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# Effect of controlled release fertilizer type and rate on mineral nutrients, non-structural carbohydrates, and field performance of Chinese pine container-grown seedlings

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#### Highlights

- We demonstrated that Chinese pine container-grown seedling nutrient status and non-structural carbohydrate content were sufficient over a wide range of fertilization rates.
- Fertilization at 80 mg N seedling<sup>-1</sup> was optimal for seedling responses in the nursery and field.
- Nursery fertilization using controlled release fertilizer (CRF) with a single coating layer yielded better seedling nursery performance than CRF with multiple coatings.

#### Abstract

Although controlled release fertilizer (CRF) with single and multiple-layer coatings are extensively used in tree seedlings, studies that compare the impact of CRF type and application rate on seedling growth, nutrient storage, and, most importantly, outplanting performance, are lacking. In the current study, container-grown Pinus tabulaeformis Carr. (Chinese pine) seedlings were fertilized with commercial CRF with either one or multiple coating layers with equivalent formulation and longevity, at six rates ranging from 40 to 240 mg N seedling<sup>-1</sup>. Seedlings were sampled for dry mass, non-structural carbohydrate (NSC) content, and mineral nutrient status at the end of the growing season in the nursery, and subsequently outplanted for one season. Compared to Chinese pine seedlings fertilized with single-layer CRF treatments, seedlings treated with multiple-layer CRF had higher starch concentrations but reduced dry mass and N, P, K concentrations in the nursery, and reduced diameter growth in the field. Fertilization rates of 80 and 120 mg N seedling<sup>-1</sup> generally vielded maximal plant dry mass and mineral nutrient content. Field survival peaked at 80 mg N seedling<sup>-1</sup>. Seedling growth, soluble sugar content, and starch concentration in the nursery and survival in the field consistently decreased at rates of 200 and 240 mg N seedling<sup>-1</sup>. In our study, optimal nursery and field performance of P. tabulaeformis were observed using single layer CRF at 80 mg N seedling<sup>-1</sup> (3.3 g CRF l<sup>-1</sup> media).

Keywords *Pinus tabulaeformis*; nursery fertilization; growth; nutrient loading; outplanting performance

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# 1 Introduction

Several research papers have documented clearly that tree seedling survival and growth performance is closely associated with seedling nutrient reserves at the time of outplanting in the field (Oliet et al. 2013; Villar-Salvador et al. 2012, 2015). Consequently, there is global interest in optimizing fertilizer application in the nursery for effective nutrient storage by seedlings (Dumroese et al. 2005; Salifu et al. 2009; Li et al. 2014).

An ideal fertilization regime promotes seedling growth and nutrient loading without toxic effects (Uscola et al. 2015). A conceptual model proposed by Timmer (1996) to optimize fertilization based on assessment of plant dry mass and mineral nutrient status (mainly N) in response to a wide range of fertilization rates has been used successfully for many tree species, including *Picea* spp., *Quercus* spp., and *Pinus* spp. using fertigation (Salifu and Timmer 2003; Salifu and Jacobs 2006; Wang et al. 2015).

Controlled release fertilizer (CRF) with a soluble nutrient core and a water-insoluble coating provides an inexpensive and simple way to supply nutrients to tree seedlings (Jacobs et al. 2005), with potential for optimizing nutrient delivery in the nursery (Haase et al. 2006). Timmer's conceptual model for optimizing fertilization based on investigating the impact of fertilization rates on plant dry mass and mineral nutrient status has not been assessed yet for CRF. Measurements of non-structural carbohydrate (NSC) levels in seedlings also appear to be useful for evaluating nursery practices (Millar and Grelet 2010; Villar-Salvador et al. 2015). The lack of research using a sufficiently broad range of CRF rates to cover nutrient deficiency to toxicity (Oliet et al. 2004, 2009; Luis et al. 2009; Dumorese et al. 2011), hinders seedling nutrient optimization. Thus, investigation of nursery fertilization using a range of CRF rates and assessing tree seedling dry mass, mineral nutrient status, and NSC content is warranted.

Nursery fertilization is effective for nutrient loading and seedling growth; however, the subsequent field response is variable because outplanted seedlings are exposed to sites that often vary in nutrient composition and moisture content. Site-specific conditions should be considered in addition to seedling response to nursery fertilization (Wang et al. 2015). Previous studies using CRF predominantly addressed effects on seedling morphology and mineral nutrient status in the nursery (Fan et al. 2004; Oliet et al. 2004; Dumorese et al. 2011). Subsequent outplanting performance in the field (Haase et al. 2006; Luis et al. 2009; Oliet et al. 2009), let alone response to a wide range of nursery CRF application rates, have not been explored fully.

Nutrient release of CRF is affected by the coating thickness and chemical composition, nutrient formulation (N:P:K), application method (topdressing vs incorporation), and the environment (Crowley et al. 1986; Ruter 1992; Jacobs et al. 2009). Recent, innovative coating techniques have produced new types of CRF with different patterns of nutrient release. A single coating product such as Osmocote Exact Standard fertilizer (SF) typifies a third generation CRF whereas Osmocote Exact Hi End fertilizer (HF), a fourth generation CRF with two coating layers of nutrients, provides a slower, sustained release pattern. (O.M. Scotts Co., Marysville, OH, USA). The variation in release patterns dictated by the number of coating layers may contribute to differences in nutrient availability and, thus, seedling development. Further exploration of the impact of the number of CRF coating layers on seedling physiology using a range of application rates may identify combinations for optimal nursery fertilization.

Chinese pine (*Pinus tabulaeformis* Carr.), a native evergreen, is the most widely planted coniferous species in northern China. As a consequence of its drought resistance and tolerance of low soil fertility, Chinese pine is planted across a wide range of site types, often exposed to environmental stress (Xu et al. 1981). In earlier studies on Chinese pine, the use of immediately soluble fertilizers during nursery growth was facilitated N storage in seedlings and improved outplanting

performance (Wang et al. 2015; Li et al. 2016). In consideration of observed differences in *Olea europaea* L. seedling growth using immediately soluble fertilizer and CRF (Fernández-Escobar et al. 2004), evaluation of CRF use for extensively planted genera such as *Pinus* spp. may provide valuable insights for fertilization regime optimization in the nursery. We hypothesized that CRF with multiple layers of nutrient coating may be more effective for nutrient loading of seedlings than CRF with a single layer, as the delayed nutrient release pattern of the former (The Scotts Company 2015) may be more coincident with Chinese pine growth phases (Zhang et al. 2014). We investigated the effects of combinations of CRF type, using a broad range of fertilization rates in the nursery on seedling mineral status, NSC content and concentration, and subsequent field performance.

# 2 Materials and methods

## 2.1 Nursery production and fertilization treatments

To test our hypothesis, we investigated independent and interacting effects of two controlled release fertilizer (CRF) nutrient release patterns for six rates of fertilization. On 22 April 2014, Chinese pine seeds collected from the 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 in plastic containers (SC10 Super; container volume, 164 ml; container depth, 21 cm; Ray Leach "Cone-tainers"<sup>TM</sup>, Stuewe & Sons, Inc., Oregon, USA). Containers were filled with a 3:1 (v:v) mixture of peat (Pindstrup Seeding; pH, 6.0; Screening, 0–6 mm) and perlite (5 mm diameter; Xinyang Jinhualan Mining Co., Henan, China).

Commercial CRF fertilizers (O.M. Scotts Co., Marysville, OH, USA) were incorporated into the growth media prior to sowing using two CRF with equivalent nutrient formulations but varying in the number of coating layers and, consequently, in nutrient release patterns. Both fertilizers contained 8.4% NH<sub>4</sub>-N and 6.6% NO<sub>3</sub>-N, 3.9% P, 9.1% K, 2% MgO, 0.45% Fe, 0.06% Mg, 0.05% Cu, 0.015% Zn, 0.03% B, and 0.02% Mo) with a longevity of 8–9 months at 21 °C. Osmocote Exact Standard fertilizer has a single coating layer releasing nutrients continuously over the expected longevity of the fertilizer (The Scotts Company 2015). Osmocote Exact Hi End has a different, predefined nutrient release pattern. It contains approximately 25% encapsulated fertilizer with an additional coating layer which delays release of this proportion of nutrients by 2 to 3 months, providing plants with lower levels of mineral nutrients during plant establishment and higher levels during the rapid growth stage.

Previously reported observations on growth and nutrient responses of Chinese pine to a range of soluble nitrogen (N) fertilizers in the nursery (Wang et al. 2015) informed our choices of N application rates. The N rates used in our study were 40, 80, 120, 160, 200, and 240 mg seed-ling<sup>-1</sup>, i.e. incorporation of 1.6, 3.3, 4.9, 6.5, 8.1, and 9.8 g CRF l<sup>-1</sup> media, respectively. These CRF application rates are consistent with other *Pinus* spp. fertilization studies (Crowley et al. 1986; Oliet et al. 2004), as well as compliant with the recommended CRF application ranges of 3-7 g l<sup>-1</sup> for HF and 3-6 g l<sup>-1</sup> for SF (The Scotts Company 2015).

Containers were inserted into Ray Leach "Cone-tainers"<sup>TM</sup> RL98 trays (98 containers per tray, 528 containers m<sup>-2</sup>; Stuewe & Sons, Inc., Oregon, USA). Trays were then placed on raised benches in a polyethylene-covered, controlled-environment greenhouse at the Chinese Academy of Forestry Sciences in Beijing (40°40′N, 116°14′E). A total of 48 trays were arranged in a completely randomized design with 4 trays for each of the 12 treatments from 2 nutrient release patterns × 6 rates. Each tray served as a replicate, resulting in 392 seedlings per treatment and 4704 seedlings in total.

Germination completed within 2 weeks after sowing, and germinants were thinned to one per container. All seedlings were irrigated to field capacity at similar rates by periodic weighing to estimate growth media water content and adding water as necessary (Ladis et al. 1989). Each tray position was rotated weekly to minimize edge effects. Temperature was measured with a JL-18 Series thermometer (Huayan Instrument and Equipment Co., Shanghai, China) at 15-min intervals throughout the entire nursery period. Monthly average temperatures in the greenhouse from May through October were 21.9, 22.9, 26.1, 24.1, 21.4, 17.5 °C, respectively.

On 27 October, seedlings were moved outdoors to initiate hardening. Irrigation was reduced to once a week. After 1 December, seedlings overwintered under snow cover. Monthly average temperature during seedling overwintering outdoors from November 2014 to February 2015 were 7.5, -0.1, -0.1, and  $1.2 \,^{\circ}\text{C}$ , respectively.

#### 2.2 Seedling outplanting trial

On 1 May 2015, seedlings from the twelve treatments were shipped and outplanted in a previously cultivated site in the Beijing Forestry University Northern Experimental Base at Pingquan, Hebei province (41°13'N, 118°40'E). The soil was ploughed prior to outplanting. This site has an average elevation of 765 m above sea level with a small slope (< 2%). The depth of soil varies between 45 and 60 cm (Li et al. 2016). Surface soil (0-20 cm) was sampled and measured following the methods of Bao (2000). The surface soil was 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 area has a temperate continental monsoon climate with typically dry winter and spring seasons. Based on the weather data between 1981 and 2010, the average monthly precipitation and air temperature is typically 8.6 mm and -0.8 °C, respectively in the cold and dry season from October to April; and 87.8 mm and 19.7 °C, respectively in the hot and moist season from May to September. During the experiments, precipitation and temperature data were recorded by an on-site weather station. From May to October 2015, the outplanting period, monthly precipitation averaged 42.5, 124.5, 90.7, 54.6, 51.5, 10.6 mm, respectively, and monthly average air temperature was 16.9, 19.6, 23.0, 22.0, 16.2, 8.7 °C, respectively.

The field experiment was a randomized complete block experimental design with four replicates. Each block measured  $9 \times 16.5 \text{ m}^2$  and was separated from adjacent blocks by 1 m buffers. Ten randomly selected seedlings from a tray per treatment were planted in single parallel rows within each block, resulting in a total of 480 seedlings planted. Seedlings were planted in  $0.40 \times 0.40 \times 0.40$  m manually dug planting holes with a  $1 \times 1.5$  m spacing. Intensive weed control was performed manually throughout the outplanting stage.

#### 2.3 Plant sampling and chemical analysis

On 27 November 2014, 8 seedlings per tray were randomly sampled (32 seedlings per treatment, 384 seedlings in total) to determine dry mass and nutrient status. Seedlings were gently removed from containers, the root systems washed free of media. Each seedling was separated into needle, stem and root tissues. Each type of plant tissue was oven-dried at 65 °C for 48h to determine dry mass. Each tissue type (needle, stem, or root) for the 8 seedlings within each tray was combined as a composite sample, ground and sieved through a 0.25 mm mesh. Approximately 0.2 g of each of the subsamples was wet digested in a sulphuric acid-hydrogen peroxide mixture using a block digester for subsequent mineral nutrient analysis (Lowther 1980). Nitrogen was determined using standard Kjeldahl digestion with water distillation by a distillation unit (UDK-152, VELP

Scientifica, Italy). Phosphorus (P) was determined using molybdenum blue method (Allen 1974) with a UV-visible spectrophotometer (Agilent 8453, Waldbronn, Germany). Potassium (K) was determined with atomic emission spectrophotometry (SpectrAA 220 Atomic Absorption Spectrometer, Varian Inc., USA).

Approximately 0.1 g of the subsamples was extracted with 80% ethanol at 80 °C for carbohydrate analysis (Wang and Huang 2015). Starch and soluble sugar concentration was determined by UV-visible spectrophotometer using amylase hydrolysis and anthrone colorimetry methods, respectively. Plant N (P, K, starch, soluble sugar) content was a total of root, stem, and needle N (P, K, starch, soluble sugar) content. Plant N (P, K, starch, soluble sugar) concentration was calculated using the specific mineral or nutrient content divided by plant dry mass.

Immediately after planting on 1 May 2015, seedling height and diameter were measured (T1). When seedling growth ceased in late October of 2015 (T2), survival, height and diameter were measured on 21 October. Height or diameter incremental growth was defined as the observed difference between T2 and T1.

## 2.4 Statistical analysis

Effects of nutrient release pattern using types of CRF at six fertilization rates, and type and rate interactions were assessed using a two-way analysis of variance (ANOVA) for a completely randomized design for the nursery experiment and for a randomized complete block design in the outplanting trial. When ANOVA results showed a significant effect, a Duncan test was carried out for multiple comparisons among treatments at  $\alpha$ =0.05. Statistical analyses were performed using SPSS 16.0 (2007, SPSS ®, Chicago, Illinois, USA). The explore function was used to examine data prior to ANOVA, and survival was arsine transformed to fulfill normality and variance homogeneity requirements.

# **3** Results

#### 3.1 Seedling growth and nutrient status in the nursery

The main effects of the CRF nutrient release patterns and fertilization rate on seedling height, diameter, and dry mass, at the time of outplanting are illustrated in Fig. 1 and 2. There were no significant interactions between fertilizer release pattern and fertilization rate for these measures of growth (p=0.279-0.729).

Fertilizer release pattern affected root and total plant dry mass (p=0.003, p=0.010, respectively). Seedlings fertilized with SF had both greater root and total plant dry mass than seedlings fertilized with HF. Fertilization rate influenced all measured growth attributes (p<0.001) except needle dry mass (p=0.216). At rates lower than 120 mg N seedling<sup>-1</sup>, height and diameter were similar (Fig. 1). At fertilization rates of 160 mg N seedling<sup>-1</sup> and above, stem, root, and total plant dry mass were lower (Fig. 2). Seedlings fertilized at rates of 40 and 80 mg N seedling<sup>-1</sup> had a lower shoot/root ratio (S/R) than seedlings fertilized at 120 mg N seedling<sup>-1</sup> and above (Fig. 2).

The main effects of the CRF release patterns and fertilization rate on plant mineral nutrient status in terms of N, P, and K content or concentration are presented in Fig. 3 and 4. There were no significant interactions between fertilizer release pattern and fertilization rate on plant nutrient status (p=0.231-0.818).

Fertilizer release pattern had a significant impact on plant mineral nutrients (p < 0.001 to p=0.006). Fertilization with SF resulted in greater N, P and K content and concentration in seed-



**Fig. 1.** Main effects of nursery fertilizer release pattern (HF = Hi·End fertilizer; SF = Standard fertilizer) and fertilization rate (mg N seedling<sup>-1</sup>) on *Pinus tabulaeformis* seedling total height, diameter, and their increments (means  $\pm$  SE) at the time of field planting and at the end of the first-year outplanting season. Error bars represent SE of total height or diameter at planting and at the end of the first-year outplanting season. Bars marked with different capital letters differ statistically for total height (diameter) and different lower case letters differ statistically for increment according to Duncan's test  $\alpha = 0.05$ .



**Fig. 2.** Main effects of fertilizer release pattern (HF = Hi·End fertilizer; SF = Standard fertilizer) and fertilization rate (mg N seedling<sup>-1</sup>) on dry mass (means  $\pm$  SE; bars) and shoot/root ratio (S/R; dots) of *Pinus tabulaeformis* seedlings at outplanting. Bars marked with different capital letters differ statistically for whole plant dry mass and different lower-case letters differ statistically for each tissue dry mass according to Duncan's test  $\alpha = 0.05$ . Dots marked with different lower-case letters differ statistically for shoot/root ratio.



**Fig. 3.** Main effects of fertilizer release pattern (HF = Hi End fertilizer; SF = Standard fertilizer) and fertilization rate (mg N seedling<sup>-1</sup>) on mineral nutrient (MN) content and concentration of whole plant (means  $\pm$  SE) of *Pinus tabulae-formis* seedlings at planting. Dots marked with different letters differ statistically according to Duncan's test  $\alpha = 0.05$ .

lings (Fig. 3). Fertilization rate also had significant effects on seedling mineral nutrient content and concentration (p < 0.001 to p = 0.021) except for P concentration (p = 0.099). Plant N concentration increased at 80 mg N seedling<sup>-1</sup> and then appeared to plateau (Fig. 3). The trend for plant K concentration was similar except for a slight decline at 240 mg N seedling<sup>-1</sup>. The maximum plant P and K contents were observed at 80 and 120 mg N seedling<sup>-1</sup>, respectively (Fig. 3).

Starch concentration in seedlings was higher for seedlings in HF treatments than those fertilized with SF (p < 0.001, Fig. 4). Fertilization rate also significantly influenced plant soluble sugar content (p < 0.001) and starch concentration (p = 0.021). Plant soluble sugar content was highest at rates of 40, 80, and 120 and reduced at 200 and 240 mg N seedling<sup>-1</sup> (p < 0.001, Fig. 4). Starch content and concentration were maximal at 40 mg N seedling<sup>-1</sup>; starch concentration decreased at fertilization rates of 160 mg N seedling<sup>-1</sup> and higher above (Fig. 4).

#### 3.2 Survival and growth performance after outplanting

The main effects of fertilizer release pattern and fertilization rate on outplanted Chinese pine seedling performance in terms of survival and growth are shown in Table 1 and Fig. 1, respectively. Survival was not affected by fertilizer release pattern (p=0.104) but it was affected by fertilization rate (p=0.010). Survival was significantly higher at 80 mg N seedling<sup>-1</sup> than at 200 and 240 mg N seedling<sup>-1</sup> (Table 1). No significant interactions between fertilizer release pattern and fertilization rate were observed for seedling survival (p=0.849) or growth (p=0.737-0.965).



**Fig. 4.** Main effects of fertilizer release pattern (HF = Hi End fertilizer; SF = Standard fertilizer) and fertilization rate (mg N seedling<sup>-1</sup>) on non-structural carbohydrate (NSC) content and concentration for the whole plant (means  $\pm$  SE) of *Pinus tabulaeformis* seedlings at planting. Bars marked with different letters differ statistically for starch and soluble sugars according to Duncan's test  $\alpha = 0.05$ , respectively.

Osmocote Exact Standard fertilizer treatments resulted in greater diameter growth and greater total diameter measured at the end of the outplanting season (Fig. 1). Fertilization rate in the nursery resulted in no significant differences in either height or diameter growth during the outplanting season(p=0.346, p=0.163, respectively). Seedlings fertilized with 120 mg N were taller but there were no differences in diameter at different rates of fertilization (Fig. 1).

**Table 1.** Main effects of nursery fertilizer release pattern (HF = Hi·End fertilizer; SF = Standard fertilizer) and fertilization rate (mg N seedling<sup>-1</sup>) on *Pinus tabulae-formis* seedling survival (means ± SE) at the end of first growing season. Different letters differ statistically according to Duncan's test  $\alpha = 0.05$  and separately to fertilizer release pattern and fertilization rate.

	Level	Survival (%)
Fertilizer release pattern	HF	93±1.85a
	SF	$97 \pm 1.15a$
Fertilization rate	40	95±2.67abc
	80	$100\pm0c$
	120	$98 \pm 1.64 bc$
	160	$98 \pm 1.64 bc$
	200	$88 \pm 3.66a$
	240	$91\pm2.95ab$

# 4 Discussion

#### 4.1 Impact of controlled release fertilizer rate on Chinese pine seedlings

Building on the results of previous studies with *Pinus* spp. (Crowley et al. 1986; Walker and Hutt 1992, 2000; Fan et al. 2004; Oliet et al. 2004), we assessed nursery and field performance in response to a range of fertilization rates to identify regimes beneficial to globally important *Pinus* spp. seedling production and quality.

Our observations of Chine pine seedling dry mass, N content and concentration in response to a broad range of controlled release fertilizer (CRF) rates applied in the nursery align well with the conceptual model proposed by Timmer (1996) to identify optimal fertilization regimes. Our data shows that a rate of 40 mg N seedling<sup>-1</sup> or greater was sufficient to maximize plant dry mass. Both the highest dry mass and N content were observed for plants fertilized with CRF at rates of 80 and 120 mg N seedling<sup>-1</sup>, consistent with our earlier work in which Chinese pine seedlings were fertilized with immediately soluble fertilizer (Wang et al. 2015). At higher fertilization rates of 200 and 240 mg N seedling<sup>-1</sup> in the nursery, seedling height and diameter at the time of field planting were reduced in comparison to seedlings fertilized at lower rates. This observation was consistent with lower seedling dry mass measured at the end growing season in the nursery. Toxicity, demonstrated by reduced growth, has been associated extensively with the use of immediately soluble fertilizer at higher fertilization rates in *Quercus rubra* L., *Quercus alba* L., and *Picea mariana* (Mill.) BSP (Phillion and Libby 1984; Salifu and Timmer 2003; Salifu and Jacobs 2006). Our observations suggest there may be similar negative effects from higher fertilization rates on Chinese pine seedling growth.

In the current study, we found fertilization rates of 40–120 mg N seedling<sup>-1</sup> yielded similar NSC content (both starch and soluble sugar) whereas rates above 160 mg N seedling<sup>-1</sup> had adverse effects on NSC accumulation. Studies with *Picea abies* L. and *Pinus pinea* L. seedlings, using low and high fertilization rates, indicated that NSC contents were positively and negatively correlated with fertilization rates, respectively (Kaakinen et al. 2004; Villar-Salvador et al. 2013). Stored N and NSC are functional attributes, indicative of seedling quality and a substantive body of evidence in the literature concludes that over-fertilization retards N storage (Millard and Grelet 2010; Villar-Salvador et al. 2015). These inconclusive NSC responses to nursery fertilization imply effects beyond N uptake and storage on carbon assimilation and sequestration. The relationship between plant NSC and a broad range of nursery fertilization treatments requires quantification for more tree species.

Consistent with studies on *Picea mariana*, *Quercus rubra* and *Quercus alba* (Salifu and Timmer 2003; Salifu and Jacobs 2006; Birge et al. 2006), in our current study, seedling N concentration increased with higher fertilization rates in Chinese pine. This response pattern did not occur for K. Potassium concentration peaked during a range of fertilization rates in our work and other studies (Hawkins et al. 2005; Salifu and Jacobs 2006; Salifu et al. 2009). High N fertilization may facilitate NH<sub>4</sub><sup>+</sup> uptake due to ion-exchange at the expense of the K<sup>+</sup> storage (Landis et al. 1989). Other possible explanations include variations in individual seedling nutrient-release rates or differences in element mobility in the growth media.

Our study observed no significant differences in Chinese pine seedlings survival and growth in the field after nursery CRF fertilization rates of 40 to 160 mg N seedling<sup>-1</sup>, consistent with our previous study using immediately soluble fertilizer (Wang et al. 2015). Average survival and total height for Chinese pine seedling peaked at 80 and 120 mg N seedling<sup>-1</sup>, respectively, at the end of the field season.

Considering three factors, overall nursery performance, as quantified by growth, mineral nutrients and NSC content; field performance in terms of survival and total height; and fertilizer

cost and potential nitrate leaching risk, we currently suggest an application rate of 80 mg N seed-ling<sup>-1</sup> for Chinese pine seedlings. This is equivalent to 3.3 g CRF l<sup>-1</sup> media and falls within the manufacturer's lower limit of the recommended rates of 3-7 g l<sup>-1</sup> for HF and 3-6 g l<sup>-1</sup> for SF (The Scotts Company 2015).

# 4.2 The impact of nutrient release pattern on nursery and field performance of Chinese pine seedlings

These two types of fertilizers used in current study were similar except for their release patterns. This additional coating with HF results in a delayed nutrient release, purportedly coincident with the onset of the critical growth phase of plant (The Scotts Company 2015). Theoretically, HF should be more effective than SF for nutrient storage by seedling. In contrast to our expectations, we observed that Chinese pine seedlings fertilized with HF had lower plant dry mass, nutrient content and concentration in the nursery, and decreased incremental growth in diameter after outplanting. It is possible that SF nutrient release may be more synchronized with the rhythm of Chinese pine seedlings growth and nutrient demand. Our hypothesis that multiple-layer-coating CRF would be more effective in supporting seedling growth, nutrient storage and outplanting performance was not supported by our study results.

Currently, we cannot provide a definitive explanation for the observed results in this study because we did not measure seedling growth dynamics over time, nor the temporal patterns of nutrient release (e.g. by measuring media electrical conductivity) of two CRF types. Our findings suggest that that assessment of crop nutrient requirements throughout development may be as important as the predicted pattern and intensity of nutrient release from a given CRF (Jacobs et al. 2005).

# 5 Conclusions

Controlled release fertilizer (CRF) of both types contributed to Chinese pine seeding performance for both nursery and field performance. The best CRF application rates observed in this study were in the lower end of the manufacturer's recommended rates and provided sufficient mineral nutrients (NPK), supported the best NSC levels observed, and field performance. Although interactions between fertilizer release pattern and fertilization rates were not evident, the combination of SF at 80 mg N seedling<sup>-1</sup> (3.3 g CRF g l<sup>-1</sup>) yielded satisfactory seedling nursery and field performance. Our results will support the optimization of seedling production for the widely planted Chinese pine and other *Pinus* species with CRF; however, further study is needed to monitor seedling growth dynamics and CRF nutrient release patterns over time to determine the optimal nutrient release pattern that synchronizes best with the growth rhythm of Chinese pine seedlings.

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# References

- Allen S.E. (1974). Chemical analysis of ecological materials. Blackwell Scientific, Oxford, UK. 575 p.
- Bao S.D. (2000). Soil and agricultural chemistry analysis. Chinese Agriculture Press, Beijing. p. 39–114. [In Chinese].
- Birge Z.K.D., Salifu K.F., Jacobs D.F. (2006). Modified exponential nitrogen loading to promote morphological quality and nutrient storage of bareroot-cultured *Quercus rubra* and *Quercus alba* seedlings. Scandinavian Journal of Forest Research 21(4): 306–316. https://doi.org/ 10.1080/02827580600761611.
- Crowley D.E., Maronek D.M., Hendrix J.W. (1986). Effect of slow release fertilizers on formation of mycorrhizae and growth of container grown pine seedlings. Journal of Environmental Horticulture 4(3): 97–101.
- Dumroese R.K., Page-Dumroese D.S., Salifu K.F., Jacobs D.F. (2005). Exponential fertilization of *Pinus monticola* seedlings, nutrient uptake efficiency, leaching fractions, and early outplanting performance. Canadian Journal of Forest Research 35(12): 2961–2967. https://doi. org/10.1139/X05-226.
- Fan Z.F., Moore J.A., Wenny D.L (2004). Growth and nutrition of container-grown ponderosa pine seedlings with controlled-release fertilizer incorporated in the root plug. Annals of Forest Science 61(2): 117–124. https://doi.org/10.1051/forest:2004002.
- Fernández-Escobar R., Benlloch M., Herrera E., García-Novelo J.M. (2004). Effect of traditional and slow-release N fertilizers on growth of olive nursery plants and N losses by leaching. Scientia Horticulturae 101(1–2): 39–49. https://doi.org/10.1016/j.scienta.2003.09.008.
- Haase D.L., Rose R., Trobaugh J. (2006). Field performance of three stock sizes of Douglas-fir container seedlings grown with slow-release fertilizer in the nursery growing medium. New Forests 31(1): 1–24. https://doi.org/ 10.1007/s11056-004-5396-6.
- Hawkins B.J., Burgess D., Mitchell A.K. (2005). Growth and nutrient dynamics of western hemlock with conventional or exponential greenhouse fertilization and planting in different fertility conditions. Canadian Journal of Forest Research 35(4): 1002–1016. https://doi.org/10.1139/ X05-026.
- Jacobs D.F., Salifu K.F., Seifert J.R. (2005). Growth and nutritional response of hardwood seedlings to controlled-release fertilization at outplanting. Forest Ecology and Management 214(1–3): 28–39. https://doi.org/10.1016/j.foreco.2005.03.053.
- Kaakinen S., Jolkkonen A., Iivonen S., Vapaavuori E. (2004). Growth, allocation and tissue chemistry of *Picea abies* seedlings affected by nutrient supply during the second growing season. Tree Physiology 24(6): 707–719. https://doi.org/10.1093/treephys/24.6.707.
- Landis T.D., Tinus R.W., McDonald S.E., Barnett J.P. (1989). Seedling nutrition and irrigation. Volume 4: The container tree nursery manual. Agriculture Handbook 674. U.S. Department of Agriculture, Forest Service, Washington, DC. 119 p.
- Li G., Wang J., Oliet J.A., Jacobs D.F. (2016). Combined pre-hardening and fall fertilization facilitates N storage and field performance of *Pinus tabulaeformis* seedlings. iForests 9: 483–489. https://doi.org/10.3832/ifor1708-008.
- Li G.L., Zhu Y., Liu Y., Wang J.X., Liu J.J., Dumroese R.K. (2014). Combined effects of prehardening and fall fertilization on nitrogen translocation and storage in *Quercus variabilis* seedlings. European Journal of Forest Research 133(6): 983–992. https://doi.org/10.1007/ s10342-014-0816-4.
- Lowther J.R. (1980). Use of a single sulphuric-hydrogen peroxide digest for the analysis of *Pinus* radiata needles. Communications in Soil Science and Plant Analysis 11(2): 175–188. https://

doi.org/10.1080/00103628009367026.

- Luis V.C., Puértolas J., Climent J., Peters J., González-Rodríguez Á.M., Morales D., Soledad Jiménez M. (2009). Nursery fertilization enhances survival and physiological status in Canary Island pine (*Pinus canariensis*) seedlings planted in a semiarid environment. European Journal of Forest Research 128(3): 221–229. https://doi.org/10.1007/s10342-009-0257-7.
- Millard P., Grelet G. (2010). Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. Tree Physiology 30(9): 1083–1095. https://doi.org/10.1093/treephys/tpq042.
- Oliet J., Planelles R., Segura M.L., Artero F., Jacobs D.F. (2004). Mineral nutrition and growth of containerized *Pinus halepensis* seedlings under controlled-release fertilizer. Scientia Horti-culturae 103(1): 113–129. https://doi.org/10.1016/j.scienta.2004.04.019.
- Oliet J.A., Planelles R., Artero F., Valverde R., Jacobs D.F., Segura M.L. (2009). Field performance of *Pinus halepensis* planted in Mediterranean arid conditions: relative influence of seedling morphology and mineral nutrition. New Forests 37(3): 313–331. https://doi.org/10.1007/ s11056-008-9126-3.
- Oliet J.A., Puértolas J., Planelles R., Jacobs D.F. (2013). Nutrient loading of forest tree seedlings to promote stress resistance and field performance: a Mediterranean perspective. New Forests 44(5): 449–469. https://doi.org/10.1007/s11056-013-9382-8.
- Phillion B.J., Libby M. (1984). Growth of potted black spruce seedlings at a range of fertilizer levels. The Plant Propagator 30(2): 10–11.
- Ruter J.M. (1992). Influence of source, rate, and method of applicating controlled release fertilizer on nutrient release and growth of 'Savannah' holly. Fertilizer Research 32(1): 101–106. https://doi.org/10.1007/BF01054399.
- Salifu K.F., Jacobs D.F. (2006). Characterizing fertility targets and multi-element interactions in nursery culture of *Quercus rubra* seedlings. Annals of Forest Science 63(3): 231–237. https:// doi.org/10.1051/forest:2006001.
- Salifu K.F., Timmer V.R. (2003). Optimizing nitrogen loading of *Picea mariana* seedlings during nursery culture. Canadian Journal of Forest Research 33(7): 1287–1294. https://doi. org/10.1139/x03-057.
- Salifu K.F., Jacobs D.F., Birge Z.K.D. (2009). Nursery nitrogen loading improves field performance of bareroot oak seedlings planted on abandoned mine lands. Restoration Ecology 17(3): 339–349. https://doi.org/10.1111/j.1526-100X.2008.00373.x.
- The Scotts Company (2015). Scotts fertilizer technical sheet. http://www.everris.com/Home/ Ornamental-Horticulture/Products/Technology/Specialty-Fertilizers/CRF. [Cited 6 Dec 2015].
- Timmer V.R. (1996). Exponential nutrient loading: a new fertilization technique to improve seedling performance on competitive sites. New Forests 13(1): 275–295. https://doi.org/10.1023/A:1006502830067.
- Uscola M., Salifu K.F., Oliet J.A., Jacobs D.F. (2015). An exponential fertilization dose-response model to promote restoration of the Mediterranean oak *Quercu ilex*. New Forests 46(5): 795–812. https://doi.org/10.1007/s11056-015-9493-5.
- Villar-Salvador P., Puértolas J., Cuesta B., Peñuelas J.L., Uscola M., Heredia-Guerrero N., Rey Benayas J.M. (2012). Increase in size and nitrogen concentration enhances seedling survival in Mediterranean plantations. Insights from an ecophysiological conceptual model of plant survival. New Forests 43(5): 755–770. https://doi.org/10.1007/s11056-012-9328-6.
- Villar-Salvador P., Peñuelas J.L., Jacobs D.F. (2013). Nitrogen nutrition and drought hardening exert opposite effects on the stress tolerance of *Pinus pinea* L. seedlings. Tree Physiology 33(2): 221–232. https://doi.org/10.1093/treephys/tps133.
- Villar-Salvador P., Uscola M., Jacobs D.F. (2015). The role of stored carbohydrates and nitrogen

in the growth and stress tolerance of planted forest trees. New Forests 46(5): 813–939. https://doi.org/10.1007/s11056-015-9499-z.

- Walker R.F., Hutt C.D. (1992). Controlled release fertilizer effects on growth and foliar nutrient concentration of container grown Jeffrey pine and singleleaf pinyon. Western Journal of Applied Forestry 7(4): 113–117.
- Walker R.F., Hutt C.D. (2000). Production of containerized Jeffrey pine planting stock for harsh sites: growth and nutrition as influenced by controlled-release fertilization. Western Journal of Applied Forestry 15(2): 86–91.
- Wang J., Li G., Pinto J.R., Liu J., Shi W., Liu Y. (2015). Both nursery and field performance determine suitable nitrogen supply of nursery-grown, exponentially fertilized Chinese pine. Silva Fennica 49(3) article 1295. https://doi.org/10.14214/sf.1295.
- Wang X.K., Huang J.L. (2015). Principles and techniques of plant physiological biochemical experiment. Higher Education Press, Beijing. p. 171–186. [In Chinese].
- Xu H.C., Sun Z.F., Guo G.R., Feng L. (1981). Geographic distribution of *Pinus tabulaeformis* Carr. and classification of provenance regions. Scientia Silvae Sinicae 17(3): 258–270. [In Chinese].
- Zhang K.Y., Zhang G.J., Yang J.M. (2014). Comparison of growth dynamics of *Pinus tabulaeformis* seedlings of different provenances. Acta Agriculturae Jiangxi 26(11): 32–35. [In Chinese].

Total of 37 references.