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Original article

Post-transplant root and shoot development in *Abies nordmanniana* Spach. seedlings after whorl bud and branch pruning

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Abstract – After lifting in a nursery, 3-year-old seedlings of *Abies nordmanniana* were treated for improvement in new leader growth after transplanting. The treatments were: (A) whorl (subapical) buds, lateral buds at the stem and whorl branch tips removed; (B) as (A), but with whorl branches removed at their junction to the stem; and (C) untreated control seedlings. After transplanting in April, plant samples were lifted in June, July and September to determine the effect of treatments on root and shoot development. Root length and number of root tips were determined by scanning and computed image analysis. Height, dry weight of shoot and root and ratio between these were determined. Removal of whorl and lateral buds and whorl branch tips had a negative effect on root and shoot growth after transplanting. Root length, number of root tips and shoot dry weight were reduced, but by September equaled the untreated control seedlings. Removal of whorl branches caused a significant reduction in both root length and shoot dry weight compared to untreated control seedlings.

root length / root:shoot / root tip scanning

Résumé – Développement des racines et des pousses après transplantation de semis d'*Abies nordmanniana* Spach. après une taille des verticilles de bourgeons et de branches. Après arrachage en pépinière, des semis âgés de 3 ans d'*Abies nordmanniana* ont été traités pour une amélioration de la croissance de la pousse après transplantation. Les traitements étaient : (A) verticilles de bourgeons (subapicaux), bourgeons latéraux sur la tige et apex des verticilles de branches enlevés ; (B) comme (A) mais avec les verticilles enlevés à leur jonction avec le tronc ; et (C) semis témoins non traités. Après transplantation en avril, des échantillons de plants étaient arrachés en juin, juillet et septembre pour déterminer les effets des traitements sur le développement des racines et des pousses. La longueur des racines et le nombre des apex étaient déterminés par numérisation et analyse d'images. La hauteur, le poids sec des pousses et des racines et leur rapport ont été déterminés. L'enlèvement des verticilles et bourgeons latéraux et des verticilles de branches en apex a eu un effet négatif sur la croissance des racines et des pousses après transplantation. La longueur des racines, le nombre d'apex racinaires et le poids sec des pousses ont été diminués, mais en septembre ont égalé les semis du traitement témoin non traités. L'enlèvement des verticilles de branches a causé une réduction significative de la longueur de racines et du poids sec des pousses comparativement aux semis du traitement témoin non traités.

longueur des racines / racine/pousse / scanner des pointes racinaires

1. INTRODUCTION

Abies nordmanniana Spach. is grown for Christmas tree production in Northern Europe, and, after transplanting in the forest or the field, leader growth is usually very poor in the first few years after transplanting [2]. New height growth is important in reducing the time from planting to final harvest [25]. Leader growth is controlled by environmental factors as light as in *Abies alba* [13] or by competition between terminal and lateral buds as observed in *Pinus strobus* [28] or both. An experiment aimed at directing more growth into the leader of seedlings by the removal of buds and branches has shown promising results [22]. The removal of whorl buds (subapical buds) in 3-year-old *A. nordmanniana* seedlings increased leader growth by up to 30% after transplanting, probably by removing competition to the apical bud [22]. The removal of a larger part, such as whorl branches reduced shoot growth after transplantning in *A. nordmanniana* [23]. The question is whether root growth will be reduced by the bud and branch treatment, as has been shown in *Abies balsamea* [15]. New roots are sinks for photosynthetic products in conifers, such as in *Picea sitchiensis* and *Pseudotsuga menziesii* [26]. Reductions in future photosynthetic areas by debudding before budburst could therefore affect new root growth. At the same time, however, debudding will increase the root to shoot ratio, which can be important for the survival and growth of transplanted *A. nordmanniana* seedlings on dry soil conditions and under competition from weeds [2].

In the present project we focused on the development in root length and in the number of root tips in a study on root growth after transplanting *A. nordmanniana* seedlings. Root fibrosity is regarded as an indicator of root growth potential [3,12]. Knowledge concerning development in root growth

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Table I. Monthly mean air and soil temperature (10 cm below ground), sum of precipitation and evapotranspiration [18] at a climatic station approximately 15 km from the experimental area (DMI).

	Air temperature	Soil temperature	Precipitation	Evapotranspiration
	(°C)		(mm)	
May	10.7	11.1	39	80
June	12.5	13.7	88	89
July	17.2	17.8	31	117
August	16.5	17.6	52	80
September	12.1	14.3	93	36
Sum			303	402

development can be used to advantage in improving the success rate of transplanting also other conifer species in the forest. Root growth studies are laborious and time consuming and are few in number, but a new procedure in which root length and number of root tips are determined by scanning renders determination of these parameters easier. The method has been used with success in other experiments [7, 10, 11, 14].

As part of a project to stimulate new leader growth the aim was to study root and shoot development of *A. nordmanniana* seedlings after transplanting, when whorl and lateral buds and whorl branch tips or whorl branches had been removed prior to transplanting, and to compare with untreated control seedlings.

2. MATERIALS AND METHODS

Three-year-old bare-rooted seedlings (3+0) of *A. nordmanniana* from the Ambrolauri provenance were lifted in April 2001 at a Danish nursery. They had been grown in rows and were root wrenched 20 cm below ground 5 weeks before lifting. After lifting, the seedlings were treated as follows: (A) whorl (subapical) buds, lateral buds at the stem and whorl branch tips were removed by hand; (B) as A, but with whorl branches removed by scissors at the junction to the stem; and (C) untreated control seedlings. After treatment samples of 10 seedlings from each treatment were taken for analysis. On average, 5% ± 0.5 of shoot dry weight was removed in treatment (A) and 20% ± 1.8 in treatment (B) compared to the shoot dry weight of control seedlings.

On the day after lifting and treatment, 50 seedlings in each treatment were transplanted, along with seedlings in other treatments not referred to here, as part of a private owners Christmastree production in the open field on a sandy loam soil (app. 10% clay; 65–70% sand) in Denmark (56° 18' N, 10° 08' E) and were neither irrigated nor fertilised. Before transplanting weeds were controlled by spraying with herbicides (Diurone 800 g ha^{-1} and terbuthylazine 2 kg ha^{-1}). Each individual seedling represented an experimental unit in a fully randomized design. Climatic conditions during the growing period were analysed from data recorded at a climate station (Danish Meteorological Institute (DMI)) approximately 15 km from the experimental field (Tab. I). Evapotranspiration was calculated from global radiation [18].

Plant samples were lifted by hand and pitch fork three times during the first growing season: on 20 June, 25 July and 27 September in 2001. Ten seedlings were lifted in each treatment on each occasion; thus, a total of 90 seedlings were lifted. Although lifting was done carefully, some fine roots were lost during the process – estimated at less than 2%, because the sandy conditions allowed the roots to slip free easily. After lifting, the seedlings were wrapped in wet paper, put in plastic bags and taken to the laboratory, where they were kept overnight at 2 °C. The roots of each seedling were gently washed before the shoot and roots were separated at the root collar scar. Roots were kept in 70% alcohol, separated into bottles and placed at 2 °C until scanning. Before scanning, the roots were washed in cold water to remove the ethanol.

Root length and number of root tips were determined on a flatbed scanner (Hewlett-Parkard Scan Jet 4c modified by Regent, Canada) with Winrhizo® software (WinRHIZO 2002a, Regent, Canada). All the lateral roots were cut from the taproot and spread on plates (20 × 30 cm) with 200 mL of de-ionized water, care being taken to avoid overlapping. Root systems were spread over up to 8 trays to keep the roots separated from each other. Preliminary studies of *A. nordmanniana* root systems in our laboratory had shown that the accuracy of determinations is increased if taproot and lateral roots are scanned separately and that a resolution at 300 dpi with black and white background and threshold automatically gives the best correlation to manual determination of root length [7]. Height was measured from root collar scar to end-bud. Dry weight (DW) of shoot and root was determined after 48 h of drying at 70 °C.

The dataset was analyzed using a general linear model (GLM SAS® Inc. Cary, NC, USA). Treatments were treated as fixed effects. The Student-Newman-Keuls test was used for evaluating treatment effects and probability values of $p < 0.05$ were considered significant. Residuals were tested for normality with Univariate analyses (SAS® Inc. Cary, NC, USA). Standard error (±) was also used.

3. RESULTS

3.1. Root length and root tips

Total root length, including length of both tap and lateral roots, doubled from time of transplanting in April to first sampling in June in untreated controls and in seedlings in which whorl and lateral buds and whorl branch tips had been removed, now significantly greater than where the whorl branches had been removed ($p = 0.0619$) (Fig. 1a). In July, 3 months after transplanting, the root length of untreated control seedlings had increased by a factor of 5 compared to length at transplanting. Only a small increase in root length was found

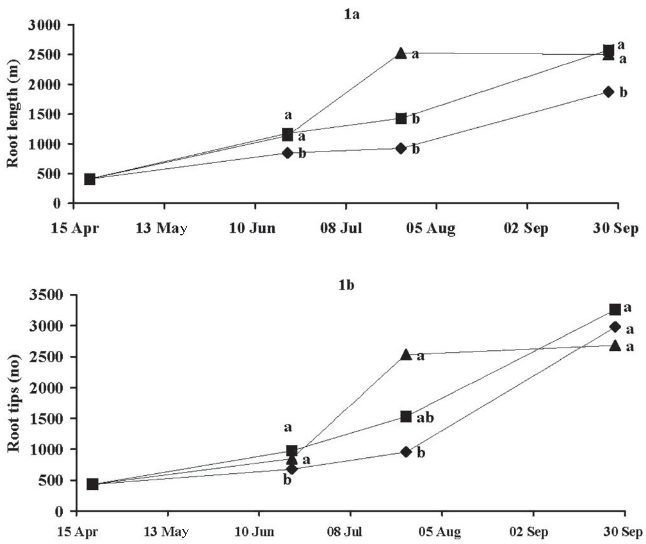

Figure 1. (a) Root length (m per plant) from transplanting to September in relation to treatment at transplanting. **(b)** Number of root tips (no per plant) from transplanting to September in relation to treatment at transplanting: Whorl and lateral buds and whorl branch tips removed A (■), whorl and lateral buds and whorl branches removed B (♦) and untreated control plants C (▲). Significant difference according to the Student-Newman-Keuls test ($p < 0.05$) is indicated by different letters.

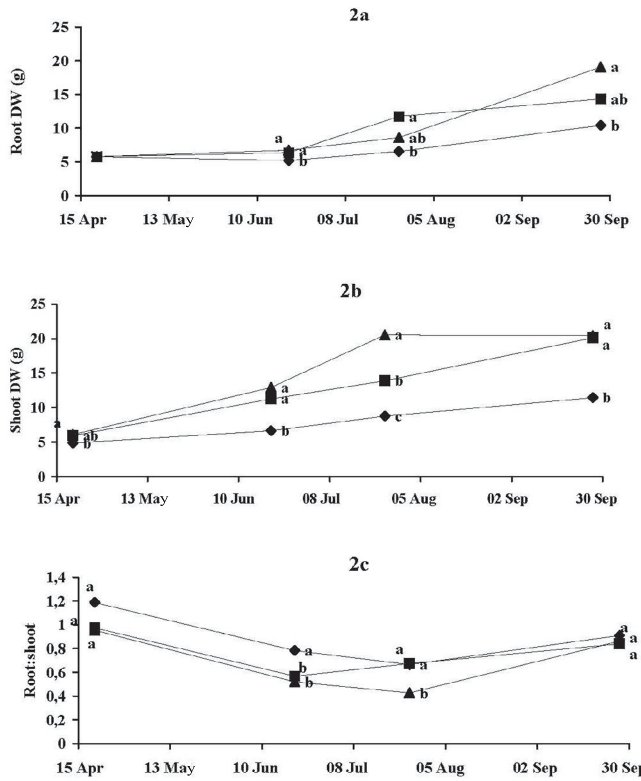

Figure 2. (a) Root DW, **(b)** shoot DW and **(c)** ratio between root and shoot from transplanting to September in relation to treatment at transplanting: Whorl and lateral buds and whorl branch tips removed A (■), whorl and lateral buds and whorl branches removed B (♦) and untreated control plants C (▲). Significant difference according to the Student-Newman-Keuls test ($p < 0.05$) is indicated by different letters.

from June to July between the two treatments, now significantly shorter than untreated control seedlings ($p = 0.0054$) (Fig. 1a). From July to September, however, root length increased in both treatments, while root length in untreated control seedlings did not change. In September, root length was not significantly different between untreated control and seedlings where the whorl and lateral buds and whorl branch tips had been removed, but was significantly lower in cases where the whorl branches had been removed in addition ($p = 0.1191$) (Fig. 1a).

At transplanting the number of root tips was 433 ± 18 as a mean of all treatments, and from transplanting until the first sampling in June this increased in all three treatments (Fig. 1b). In July, the number of root tips was significantly greater in untreated control than in the treatment where whorl and lateral buds and whorl branches had been removed ($p = 0.0172$), but by September the number was not significantly different between the two treatments and untreated control ($p = 0.6243$).

3.2. Height and dry weight

Seedling height increased from time of transplanting up until September, when a not-significant difference between the treatments was observed ($p = 0.6830$). Height increment was 17% ± 1.2 where whorl and lateral buds and whorl branches had been removed and 12% ± 1.4 where whorl and lateral buds and whorl branch tips had been removed compared to 11% ± 1.5 in untreated control seedlings (data not shown).

The development in root DW remained low in the period from transplanting to the first sampling in June (Fig. 2a). From June to July an increase was observed in the treatment where whorl and lateral buds and whorl branch tips had been removed, significantly different to the other treatment ($p = 0.0212$), but not to untreated controls. A relatively large increase in root DW was found from July to September in all treatments, with root DW significantly greater in untreated controls than in seedlings in which whorl branches had been removed ($p = 0.001$) (Fig. 2a). The increase in root DW from transplanting until September was 229% in untreated controls, 147% in seedlings in which whorl and lateral buds and whorl branch tips had been removed and 79% where whorl and lateral buds and whorl branches had been removed.

From transplanting until July, a large increase (338%) in shoot DW was observed in untreated control seedlings as the new shoots and buds developed, but there was no development thereafter (Fig. 2b). In the treatment in which whorl and lateral buds and whorl branch tips had been removed, a near constant increase in shoot DW was found during the growing season until September and shoot DW ended up not significantly different from that of the untreated control seedlings. Removal of whorl and lateral buds and whorl branches reduced shoot

DW, which was significantly less than that of the untreated control seedlings throughout the growing season ($p = 0.0019$) (Fig. 2b).

A drop in root and shoot ratio was observed in all treatments during the two summer samplings in June and July (Fig. 2c). However, at the final sampling in September, root:shoot was improved in all treatments compared to July and not significantly different between the two treatments and the untreated controls ($p = 0.5201$) (Fig. 2c).

Evapotranspiration was higher than precipitation in the period from transplanting until September at the climate station 15 km away from the experimental field (Tab. I). In July the deficit was large (86 mm), while the total water deficit from May to the end of September was 99 mm.

4. DISCUSSION

The total height of the seedlings was greater by up to 17% than the initial height where whorl branches had been removed and less in the other treatments. Removal of branches could have caused an increase in the leader growth as observed in *Nothofagus dombeyi* [21]. Debudding removes apical inhibition caused by cytokinin in the whorl buds and branches [16]. However, the increment in height was much less compared to new leader growth, which was increased by 30% [22]. Hence, height increment should be further improved to comply with the costfull operation by debudding. The removal of whorl and lateral buds and whorl branch tips reduced root and shoot development, i.e. root length, root tips and DW in accordance with results in *A. menziesii* [15]. However, the root and shoot development equaled up by September, when root length and shoot DW were similar to those of the untreated controls (Figs. 1 and 2b). The sequence in root length suggests that removal of buds at the same time removed a shoot-to-root signal, which had to be synthesized before root elongation could continue [1,5,20]. Conifer buds are high in auxin, which might be lowered by the debudding proces [1, 6, 27]. Removal of the whorl and lateral buds and whorl branches had a more severe effect on plant growth and caused a reduction in root length and DW. Removal of a significant part of the photosynthetic area was the likely reason for the reduction in root and shoot growth, which was in accordance with [26], who observed that current photosynthesis was responsible for new root growth in conifer species, and with [24], who found similar results in *Pinus taeda* L.

No straightforward relation was observed between number of root tips and root growth after transplanting, which is contrary to the findings of others [8, 12]. Surprisingly, root length was improved without root DW being affected at the first sampling in June in both treatments and control (Figs. 1 and 2a). Later, from July to September, root length in untreated control seedlings did not increase to the extent that root DW did, which suggests that carbohydrates accumulated as storage in the roots after shoot growth had ceased [9]. Hence *A. nordmanniana* showed a plasticity in root development as found in beech but not in *Pinus* [11].

Shoot DW was affected similarly to root DW in relation to treatments before transplanting. Removal of whorl and lateral buds and whorl branch tips changed the shoot DW accumulation probably in parallel with loss of the photosynthetic area in the removed buds [23] (Fig. 2b). At the final sampling in September, shoot DW had equaled that of the untreated control seedlings, which is in accordance with the results of [22]. Shoot growth fell off in untreated control plants from July to September, whereas root DW continued to accumulate (Fig. 2a). Evapotranspiration was high in July compared to precipitation and might have affected the untreated control seedlings with a lower root:shoot. In addition to initial root collar diameter [17], root:shoot can be a predicter of growth after transplanting in many conifer species [2, 19]. However, the change in root:shoot during the growing season in *A. nordmanniana* and the possible signalling between root and shoot should be given more attention in future work [4].

As concluding remarks, the new method to increase leader growth did not significantly increase height in the present experiment, and further studies aimed at optimizing leader growth after transplanting should focus on further height increment and methods to employ in practice.

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