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Effect of nursery nitrogen application of bare-root *Larix olgensis* seedlings on growth, nitrogen uptake and initial field performance

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Abstract

Nursery nitrogen application has been used to improve seedling quality. The technique has received little attention with bare-root seedlings and their subsequent field performance on weed competition sites. Our research objective was to examine responses of one- and two- year-old bare-root Olga Bay larch (*Larix olgensis* Henry) seedlings to nursery nitrogen supplements and subsequent one-year field performance on a competitive site. The fertilizer levels (kg N ha^{-1}) were 0 (control), 60 (conventional fertilization, 60 C), 120 (additional nitrogen applied two times, 120 L), 180 (additional nitrogen applied three times, 180 L) and N were applied in increments of 30 kg ha^{-1} at 15-day interval to maintain a base nutrient level. Although pre-planting morphological attributes and nitrogen status of one-year-old (1a) seedlings were more sensitive to 60 C than for two-year-old (2a) seedlings, the conventional application failed to enhance their field survival(15.6% vs 17.8%), relative height growth (89.0% vs 79.6%), and relative diameter growth (17.0% vs 22.9%). The 1a seedlings' field survival (15.6% for 0, 17.8% for 60 C) and 2a seedlings' relative height growth rate (11.0% for 0, 8.9% for 60 C) were not increased significantly until they were provided the 120 L (survival of 23.3% for 1a, relative height growth rate of 15.0% for 2a). According to pre-planting attributes and field performance, optimum nursery nitrogen application was 120 L for the 2a seedlings and 180 L for 1a seedlings. Except for component nitrogen concentration, pre-planting morphological attributes and component N content for the 2a seedlings were as much 3.3 to 37.7 times that of 1a seedlings. In conclusion, the contrasting survival of poor (15.6%-28.9%) for 1a seedlings and high (84.4%-91.1%) for 2a seedlings indicated that additional nitrogen fertilizer would not equal the benefits of an another year's growth in the nursery. Successful reforestation could not be fulfilled by 1a seedlings regardless of their pre-nutrients. An alternative technique for sites with competing vegetation was to apply 120kg N ha^{-1} in the nursery during July and August on 2a seedlings.

Key words

Larix olgensis, Nitrogen application, Seedling age, Weed competition, Field performance

Introduction

In recent years restoration on harsh sites has attracted increasing attention. Intensive techniques for producing and evaluating stocktype are needed to meet these demands (South and Mitchell, 1999; Folk and Grossnickle, 2000; Oliet *et al.,* 2009). Herbaceous vegetation (weeds) can seriously affect the survival and growth of seedlings through interspecific competition for light, water and nutrients (Wagener *et al.,* 1989). Along with adopting vegetation control to improve field performance (Nordborg and Nilsson, 2003; Way *et al.,* 2007; Johansson *et al.,* 2007; Jacobs *et al.,* 2005), alternative ways are to enhance the ability of the seedling to compete while still in the nursery by either applying additional fertility or by prolonging the culture period (South *et al.,* 2005; Johansson *et al.,* 2007). Recommendations for seedlings to overcome competing vegetation may be by achieving greater size (Burdett, 1990), or by providing an additional year's growth for bare-root stocks in the nursery. This has been an operational practice over the last three or more decades as a means to achieve a desired size and improve reforestation success (Newton *et al.,* 1993; Noland *et al.,* 2001).

In competitive environments more nitrogen (N) is retranslocated from older tissues to actively growing tissues. Thus additional N fertilizer in nursery culture has been viewed as an effective solution in recent years for enhanced field performance and for economic purposes (Malik and Timmer, 1998; Imo and Timmer, 2001). Several bioassays found one-year-old (1a) container seedlings are capable of performing well with neighboring vegetation under greenhouse when they are provided nursery additional N in the previous year (Malik and Timmer, 1995; Imo and Timmer, 2001, 2002). While bareroot seedlings may be more sensitive to vegetation competition because they take longer to establish good contact between roots and soil after planting (Nilsson and Örlander, 1995; Landis *et al.,* 2010), and have an inferior competitive ability to their container counterparts in the first two years after planting (Nilsson and Örlander, 1999). 1a bare-root seedlings that have received sufficient supplemental N while in the nursery can improve subsequent field establishment success when weed competition is controlled using herbicides (Salifu *et al.,* 2009). The question is whether they would also establish successfully on more competitive sites if in the absence of herbicides they were provided sufficient fertility in the nursery.

Though we have several studies that have dealt with the effect of seedling size and nutritional status on the field performance for a particular age seedling, and with the relation between field performance and seedling size caused by seedling age (Newton *et al.,* 1993; Puértolas *et al.,* 2003; Renou-Wilson *et al.,* 2008), the question remains for competitive sites whether better success is dependent on seedling age or nutrient reserves.

Olga Bay larch (*Larix olgensis* Henry) is one of the most important timber species grown in northeastern China. Compared to other softwoods, it is valued for its rapid early growth, dense cellular structure, and for its characteristic strength and resistance to rot. The predominant nursery stocktypes for this species are 1+0 (1a) and 1+1(2a) bareroot seedlings. So far no information is available on how these two kinds of stocktypes respond relative to each other on weed-free sites. In this study, we extended N fertilizer supplements to test the effect of additional N on the pre-planting attributes and initial field performance in the presence of competing herbaceous vegetation.

Materials and Methods

Nursery phase : Bare-root seedlings were grown at the Jiangmifeng nursery, Jilin City, China (126°482' E, 43°572' N; elevation of 233 m). The area is characterized by a temperate continental monsoon climate with a mean annual air temperature of 6.4 °C and an average annual precipitation of 645 mm. The soil class (0-15 cm) is 79% sand, 11% silt, and 10% clay with a pH of 5.1 and soil organic carbon of 0.8%. Average total N, available P and available K were 770, 37 and 77 mg kg^{-1}, respectively.

Seeds of *L. olgensis* were collected from the Baishishan seed orchard. On 10th May 2008, the 2a seedlings were first operationally broadcast sown by one crew. After one month germinants were thinned to a final density of 400 seedlings m^{-2}. A total of 60 kg ha^{-1} of N was applied in 2 increments of 30 kg ha^{-1} on the July 1 and 16. Urea (46-0-0) was weighed and dissolved in water and the solution was applied to the seedbeds manually with a sprayer. On 15th October seedlings were undercut, lifted, and stored in bags in a cooler at a temperature of 2°C.

After one year, 2a seedlings were transplanted to a spacing of 200 seedlings m^{-2} and 1a seedlings were sown. All standard nursery practices for seedling production were followed except for fertilizer levels. Seedlings of both types received four fertility levels. The seedlings were not fertilized with N during the whole growing season (control). The conventional fertilization level was supplied with 60 kg N ha^{-1} in July (60 C). The level of 120 kg N ha^{-1} was applied in July and August (120 L). And the level of 180 kg N ha^{-1} was applied in July, August and early September (180 L). All levels were split equally at 15 day intervals. A randomized complete-block experimental design was used with three replicates. Triple superphosphate fertilizer was applied to all the seedlings at the rate of 150 kg P_2O_5 ha^{-1}.

Field study : Nursery-reared seedlings were outplanted on 22nd April 2010 in the Jiangmifeng Forest Center (126°472' E, 43°572' N; elevation of 289 m). The site is on a hillside with a northwest aspect and a moderate slope of 17°. The soil depth varied between 45 and 50 cm over an igneous rock layer. Texture was a clay loam with a pH of 5.0. The surface layer (0-15 cm) averaged 3 g kg^{-1} N, 4 mg kg^{-1} available P, and 322 mg kg^{-1} available K. And the site is considered to be suitable for the establishment of *L. olgensis* as a plantation crop.

A split-plot experimental design with a 2 × 4 factorial treatment structure was replicated in three blocks. The main plots were the seedling age at two levels, and the subplots were the four nursery fertility levels. Each block measured 24 × 24 m and was divided as two main plots, one for 1a seedlings (size 24 × 16 m), and another for 2a seedlings (size 24 × 8 m). The four fertility levels were represented by eight and four rows within 1a and 2a seedlings, respectively. Weed was free after planting to examine effects of prior nursery fertilization on seedling field performance in the weed-prone sites.

Plant sampling, chemical and statistical analyses : 15 interior seedlings per block were excavated from the nursery beds manually on 15th October, 2009. Seedlings were determined for height (cm), RCD (mm), number of first order lateral roots (FOLR) with a diameter larger than 1 mm at the tap root junction, component dry mass, and component N concentration (Li *et al.,* 2012).

Survival, height and RCD were determined for the field subjects on 1st November 2010. Relative growth rate (RGR) of height or RCD was calculated by the following formula.

$$\mathrm{RGR} = \frac{\mathrm{Size\ 2 - Size\ 1}}{Size\,1} \times 100\%$$

Where Size 1 and Size 2 were height or RCD at the planting date of 22nd April 2010, and the concluding measurement date of November 1.

Means of the variables were calculated using the SPSS program (Chicago, USA). Treatment means computed from individual seedlings were used for height and diameter analysis. For the biomass, N response in the nursery, and survival in field phases, data were analyzed from a composite sample for each treatment from a block. A T-test was used to compare the effect of seedling age on morphological and nutrient attributes within the nursery. To further analyze the effect of seedling age and fertilization on field performance, block means of survival, height and RCD and their RGR in the split-plot design were used. For each analysis, when fixed effects were significant (α=0.05), a Duncan's multiple comparison test was conducted to test the significance of differences among fixed means.

Results and Discussion

Nursery phase : The 1a seedlings were more sensitive to nursery N applied than 2a seedlings. For 1a seedlings, compared to unfertilized seedlings (control), fertilization increased morphological and nutrient content for 8 of 12 attributes significantly, except for shoot dry mass, N concentration and content, and root N concentration. However, no attribute other than root N concentration and content, and plant N concentration was enhanced when 2a seedlings were fertilized (Table 1; Fig. 1-3). N demand of 1a seedlings was higher than for the 2a seedlings. For 2a seedlings, a maximum of RCD, shoot dry mass, plant dry mass, root N content and plant N content was exhibited when applied N was 120 kg ha^{-1} (Table 1; Fig. 1-3). While for 1a seedlings a maximum for RCD, FOLR, root N concentration, plant N concentration and content was not attained until additional N was 180 kg ha^{-1}. N applied could enhance root N concentration of both 1a and 2a seedlings, but not shoot (Fig. 2). Compared to conventional fertilization (60 C), significant increases of root N concentration were found in 1a seedlings.

Although there were no differences in root, shoot and plant N concentration between 1a and 2a seedlings, other attributes of 2a seedlings were higher than 1a seedlings (Table 2). For example, height, RCD, FOLR, plant dry mass and plant N content of 2a seedlings were as much as 5.8, 3.3, 11.3, 28.9 and 25.7 times that of 1a seedlings, indicating that by prolonging the cultural period we could enhance morphological attributes and N reserves.

Field performance : For 1a seedlings nursery fertilization promoted field survival relative to control at the end of growing season, and additional N of 180 L in the nursery could enhance survival relative to 60 C (Table 3). Survival as low as 15.6-28.9% showed that 1a seedlings would not establish successfully on weed-prone sites regardless of initial size and N reserves.

Although additional nursery fertilization could not facilitate seedling survival for 1a seedlings, survival as high as 84.4-91.1% indicated that 2a seedlings could compete over weed competition (Table 3). Compared to control, 120 L and 180 L nursery fertilization should result in greater height RGR, indicating that additional N applied is superior to conventional applications. While the seedlings with greater initial height maintained their height dominance, there was no difference of height RGR between 120 L and 180 L, showing that 120 L was the optimum level of N applied for 2a seedlings (Table 1, 3).

Significant differences were found in survival, height, RCD and their RGR between 1a and 2a seedlings (Table 4). Both survival and RCD RGR for 2a seedlings were higher than for 1a (Table 3-4), while height RGR was lower for 2a seedlings than for 1a seedlings.

When field performance was surveyed at the end of the growing season, weed competition was 130 cm in height and had covered 90% on this fertile and moist site. In this study, low survival 15.6-28.9% indicated that 1a seedlings could not establish successfully on a competitive site regardless of pre-planting nutrient level. However, in a previous study 1a seedlings' survival surpassed 90% at the end of the growing season when herbicides were successfully used to control weeds (Li *et al.,* 2011). These contrasting facts show that 1a bare-root seedlings are more sensitive to competing vegetation. And that weed control after planting should be considered for competing vegetation sites even if seedlings are provided with additional N while in the nursery.

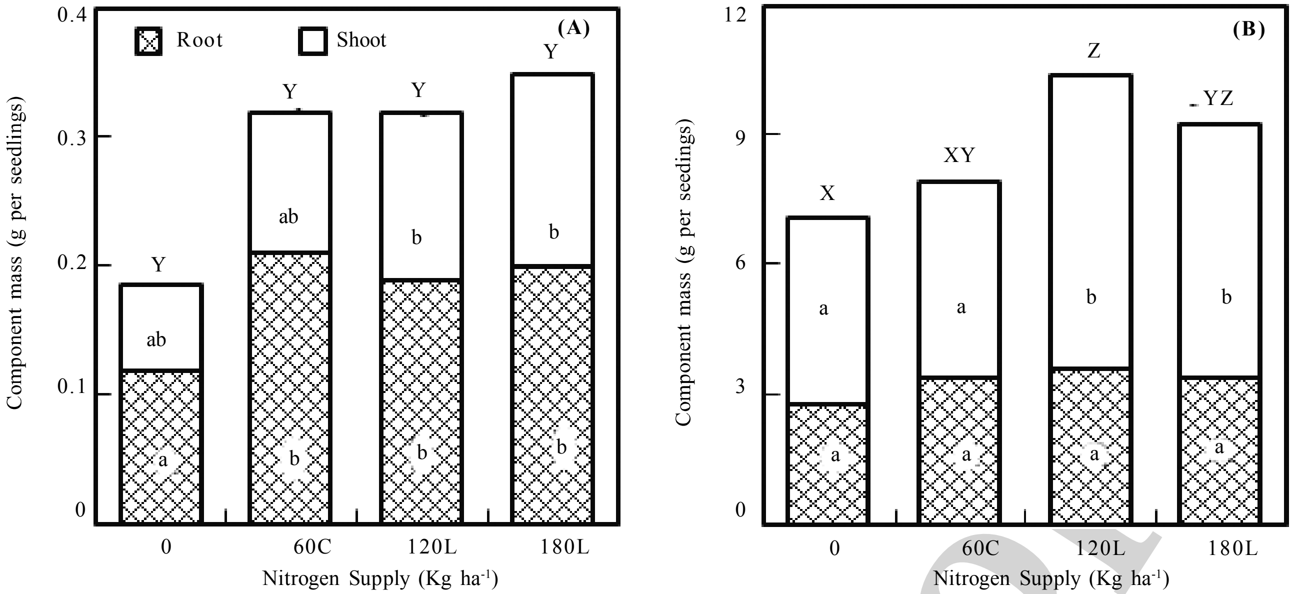

Fig. 1 : Component dry mass of (A and B) seedlings exposed to increasing fertility treatments during nursery culture (n = 3). Treatments marked with different letters differ statistically for each component according to Duncan's multiple range test at the 0.05 probability level

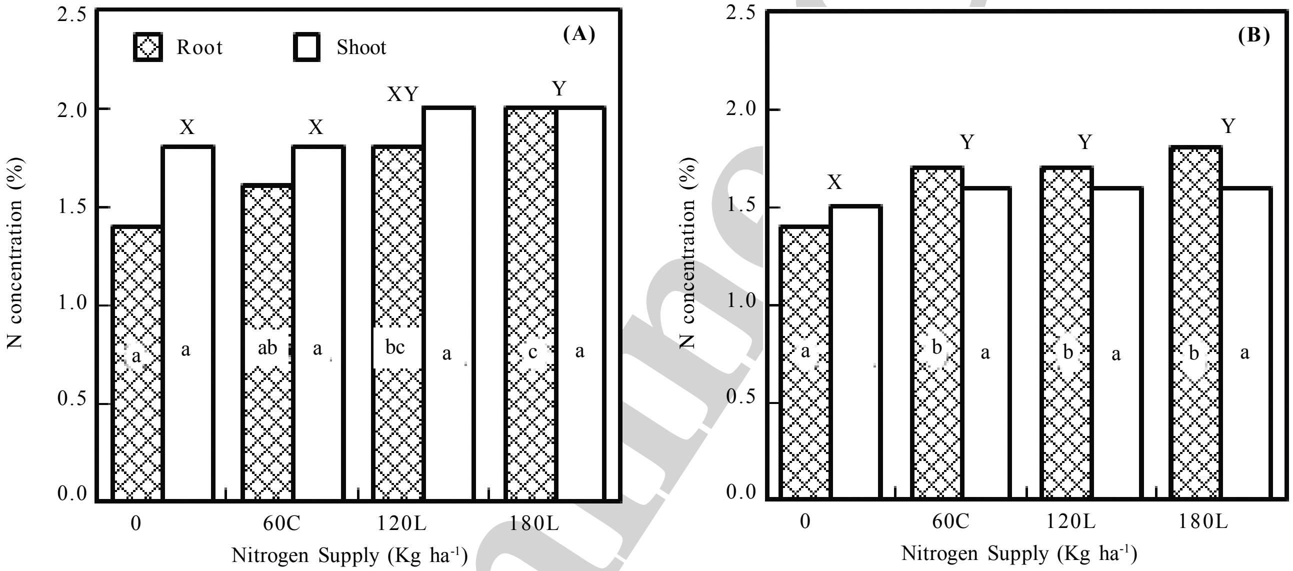

Fig. 2 : Component nitrogen concentration of (A and B) seedlings exposed to increasing fertility treatments during nursery culture (n=3). Treatments marked with different letters differ statistically for each component according to Duncan's multiple range test at the 0.05 probability level

Because planted seedlings were most affected by competing vegetation in either the first, or first and second years after planting (Garau *et al.,* 2009; Nilsson and Örlander, 1999), size becomes the principal consideration with survival (Burdett, 1990). Larger size (Grossnickle, 2000) and a higher nutrient content (Oliet *et al.,* 2009) could substantially improve 2a seedlings competitive ability with weeds. In addition, FOLR was closely related with field performance (Thompson and Schultz, 1995; Jacobs *et al.,* 2005) and 2a seedlings with more lateral roots should be expected to absorb more water and nutrient from the soil than 1a seedlings when established. Although 2a seedlings (56.9-68.1 cm) were overwhelmed by the heavy weed competition (130 cm), they still displayed superior survival 84.4%-91.1% over 1a seedlings (Table 3). Thus it was deduced that successful establishment on competitive sites was more dependent on seedling age than N reserves.

Unlike container seedlings (Malik and Timmer, 1998; Imo and Timmer, 2001), bare-root stock takes longer to

Table 1 : Mean height (cm), RCD (mm) and FOLR of 1a (n = 45) and 2a (n = 30) seedlings under conventional (C) or additional (L) fertilization regimes during nursery culture

Stock/Treatment	**1a**				**2a**			
	0	**60 C**	**120 L**	**180 L**	**0**	**60 C**	**120 L**	**180 L**
Height	6.3a	9.6b	11.0b	10.1b	51.3a	53.4a	54.6ab	58.0b
RCD	1.95a	2.20b	2.28bc	2.44c	6.72a	6.80a	7.01b	7.28b
FOLR	0.4a	1.0b	1.2b	1.8c	11.8a	12.0a	13.8a	12.0a

For each parameter, values in the same seedling age with different letters differ statistically according to Duncan's multiple range test at the 0.05 probability level

Table 2 : Results of t-tests on the effects of the seedling age on the mean of height (cm), RCD (mm) and FOLR by individuals (n = 180 for 1 a seedlings and n = 120 for 2 a seedlings), and mean (SE) component dry mass (g seedling^{-1}), N concentration (%), and N content (mg seedling^{-1}) by treatment and block (n = 12)

Morphological attributes	**1a**	**2a**	**F**	**2a/1a**	**Nutrient attributes**	**1a**	**2a**	**F**	**2a/1a**
Height	9.3	54.3	85.895***	5.8	Root N concentration	1.7	1.6	3.470	0.9
RCD	2.12	6.95	108.066***	3.3	Shoot N concentration	1.9	1.6	1.011	0.8
FOLR	1.1	12.4	128.910***	11.3	Plant N concentration	1.8	1.6	3.558	0.9
Root dry mass	0.18	3.29	12.096**	18.3	Root N content	3.15	54.54	20.628***	17.3
Shoot dry mass	0.12	5.38	79.983***	44.8	Shoot N content	2.23	83.97	74.246***	37.7
Plant dry mass	0.30	8.67	41.176***	28.9	Plant N content	5.38	138.51	33.879***	25.7

Significant at $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$

Table 3 : Mean survival percentage by block (n = 3), mean height (cm) and RCD (mm), and their RGR (%) by individual of 1a (n = 30) and 2a (n = 15) seedlings for each fertility treatment at the end of first year growing season on a weed-prone site

Age/Treatment	**1a**				**2a**			
	0	**60 C**	**120 L**	**180 L**	**0**	**60 C**	**120 L**	**180 L**
Survival	15.6a	17.8ab	23.3bc	28.9c	84.4a	84.4a	88.9a	91.1a
Height	11.9a	17.2b	18.5b	17.8b	56.9a	58.1a	62.8b	68.1b
RCD	2.28a	2.70b	2.80b	2.85b	9.02a	9.45a	9.92a	9.82a
RGR of height	89.0a	79.6a	67.7a	76.3a	11.0a	8.9a	15.0b	17.4b
RGR of RCD	17.0a	22.9a	22.8a	16.6a	34.2a	39.0a	41.5a	34.9a

For each parameter, values in the same seedling age with different letters differ statistically according to Duncan's multiple range test at the 0.05 probability level

Table 4 : Summary of analysis of variance, testing effects of single and factorial treatments (seedling age and fertilization) on mean survival, height, RCD, and their RGR by block under field conditions (n = 3)

Source	**df**	**MS**				
		Survival	**Height**	**RCD**	**RGR of height**	**RGR of RCD**
Main plot comparisons						
Seedling age (S)	1	3.471**	12195.042**	283.250**	2.395**	0.196***
Block (B)	2	0.082	30.476	1.378	0.065	0.063**
Main plot error (S×B)	2	0.045	8.618	0.454	0.015	0.001
Subplot comparisons						
Fertilization (F)	3	0.024*	85.712***	0.703**	0.004	0.008
S × F	3	0.006	24.743***	0.065	0.016	0.001
Subplot error	12	0.003	2.444	0.106	0.019	0.005

Seedling age = 1a and 2a; Fertilization = 0, 60 C, 120 L, and 180 L. MS = Mean square. Significant of $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$

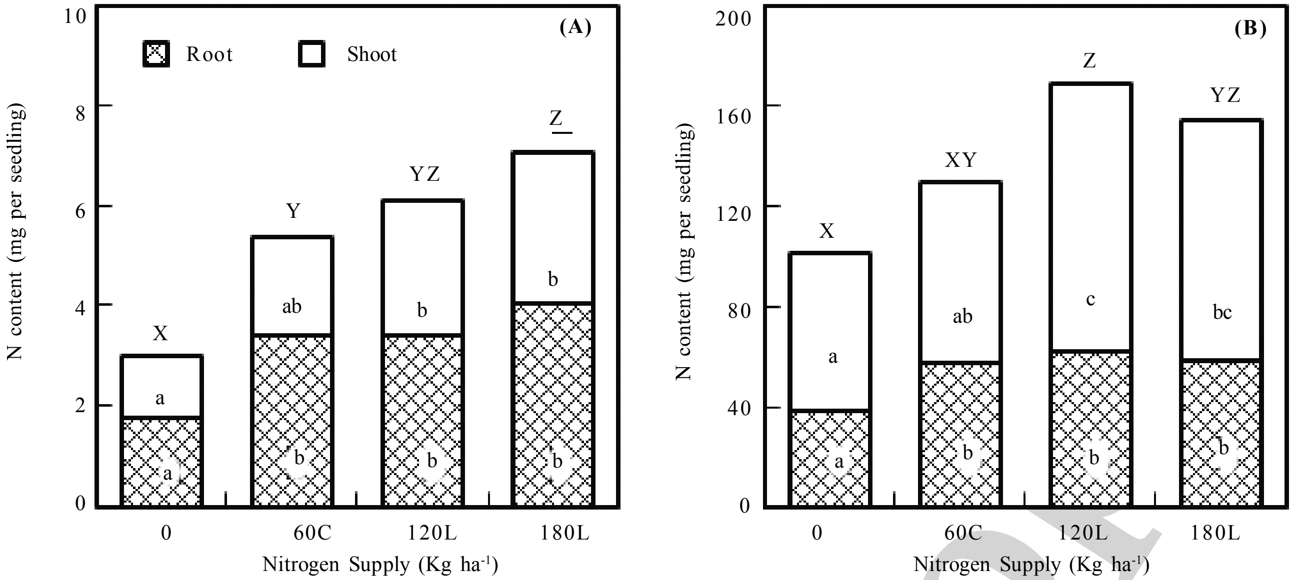

Fig. 3 : Component nitrogen content of (A and B) seedlings exposed to increasing fertility treatments during nursery culture (n = 3). Treatments marked with different letters differ statistically for each component according to Duncan's multiple range test at the 0.05 probability level.

establish good root to soil contact and has inferior competitive ability in the first two years after planting (Nilsson and Örlander, 1995, 1999; Landis *et al.,* 2010). Several bioassay studies have shown that 1a container seedlings could establish effectively when seedlings of high N reserves were transplanted to potted substrates under greenhouse conditions (Malik and Timmer, 1998; Imo and Timmer, 2001). Thus further work should test whether reforestation success on competing vegetation sites may be best accomplished by using 1a stock transplanted into a container.

In some studies exponential fertilization has been tested to be more effective and less toxic because enrichment is raised progressively to match exponential growth and nutrient demand (McAlister and Timmer, 1998; Qu *et al.,* 2003; Birge *et al.,* 2006). Contradictions do exist as some published papers have shown that the exponential regime did not increase uptake efficiency compared to conventional fertilization (Salifu and Jacobs, 2006; Oliet *et al.,* 2009). As a culture protocol, bare-root seedlings were most applied in equal doses at regularly spaced intervals (McKay and Morgan, 1999; Heiskanen *et al.,* 2009).

According to nursery and field performance, nursery applied N was optimum at 180, 120 kg ha^{-1} for 1a, 2a seedlings, respectively. Poor survival showed that additional N failed to make 1a seedlings more competitive on weedy sites. In contrast, 2a seedlings had survival as high as 84.4-91.1%. Seedling age had a significant effect on both field survival and growth performance. Thus, we conclude that urgent work needs to be done to test whether nursery exponential fertilization could significantly promote a 1a seedling's survivability over weed competition.

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