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UNIVERSITY OF ALBERTA

THE INTERACTION OF NITROGEN SUPPLY AND WATER STRESS IN PRECONDITIONING LODGEPOLE PINE SEEDLINGS

BY

JAMES DOUGLAS STEWART

A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ENTITLED...THE INTERACTION OF NITROGEN SUPPLY AND WATER

STRESS IN PRECONDITIONING LODGEPOLE PINE SEEDLINGS

SUBMITTED BY...JAMES DOUGLAS STEWART

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

Victor J. Lieffers

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Date: March 26, 1991

ABSTRACT

The interaction between nitrogen relative addition rate (N Ra) and polyethylene glycol (PEG) 8000-induced osmotic stress was determined in three-month-old seedlings of lodgepole pine (*Pinus contorta* var. latifolia Englm.) grown in solution culture. The lowest (2 % day^{-1}) N Ra increased the effect of -0.5 MPa and decreased the effect of -1.0 MPa solution osmotic potentials on relative growth rate (Rg). Leaf:root ratio was highest at the intermediate osmotic stress level, except when also fed nitrogen at 10 % day^{-1}. No interaction was found for water content, xylem pressure potential (XPP), or foliar nutrient concentrations. Stomatal conductance was highest at 4 % day^{-1} N, and XPP increased with N Ra. Foliar N concentration was reduced by osmotic stress.

The effects of preconditioning lodgepole pine seedlings with combinations of N Ra and PEG 8000-induced osmotic stress in solution culture were determined. Seedlings were preconditioned at 4 and 8 % day^{-1} N Ra for 11 weeks, including a 2 week -0.4 MPa osmotic stress. Seedling performance and characteristics were assessed after 6 weeks of simulated field growth at 2 and 4 % day^{-1} N Ra, including 2 weeks of -0.4 and -0.8 MPa osmotic stress. Seedlings preconditioned at -0.4 MPa or at 4 % day^{-1} N Ra temporarily maintained higher Rg during the second phase osmotic stress, than those preconditioned at 8 % day^{-1} N Ra or without osmotic stress. However, the stress preconditioned seedlings were smaller. A 7.5 % reduction in water content due to preconditioning osmotic stress was maintained until harvest. Foliar N concentrations remained 9 % lower in seedlings preconditioned with osmotic stress.

Five-week-old containerized lodgepole pine seedlings were preconditioned for 6 weeks at 3 % day^{-1} and 6 % day^{-1} N Ra, and were either droughted or well-watered. This was followed by a second 6 week phase in which seedlings were distributed among the same treatment combinations, to give a 2^4 factorial design. Interaction between nitrogen and watering treatments applied simultaneously was found only in Rg. An interaction between preconditioning N Ra and second phase water supply was found in root water content and XPP. Elevated foliar reducing sugar concentration resulted from low nitrogen preconditioning, and persisted through the 6 week second phase. Preconditioning at the lower N Ra increased subsequent XPP, root weight ratio, and reducing sugars concentration, which could contribute to drought resistance.

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CHAPTER I. INTRODUCTION.

The Problem

An objective of forest regeneration programs is to produce seedlings which survive and grow well on cut-over areas. Artificial regeneration using nursery-grown seedlings must be used in cases where natural regeneration is insufficient or uncertain. Between 1981 and 1985, nursery-grown seedlings were used to restock over 1.2 million ha in Canada. This area represents more than one quarter of the forest area harvested, and is steadily increasing (Kuhnke 1989). For artificial regeneration to succeed, nursery seedlings require appropriate physiological characteristics which will allow them to survive and grow under field conditions. As long as 40 years ago it was known that size and vigour of seedlings in the nursery did not always translate into good field performance (Wakeley 1948). First attempts at seedling preconditioning using nutrient deficient conditions in order to acclimate them to field conditions were unsatisfactory (Duryea and McClain 1984). Work in the past few decades has shown that various kinds of temporary preconditioning stress treatments in the nursery can improve seedling physiological and morphological condition, and therefore field performance (see Duryea and Brown 1984 for review).

I chose to study nitrogen and water availability as preconditioning agents because they are major resources for plant growth, are important nursery management tools, and have been demonstrated to have useful preconditioning effects in conifers (Carlson 1983, Duryea and McClain 1984, Hennessey and Dougherty 1984, Aussenac and El Nour 1985). In addition, I investigated the effect of the interaction of moisture and nutrition on seedling performance, which few studies have examined.

Nitrogen supply

Nitrogen fertilization can improve the growth both in the nursery, and in the field after transplanting, of Pseudotsuga menziesii, Picea sitchensis, Pinus contorta (van den Driessche 1980, 1982), and Pinus taeda seedlings (Switzer and Nelson 1963, Mullin and Bowdery 1977). Nitrogen content of foliage generally increases with nitrogen fertilization in conifer seedlings (Schomaker 1969, McClain and Armson 1976, van den Driessche 1980), though Armson (1968) found that nitrogen concentration in Pinus resinosa seedlings did not change with soil nitrogen concentration, but dry weight increase appeared to keep pace with increased nitrogen uptake. These foliage nitrogen contents can affect performance of the seedling in the field. Duryea and McClain (1984) reviewed the literature and found that 1.7 to 2.3 % foliar nitrogen was optimal for growth and survival of seedlings after outplanting.

Ingestad (1982) has proposed a model of the relationship between the rate of nitrogen supply, the internal concentration of nitrogen, and plant growth rate. Where nitrogen supply increases exponentially with plant growth, the relative rate of supply determines the internal nitrogen content, which in turn determines the relative growth rate. Fertilization at rates of N supply which are based on the plant's demand for N avoid the confounding problems of over-supply and potential toxicity in smaller seedlings, and under-supply and potential N deficiency in larger seedlings. This technique allows a more efficient and effective use of fertilizer N in rearing nursery seedlings (Timmer and Armstrong 1989).

Absolute control of growth rate of containerized seedlings may not always be possible, but modification of growth, nutrient status and morphology can be achieved by relative nutrient addition treatments (Troeng and Ackzell

1988). Nutrient addition at exponential rather than constant rates resulted in larger seedlings with lower shoot:root ratios in container-grown Pinus resinosa (Timmer and Armstrong 1989). Root:shoot ratio was little altered by differences in nitrogen fertilization at constant supply rates, except at very high levels (van den Driessche 1982).

Water stress

Preconditioning with water stress has been shown to be beneficial despite its negative effect on plant growth. Drought preconditioning enhances performance of transplanted conifer seedlings by reducing shoot:root ratio, and stimulating root growth in the field (Rook 1972, Aussenac and El Nour 1985, Kaushal and Aussenac 1989). An exception to this appears to be Pinus taeda L., in which root growth is more sensitive to water stress than shoot growth (Seiler and Johnson 1988). Both photosynthesis and respiration are sensitive to water stress, though photosynthesis less so (Brix 1962). Acclimation of photosynthesis to water stress occurs through both stomatal and non-stomatal mechanisms. Preconditioning increased stomatal sensitivity to water stress in Pinus radiata D. Don (Rook 1972), and decreased non-stomatal inhibition of photosynthesis by water stress in Pinus taeda L. (Seiler and Johnson 1988). In Picea mariana Mill. BSP, osmotic stress preconditioning did not modify photosynthetic rates, but maintained higher osmotic and turgor potentials, and reduced stomatal conductance and increased stomatal sensitivity to osmotic stress (Zwiazek and Blake 1989).

Interaction of nitrogen supply and water stress

The interaction of nutrient and moisture regimes has been examined in relatively few instances. In Pinus strobus, growth decreased and foliar nutrient concentration increased with decreased fertility and moisture regimes, though total nutrient contents decreased; however, a synergistic interaction was found at high water and high fertility conditions (Schomaker 1969). Brown (1970) found growth responses to moisture and fertility varied with provenance of *Pinus sylvestris*. Northern seedlings exhibited maximum growth at medium moisture and fertility levels, while southern seedlings grew best at low fertility and high moisture conditions. Nutrient-water interactions were also significant within each provenance. Interaction effects were significant in the growth response of *Picea glauca* and *Picea mariana*, but not of *Pinus banksiana* (McClain and Armson 1975). For example, although all three species grew best at the highest moisture level and moderate fertility level, the response of *Picea mariana* to increasing fertility was severely limited in all but the highest moisture condition, and the response to increasing moisture was minimal in unfertilized *Pinus banksiana* seedlings.

Shoot:root ratio in conifers is also affected by the interaction of moisture and fertilization. In *Pinus banksiana*, *Picea glauca* and *Picea mariana*, reduction in either fertility or moisture reduced the seedlings response to the other factor similar to the height growth responses noted above (McClain and Armson 1975). However, root area index in *Picea glauca* and *Picea mariana* had different fertility optima at the different moisure levels used. Although reduction in either fertilization or moisture usually decreases shoot:root ratios (Pharis and Kramer 1964, McClain and Armson 1975), under moisture stress shoot:root ratios increase in *Juglans nigra* (Dickson 1971) and *Pinus taeda* L. (Seiler and Johnson 1988).

Nitrogen nutrition can moderate the effects of water stress. In shoots of *Zea mays*, relative water content and water potential were higher when grown at lower N fertilization rates (Tesha and Eck 1983). In *Festuca* *arundinacea*, a high N fertilization rate maintained over many drought cycles ameliorated yield reduction due to water stress (Belesky et al. 1984). However, drought conditions in this field experiment were exacerbated by increased leaf area and stomatal conductance under the high-N treatment. In their study of *Pinus taeda*, Pharis and Kramer (1964) concluded that nitrogen concentrations which were 'optimal' under no water stress conferred the greatest drought resistance, and that greater or lesser concentrations of nitrogen reduced drought resistance. High levels of nitrogen fertilization reduced drought resistance in *Pinus contorta* (Etter 1969), and reduces growth in *Picea glauca* seedlings after outplanting (McClain 1983). Nitrogen deficiency in cotton (*Gossypium hirsutum* L.) produces a suite of responses usually associated with drought resistance, including less water loss with decreasing water potential, osmotic adjustment and greater cell wall rigidity, reduced leaf area, and increased stomatal sensitivity to water stress (Radin and Parker 1979a, 1979b).

Water stress and nitrogen supply could also interact at the biochemical level. Foliar concentrations of soluble proteins increase, and free amino acids decrease with increasing N supply (Tesha and Kumar 1978, Gezelius 1987), but not in lodgepole pine (*Pinus contorta*) where soluble protein decreases and amino N increases with increasing N supply (Etter 1969). Water stress also increases amino acid concentrations, especially proline, and decreases soluble protein (Taylor *et al*. 1982, Handa *et al*. 1983, Vance and Zaerr 1990). Water stress is thought to affect metabolism by limiting enzyme synthesis or activity (Taylor *et al*. 1982, Vance and Zaerr 1990) and by degrading membrane integrity and function (Kluge 1976). Nitrogen supply effects appear to operate through effects on carbon assimilation; N limitation reducing chlorophyll concentration and Rubisco activity (Tesha and Kumar 1978, Gezelius 1987), which could result in increased competition for reducing power and photoassimilate between C and N metabolic pathways (Etter 1969, Kluge 1976).

Lodgepole pine

Lodgepole pine (Pinus contorta var. latifolia Englm.) is one of the two major commercial tree species which are nursery-raised and outplanted for reforestation in Alberta. It often grows on sites where levels of nutrients or water or both are deficient. Cannell et al. (1978) showed a strong decrease in growth of lodgepole pine seedlings with increase in water stress; under stress conditions, growth was highly correlated with soil moisture. Kaufmann (1968) showed delayed root regeneration and decreased root growth in Pinus contorta with decreased soil water potential. The interaction of water and nitrogen pre-conditioning in lodgepole pine has not been studied, with one exception noted previously (Etter 1969). Experiments which control both water and nitrogen stress, using current methods of mineral nutrition and water potential control, are required to elucidate their interactive effects on lodgepole pine, and to quantify the potential of pine seedlings to acclimate to these stresses.

Research objectives

The research reported here investigated the growth and physiological responses of Pinus contorta to the interacting factors of nitrogen fertilization and moisture, both the immediate responses and the long-term preconditioning effects. The objectives of this study were: 1) to determine the effect of the interaction of water stress and nitrogen supply on the growth, nutritional status, and water relations of lodgepole pine seedlings; 2) to determine the effect of these factors as preconditioning agents in improving long-term performance of seedlings grown in both solution culture and containers; and 3) to determine the interaction between preconditioning and subsequent treatments of nitrogen fertilization and water stress.

The measured growth and physiological parameters may be affected by nitrogen and water stress factors singly, or in combination. Main effects can be independent of each other (i.e., additive) or they can interact, either positively, where the effect of one factor is enhanced at some levels of the second factor (synergism), or negatively, where the effect of a factor is diminished at some levels of the second factor (i.e., antagonism).

The first objective was addressed by growing seedlings in a controlled environment in nutrient solution culture, applying wide ranges of nitrogen and osmotic stress concurrently. The second objective was met by another solution culture experiment, using a 2 by 2 subset of the original treatments, and completely crossing the 4 treatments in a pre-conditioning phase with 4 treatment combinations in a second evaluation phase. The third experiment followed the design of the second, but used a solid medium, fertilization and irrigation in a greenhouse setting to achieve the stress treatments in a manner which more closely approximated normal nursery conditions. Results from the first two experiments in well-controlled, though artificial, conditions can be used to evaluate the results of the latter, less controlled experiment and, by extrapolation, to suggest appropriate conditioning methods to be field-tested in operational trials.

Abbreviations used

D, droughted
DW, dry weight
FW, fresh weight
N, nitrogen
PEG, polyethylene glycol
Ra, relative addition rate (% day^{-1})
Rg, relative growth rate (% day^{-1})
SOPD, solution osmotic potential depression
W, well-watered
XPP, xylem pressure potential

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CHAPTER II. INTERACTION BETWEEN NITROGEN AND PEG-INDUCED OSMOTIC STRESS IN LODGEPOLE PINE SEEDLINGS

INTRODUCTION

Growth and physiology of conifers are affected by soil moisture (Hennessey and Dougherty 1984) and nitrogen (N) nutrition (Duryea and McClain 1984). The interaction of water stress and N supply is of particular interest in tree nurseries, where watering and fertilization are controlled to produce seedlings of specific size and proportions. Studies of nitrogen fertilization on conifer seedlings have addressed aspects such as nitrogen source, rate of supply, and time and frequency of application, and their effects on growth and survival in the field (McClain and Armson 1975, Brix 1981, van den Driessche 1982, 1988). Similarly, the role of water supply has been studied to determine its effects on growth and drought resistance (Rook 1972: Roberts and Dumbroff 1986, Seiler and Johnson 1988, Zwiazek and Blake 1989) However, most studies on seedling response to moisture and nutrition have not considered how these two factors interact in their effect upon seedlings.

Moisture and fertilization interactions have been reported in tree seedling growth and leaf:root ratios (Pharis and Kramer 1964, Schomaker 1969, Dickson 1971, McClain and Armson 1975). Nitrogen fertilization has been shown to improve growth, but apparently reduces drought resistance in pines (Pharis and Kramer 1964, Etter 1969). These studies clearly show the potential for interaction between N fertility and water stress, and the need to simultaneously take both factors into account in understanding seedling responses.

Lodgepole pine (Pinus contorta var. latifolia Englm.) is an important commercial tree species in Alberta, and often grows on sites which are deficient in water, nutrients or both (Lotan and Perry 1983). The objective of this experiment was to determine the effects of nitrogen supply and water stress applied concurrently on the growth, nutritional status, and water relations of lodgepole pine seedlings.

Previous studies have suffered from three methodological deficiencies: 1) constant nutrient solution concentration did not match the nutrient demand of exponentially growing seedlings, causing seedlings to experience over- and then under-supply of nutrients (*cf.* Ingestad and Lund 1986); 2) since imposition of water stress by drying of the rooting medium is difficult to control, the rate of drying depended on seedling size and growing conditions, resulting in either variation of drought duration or intensity among treatments (Krizek 1985); and 3) interactions among treatments and/or growing conditions may have occurred in the growth medium rather than in the plant.

In solution culture, treatments are applied to the plant roots, rather than being mediated by the soil or potting mix used, eliminating confounding effects of nutrient adsorption and release, bulk flow differences, and gradients of soil water potential. Constant water stress treatments can be achieved in solution culture by the use of a relatively inert, non-ionic osmotic agent. Polyethylene glycol (PEG) is less harmful than inorganic salts, and does not contribute to metabolism as do absorbable organic compounds such as sucrose and mannitol (Cress adn Johnson 1987). As well, the potential side-effects of PEG, such as toxicity and uptake into the plant, and insufficient aeration of PEG solutions, are well documented (Krizek 1985). These side-effects can be minimized by using the higher molecular weight forms, restricting the concentrations used, aerating the solutions, and by using semi-permeable membranes to separate PEG from plant roots. However, this latter method is not appropriate to solution culture studies, because it concentrates the nutrient salts surrounding the roots. Despite its drawbacks, PEG remains, arguably, the best and most commonly used osmotic agent (Buxton et al. 1985, Rego et al. 1986, Talouizite and Champigny 1988).

Nutrient supply can be matched to the demand of exponentially growing plants by adding nutrients at an exponentially increasing rate. One method of achieving this is to add N as a constant percentage of internal N content per unit time (relative addition rate; Ingestad and Lund 1986). Plant growth rate and internal nutrient concentrations adjust to the rate of supply during a lag phase, and eventually attain a steady-state condition. Since an imposed osmotic stress was expected to alter the nutrient supply - growth rate relationship in the seedling, a steady-state condition during the osmotic stress period could not be maintained. However, the different rates of supply reduce the confounding effect of seedling size among nutrient treatments, and provide different growth rates and nutrient stresses. A similar combination of techniques has been used by Rego et al. (1986, 1988) to study N supply and osmotic stress effects in Sorghum.

MATERIALS AND METHODS

Species and conditions

Lodgepole pine seeds from northwestern Alberta were germinated in washed fine silica sand in a growth chamber using a 16 h photoperiod, a photosynthetically-active photon flux density (PPFD) of 380 - 550 μmol m^{-2} s^{-1}, 30/20^{o}C day/night temperature regime, and relative humidities between 90 and 100%. Trays were misted twice daily with tap water until roots were established, and then every second day. Seedlings were transplanted into solution culture pots when roots were long enough to reach the nutrient solution (approx. 7 weeks after planting).

The solution culture pots were 21 cm diam., 4.55 l dark brown plastic pails fitted with white plastic lids with 4 slots to hold seedlings supported by closed-cell foam collars. Compressed air was bubbled through an aquarium airstone. An inverted polypropylene funnel above the airstone minimized splashing, foaming and root disturbance. Pots were filled with 4.1 l of quarter-strength Long Ashton no-N formulation (Hewitt 1966), modified to provide constant proportions of mineral nutrients in optimal ratios for lodgepole pine (Ingestad and Kahr 1985). This solution contained 107.18 mg l^{-1} KH_2PO_4, 25.09 mg l^{-1} K_2SO_4, 18.50 mg l^{-1} $CaCl_2$, 16.05 mg l^{-1} $MgSO_4$, 11.21 mg l^{-1} MicroMix (chelated micronutrient mixture, Plant Products Co. Ltd., Bramalea, Ont.), adjusted to pH 5.5. The addition of nitrogen as NH_4NO_3 minimizes the problem of net pH changes occurring when one of the ionic species is taken up in excess of the other, and the consequent need for constant pH control which introduces unwanted variation in counter-ion concentrations. Distilled water was added weekly to replace evaporation and transpiration losses. The growth room had a 16 h photoperiod, with a PPFD of 400 to 550 μmol m^{-2} s^{-1}, and day/night humidities and temperatures of 60/70% RH and $25^{o}/20^{o}C$. Pot position was randomized twice weekly.

Treatments

The experiment was a 4 by 3 factorial in a randomized complete block design, with 4 levels of N relative addition rate (N Ra) and 3 levels of solution osmotic potential depression (SOPD). Each treatment combination was replicated 5 times in the course of 3 sequential runs (5/3 replication), for a total of 60 experimental units (pots).

Each week, seedlings were weighed and N additions for each pot were calculated based on the weight of the four seedlings per pot. Damage to roots during the weighings was minimized by gentle handling during transfers, and blotting roots between layers of soft cotton towelling, though some reduction in growth due to handling was unavoidable. The total fresh weight of seedlings was multiplied by the estimated N concentration (0.3% of fresh weight, Ingestad and Kahr, 1985). Nitrogen was added thrice weekly as NH_4NO_3, according to the following equation:

$$A_N=N_i(e^{Ra*Ta})(e^{Ra*Tb}-1) \qquad [1]$$

where A_N is the mass of nitrogen to be added, N_i is the internal nitrogen content at the time of weighing, Ra is the treatment variable relative addition rate (2, 4, 6 or 10% day^{-1} of internal N content), Ta is the time in days between weighing and nitrogen addition, and Tb is the time in days between present and subsequent additions (adapted from Ingestad 1982). The other nutrients were added at the same time, in the proportion to N as suggested in Ingestad and Kahr (1985). The Ra's of 10, 6, 4 and 2 % day^{-1} were chosen as being super-optimal, near optimal, and 2 levels below optimal, respectively, based on earlier growth trials with this system.

Nitrogen treatments began 1 week after transplanting. The lag phase, during which relative growth rate (Rg) adjusted to N Ra, lasted 5, 6, and 9 weeks (respectively, for the 3 runs); the duration determined by variation in growth room conditions and temporary algal contamination (run 3). Therefore, the experiment did not control for chronological age of the seedlings, but the slower growth in the longer experimental run meant that developmental ages did not differ as much as the chronological ages. Once Rg's in various N Ra treatments had diverged and leveled off, the SOPD treatments were imposed using PEG 8000 (BDH Chemicals) to lower solution osmotic potentials by 0, 0.5 (168 g l^{-1}) and 1.0 MPa (230 g l^{-1}). The mass of PEG required to achieve the required SOPD was calculated using equation 1 of Michel (1983). Flaked PEG was added without stirring to the pots in 3 equal aliquots over 5 days. Total dissolution, and hence the total osmotic effect of each PEG aliquot, was achieved after approximately 24 hours. Senescent seedlings (21 out of 240, equally distributed among the osmotic stress treatments) were removed from the experiment, and pot mean values were calculated from the remaining, healthy seedlings. Solution aeration was checked with a YSI model 51B dissolved oxygen meter, and was > 5.0 mg O_2 l^{-1} in PEG solutions compared to 7.2 mg O_2 l^{-1} in nutrient solution without PEG. After 2-1/2 weeks of the osmotic stress treatment, all seedlings were harvested.

Stomatal conductance was measured on 1 seedling per pot using a LI-COR 1600 Steady-state Porometer and conifer cuvette, at approximately 1000h on the day before harvest. Leaf areas were estimated later from dry weight measurements, using a relationship determined for needle dry weight and surface area in non-experimental seedlings grown in the same conditions. Previous measurements had produced the following relationship for needle dry weight and length:

$$\mathrm{Len(mm)=DW(mg)*7.901+15.217,\ N=87,\ R^2=0.91} \qquad [2]$$

The glass bead method (Thompson and Leyton, 1971) was used to relate needle surface area to length as follows:

$$\mathrm{Area(mm^2)=Len(mm)*0.028186-0.22578,\ N=60,\ R^2=0.98} \qquad [3]$$

Pre-dawn xylem pressure potential (XPP) was measured on the same seedlings on harvest day (Ritchie and Hinckley 1975). All seedlings were harvested and weighed fresh and after 48 hours of drying at 70°C to give dry weights and water contents ((FW-DW)/DW). Dried leaf tissue was ground with mortar and pestle, digested with H_2SO_4 and H_2O_2 (Thomas _et al_. 1967), and analysed for total N and P using standard autoanalyser methods (Technicon Industrial Method No. 334-74W/B+, 1977), and for K and Mg using a Perkin-Elmer model 500 atomic absorption spectrophotometer (McKeague 1976).

Dry weights during the experimental period were estimated from the weekly fresh weight measurements and water contents extrapolated from harvest data using 0 MPa water content for all pre-PEG estimates, and assuming a linear decrease over time. Relative growth rate (expressed as % day^{-1}) was calculated from the dry weight estimations as:

$$Rg=(\log DW_2-\log DW_1)/(T_2-T_1) \qquad [4]$$

where DW_1 and DW_2 are seedling dry weights at times T_1 and T_2.

Analysis

The data was analysed by ANOVA for unbalanced designs using the GLM procedure in SAS. The observed responses of the seedlings (y; averaged for each pot) were a linear function of the run and treatment effects:

$$y = \text{Grand mean} + \text{Run} + \text{N Ra} + \text{SOPD} + \text{interactions} + \text{error} \qquad [5]$$

Since pots were treated independently, and randomly assigned to the treatment combinations, they were the experimental units. The variance among pots within runs and nitrogen and water stress levels is the experimental error, and therefore the appropriate error term for testing significance of the treatment effects (Steel and Torrie, 1980, Milliken and Johnson, 1984). Data were transformed as necessary to minimize heterogeneity of variance. Sixteen out of 60 stomatal conductance measurements were near the lower limits of accuracy of the porometer, and were replaced by a threshold value set at two orders of magnitude less than the maximum value obtained. This level represented effective stomatal closure.

RESULTS

Mean Rg was related to N Ra prior to PEG addition (Table II-1); however, the slope of the relationship of Rg _vs_. N Ra was less than the theoretical optimum of 1.0 (Fig. II-1) (Ingestad 1982, Ingestad and Lund 1986). Although this optimum has been attained in aeroponic systems with computer-aided control of nutrient levels (Ingestad and Lund 1979, Ingestad and McDonald 1989), my less sophisticated control system involved nutrient supply fluctuations on the scale of 2-3 days and lower mixing rates. As well, the seedling N content estimates (0.3 % FW) were likely over-estimates at the lower N Ra's. Growth rates in osmotically stressed seedlings declined steadily to the harvest date. At harvest, Rg was about 0 in all of the 0.5 MPa SOPD treatments, and between -2 and -4 % day^{-1} in the 1.0 MPa SOPD treatments. The exception was the combination of 2% N Ra with 0.5 MPa SOPD. Here, Rg was as negative as the 1.0 MPa SOPD treatment, which itself was higher than in the other N Ra levels (Rg pre-harvest, Table II-1; Fig. II-2). Negative growth rates were not due to needle loss, but may have been due to sloughing of root tissue, increased maintenance respiration, or a combination of both.

Dry weight accumulation during the 3 week period of both PEG and N treatments was significantly affected by both N Ra and SOPD, but not by their interaction (Table II-1). Total dry weight at harvest, the majority of which was produced during this period, is shown partitioned into shoot and root (Fig. II-3). The apparent non-additive response to N Ra and SOPD in Fig. II-3 was a consequence of dry weight being an integral over time of exponential growth rate, which is adjusted for by the log transformation of the data prior to analysis (Table II-1).

Dry weight of both shoot and root were reduced by N and osmotic stresses (Fig. II-3). Leaf:root ratio doubled from the 2% to the 10% N Ra level (Table II-1, Fig. II-3). In the osmotic stress treatments, the maximum leaf:root ratio was at 0.5 MPa SOPD, except for the 10 % day^{-1} N Ra seedlings, which had their minimum value at 0.5 MPa SOPD.

Seedling water content dropped from approximately 5 g g^{-1} DW in the 0 MPa SOPD treatments to about 3.3 and 2.6 g g^{-1} DW at the 0.5 and 1.0 MPa SOPD levels, respectively (Fig. II-4) Water content was unaffected by N treatments (Table II-2), and there was no significant N Ra by SOPD interaction. Pre-dawn xylem pressure potential decreased slightly at 1.0 MPa SOPD, compared to 0 and 0.5 MPa SOPD treatments (Table II-2; Fig. II-4). At the low N Ra, seedlings maintained XPP about 0.2 to 0.4 MPa lower than that in the high N Ra seedlings, at all SOPD levels (Fig. II-4). There was no significant N Ra by SOPD interaction (Table II-2). Mid-morning stomatal conductances at 0.5 MPa SOPD were about 10 % to 33 % of the unstressed values, and were reduced to less than 10 % of the unstressed values at 1.0 MPa SOPD (Table II-2). Stomatal conductance was affected to a lesser degree by N Ra, with maximum conductances at 4 % day^{-1}.

Foliar N concentration increased with N Ra (Table II-3, Fig. II-5). At 2% day^{-1} N Ra, N content was 0.9 to 1.4 % of dry weight, while at 10% day^{-1} N Ra, N was 2.0 to 2.2% of dry weight; the former group probably experienced N deficit and the latter luxury consumption. Foliar N declined with increasing SOPD, but no significant interaction between N Ra and SOPD was observed. Foliar P, K and Mg concentrations varied inversely with N Ra, regardless of SOPD level (Table II-3, Fig. II-5). As these nutrients were not limiting growth, the increase in their concentrations as N Ra decreased may reflect luxury consumption of P, K and Mg at lower Rg.

DISCUSSION

This experiment was designed to test for interaction between N supply and water stress treatments on growth and physiology of lodgepole pine seedlings. Despite reports that such interactions exist for lodgepole pine (Etter 1969) and for other pines (Pharis and Kramer 1964, McClain and Armson 1975), I found only a few. Interactions were observed for Rg, leaf:root ratio and possibly for stomatal conductance. For the Rg interaction, in the 2 % day^{-1} N Ra treatments, the 1.0 MPa SOPD did not cause any more reduction in Rg than the 0.5 MPa SOPD (Rg approx. -2 % day^{-1}). At all of the higher N Ra levels, the greater osmotic stress caused the greater reduction in Rg (in all cases, between -3 and -4 % day^{-1}). It is not clear why the lowest N Ra treatment conferred some advantage for sustaining tissue during water stress, but it has been reported that very low N supply can mitigate the influence of water stress on growth rate (Pharis and Kramer 1964, Etter 1969). However, as observed by Pharis and Kramer

(1964), because growth rates are so low at the low N rates, improvements in the ability to withstand water stress may be of little practical value, except in cases where drought resistance is more important than competitive ability.

The decrease in leaf:root ratio with decreasing N Ra reported here reflects a general trend in plants, where deficiency in a resource enhances the growth of the organ responsible for the acquisition of that resource, relative to the rest of the plant (Hunt and Nicholls 1986). In the case of lodgepole pine, a N stress could improve the seedlings capability to exploit other soil resources, such as water. In the N rate by osmotic stress interaction for leaf:root ratio, both root and leaf mass were lower for seedlings subjected to water stress. However, under moderate osmotic stress (0.5 MPa SOPD), there was a greater reduction of root mass than of shoot mass (being mostly leaf), for seedlings grown at the lower N Ra levels (Fig. II-3). These data suggest that root growth inhibition by at moderate water stress is inhibited more at lower N Ra than at high N Ra. With a further reduction in osmotic potential to 1.0 MPa SOPD, the reductions were equivalent for both shoot and root, and leaf:root ratios were similar to those of unstressed seedlings (Fig. II-3). Greater inhibition of root growth (relative to shoot growth) by water stress has been found for other pines (Squire _et al_. 1987, Seiler and Johnson 1988), in contrast to the shift in biomass partitioning in favour of roots observed in most genera (Stocker 1960). This differential response may be caused by osmotic stress causing dormancy in root meristems (Kaufmann 1968, Squire _et al_. 1987), and thereby restricting root growth. Alternatively, the yield threshold for expansion of cell walls may be higher in roots than in shoots, or the stress may be greater in roots than in shoots. It is not clear if this root growth inhibition gives seedlings at low N Ra any advantage in surviving drought stress; however, my growth rate results (Fig. II-2), and those of Etter (1969), suggest that it may be beneficial. It is also possible that PEG toxicity to roots may have been more of a problem to seedlings fed at low N Ra. However, I discount this because I saw few symptoms of PEG toxicity (e.g., reddening, necrosis), and the root mass actually decreased; thus PEG was probably not taken up significantly.

This study and that of Ingestad and Kahr (1985) suggest that lodgepole pine can attain high growth rates under optimal conditions; however, it is sensitive to moderate water stress. I observed pronounced stomatal closure and a virtual cessation of growth at 0.5 MPa SOPD, when XPP was still greater than -1.0 MPa, and virtually complete stomatal closure at 1.0 MPa SOPD. This corresponds with results from Lopushinsky (1975) for field grown lodgepole pine. As well, root growth was more affected by mild osmotic stress than shoot growth, which may further enhance the water deficit. Given this sensitivity of root growth to water stress, the stomatal sensitivity is necessary for water retention during water stress and, thus, for survival. The decrease in XPP at 1.0 MPa SOPD, which was not accompanied by a decrease in water content of the same magnitude, suggests that some degree of osmotic adjustment or change in cell wall elasticity may be occurring.

A negative relationship between nitrogen fertilization and drought resistance in lodgepole pine seedlings was not well established in my experiment, despite the evidence for it in other studies of pine (Pharis and Kramer 1964, Etter 1969). In my experiment, seedlings fed at high N rates generally had lower stomatal conductances and higher shoot:root ratios, which would promote water conservation and acquisition. However, in contrast to these benefits, these seedlings also had less negative xylem pressure potentials, i.e., they did not maintain as large a water potential differential between the solution and the seedling as those fed low N rates, and their water content dropped with increasing osmotic stress in all the N Ra treatments (Fig. II-4). It is not clear whether high rates of N supply have a net positive or negative effect on lodgepole pine seedling drought resistance.

The relationship between foliar N concentration and Rg held only for seedlings which were not osmotically stressed. The correlation between N supply and internal N concentration was maintained in all treatments, although both levels of PEG-induced osmotic stress diminished the foliar N concentration by about 6 % (Figure 5); however, growth rate during the period of PEG treatment was controlled by the level of osmotic stress and not by N (Table II-1). The decrease in Rg could be attributed in part to the reduction in gas exchange, but this does not entirely account for the lower Rg at the 1.0 MPa, compared to the 0.5 MPa SOPD level. Between 0.5 and 1.0 MPa SOPD, changes in osmotic potential or wall elasticity, as postulated above, or some other metabolic change could have increased the maintenance respiration requirements in the seedlings, and thus lowered Rg. In addition to the marked effect of osmotic stress on stomatal conductance (Figure II-4), either the osmotic stress or the PEG itself inhibited the uptake and/or transport of N, but had no effect on P, K and Mg (Table II-3). It is not known what relationship exists between internal N and growth rate in this case, as internal N concentration is not driving growth rate (Ingestad 1982), and N is not taken up in response to the demands of growth.

Table II-1. Results of ANOVA of nitrogen addition rate and PEG-induced osmotic stress on growth of lodgepole pine seedlings. Each effect was tested against the mean square for error (MSE). The 3 interaction terms between run and treatments are pooled where there were no significant differences, and presented separately where different.

Variable	Effect	DF	F	p
Rg (pre-PEG)	Run	2	25.42	0.0001
	N Ra	3	19.49	0.0001
	Run*N Ra	6	1.69	0.1433
MSE=136.65		48		
Rg (pre-harvest)	Run	2	1.53	0.2371
	N Ra	3	1.54	0.2290
	SOPD	2	117.30	0.0001
	N Ra*SOPD	6	3.09	0.0219
	Run interactions	22	0.79	
MSE=928.41		24		
Dry weight change	Run	2	17.92	0.0001
	N Ra	3	29.02	0.0001
	SOPD	2	18.90	0.0001
	N Ra*SOPD	6	1.31	0.2919
	Run*N Ra	6	2.87	0.0297
	Run*SOPD	4	0.37	0.8274
	Run*N Ra*SOPD	12	0.53	0.8723
MSE=$1.7276*10^{-2}$		24		
Leaf:root mass ratio	Run	2	136.21	0.0001
	N Ra	3	118.62	0.0001
	SOPD	2	10.71	0.0010
	N Ra*SOPD	6	3.79	0.0141
	Run interactions	20	3.32	
MSE=$9.2373*10^{-3}$		17		

Table II-2. Results of ANOVA of nitrogen addition rate and PEG-induced osmotic stress on the water relations of lodgepole pine seedlings. Each effect was tested against the mean square for error (MSE). The 3 interaction terms between run and treatments are pooled where there were no significant differences, and presented separately where different.

Variable	Effect	DF	F	p
Water content	Run	2	2.66	0.0906
	N Ra	3	1.57	0.2225
	SOPD	2	125.47	0.0001
	N Ra*SOPD	6	1.14	0.3675
	Run interactions	22	0.59	
MSE=12.802		24		
Xylem pressure potential	Run	2	9.51	0.0013
	N Ra	3	3.81	0.0261
	SOPD	2	23.06	0.0001
	N Ra*SOPD	6	0.30	0.9300
	Run interactions	22	0.95	
MSE=$2.7354*10^{-3}$		20		
Stomatal conductance	Run	2	4.51	0.0217
	N Ra	3	3.57	0.0289
	SOPD	2	335.50	0.0001
	N Ra*SOPD	6	2.25	0.0727
	Run interactions	22	1.49	
MSE=$1.3096*10^{-4}$		24		

Table II-3. Results of ANOVA of nitrogen addition rate and PEG-induced osmotic stress on the mineral nutrient content of lodgepole pine seedlings. Each effect was tested against the mean square for error (MSE). The 3 interaction terms between run and treatments are pooled where there were no significant differences, and presented separately where different.

Variable	Effect	DF	F	p
Foliar [N]	Run	2	27.46	0.0001
	N Ra	3	51.70	0.0001
	SOPD	2	5.35	0.0120
	N Ra*SOPD	6	0.40	0.8712
	Run interactions	22	1.01	
MSE=0.87026		24		
Foliar [P]	Run	2	6.18	0.0068
	N Ra	3	3.98	0.0197
	SOPD	2	1.57	0.2296
	N Ra*SOPD	6	0.45	0.8358
	Run interactions	22	0.32	
MSE=$3.8242*10^{-4}$		24		
Foliar [K]	Run	2	0.31	0.7334
	N Ra	3	8.22	0.0006
	SOPD	2	1.05	0.3641
	N Ra*SOPD	6	0.58	0.7399
	Run interactions	22	1.35	
MSE=$1.6359*10^{-3}$		24		
Foliar [Mg]	Run	2	11.04	0.0004
	N Ra	3	8.48	0.0005
	SOPD	2	0.45	0.6420
	N Ra*SOPD	6	0.29	0.9364
	Run interactions	22	0.92	
MSE=$8.913*10^{-5}$		24		

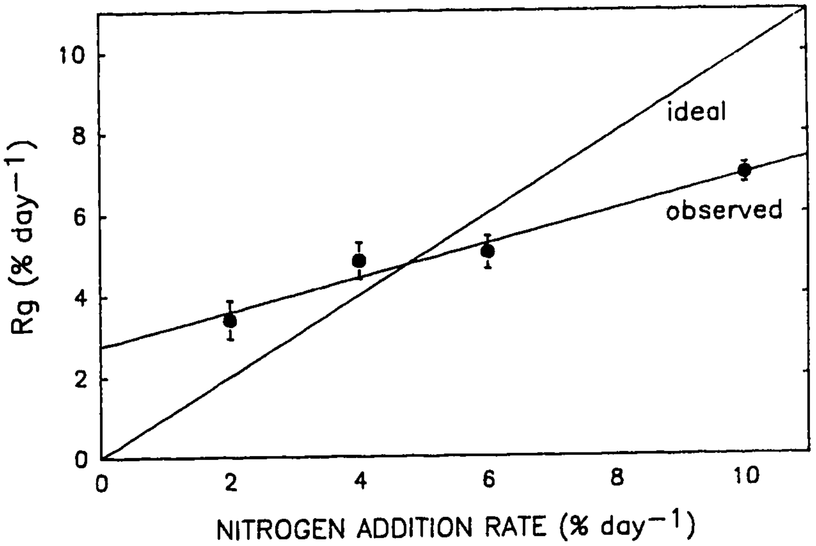

Fig. II-1. Steady-state relative growth rates (FW basis) of lodgepole pine seedlings grown at 4 exponential N addition rates in solution culture compared with the ideal relationship (Ingestad 1982). Data represent means and standard errors from 15 replicates.

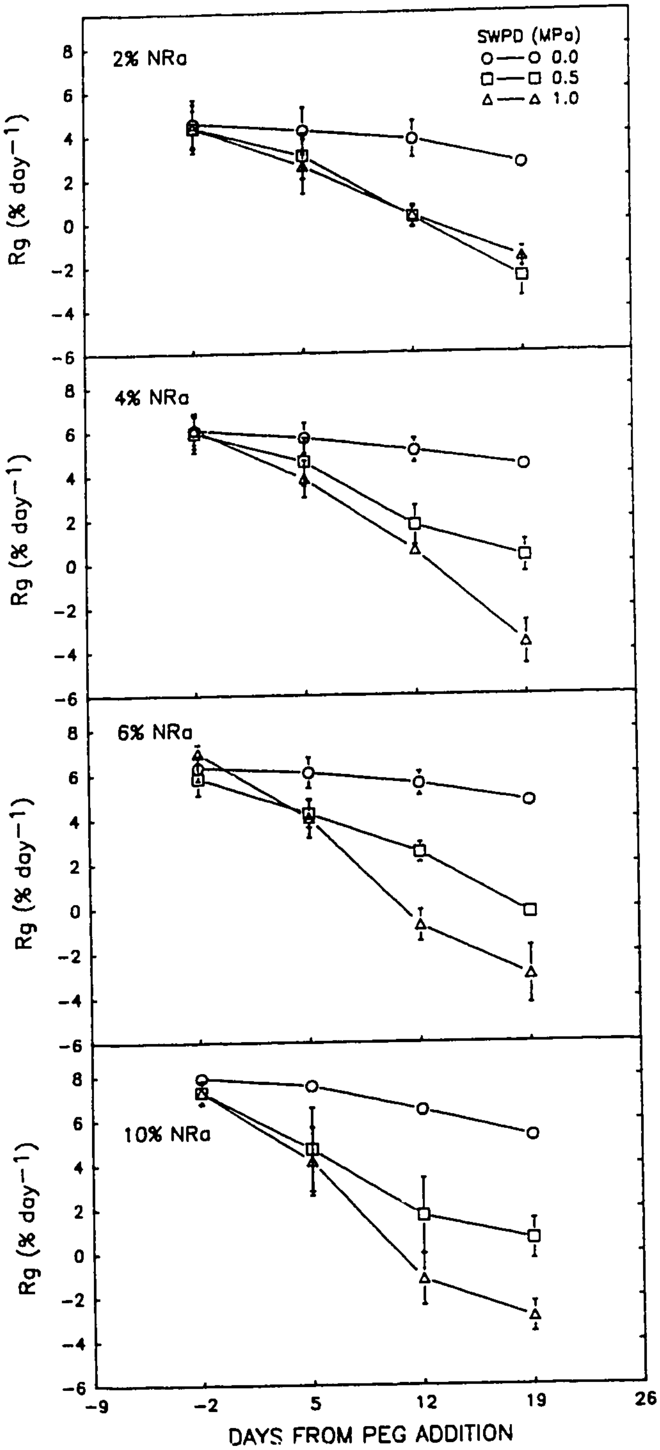

Fig. II-2. Relative growth rates (DW basis) of lodgepole pine seedlings grown in solution culture at 4 exponential N addition rates and 3 levels of water potential. PEG 8000 was administered on days 0, 2 and 4. Data represent means and standard errors from 5 replicates.

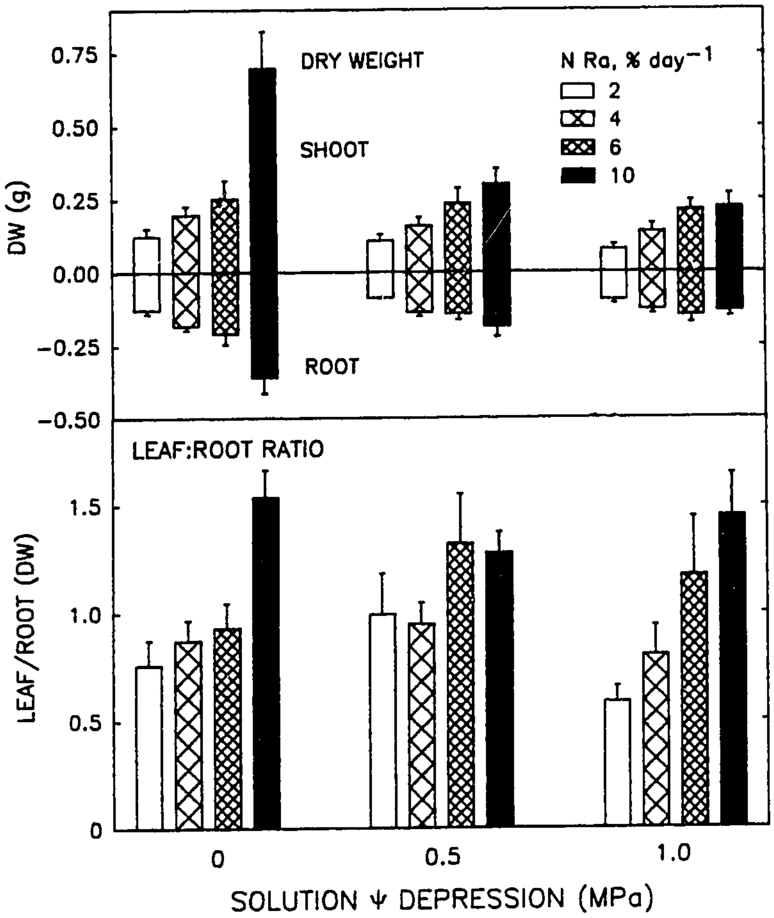

Fig. II-3. Shoot and root biomass, and leaf:root mass ratio of lodgepole pine grown in solution culture at 4 exponential N addition rates and exposed to 3 levels of osmotic stress for 2-1/2 weeks. Data represent means and standard errors from 5 replicates.

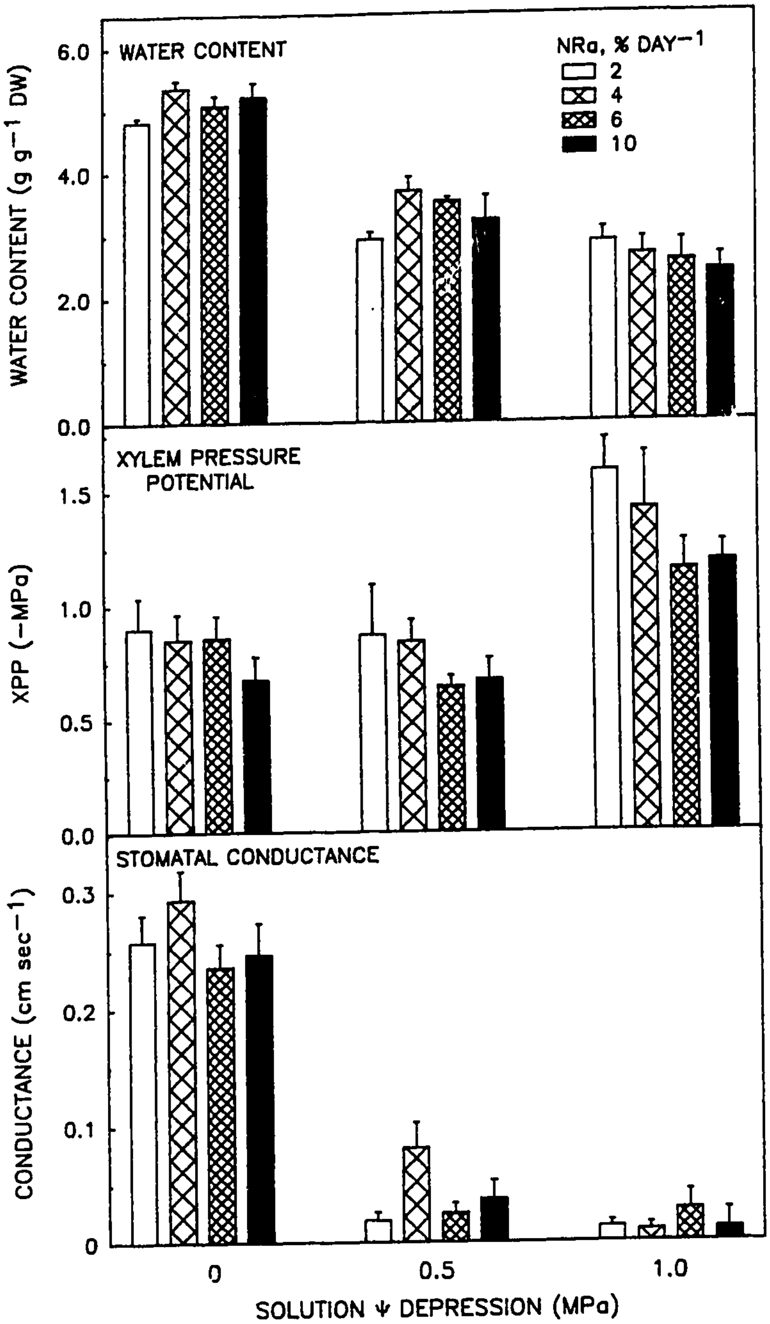

Fig. II-4. Seedling water content, pre-dawn xylem pressure potential, and stomatal conductance of lodgepole pine grown in solution culture at 4 exponential N addition rates and exposed to 3 levels of osmotic stress for 2-1/2 weeks. Data represent means and standard errors from 5 replicates.

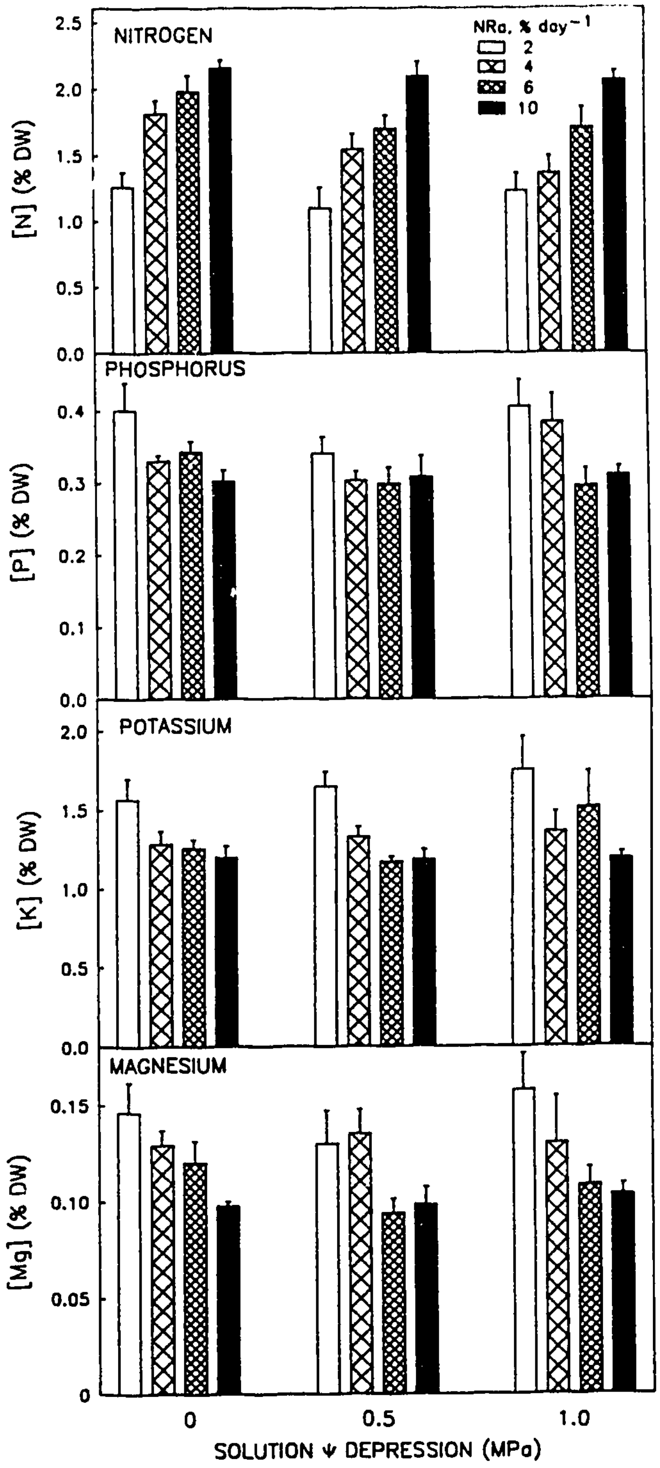

Fig. II-5. Foliar nutrient concentrations in lodgepole pine grown in solution culture at 4 exponential N addition rates and exposed to 3 levels of osmotic stress for 2-1/2 weeks. Data represent means and standard errors from 5 replicates.

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CHAPTER III. PRECONDITIONING LODGEPOLE PINE SEEDLINGS WITH NITROGEN AND OSMOTIC STRESS

INTRODUCTION

Work in the past few decades has shown that various kinds of temporary preconditioning stress treatments in the nursery can improve seedling physiological and morphological condition, and therefore field performance (see Duryea and Brown 1984 for review). Preconditioning water stress reduces transpiration and mesophyll resistance, and increases water use efficiency in loblolly pine (*Pinus taeda* L.; Seiler and Johnson 1988), enhances root regeneration and reduces duration of internal water stress after transplanting of Corsican pine (*Pinus nigra* Arn. ssp *laricio* Poiret var. Corsicana) and Cedar of Atlas (*Cedrus atlantica* Manetti; Kaushal and Aussenac 1989), and improves turgor maintenance of black spruce (*Picea mariana* Mill. BSP) under water stress (Zwiazek and Blake 1989). Enhanced nitrogen supply in the nursery improves growth and survival of outplanted conifer seedlings, though superoptimal fertilization reduces survival and growth of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (van den Driessche 1980, 1982).

Nitrogen nutrition can also moderate the effects of water stress. In shoots of *Zea mays* L., relative water content and water potential are higher when grown at lower N fertilization rates (Tesha and Eck 1983). In loblolly pine, nitrogen concentrations that are 'optimal' under no water stress confer the greatest drought resistance, and greater or lesser concentrations of nitrogen reduce drought resistance (Pharis and Kramer 1964).

Lodgepole pine is an important commercial tree species of dry and nutrient-poor sites in western North America

(Lotan and Perry 1983). Nitrogen fertilization of lodgepole pine nursery seedlings increases shoot growth but not survival after transplanting (van den Driessche 1982), likely due to the negative effect of a perhaps superoptimal N fertilization on seedling drought resistance (Etter 1969). Nitrogen addition rate and water stress acted independently on water relations and nutrient status, but not on growth, of lodgepole pine seedlings (chapter II).

In this study I grew lodgepole pine seedlings in controlled environment solution culture to simulate the transition from one set of conditions to another set, such as occurs when nursery seedlings are transplanted into field sites. The effects of initial preconditioning treatments were evaluated under various treatment combinations in a second, subsequent phase in which N and osmotic stresses were used to simulate stress on a planting site. Controlling N availability by using relative addition rate (Ingestad 1982) offers many advantages over the traditional method of imposing N stress by withholding N fertilizer, because it allows for constant stress, and does not result in depletion of seedling N content. My objective was to determine the effect of the preconditioning nitrogen relative addition rate and polyethylene glycol-induced osmotic stress on the subsequent growth, water relations and nutrient status of the seedlings during the subsequent evaluation phase.

MATERIALS AND METHODS

Species and conditions

Lodgepole pine seeds from northwestern Alberta were germinated in washed fine silica sand in a growth chamber using a 16 h photoperiod, a photosynthetically-active photon flux density (PPFD) of 380 - 550 μmol m^{-2} s^{-1}, 30/20^{o}C day/night temperature regime, and relative humidities between 90 and 100%. Trays were misted twice daily with tap water until roots were established, and then every second day.

Seedlings were transplanted to solution culture pots when roots were long enough to reach the nutrient solution (approx. 9 weeks after planting), and acclimated for 2 weeks before N addition treatments began. Solution culture pots were 21 cm diam., 4.55 l dark brown plastic pails fitted with white plastic lids with 4 slots to hold seedlings supported by closed-cell foam collars. Compressed air was bubbled through an airstone. An inverted polypropylene funnel above the airstone minimized splashing, foaming and root disturbance. Pots were filled with 4.1 l of quarter-strength Long Ashton no-N formulation (Hewitt 1966), modified to provide constant proportions of mineral nutrients in optimal ratios for lodgepole pine (Ingestad and Kahr 1985). This contained 107.18 mg l^{-1} KH_2PO_4, 25.09 mg l^{-1} K_2SO_4, 18.50 mg l^{-1} $CaCl_2$, 16.05 mg l^{-1} $MgSO_4$, 11.21 mg l^{-1} MicroMix (chelated micronutrient mixture, Plant Products Co. Ltd., Bramalea, Ont.), adjusted to pH 5.5. Distilled water was added weekly to replace evaporation and transpiration losses. Nutrient solutions were normally replaced when treatment conditions changed (2 and 6 week intervals), but were also replaced at other times to prevent buildup of algae, and root exudates and dead material. The growth room had a 16 h photoperiod, with a PPFD of 400 to 550 μmol m^{-2} s^{-1}, and day/night humidities and temperatures of 60/70% RH and $25^{o}/20^{o}C$. Pot position was randomized twice weekly.

Treatments

The treatment structure of the experiment was a 2^4 full factorial combination of nitrogen relative addition rate (N Ra) and solution osmotic potential depression (SOPD), crossed in two sequential phases. The initial, or preconditioning, phase was a 2 by 2 factorial design, with 4 and 8 % day^{-1} N Ra and 0 and 0.4 MPa SOPD. In the second, or evaluation, phase, seedlings from each of the four first phase treatment combinations were divided among the 2 by 2 factorial treatments of 2 and 4 % day^{-1} N Ra and 0.4 and 0.8 MPa SOPD (Fig. III-1). Each of the 16 treatment combinations was replicated twice in each of two sequential runs.

Preconditioning N treatments began when seedlings were 11 weeks old, and lasted until they were 22 weeks old. Seedling internal nitrogen content and growth rate adjusted to the N Ra treatments until steady-state nutrition conditions had been achieved. Polyethylene glycol (PEG) 8000 was added during the 19th week and removed when seedlings were 20 weeks old. Second phase N Ra treatments started when seedlings were 22 weeks old, and PEG was added during the 27th week; both continued until the seedlings were harvest at 28 weeks old.

Seedlings were weighed each week, and the amounts of NH_4NO_3 to be added to each pot in three aliquots per week were calculated according to the following equation:

$$A_N=N_i(e^{Ra*Ta})(e^{Ra*Tb}-1) \qquad [1]$$

where A_N is the mass of nitrogen to be added, N_i is the estimated internal nitrogen content (0.3 % of fresh weight; Ingestad and Kahr 1985) at the time of weighing of the four seedlings per pot, Ra is the treatment variable relative addition rate (2, 4, or 8 % day^{-1}), Ta is the time in days between weighing and nitrogen addition, and Tb is the time in days between present and subsequent additions (adapted from Ingestad 1982). The other nutrients were added at the same time, in the proportion to N as suggested in Ingestad and Kahr (1985). Relative growth rate (Rg, expressed as % day^{-1}) was calculated from the weekly fresh weight measurements as:

$$Rg=(\log FW_2-\log FW_1)/(T_2-T_1) \qquad [2]$$

where FW_2-FW_1 is seedling fresh weight difference between weighings, and T_2-T_1 is the number of days between weighings.

The mass of PEG required to achieve the SOPD (151 g L^{-1} for -0.4 MPa, and 208 g L^{-1} for -0.8 MPa) was calculated using equation 1 of Michel (1983). Flaked PEG was added without stirring to the pots in 6 equal aliquots over 6 days. Total dissolution, and hence the total osmotic effect of each PEG aliquot, was achieved after approximately 24 hours. The osmotic contribution of the nutrient ions was minute; the largest aliquot of N added (44 mg, in the last week of the experiment) was calculated to reduce osmotic potential 0.64 kPa, a negligible amount. Solution aeration was checked with a YSI model 51B dissolved oxygen meter, and was > 5.0 mg O_2 l^{-1} in PEG solutions compared to 7.2 mg O_2 l^{-1} in nutrient solution without PEG.

Senescent seedlings (8 total) were removed from the experiment, and mean values per pot were calculated from the remaining, healthy seedlings.

Stomatal conductance, based on a fixed leaf area, was measured on 1 seedling per pot with a LI-COR 1600 steady-state porometer and conifer cuvette (LI-COR Inc., Lincoln, Nebraska), at approximately 1000 h on the day before harvest. Leaf areas were estimated from dry weight measurements, using a relationship determined for needle dry weight and surface area in non-experimental seedlings grown under the same conditions (glass bead method, Thompson and

Leyton 1971). Pre-dawn xylem pressure potential (XPP) was measured on harvest day on the same seedlings (Ritchie and Hinckley 1975).

All seedlings were harvested and weighed fresh and after drying for 48 hours at 70°C. Dried leaf tissue was ground with mortar and pestle, digested with H_2SO_4 and H_2O_2 (Thomas et al. 1967), and analysed for total N and P using standard autoanalyser methods (Technicon Industrial Method No. 334-74W/B+, 1977), and for K and Mg using a Perkin-Elmer model 500 atomic absorption spectrophotometer (McKeague 1978).

Analysis

The experimental design was a randomized complete block, with all treatment combinations replicated twice in each of two blocks (runs). The observed responses of the seedlings (y) at harvest were a linear function of the run (random effect) and the first and second phase N Ra (N) and SOPD (O) treatment (fixed effects):

$$\begin{aligned} y = {} & \text{Grand mean} + \text{run} + N1 + O1 + N2 + O2 \\ & + 2 \text{ way interactions} + 3 \text{ way interactions} \\ & + 4 \text{ way interactions} + 5 \text{ way interaction} + \text{error} \end{aligned} \qquad [3]$$

The data were analysed using the SAS ANOVA procedure as a 5-way factorial ANOVA, using variation among pots within treatments and runs as the error variance. Data were transformed as necessary to minimize heterogeneity of variance before ANOVA.

RESULTS

There were no significant interactions among the treatments i.e., the effects of the treatments on the measured characteristics were only additive. Significant preconditioning effects indicate that responses to these treatments persisted through the second phase, independent of the second phase treatments. Treatment effects were also independent of run (i.e., there were no significant interactions between run and treatments in any of the analyses). I therefore present the results as main effects only, with data pooled from the two runs.

In 26-week-old seedlings, just before the second PEG addition, relative growth rate was controlled by the second phase nitrogen relative addition rate (N Ra) (2.6 and 3.0 % day^{-1} relative growth rate (Rg), at 2 and 4 % day^{-1} N Ra, respectively; Fig. III-2C). In 28-week-old seedlings, osmotic stress reduced Rg to about 0.5 % day^{-1} at 0.4 MPa and to about 0 % day^{-1} at 0.8 MPa solution osmotic potential depression (SOPD), overwhelming all the other treatment effects (Fig. III-2D). In the week between (i.e., week 27), as control of Rg passed from N supply to osmotic stress, significant effects of both preconditioning N Ra and SOPD on Rg were seen in the first week of the final osmotic stress (Fig. III-2A and III-2B). Though Rg of all seedlings was reduced by the second phase osmotic stress, those seedlings which had experienced a preconditioning osmotic stress maintained, on average, 0.52 % day^{-1} higher Rg in week 27 compared to the previously unstressed seedlings (Fig. III-B). Seedlings preconditioned at the 8 % day^{-1} N Ra had, on average, 0.34 % day^{-1} lower Rg at week 27 than those preconditioned at 4 % day^{-1} (Fig. III-2A).

At harvest, shoot dry weight response was the summation of the main effects of second phase N Ra, and preconditioning N Ra and SOPD (Table III-1). Lower N Ra resulted in lower dry weights at harvest, as did preconditioning osmotic stress (Table III-1). The lack of effect of second phase SOPD may have been due to insufficient time for a significant response, because growth rates were low at both SOPD levels. Root dry weight was not affected by second phase treatments; however, seedlings grown at the lower levels of both preconditioning N Ra and SOPD had resulted in about 800 mg less root DW compared with the less stressed seedlings (Table III-1). Roots responded more than shoots to osmotic stress, and less to N supply (see the proportional changes in Table III-1). This resulted in shoot:root ratio increasing with both the preconditioning and second phase N Ra, and with preconditioning osmotic stress (Table III-1). The lack of response of shoot and root dry weight to second phase SOPD was also seen in a lack of response in shoot:root ratio. There were no significant interaction effects for either DW or shoot:root ratio.

Water content at harvest, was reduced by the preconditioning osmotic stress treatments from 3.56 to 3.29 g g^{-1} DW (for 0.0 and 0.4 MPa, respectively), and by the second phase osmotic stress from 3.60 to 3.25 g g^{-1} DW (for 0.4 and 0.8 MPa, respectively) (Table III-2). Pre-dawn XPP decreased from -0.58 MPa to -0.90 MPa at the second phase 0.4 and 0.8 MPa SOPD levels at the time of harvest, and showed no other treatment effects (Table III-2). Stomatal conductance at 0.8 MPa SOPD was half that at 0.4 MPa (0.37 and 0.78 mm s^{-1}, respectively; Table III-2), and showed no effect of preconditioning treatments.

In 28-week-old seedlings. Foliar N concentration was higher (1.84 % DW) in seedlings grown in the second phase N Ra of 4 % day^{-1} than in those grown at 2 % day^{-1} (1.63 % DW) (Table III-3). Foliar N was similarly affected by preconditioning SOPD (1.81 and 1.65 % DW, respectively at 0.0 and 0.4 MPa SOPD; Table III-3). Foliar P concentration responded negatively to preconditioning N Ra (0.204 % and 0.186 % DW, respectively, at 4 and 8 % day^{-1}; Table III-3).

Foliar Mg concentration was reduced from 0.16 to 0.14 by increasing the second phase N Ra from 2 to 4 % day^{-1}; however, it also responded negatively to preconditioning N Ra, and positively to preconditioning osmotic stress (Table III-3). Foliar K concentration averaged 0.97 % DW, and was not significantly affected by any of the experimental treatments (data not shown).

DISCUSSION

Preconditioning effects of nitrogen relative addition rate (N Ra) and osmotic stress (solution osmotic potential depression, SOPD), or both, were observed to persist at least 6 weeks in most physiological and biomass characteristics of lodgepole pine seedlings examined. However, the effects of N supply and osmotic stress acted independently of each other. Also, there were no interactions between preconditioning and second phase treatments. Given the independence of N Ra and SOPD, their effects on seedling growth and physiology can be discussed separately.

Preconditioning lodgepole pine seedlings with reduced (suboptimal) rates of N supply produced smaller seedlings, but these had higher concentrations of P and Mg, lower shoot:root ratios, and higher growth rates under initial periods of water stress. These characteristics, especially the latter two, could contribute to the performance of outplanted lodgepole pine seedlings, and could be especially important during periods of water and/or nutrient stress. An inverse relationship of N fertilization and drought resistance has been observed in loblolly and lodgepole pines (Pharis and Kramer 1964, Etter 1969, respectively). Van den Driessche (1982) found nursery fertilization improved field growth, but not survival of outplanted lodgepole pine seedlings. The difference between his results and ours may stem from greater and more varied stresses occurring in the field, compared to the controlled environment conditions of this experiment. As well, the results of preconditioning with N applied at relative rates may not be directly comparable to preconditioning with constant rates; Troeng and Ackzell (1988) reported that shoot growth of transplanted Scots pine (Pinus sylvestris L.) seedlings were increased by higher N Ra, but not by the greatest total amount of N when applied at a constant rate.

Preconditioning with water stress produced smaller seedlings with lower water content and foliar N concentration, and with higher shoot:root ratios. These characteristics would normally contribute to decreased drought resistance; however, as with N preconditioning, relative growth rate (Rg) was higher during the first week of water stress at the end of the experiment. Although this growth effect was small and transitory, seedlings in the field experience multiple drought cycles between rains, and the cumulative effect over the whole growing season of maintaining even slightly higher Rg could be a considerable advantage. Others have also reported transitory effects (on the scale of days) of water stress preconditioning, e.g., shoot water potential in radiata pine (Pinus radiata D. Don) (Squire et al. 1987), transpiration in loblolly pine (Seiler and Johnson 1988) and radiata pine (Rook 1972), and stomatal conductance, photosynthetic rate and shoot water potential in black spruce (Picea mariana Mill. B.S.P.; Zwiazek and Blake 1989). However, Aussenac and El Nour (1985) and Rook (1972) reported stimulated root growth up to 40 days after transplanting of water stress preconditioned Corsican pine and radiata pine.

The effects of preconditioning N Ra and osmotic stress on the DW of both shoots and roots at the end of the experiment can be attributed largely to the fact that biomass accumulates. The response to early conditioning treatments was therefore still in existence at the time of measurement, and the residual effect shows that this response was not overwhelmed by later treatments. Of greater interest are those preconditioning effects which persist after the conditioning treatment. Such residual effects on physiological variables are the result of physiological or developmental changes induced during the preconditioning treatment. In my study, preconditioning N Ra and SOPD had such a residual effect on Rg, water content, and foliar nutrient concentrations. Since Rg tends to decrease with size, the enhanced Rg may be due to the smaller size of low N Ra or osmotic stress preconditioned seedlings. Persistant preconditioning effects on non-cumulative characteristics, such as water content or nutrient concentration, cannot be explained in this same way.

Nitrogen deficient plants exhibit xeromorphic characteristics (Stocker 1960), including such structural characteristics as smaller cell size and thicker walls (Shimshi 1970, Radin and Parker 1979). Decreased cell size and greater wall thickness increases the osmotic fraction of total water volume and cell density, both of which improve plant water relations, particularly water conservation under water stress (Cutler _et_ _al_. 1977). Stress reduces growth rate in white pine and produces smaller cells (Schomaker 1969). Smaller cell size could account for the observed persistence of reduced water content and foliar N concentration in lodgepole pine. Lower N concentration could be due to the lower proportion of N-rich cell contents to (relatively) N-poor cell wall (Schomaker 1969). Etter (1969) reported that water content of lodgepole pine seedlings increased with increased nitrate in the nutrient solution, from 0.4 to 4.0 meq l^{-1}. Similarly, the higher initial growth rate under the initial period of osmotic stress (week 27) of seedlings which were preconditioned with osmotic or N stress could be attributed to this same anatomical change. Tibbits and Bachelard (1981) found that drought resistance was enhanced by reduced fertilization in *Angophora costata* Britten and by water stress in *Banksia serrata* L. f. They suggested that reduced growth rate itself, rather than the particular treatments used, was the critical factor in drought resistance. This could have been a cell structure change initiated by either nutrient and water stress. This hypothesis that N and water stress alter cell structure is consistent with the literature and with the present results, but requires experimental corroboration.

My results demonstrate the potential of lodgepole pine seedlings to be affected by preconditioning water and N stress. Despite its positive effect on Rg, osmotic stress appeared to have some serious drawbacks as a preconditioning treatment for lodgepole pine seedlings (*i.e.*, lower water content and foliar [N], and higher shoot:root ratio). Many studies have demonstrated beneficial effects of water stress preconditioning on the physiology of pines (*e.g.*, Rook 1972, Aussenac and El Nour 1985); however, further study is necessary to determine the cause of these apparent inconsistencies. Such knowledge will assist in the development of water stress regimes for preconditioning lodgepole pine seedlings. Since water stress and fertilization affect partly overlapping sets of seedling characteristics, water stress preconditioning might be best used to modify the results of other treatments, such as fertilization.

My results indicate that N addition rate may be an effective preconditioning agent for lodgepole pine seedlings. In nurseries, high N is supplied during the exponential growth phase, followed by 6 to 7 weeks of low N, high P and K, during the hardening phase of seedling production (Carlson 1983). It is probable that the latter phase of low N would not be long enough to fully precondition developing tissue, since the combination of nutrient, temperature and photoperiod regimes inhibits leaf growth and promote bud set and dormancy. However, an intermediate phase of N fertilization at a lower exponential rate, which would replace parts of the growth and hardening phases, would still allow for seedling growth as well as conditioning. If growth rate, anatomy and drought resistance are correlated, then the intermediate phase should be long enough to permit the majority of cells to become preconditioned. Allowing for biomass to double twice during this preconditioning phase would result in preconditioned cells making up at least 75% of the total, and take, for example, 46 days at 3 % day^{-1} Rg. This suggested modification of production schedules requires testing to prove its efficacy, since the effect of the intermediate phase could be altered by the hardening process. In addition, a phase of reduced N Ra during the exponential growth phase will likely alter the hardening process.

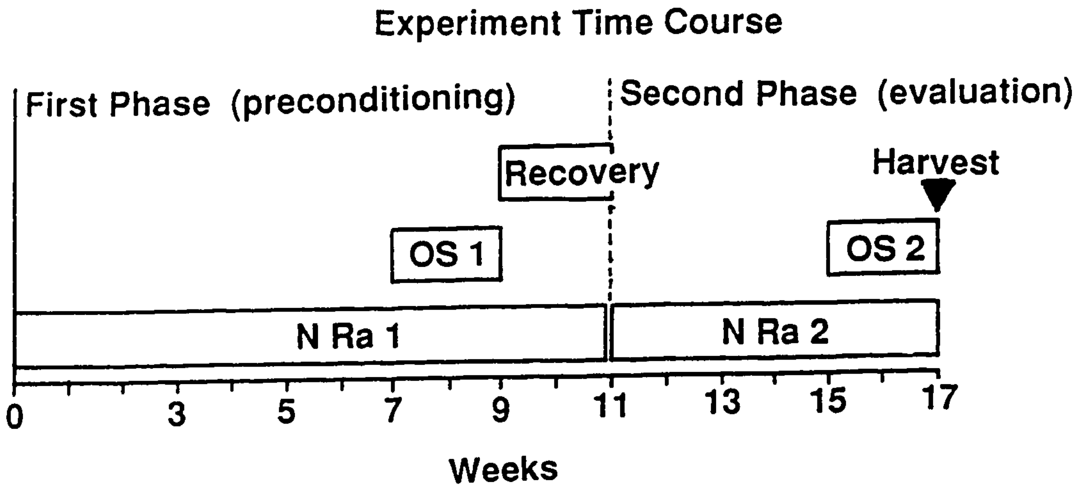

Figure III-1. Time course of the experiment showing the schedule of treatments and the harvest. N Ra is nitrogen relative addition rate, OS is polyethylene glycol-induced osmotic stress. Preconditioning phase treatments are denoted by 1, and second phase treatments by 2.

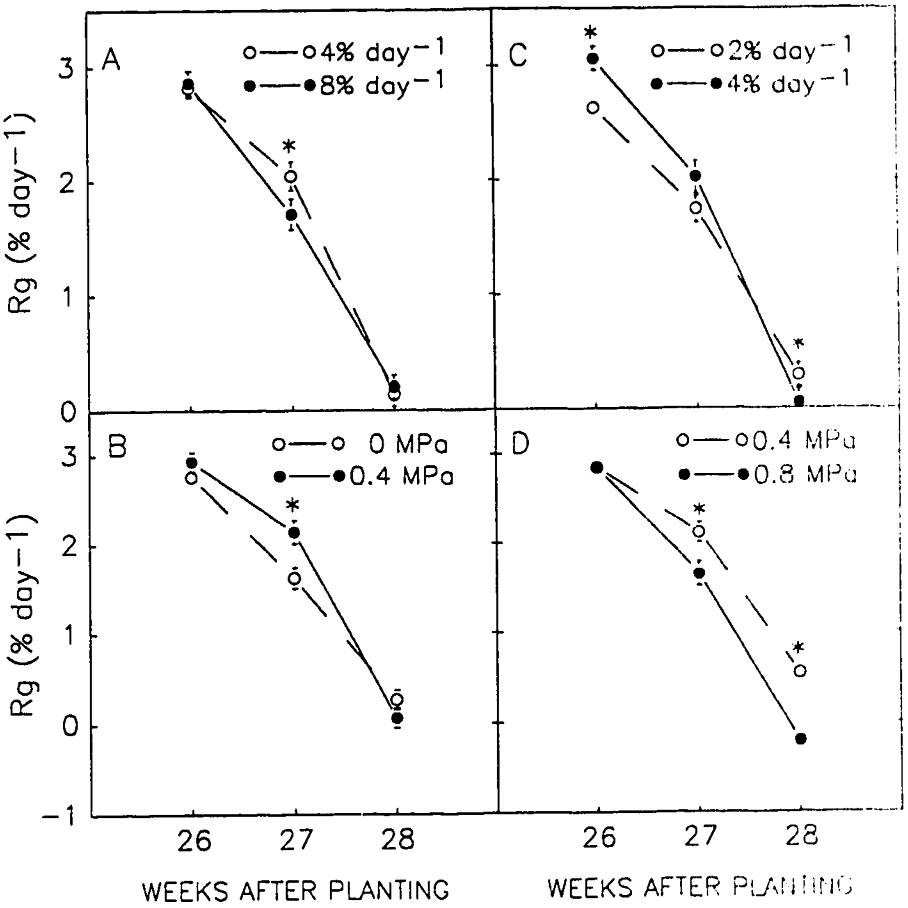

Figure III-2. Relative growth rate during the final 3 weeks of the study of lodgepole pine seedlings which had been preconditioned with nitrogen supply and osmotic stress, and subsequently grown at 2 nitrogen relative addition rates. Polyethylene glycol induced osmotic stress was applied during week 26, and maintained until harvest at the end of week 28. There were no significant interaction effects. Means and standard errors for the main effects are presented: A) first phase nitrogen supply, B) first phase osmotic stress, C) second phase nitrogen supply, D) second phase osmotic stress. Asterisks indicate the weeks in which the difference between the treatment levels is significant at $p \leq 0.05$.

Table III-1. Biomass characteristics (mg) of lodgepole pine seedlings as affected by nitrogen addition rate (N Ra) and PEG-induced osmotic stress (SOPD) applied in two sequential phases. Means and standard errors (n=32), and probability (p) levels from ANOVA. No interactions were significant, and thus are not presented.

Phase	Effect	Level	Mean	S.E.	p
Shoot DW					
first	N Ra	[illegible] day^{-1}	829	86.9	0.0001
		[illegible] % day^{-1}	1646	121.8	
	SOPD	0 MPa	1489	139.6	0.0001
		0.4 MPa	987	97.9	
second	N Ra	2 % day^{-1}	1081	119.0	0.0077
		4 % day^{-1}	1394	131.9	
	SOPD	0.4 MPa	1247	130.3	0.8226
		0.8 MPa	1229	127.1	
Root DW					
first	N Ra	4 % day^{-1}	1097	98.6	0.0001
		8 % day^{-1}	1869	150.7	
	SOPD	0 MPa	1902	145.4	0.0001
		0.4 MPa	1064	97.9	
second	N Ra	2 % day^{-1}	1464	153.	0.7037
		4 % day^{-1}	1502	136.3	
	SOPD	0.4 MPa	1449	139.8	0.7800
		0.8 MPa	1517	149.8	
Shoot:root ratio					
first	N Ra	4 % day^{-1}	0.786	0.036	0.0018
		8 % day^{-1}	0.913	0.034	
	SOPD	0 MPa	0.770	0.035	0.0002
		0.4 MPa	0.928	0.033	
second	N Ra	2 % day^{-1}	0.767	0.035	0.0001
		4 % day^{-1}	0.932	0.032	
	SOPD	0.4 MPa	0.866	0.036	0.3666
		0.8 MPa	0.832	0.037	

Table III-2. Water content (g g^{-1}DW), xylem pressure potential (-MPa), and stomatal conductance (mm sec^{-1}) of lodgepole pine seedlings. Conditions were as for Table 1. No interactions were significant.

Phase	Effect	Level	Mean	S.E.	p
Water content					
first	N Ra	4 % day^{-1}	3.44	0.055	0.7045
		8 % day^{-1}	3.42	0.060	
	SOPD	0 MPa	3.56	0.052	0.0001
		0.4 MPa	3.29	0.051	
second	N Ra	2 % day^{-1}	3.40	0.060	0.3604
		4 % day^{-1}	3.45	0.054	
	SOPD	0.4 MP	3.60	0.052	0.0001
		0.8 MPa	3.25	0.043	
Xylem pressure potential					
first	N Ra	4 % day^{-1}	0.75	0.037	0.3655
		8 % day^{-1}	0.73	0.033	
	SOPD	0 MPa	0.74	0.040	0.8555
		0.4 MPa	0.74	0.029	
second	N Ra	2 % day^{-1}	0.73	0.035	0.3655
		4 % day^{-1}	0.75	0.036	
	SOPD	0.4 MPa	0.58	0.018	0.0001
		0.8 MPa	0.90	0.021	
Stomatal conductance					
first	N Ra	4 % day^{-1}	0.640	0.0721	0.1064
		8 % day^{-1}	0.501	0.0764	
	SOPD	0 MPa	0.636	0.0832	0.1428
		0.4 MPa	0.505	0.0645	
second	N Ra	2 % day^{-1}	0.615	0.0721	0.2940
		4 % day^{-1}	0.526	0.0777	
	SOPD	0.4 MPa	0.775	0.0824	0.0001
		0.8 MPa	0.366	0.0431	

Table III-3. Foliar nitrogen, phosphorus, and magnesium concentrations (% DW) of lodgepole pine seedlings. Conditions were as for Table 1. No interactions were significant.

Phase	Effect	Level	Mean	S.E.	p
Nitrogen					
first	N Ra	4 % day^{-1}	1.71	0.051	0.5290
		8 % day^{-1}	1.75	0.044	
	SOPD	0 MPa	1.81	0.045	0.0124
		0.4 MPa	1.65	0.046	
second	N Ra	2 % day^{-1}	1.63	0.042	0.0011
		4 % day^{-1}	1.84	0.046	
	SOPD	0.4 MPa	1.72	0.044	0.5631
		0.8 MPa	1.75	0.051	
Phosphorus					
first	N Ra	4 % day^{-1}	0.204	0.004	0.0010
		8 % day^{-1}	0.186	0.003	
	SOPD	0 MPa	0.194	0.003	0.7939
		0.4 MPa	0.196	0.005	
second	N Ra	2 % day^{-1}	0.192	0.004	0.2044
		4 % day^{-1}	0.198	0.004	
	SOPD	0.4 MPa	0.200	0.005	0.0877
		0.8 MPa	0.191	0.003	
Magnesium					
first	N Ra	4 % day^{-1}	0.15	0.005	0.0366
		8 % day^{-1}	0.14	0.004	
	SOPD	0 MPa	0.14	0.005	0.0518
		0.4 MPa	0.15	0.004	
second	N Ra	2 % day^{-1}	0.16	0.005	0.0138
		4 % day^{-1}	0.14	0.004	
	SOPD	0.4 MPa	0.15	0.004	0.5399
		0.8 MPa	0.15	0.005	

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CHAPTER IV. LONG-TERM PRECONDITIONING EFFECTS OF NITROGEN ADDITION RATE AND DROUGHT STRESS ON CONTAINER-GROWN LODGEPOLE PINE SEEDLINGS.

INTRODUCTION

Reforestation of cut-over sites relies increasingly upon containerized nursery seedlings. The impact of various nursery conditions and treatments has been the object of much research in the past few decades (Duryea and Brown 1984, Burdett 1990, Margolis and Brand 1990). Two of the most important, and most studied, of these nursery treatments are nitrogen supply and water stress. They have been seldom examined together as simultaneous treatments, to assess their interaction. Individually, their effectiveness as preconditioning agents for improving subsequent survival and performance of seedlings has been studied in many tree species. However, the bulk of this work has looked at short term effects (see Burdett 1990), and the persistence of preconditioning effects over spans of weeks or months is not well known. It has been shown that the effects of preconditioning can change with time (e.g., Rook 1972), and that preconditioning treatments might have different effects on survival than on the subsequent performance of the survivors (van den Driessche 1984).

In this study, I investigated the interaction of N addition rate and drought stress in the preconditioning of container-grown lodgepole pine (_Pinus_ _contorta_ var. latifolia Englm.) seedlings, an ecologically and commercially important tree species of northwestern North America.

MATERIALS AND METHODS

Seeds from a lodgepole pine stand in northwestern Alberta were planted in moist horticultural peat in Spencer-Lamaire Deep 5 containers (2.5x2.5x12.5 cm plug size). Seedlings were placed in a greenhouse in a 18/6 h photoperiod, at 25/15°C, and humidity varying from 35 to 70 %RH. Sodium HID lamps were used to supplement natural lighting as necessary. A polyethylene covered cold frame was initially placed over the trays for the first 3 weeks, to improve humidity and temperature conditions for germination. Seedlings were watered thrice weekly with nutrient solution containing 80 mg l^{-1} NH_4NO_3, 107.18 mg l^{-1} KH_2PO_4, 25.09 mg l^{-1} K_2SO_4, 18.50 mg l^{-1} $CaCl_2$, 16.05 mg l^{-1} $MgSO_4$, 11.21 mg l^{-1} MicroMix (chelated micronutrient mixture, Plant Products Co. Ltd., Bramalea, Ont.). Roots appeared at the bottom of the peat plug (about 5 weeks), at which time the trays were flushed repeatedly with an N-free nutrient solution to reduce residual N in the system prior to the experimental treatments. Tray positions on the bench were randomized each week.

The experiment was a 2^4 full factorial design, with 2 levels of nitrogen addition rate (N Ra; 3 and 6 % day^{-1}) and 2 levels of water stress (well-watered, and cyclical droughted), applied in each of two sequential phases, an initial preconditioning phase and a second evaluation phase. Each drought cycle began with all containers being thoroughly flushed with fertilizer solution, with the intent to replace the exhausted nutrient solution with the new solution. The containers which received the drought treatment were then allowed to dry out until xylem pressure potential (XPP) in seedlings indicated a significant internal water stress (see second phase drought treatment, Table 1), and the peat plug was dry to the touch. To replace water lost through evapotranspiration, without leaching out nutrients, the non-drought containers were allowed to rehydrate completely by taking up deionized water from shallow pans thrice weekly. At the end of each drought cycle, all containers were brought to field capacity by this method. The 4 treatment combinations were used in the preconditioning phase of 3 drought cycles of approximately 12 days each, and crossed with the same 4 treatment combinations in the second phase, comprising three more drought cycles.

Prior to each fertilization, a sample of seedlings were harvested to determine the mean dry weight of the seedlings. Nitrogen addition rates were calculated according to the following equation:

$$C_N = DW*0.02*(e^{Ra*Ta})(e^{Ra*Tb}-1)/V \qquad [1]$$

where C_N is the concentration of nitrogen in the nutrient solution to be added, DW is the mean dry weight of a seedling just prior to fertilization, 0.02 is the approximate internal nitrogen content, Ra is the relative addition rate (3, or 6 % day^{-1}), Ta is the time in days between weighing and nitrogen addition,Tb is the time in days between present and subsequent additions, and V is the estimated volume of solution held in the peat plug (45 ml in this case)(adapted from Ingestad 1982). The nitrogen was added as NH_4NO_3 in the standard nutrient solution mentioned above, which provided the other necessary nutrients in moderate excess.

Samples of seedlings were harvested (after re-watering) at the end of phase 1 (3 drought cycles) on April 21, and at the end of phase 2 (3 more drought cycles) on May 29. Seedlings were weighed fresh and after 48 hours of drying at 70°C. Pre-dawn xylem pressure potential was monitored during each drought cycle, on 4 seedlings periodically during the drought cycle, and on 16 (phase 1) or 32 (phase 2) seedlings on the last day of each drought.

Harvested seedlings were analyzed for foliar N concentration. Dried leaf tissue was ground with mortar and pestle, digested with H_2SO_4 and H_2O_2 (Thomas et al. 1967), and analyzed for total N using standard autoanalyser methods (Technicon Industrial Method No. 334-74W/B+, 1977).

Peats were extracted with KCl and assayed as above. The fertilizer nitrogen did not accumulate in the peats, which contained on average 14 ppm nitrate and 24 ppm ammonium in most treatments after the final drought cyle. This amount of N (about 0.22 mg per peat plug) is 12% or less of the 1.9 to 5.9 mg N per plug added in the fertilizer solution at the beginning of the final drought cycle. Only the peat from the second phase 6 % day^{-1} N Ra, drought treatment which had received 6 % day^{-1} preconditioning N Ra had substantially higher N levels (46 ppm nitrate and 50 ppm ammonium), but this was still only 9 % of the total added in the final fertilization. Mineralization controls (treated indentically to the experimental trays, except that no nitrogen was added) showed peat N concentrations similar to the former experimental peats. Enough N was mineralized from the peat to support the growth of seedlings with an average of 1.3 mg total foliar N. Total foliar N in experimental seedlings ranged from 2.4 to 6.2 mg per seedling. Therefore mineralized N contributed about 21 to 55 % of total N in the experimental seedlings. Although mineralization of peat N did reduce the intended relative difference between the high and low N treatments, this did not confound the N Ra and watering treatments, since the amount of N mineralized was independent of watering level.

Shoots were cut under water from a different sample of seedlings, placed in closed tubes with their stems in deionised water, and stored overnight in the dark at 23°C.

Pressure volume curves were obtained the following day by repeated weighings and water potential measurements as the shoots dried on the bench and in a desiccator (Tyree and Jarvis 1982). Due to the intensity of effort required to produce pressure-volume curves, and the complexity of the experiment, only two subsets of the treatments were analyzed at the second phase harvest. The data was analyzed using Schulte's Pressure-Volume Curve Analysis Program (version 5.2, 8-12-88) which fit linear and non-linear curves to the data and calculated osmotic potentials at saturation and the turgor loss point, relative water content at the turgor loss point point, symplastic fraction and bulk modulus of elasticity from the fitted curves (Schulte and Hinckley 1985).

Bulk leaf samples were also harvested, frozen in liquid nitrogen, and stored at -70°C for later biochemical analysis. Frozen leaf samples (about 200 mg) were ground, first under liquid nitrogen, then with sea sand and methanol-chloroform-water (MCW, 12:5:3/v), extracting 3 times with a total of 6 ml MCW, centrifuging at 10 min at 8,000 g (Dickson 1979). The supernatant was separated into organic and aqueous phases by adding 2 ml chloroform and 1.5 ml water, mixing and centrifuging 20 min at 25,000 g (Haissig 1982). The optical density of the organic phase was read at 663 and 645 nm with a chloroform blank, and chlorophyll concentration calculated from Arnon (1947). The remaining supernatant was heated at 100°C for 2 min, and centrifuged 10 min at 8,000 g. The supernatant was assayed colourimetrically for total alpha-amino N using ninhydrin, standardized to l-alanine, with absorbance read at 570 nm (Rosen 1957); and for total reducing sugars using dinitrosalicylate, standardized to D-glucose, with absorbance read at 570 nm (Chaplin 1986). For starch determination, the pellet was suspended in 3.0 ml of 0.2 M acetate buffer (pH 4.7) and 1.0 ml of amyloglucosidase

(Boehringer Mannheim; 30 Units l^{-1}) in 0.2 M acetate buffer (pH 4.7), capped with a marble and digested at 50°C for 3 hours. Aliquots were removed, heated at 100°C for 2 min, centrifuged at 12,000g for 5 min in a microcentrifuge, and the supernatant assayed for glucose residues of starch using the glucose oxidase - peroxidase method, standardized to D-glucose, with absorbance read at 450 nm (Sigma method 510; Sigma Diagnostics, St. Louis)

Results were analyzed by 2-way and 4-way analysis of variance using the ANOVA procedure in SAS (SAS Institute, Cary, NC). The observed responses of the seedlings (y) at the final harvest were a linear function of the preconditioning and second phase N Ra (N) and watering (W) treatment effects:

$$\begin{aligned} y = {} & \text{Grand mean} + N1 + W1 + N2 + W2 \\ & + \text{2-way interactions} + \text{3-way interactions} \\ & + \text{4-way interactions} + \text{error} \end{aligned} \qquad [2]$$

The analysis model for the first harvest results did not include second phase treatment effects, or 3- and 4-way interactions.

RESULTS

Maximum pre-dawn xylem pressure potential (XPP) of seedlings during the last drought cycle of both the first and second phases shows the effect of withholding water as a treatment. The last drought cycle of the preconditioning phase reduced XPP from -0.17 MPa (unstressed) to -1.03 MPa (stressed)(Table IV-1). The last drought cycle of the second phase reduced XPP from -0.22 MPa to -1.51 MPa (Table IV-1). The differences were likely due to the seedlings being at different developmental stages; the larger seedlings encountered greater water stress due to their greater demand. The effect of preconditioning N Ra on XPP during the last drought cycle of the experiment showed up both as a main effect and in interaction with second phase water regime (Table IV-1). Seedlings grown with the higher preconditioning N Ra experienced greater water stress under the drought conditions than those preconditioned with the lower rate.

Water content of rehydrated seedlings at the first phase harvest was not affected by drought, but leaf water content was 0.29 g g^{-1} DW lower and root water content was 0.21 g g^{-1} DW lower in seedlings grown at 3 % day^{-1} N Ra than at 6 % day^{-1} N Ra (Table IV-1). At the second phase harvest, leaf water content was also reduced by 0.30 g g^{-1} DW in the lower N Ra treatment, and the effect of preconditioning N Ra also persisted, reducing leaf water content by 0.13 g g^{-1} DW at the lower N Ra. Despite the lack of an effect of the current moisture conditions, at the end of the experiment seedlings which had been preconditioned with drought stress maintained 0.11 g g^{-1} DW higher leaf water content.

In the second phase harvest, root water content of rehydrated seedlings was about 1.5 g g^{-1} DW higher than first harvest root water contents (Table IV-1), as well as higher than leaf water content. Preconditioning water stress resulted in a 0.49 g g^{-1} DW decrease, and second phase water stress a 1.73 g g^{-1} DW decrease in root water content at the second phase harvest. However, both preconditioning N Ra and drought interacted with second phase drought, whereby seedlings preconditioned at 3 % day^{-1} N Ra or with drought had lower root water content (by 0.65 and 1.01 g g^{-1} DW, respectively) in the second phase moist treatment (Table IV-1).

Mean seedling relative growth rate (Rg) during the first phase was 6.5 % day^{-1} and 6.7 % day^{-1} for the 3 and 6 % day^{-1} N Ra treatments, respectively, and not affected by the watering treatments (Table IV-2). Mean Rg during the second phase was greater at the higher levels of N and water supply used during that time (Table IV-2). The preconditioning treatments affected the magnitude of the difference. The 6 % day^{-1} preconditioned seedlings had much higher Rg (1.62 % day^{-1}) when they also received preconditioning drought stress, but a lower Rg (0.99 % day^{-1}) without preconditioning drought stress (Table IV-2). The 3 % day^{-1} preconditioned seedlings had intermediate Rg (about 1.2 % day^{-1}) regardless of the preconditioning water regime.

Root weight ratio (RWR=root mass/total seedling mass) at the first harvest was not significantly affected by either N Ra or watering treatments (Table IV-2). At the second harvest, however, the partitioning of biomass in the seedlings was primarily influenced by the preconditioning treatments, where preconditioning drought and 6 % day^{-1} N Ra decreased root weight ratio (Table IV-2). Second phase N Ra had the same effect as preconditioning N Ra on root weight ratio.

At the first harvest, foliar N concentration was 0.81 mg g^{-1} DW and 1.03 mg g^{-1} DW in the 3 and 6 % day^{-1} N Ra treatments, respectively, and watering had no effect (Table IV-3). Foliar N concentrations were higher at the second harvest, and varied with both preconditioning and second phase N supply, although the magnitude of effect of preconditioning was less (Table IV-3). The seedlings grown in the second phase 3 % day^{-1} treatments had 1.05 mg g^{-1} DW N, regardless of the preconditioning N Ra. Those grown at 6 % day^{-1} second phase had higher foliar N concentrations (1.70 mg g^{-1} DW) when preconditioned with 6 % day^{-1} N Ra, than when preconditioned with 3 % day^{-1} N Ra (1.48 mg g^{-1} DW; Table IV-3).

Chlorophyll content of the leaves at the first harvest increased from 1.81 mg g^{-1} DW at 3 % day^{-1} N Ra to 2.39 % day^{-1} N Ra at 6 % day^{-1}. At the second harvest, it increased with increased second phase N Ra (2.44 to 3.27 mg g^{-1} DW at 3 and 6 % day^{-1}, respectively) and was unaffected by other treatments (Table IV-3).

Foliar starch concentration was not affected by preconditioning treatments at either harvest, but was decreased about 23 mg g^{-1} DW by second phase treatments of 6 % day^{-1} N Ra and drought stress (Table IV-4). Foliar concentrations of reducing sugars at the first harvest were reduced from 239 mg g^{-1} DW to 179 mg g^{-1} DW by the higher (6 % day^{-1}) preconditioning N Ra (Table IV-4). The effect of the preconditioning N Ra persisted at the same level through the second phase, as seen in the second harvest, but second phase N Ra and all water treatments did not affect reducing sugars.

In the first harvest, foliar alpha-amino N were decreased from 1.97 to 1.42 mg g^{-1} DW by drought stress treatment, and this effect persists to a lesser degree through to the second harvest (Table IV-4). Alpha-amino N concentrations were increased by the high (6 % day^{-1}) second phase N Ra to 1.95 mg g-1 DW, compared with 1.12 mg g-1 DW for the 3 % day^{-1} N Ra second phase treatment (Table IV-4).

Pressure-volume analysis from the first phase harvest showed changes in cell wall elasticity; bulk modulus at 0 turgor was 2.75 MPa in droughted seedlings and 2.03 MPa in non-stressed seedlings, and the slope of the relationship between bulk modulus of elasticity and turgor was steeper in droughted than in non-droughted seedlings (Table IV-5). This indicates that cell walls were less elastic at all positive turgor potentials in the droughted seedlings. Relative water content at turgor loss was higher in drought stressed seedlings (0.74) than in non-stressed seedlings (0.67), and the symplastic fraction was lower (0.73 and 0.84, respectively; Table IV-5). There was no indication of osmotic adjustment since osmotic potential averaged -1.46 MPa at saturation, and -2.34 MPa at the turgor loss point, and neither parameter was significantly affected by the experimental treatments (data not shown). At the second harvest, water relations parameters derived from pressure-volume analysis were tested for effects of the 4 second phase treatments on seedlings which had been preconditioned at 6 % day^{-1} N Ra with no drought, and for the effects of the 4 preconditioning treatments on seedlings growing in the 3 % day^{-1} N Ra plus drought second phase treatment combination. In both cases, no significant differences were found for osmotic potential, either at saturation or at the turgor loss point, nor for cell wall elasticity or relative water content at the turgor loss point. Given the positive results found from the first harvest, the lack of significant results here may be due to the low level of replication in my analysis (n=3).

DISCUSSION

Nitrogen preconditioning effects were found in biomass variables (Rg and RWR), water relations (XPP and leaf water content), and metabolic pools (foliar N and reducing sugars). Drought preconditioning persisted in a somewhat different set of variables (Rg, RWR, leaf and root water content, and foliar alpha-amino N concentration). These preconditioning effects were observed for at least 6 weeks after the end of the treatments. Such long term persistence (on the scale of weeks or longer) of drought preconditioning effects has been observed in _Pinus radiata_ (Rook 1972), and in _Cedrus atlantica_ Manetti and _Pinus nigra_ Arn. ssp.

nigricans Host. (Aussenac and El Nour 1985); of N supply preconditioning effects in Picea sitchensis (Benzian et al. 1974), Pseudotsuga menziesii (van den Driessche 1980, 1984) and Pinus silvestris (Troeng and Ackzell 1988); and of both in P. contorta (chapter III). Such persistence illustrates the potential for modifying the performance of conifer seedlings that survive the initial stress of transplanting.

Only Rg showed an interaction between nitrogen and watering treatments applied simultaneously. I expected an N by water stress interaction in biomass variables, as had been demonstrated in other conifer species (Pharis and Kramer 1964, Schomaker 1969, McClain and Armson 1975, 1976). This interaction could occur in biomass accumulation without an interaction effect on growth rate, and its underlying physiological processes. However, my results showed an interaction effect in Rg, as had my previous work with lodgepole pine seedlings grown in solution culture. The interaction effect on Rg could have been due to drought affecting nutrient availability in the peat (Viets 1972, Timmer and Armstrong 1989), but, since the interaction was also seen in solution culture, and foliar [N] was not subject to an interaction effect, it appears that some internal physiological processes of the seedlings are being affected. Nitrogen x water stress interaction on foliar [N] were reported for loblolly pine (Pinus taeda L.; Pharis and Kramer 1964) and black walnut (Juglans nigra; Dickson 1971). Both of these studies were carried out in solid growth media, which could have led to drought limiting N availability.

Root water content and XPP both showed an interaction between preconditioning N Ra and second phase water supply. Smaller cells produced in seedlings subjected to reduced N supply or drought would have higher turgor and lower water contents when fully hydrated because of their larger surface to volume ratio (Cutler et al. 1977). Similarly, such cells would retain higher water contents under water stress, by virtue of their stiffer walls promoting turgor loss at higher water contents. These characteristics are seen in the results for bulk modulus of elasticity and relative water content at the turgor loss point (Table IV-5), but only in response to water stress and not N Ra. However, if drought had progressed to the point where the majority of cells in seedlings of both high-N and low-N preconditioning treatments were at or near the turgor loss point, then the seedlings which had lost the least amount of water should have the higher water potentials. This was borne out in my XPP results (Table IV-1). Given the fixed volume of peat in the containers, the larger seedlings of the high [illegible] and high moisture treatments likely had a higher demand for the finite amount of water available. However, since the peat was kept moist, and XPP in the watered seedlings remained above -0.3 MPa, it is unlikely that any of the seedlings in the non-drought treatment experienced any water deficit.

My results corroborate Lopushinsky's (1975) assessment of lodgepole pine as being a water conservative species. Its strategy of drought resistance seems to be to conserve water at the expense of net assimilation, i.e., lodgepole pine loses turgor and reduces gas exchange while water content is still relatively high, as seen in high relative water content at turgor loss, cell wall elasticity decreasing with drought (this paper), and stomatal closure at relatively high water potentials (Lopushinsky and Klock 1974, chapter II). This corresponds more closely to a strategy of drought avoidance rather than drought tolerance in the sense of Levitt (1980). The lack of osmotic adjustment does not detract from this model since osmotic adjustment is a means of maintaining water uptake in a drying soil, and not a water conservation mechanism.

Preconditioning N Ra stress also diminished the response of foliar [N] to second phase N Ra treatments. This interaction is difficult to attribute to an artefact of peat culture, and it suggests a plastic change in the nitrogen growth relationship and N metabolism caused by the preconditioning N supply. Lodgepole pine in solution culture showed no interactions in the seedlings between preconditioning and second phase treatments of N Ra or water stress (chapter III).

The extra reducing sugars produced by the first phase 3 % day^{-1} N Ra treatment could have lowered leaf osmotic potential by approximately 0.3 MPa, but this was not observed. It may be that this increase in sugars (predominantly glucose and fructose; Etter 1971, van den Driessche 1978) was compensated by a decrease in inorganic ions, organic acids or other osmotically active compounds (Handa et al. 1983). Also, the assay does not detect sugar alcohols and non-reducing sugars such as sucrose. It is astonishing that this elevated concentration of sugars should be maintained for 6 weeks, since sugar concentrations vary with cell cycle (Handa et al. 1983), development (Haissig 1984) and season (Ericsson 1979). If the reducing sugars occurred in the cytoplasm, the maintenance of such high levels of sugars implies a homeostatic setpoint in the regulation of carbon metabolism which can be altered by N supply and only slowly returns to the original setpoint, despite the normally high rate of turnover of glucose and fructose in photosynthetic cell metabolism. If the excess sugars were entirely sequestered in the vacuole, some regulatory process must be responsible for production and storage of sugars in cells developing after the inducing treatment has ended. Interestingly, Etter (1971) found no significant change in individual sugars with N deficiency, although the total of sucrose, fructose and glucose decreased and starch increased.

The elevated alpha-amino N concentration at 6 % day^{-1} N Ra (phase 2) may be due to the high N supply and low Rg promoting an excess of N in the seedling, which would be transported and stored as amino acids or amides. The negative response of amino N to water stress is contrary to the usual observation of amino N (mostly amino acids) increasing with water stress (Taylor et al. 1982, Rhodes et al. 1986, Vance and Zaerr 19[illegible]). Individual amino acids may have shown different responses which were not detected in the bulk assay, and proline, which has been implicated in water stress adaptation, is not quantitatively detectable by the assay used here.

The sensitivity of seedlings to stress apparently changed over the course of the experiment. The seedlings responded differently to the current treatments in the two harvests (compare preconditioning effects in harvest 1 to second phase effects in harvest 2). The measured variables fell into 3 groups: 1) those which showed the same effects to the same degree in both harvests (leaf water content and chlorophyll concentration, both increased with N Ra); 2) those which showed an increased response at harvest 2 to the same effects noted in harvest 1 (XPP response to drought, and the responses of Rg, RWR, and foliar N concentration to N Ra); and 3) those which responded to different stresses in the two harvests. Of the latter group, root water content, Rg and foliar starch concentration responded to drought stress, and starch and alpha-amino N concentrations responded to N supply, only at the second harvest. The reverse occurred in only two instances; reducing sugars and alpha-amino N responded to N supply and drought, respectively, only at the first harvest. The general trend of these results suggests that the seedlings became more sensitive to water and nutrient stress as they developed. This can also be seen in seedling Rg in phase 1, when Rg was high, and differed only by about 3 % between the N treatments, and not all between watering treatments. The effects of both N and water supply on Rg were much greater during phase 2. It possible that seed N reserves could be buffering the effects of N treatments early in development; however, the amelioration of response to water stress is difficult to explain.

In addition to positive effects on XPP, root weight ratio, and reducing sugar concentration, which may contribute to drought resistance, the use of lower rates of nitrogen supply to precondition seedlings has the disadvantage of requiring more time to produce seedlings which meet present size criteria. It is up to the sivicultural manager to weigh the cost of such a treatment against the potential benefits. The other major disadvantage of reduced N Ra preconditioning was low foliar N concentration, but this effect was manifested mainly when second phase N supply was large, an uncommon occurrence in the field. In a real field situation, low N preconditioning may confer some advantage if N availability is generally low. For example, Scots pine seedlings which were preconditioned at 3.5 and 7 % day^{-1} N Ra and planted into a sandy, presumably N-poor site, showed a decline in shoot N concentration during field growth, and more so in seedlings preconditioned with the higher N rate (Troeng and Ackzell 1988).

Preconditioning nursery seedlings with water stress involves a trade-off in subsequent performance, between the positive effects on Rg and leaf water content, and the negative effects on root water content, root weight ratio and foliar alpha-amino N concentration. Preconditioning water stress had a positive effect on Rg when applied in conjunction with high rates of preconditioning N, but no effect on Rg with low rates of preconditioning N. The effectiveness of water stress in preconditioning lodgepole pine seedlings in the nursery will depend on the fertilization regime that is being applied at the same time, and vice versa. The general effect of both low N and drought preconditioning seems to be to lessen the response of seedlings to subsequent drought which agrees with the results for <u>Angophora</u> <u>costata</u> and <u>Banksia</u> <u>serrata</u> (Tibbits and Bachelard 1983).

Table IV-1. Plant water characteristics of lodgepole pine as affected by nitrogen addition rate (N Ra; 3 and 6 % day^{-1}) and watering regime (D=cyclically droughted, W=well-watered) applied in two sequential phases (1 and 2). Measurements were taken twice, at a mid-experiment harvest (end of phase 1) and at a final harvest (end of phase 2). Pressure potential was measured at the maximum of drought stress; all other data was measured after rehydration. Means, standard errors and probability (p) levels from a two-way ANOVA of the mid-experiment harvest data, and a four-way ANOVA of the final harvest data. For first phase main effects, n=8 (except n=16 where noted for biomass measurements), and for second phase harvests, n=32 (main effects) and n=16 (2-way interactions). Only significant interactions are presented.

Harvest	Treatment	Level	Mean	S.E.	p
Xylem pressure potential (-MPa)					
mid	N Ra 1	3	0.539	0.1459	0.5426
		6	0.654	0.2214	
	Water 1	D	1.026	0.1341	0.0001
		W	0.165	0.0171	
final	N Ra 1	3	0.734	0.165	0.0581
		6	0.999	0.213	
	Water 1	D	0.759	0.187	0.1192
		W	0.973	0.1961	
	N Ra 2	3	0.856	0.188	0.8720
		6	0.877	0.199	
	Water 2	D	1.511	0.140	0.0001
		W	0.222	0.012	
	N Ra 1 X	Water 2			0.0550
	3	D	1.244	0.206	
		W	0.224	0.021	
	6	D	1.778	0.145	
		W	0.220	0.012	

Leaf water content (g g^{-1}DW: n=16)

mid	N Ra 1	3	2.29	0.068	0.0127
		6	2.58	0.080	
	Water 1	D	2.48	0.072	0.3864
		W	2.39	0.091	
final*	N Ra 1	3	2.65	0.038	0.0253
		6	2.78	0.057	
	Water 1	D	2.77	0.046	0.0286
		W	2.66	0.052	
	N Ra 2	3	2.57	0.038	0.0001
		6	2.87	0.046	
	Water 2	D	2.69	0.038	0.3951
		W	2.74	0.060	

Root water content (g g^{-1}DW: n=16)

mid	N Ra 1	3	2.54	0.063	0.0289
		6	2.75	0.067	
	Water 1	D	2.57	0.068	0.1143
		W	2.72	0.068	
final	N Ra 1	3	4.06	0.194	0.3850
		6	4.24	0.245	
	Water 1	D	3.90	0.185	0.0247
		W	4.39	0.245	
	N Ra 2	3	4.05	0.224	0.3611
		6	4.24	0.217	
	Water 2	D	3.28	0.145	0.0001
		W	5.01	0.169	
	<u>N Ra 1</u> X	<u>Water 2</u>			0.0319
	3	D	3.42	0.230	
		W	4.69	0.220	
	6	D	3.14	0.176	
		W	5.34	0.238	
	<u>Water 1</u> X	<u>Water 2</u>			0.0160
	D	D	3.30	0.212	
		W	4.51	0.218	
	W	D	3.26	0.204	
		W	5.52	0.193	

Table IV-2. Mean relative growth rate and root weight ratio of lodgepole pine seedlings. Conditions were as for Table 1. Only significant interactions are presented.

Harvest	Treatment	Level	Mean	S.E.	p
Mean relative growth rate (% d^{-1} DW; n=16)					
mid	N Ra 1	3	6.515	0.0697	0.0437
		6	6.712	0.0615	
	Water 1	D	6.563	0.0672	0.2893
		W	6.664	0.0713	
final	N Ra 1	3	1.246	0.0891	0.6595
		6	1.305	0.1220	
	Water 1	D	1.427	0.1036	0.0288
		W	1.125	0.1033	
	N Ra 2	3	1.079	0.1113	0.0050
		6	1.473	0.0894	
	Water 2	D	1.118	0.0991	0.0225
		W	1.433	0.1069	
	<u>N Ra 1</u> X	<u>Water 1</u>			0.0155
	3	D	1.229	0.1276	
		W	1.263	0.1284	
	6	D	1.624	0.1514	
		W	0.987	0.1582	
Root weight ratio (n=16)					
mid	N Ra 1	3	0.386	0.0151	0.0834
		6	0.352	0.0119	
	Water 1	D	0.382	0.0144	0.1777
		W	0.356	0.0134	
final	N Ra 1	3	0.355	0.0138	0.0294
		6	0.316	0.0127	
	Water 1	D	0.311	0.0113	0.0085
		W	0.360	0.0145	
	N Ra 2	3	0.355	0.0132	0.0309
		6	0.316	0.0134	
	Water 2	D	0.341	0.0110	0.4343
		W	0.330	0.0159	

Table IV-3. Foliar nitrogen and chlorophyll concentrations of lodgepole pine. Conditions were as for Table 1. Only significant interactions are presented.

Harvest	Treatment	Level	Mea	S.E.	p
Nitrogen (% DW)					
mid	N Ra 1	3	0.81	0.026	0.0006
		6	1.03	0.038	
	Water 1	D	0.92	0.042	0.8960
		W	0.93	0.062	
final	N Ra 1	3	1.27	0.052	0.0484
		6	1.37	0.067	
	Water 1	D	1.34	0.061	0.4075
		W	1.30	0.060	
	N Ra 2	3	1.05	0.021	0.0001
		6	1.59	0.047	
	Water 2	D	1.34	0.061	0.3666
		W	1.30	0.059	
	N Ra 1 X	N Ra 2			0.0129
	3	3	1.07	0.034	
		6	1.48	0.065	
	6	3	1.04	0.025	
		6	1.70	0.057	
Chlorophyll (mg g^{-1} DW)					
mid	N Ra 1	3	1.811	0.0595	0.0006
		6	2.392	0.1041	
	Water 1	D	2.070	0.1458	0.6287
		W	2.133	0.1304	
final	N Ra 1	3	2.759	0.0986	0.0814
		6	2.952	0.1098	
	Water 1	D	2.874	0.1086	0.7400
		W	2.837	0.1028	
	N Ra 2	3	2.442	0.0781	0.0001
		6	3.269	0.0726	
	Water 2	D	2.797	0.0945	0.2882
		W	2.914	0.1150	

Table IV-4. Foliar starch, reducing sugars, and alpha-amino N concentrations (mg g^{-1} DW) of lodgepole pine. Conditions were as for Table 1. No interactions were significant.

Harvest	Treatment	Level	Mean	S.E.	p
Starch					
mid	N Ra 1	3	125.2	14.32	0.4644
		6	108.4	19.54	
	Water 1	D	136.4	15.14	0.1033
		W	97.2	16.37	
final	N Ra 1	3	27.23	4.918	0.8721
		6	27.38	4.483	
	Water 1	D	23.50	3.088	0.1850
		W	31.10	5.815	
	N Ra 2	3	38.65	5.712	0.0001
		6	15.92	1.815	
	Water 2	D	15.63	1.821	0.0001
		W	38.98	5.672	
Reducing sugars					
mid	N Ra 1	3	238.8	13.07	0.0173
		6	179.4	17.77	
	Water 1	D	192.2	13.70	0.1419
		W	226.0	21.65	
final	N Ra 1	3	230.0	13.74	0.0062
		6	178.0	10.76	
	Water 1	D	211.6	14.52	0.4969
		W	196.4	11.57	
	N Ra 2	3	214.8	15.11	0.3601
		6	193.2	10.61	
	Water 2	D	205.6	12.06	0.6627
		W	202.4	14.24	

Alpha-amino N

mid	N Ra 1	3	1.614	0.1249	0.5119
		6	1.772	0.2300	
	Water 1	D	1.419	0.0834	0.0371
		W	1.968	0.2045	
final	N Ra 1	3	5.220	0.5277	0.6743
		6	6.294	0.9572	
	Water 1	D	5.249	0.8546	0.0640
		W	6.266	0.6828	
	N Ra 2	3	3.984	0.3792	0.0069
		6	7.530	0.9310	
	Water 2	D	6.573	0.9658	0.3997
		W	4.491	0.4875	

Table IV-5. Water relations characteristics of lodgepole pine derived from pressure-volume analysis. Conditions were as for Table 1. No interactions were significant.

Harvest	Treatment	Level	Mean	S.E.	p
Bulk modulus of elasticity at 0 turgor					
mid	N Ra 1	3	2.313	0.2295	0.5527
		6	2.463	0.2026	
	Water 1	D	2.750	0.1783	0.0121
		W	2.025	0.1612	
Slope coefficient of bulk modulus of elasticity <u>vs</u>. turgor					
mid	N Ra 1	3	3.84	0.260	0.6053
		6	3.98	0.224	
	Water 1	D	4.33	0.189	0.0084
		W	3.50	0.185	
Relative water content at turgor loss					
mid	N Ra 1	3	0.699	0.0237	0.4469
		6	0.717	0.0182	
	Water 1	D	0.743	0.0150	0.0109
		W	0.674	0.0188	
Symplastic fraction					
mid	N Ra 1	3	0.770	0.0437	0.5893
		6	0.799	0.0408	
	Water 1	D	0.728	0.0429	0.0495
		W	0.841	0.0297	

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CHAPTER V. GENERAL DISCUSSION AND CONCLUSIONS.

A primary objective of this research was to assess the extent of interactions between nitrogen supply and water stress in the growth and physiology of lodgepole pine seedlings. I found such interactions for relative growth rate (Rg) in the first solution culture experiment and in the container-grown seedlings, but none in the second solution culture experiment. In both cases of an interaction effect on Rg, the lower rate of nitrogen supply limited the negative effect of water stress on Rg. The reason for the lack of an interaction in Experiment 2 is not known. It is possible that the treatments used were not in the range which was most affected in the interaction.

An interaction between N and water stress was also found for leaf:root ratio in Experiment 1, but not for shoot:root ratio in the subsequent two experiments. Otherwise there was no interaction found in nutrient status, or water relations (with stomatal conductance in experiment 1, chapter II, being a possible exception). Thus conclusions drawn from other studies where nitrogen and water stress have not both been used as treatments are not invalidated; the effects of N or water supply are interdependent only for Rg.

A different kind of interaction was found where the lower preconditioning N Ra in combination with later water stress in the second phase resulted in higher root water content and xylem pressure potential than if the two effects were only additive (Experiment 3). Is this the same interaction as when these two factors are applied simultaneously? Probably not, as the characteristics affected were both aspects of seedling water relations, and not growth as described above. Also, the converse interaction, of preconditioning water stress with second phase N Ra, did not occur.

Further to this general theme, I did find N supply affected seedling water relations, and water stress affected seedling nutrient status. These were not interactions in the statistical sense, but might be thought of as cross effects. Foliar N concentration declined with water stress, both as an immediate response and in response to preconditioning water stress. This can be interpreted as the same effect persisting beyond the period of treatment. However, the effect of N supply on water relations would not support this. In this case, the direct effect of increasing N Ra is an increase in XPP, but the effect of increased N Ra as a preconditioning treatment is to decrease XPP. It is most likely that two different mechanisms are responsible for these contradictory responses.

The overall effect of preconditioning with reduced rates of N supply is to promote characteristics associated with drought resistance (low shoot:root ratio, less negative XPP, root water content and reducing sugars concentration) and to maintain higher growth rate under subsequent water stress. The only drawback to such preconditioning is the smaller size of the seedlings. The use of a period of reduced N Ra in the operational production of nursery seedlings would require prolonging the rearing period in order to meet the size criteria presently in effect. This would increase the cost per seedling, and may preclude the rearing of a third seedling crop each year in places where this is done. In order to minimize the extra costs of longer rearing schedules, it is crucial that relative rather than constant rates of nutrient supply be used, in order to maximize the early part of the exponential growth phase of the seedlings, before the later phase of reduced exponential growth rate. Relative addition rate is also more cost-effective than constant rates, in that the seedlings make the most efficient use of nutrients when supplied in proportion to their demand (Timmer and Armstrong 1989).

The overall effect of water stress preconditioning on the subsequent condition of lodgepole pine seedlings was to reduce both water content and foliar nutrient concentration, and to increase the shoot:root ratio; these characteristics are not expected to improve drought resistance. However, just as with low-N preconditioned seedlings, water stress preconditioned seedlings maintained higher Rg in the first week of a later water stress in solution culture. This effect persisted through the multiple drought cycles of the container experiment only where seedlings had been preconditioned with high N Ra. The utility of water stress preconditioning appears to be limited and most effective when used in conjunction with N supply precding.

This research has generated three hypotheses which contribute to our understanding of stress physiology. First, the mechanism in which internal N concentration is the driving variable for growth rate (Ingestad 1982) is disconnected by water stress (chapter II). Second, the mechanism by which both N supply and water stress preconditioning affect later growth and physiological conditions may be based on the alteration of the size and wall thickness of the plant cells (chapter III). Third, lodgepole pine seedlings are more sensitive (respond more strongly) to environmental stress, such as N supply and water stress, as they grow older (chapter IV). These hypotheses should be the foci of future research on plant stress physiology and conifer nursery production techniques.

In addition to the above research directions, the results of the container experiment should be extended to operational trials using normal operating procedures and greenhouse conditions (e.g., for hardening) where they do not conflict with the application of N at relative rates, and planting out the seedlings for assessment.

Evidence from this study adds to evidence from other studies (Lopushinsky 1975, Squire _et al_. 1987, Seiler and Johnson 1988) which indicate that the strategy of lodgepole pine under water stress is to conserve water rather than to augment exploration for and uptake of water. Research on this topic would concentrate on the extent and structure of root development, and changes in hydraulic conductivity under water stress. Hormones should be investigated for their role in controlling the suite of responses to stress (cf. Chapin 1991).

The effects of water stress on plant growth and physiology has been studied largely from the perspective of responses and acclimation during the onset of water stress. Acclimation may operate on the recovery of plants from water stress, and the rate and extent of recovery from water stress may be as important to plant success as the ability to tolerate the stress. Attempts to precondition seedlings might better focus on the effect of preconditioning treatments on recovery from stress encountered in the field. This is an important aspect of a strategy of stress avoidance, rather than stress tolerance.

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