cultural systems.

In recent years, there has been a trend to determine nursery fertilization in light of field performance on a specific site condition as well as seedling biomass and nutrient reserves accumulated during nursery growth (Hawkins et al. 2005, Heiskanen et al. 2009, Oliet et al. 2009, Jonsdottir et al. 2013, Villar-Salvador et al. 2013). For example, in very dry Mediterranean environments some studies showed that seedlings

with low nutrient concentration or content performed better (Trubat et al. 2008, 2010, Cortina et al. 2013), whereas other studies indicated that increased tissue nutrient reserves improves seedling field performance (Luis et al. 2009, Cuesta et al. 2010, Villar-Salvador et al. 2012, Oliet et al. 2013). These contrasting results suggest that insight into nursery fertilization and field outplanting should be further tested, in particular with relation to climatic planting conditions or soil characteristics. In the present study, greater plant N reserves induced by the highest exponential fertilization rate did not promote survival and growth performance in the following two growing seasons. In contrast, the lowest pre-hardening fertilization rate (40 mg) yielded desirable growth performance. These findings suggest that, under the relatively dry conditions of our study, rational reduction of nursery fertilization might be a choice to improve seedling outplanted performance once these nursery fertilization rates are within the luxury range.

### *Effect of fall fertilization on nursery and field performance*

The benefit of fall fertilizer on enhancement of foliage N concentration was found in Chinese pine as well as other tree seedlings (Margolis & Waring 1986a, Sung et al. 1997, Irwin et al. 1998, Heiskanen et al. 2009, Jonsdottir et al. 2013). This result is consistent with the goal of fall fertilization to nutrient load by enhancing foliar N concentration (Dumroese 2003). Increased foliar N concentration in fall-fertilized Chinese pine seedlings did not facilitate foliar N content, in accordance with that reported for holm oak seedling (Oliet et al. 2011) but in contrast to results for Douglas-fir, loblolly pine, and Norway spruce seedlings (Margolis & Waring 1986a, Sung et al. 1997, Heiskanen et al. 2009). Lack of increased foliar N content could be attributed to the absence of increasing foliar biomass of fall-fertilized seedlings, as per other previous studies (Irwin et al. 1998, Oliet et al. 2011).

The present and earlier findings of fall fertilization indicated mixed survival and growth responses to increased foliar N concentration. Fall fertilization had no effect on survival of Chinese pine, corresponding to results for Norway spruce, Douglas-fir, and Lutz spruce (Van Den Driessche 1988, Heiskanen et al. 2009, Jonsdottir et al. 2013). However, reduced survival was reported in fall-fertilized longleaf pine (*Pinus palustris* Mill.) seedlings (Shoulders 1959). Pre-planting diameter and height of fall-fertilized seedlings was similar to that of non fall-fertilized seedlings for Chinese pine and several other species (Margolis & Waring 1986b, Irwin et al. 1998, Heiskanen et al. 2009, Oliet et al. 2011). These trends were maintained in first-year diameter for Chinese pine, which was contrary to that reported for slash pine seedlings (Irwin et al. 1998). For height, favorable effects of fall fertilization did not appear until the se-

cond growing season in our study, despite frequent reports of improved first-year height growth in fall fertilized seedlings (Margolis & Waring 1986b, Irwin et al. 1998, Heiskanen et al. 2009). Inconclusive growth performance across these studies again demonstrates that comprehensive consideration of both nursery and field performance determines rational N supply in the nursery for seedling production to meet a particular site condition. Overall, 12 and 24 mg N fall fertilization rates are more effective during hardening for Chinese pine seedling in terms of foliar N concentration prior to planting, and total height and height increment at the second year after outplanting.

### *Interaction between pre-hardening and fall fertilization on nursery seedling N storage and subsequent field performance*

In previous studies investigating fall fertilization, seedlings generally received the same dose of pre-hardening fertilization and were subsequently fall-fertilized at multiple treatment rates (Van Den Driessche 1988, Irwin et al. 1998, Heiskanen et al. 2009, Jonsdottir et al. 2013). This precludes the ability to distinguish how pre-hardening fertilization affects fall fertilization; and the use of a single pre-hardening fertilization rate potentially contributes to the inconsistent results of fall fertilization among studies. For black spruce seedlings, interactions influenced both N concentration and content of the whole plant (Boivin et al. 2004). Interaction only occurred in root N concentration of Chinese cork oak seedlings (Li et al. 2014) and in N content of the whole plant for Chinese pine in our study. Additionally, fall fertilization had adverse effects on N content when seedlings were fertilized at the pre-hardening rate of 120 mg N, indicating that this rate may have the approximated optimal nutrient input whereas additional fertilization has proven to induce some toxicity (Jacobs & Timmer 2005).

First-year height after outplanting indicated that fall fertilization had a slightly advantageous effect under 40 and 80 mg N per seedling of pre-hardening fertilization regimes, whereas negative impacts were observed at the highest rate. This could be related to incipient toxicity for seedlings fertilized at 120 mg that were subsequently fertilized with additional N in the fall. This interactive effect of pre-hardening and fall fertilization was transitory and vanished at the second-year of outplanting.

## Conclusions

Based on nursery seedling growth and N content during the pre-hardening period, the three pre-hardening fertilization rates (40, 80 and 120 mg N) induced luxury consumption of Chinese pine seedlings. Fall fertilization of seedlings that received 40-80 mg N per seedling during pre-hardening further N loaded Chinese pine. For the

highest pre-hardening fertilization rate, however, fall fertilization decreased N content.

Maximum diameter was observed in the lowest nursery exponential fertilization rate at the second year after outplanting. Supplemental fertilization during fall with 12 and 24 mg N per seedling were more effective in enhancing both total height and height increment at the second year after outplanting. Considering nursery and field performance, fertilization cost and potential nitrate leaching risks, combining pre-hardening fertilization with fall fertilization may serve as a more effective means to promote N storage and outplanting growth as compared with pre-hardening fertilization alone. Further research is needed, however, to optimize application rates under this fertilization scheme for Chinese pine and other important reforestation species under varying cultural regimes.

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