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The effect of carbon and nutrient loading during nursery culture on the growth of black spruce seedlings: a six-year field study

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Abstract We tested the effects of exponential nutrient loading and springtime carbon loading during nursery culture on the field performance of black spruce (*Picea mariana* (Mill.) B.S.P.). Seedlings were grown from seed with a conventional, fixed dose fertilizer (10 mg N seedling^{-1}) or an exponential nutrient loading regime (75 mg N seedling^{-1}). The following spring, seedlings were exposed for two weeks to either ambient (370 ppm) or elevated levels of CO_2 (800 ppm) and then planted in the field; seedling growth was followed for the next six years. Exponential nutrient loading increased seedling height, stem diameter and leader growth, with the largest increases in height and leader length occurring in the first three years after outplanting. Carbon loading increased seedling height and leader length, but only in seedlings that had been exponentially nutrient loaded. A combination of carbon and nutrient loading increased shoot height 26%, stem diameter 37% and leader length 40% over trees that received neither treatment. These results demonstrate that the growth enhancement seen under exponential nutrient loading is maintained under field conditions for at least six years. Carbon loading just before outplanting was a useful supplement to nutrient loading, but was ineffective in the absence of nutrient loading.

Keywords Nitrogen · Exponential nutrient loading · Fertilizer · CO_2 · *Picea mariana*

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Introduction

Nursery-grown conifer seedlings face many challenges in the field, including competition, drought, heat stress and nutrient deficiency (Grossnickle 2000). If nurseries could enhance non-structural carbohydrate and nutrient reserves in tree seedlings, these could stimulate growth and stress tolerance following outplanting to the field. Increased root growth would improve water and nutrient access, while greater shoot growth would increase light interception and reduce the severity of competition. Optimized fertilization can build internal nutrient stores; however it is unclear whether high CO_2 exposure can build non-structural carbohydrate pools (a procedure termed carbon loading).

Exposure to elevated CO_2 can increase photosynthesis and starch accumulation (Moore et al. 1999). Continuous exposure to high CO_2 enhances growth and is often used in greenhouses to increase biomass and yield (Mortensen 1987; Campagna and Margolis 1989). Short pulses of elevated CO_2 may also promote growth and carbohydrate reserves, while reducing costs and the risk of acclimation due to long-term high CO_2 exposure. Acclimation to high CO_2 reduces photosynthetic capacity, which would be detrimental to seedlings outplanted into normal CO_2 levels in the field. Photosynthetic acclimation is common under low nitrogen conditions and is aggravated in small containers and in nursery culture (Mcconnaughay et al. 1993; Sage 1994; Moore et al. 1999). High nutrient supply weakens the acclimation response, and may therefore be a prerequisite if carbon loading during nursery culture is to improve seedling performance upon outplanting.

Continuous growth at high nutrient supply inhibits root development in seedlings during nursery culture (Timmer 1997). However, if nutrients are supplied in proportion to seedling size (a technique termed exponential nutrient loading) then nutrient levels can be increased without adverse effects (Timmer 1997). When nutrient delivery matches the increase in the absolute rate of seedling growth, steady-state nutrient concentrations are maintained in the plants, preventing nutrient toxicity early in development and nutrient deficiency later in the season (Timmer 1997). Greenhouse studies and one three-year field study have shown that exponentially nutrient loaded seedlings have greater height, shoot biomass and nutrient uptake than conventionally fertilized trees following outplanting (Malik and Timmer 1995; Malik and Timmer 1998). No study has examined the field response of carbon loading. Here, we study the effect of both carbon and exponential nutrient loading on black spruce grown in the field for six years. This represents the longest field trial of nutrient loaded seedlings, and the only field trial of carbon loaded seedlings.

Materials and methods

In spring 1999, black spruce seeds (from seed zone 25) were planted in 40 mL cavities in styroblock trays at a polyethylene covered, ventilated greenhouse (North Gro, Kirkland Lake, ON). Seedlings were grown in 4:1 v/v peat moss/vermiculite for 16 weeks at 22:15°C day:night temperatures under natural light. Water was added as needed to keep the potting medium moist without leaching nutrients, and sodium vapor lamps provided supplementary light to ensure a 16-hour photoperiod of at least 250 μmol photons m^{-2} s^{-1}. A soluble fertilizer (20:20:20 N-P_2O_5-K_2O plus micronutrients, Plant Products, Brampton, ON) was sprayed weekly as a pre-mixed solution for 12 weeks, starting two weeks after germination. Conventional fertilizer (10 mg N seedling^{-1}) was applied as repeated applications of a weekly fixed dose (0.83 mg N week^{-1}). The exponential nutrient loading procedure (75 mg N seedling^{-1}) was applied weekly as:

$$N_T = N_s\left(e^{rt} - 1\right) \tag{1}$$

where r is the relative rate of addition (0.46) used to raise the initial nitrogen concentration (N_s, 0.3 mg) to a final level ($N_T + N_s = 75$ mg), over t applications (Timmer 1997). The amount of nitrogen to be applied in a given week (N_t) was then calculated as:

$$N_t = N_s\left(e^{rt} - 1\right) - N_{t-1} \tag{2}$$

where $N_{t\text{-}1}$ is the cumulative fertilizer applied up to a given week's application (Timmer 1997).

Seedlings were then placed in an unheated greenhouse for bud set and hardening. In November, 1999, four trays (760 seedlings) of each treatment were wrapped and boxed and put in a dark 3°C cold room. In June 2000, the seedlings were replanted into styroblock trays and moved for two weeks to a Conviron growth chamber (E-36) with 21:14°C day:night temperatures and a 16-hour photoperiod at 400 ± 40 μmol photons m^{-2} s^{-1}. Six seedlings per treatment were sampled for N, P and K as per Malik and Timmer (1996). Two trays from each nutrient treatment were put in an ambient CO_2 (370 ± 20 ppm) growth chamber; the other two trays from each nutrient treatment were put in a high CO_2 (800 ± 20 ppm) chamber. High CO_2 was controlled with an infrared gas analyzer (WMA-3, PP Systems, Haverhill, MA) regulating a solenoid valve on an air-line from a high-pressure CO_2 cylinder. Ambient CO_2 was maintained by blowing air through a soda lime scrubber using a WMA-3 controller to activate fans. CO_2 treatments lasted two weeks, and seedlings began flushing in the chambers; after one week, trays and CO_2 treatments were transferred between chambers. All other conditions were the same as in the initial two-week period. This generated four treatments: conventional fertilizer, ambient CO_2 (CA); conventional fertilizer, high CO_2 (CH); nutrient loaded, ambient CO_2 (NA); nutrient loaded, high CO_2 (NH).

In July 2000, seedlings were planted into a plowed field with a clay-loam soil at the University of Toronto's Koffler Scientific Reserve (44°03' N, 79°29' W). Trees on the edges of the styroblocks, and the smallest and largest 10% of the trees were not used; seedlings planted were randomly selected from the remaining trees. Four blocks of 64 seedlings each were planted; a second plot, with three blocks of 40 trees, was planted for root analysis. Seedlings from each treatment were randomly planted within each block in rows 1 m apart from neighboring trees. Measurements were made during the growing seasons of 2000 to 2005 at the start and end of each growing season; the site received no maintenance during the study period. Shoot height was measured from the soil to the top of the main leader. Leader length was measured as the length of new growth at the tree tip. Stem diameter at soil level was assessed with a digital caliper. Root growth was measured four and seven weeks after planting by excavating and washing the roots free of soil. The length of all new roots (>1 cm) was measured on each tree; new root growth was the sum of these lengths per seedling. Nutrient content and root growth were analyzed with an ANOVA and shoot growth data were analyzed with a repeated measures ANOVA using nutrient and carbon loading as factors; Tukey's HSD was used to compare means (JMP 4.0.2, SAS Institute).

Results

At the time of CO_2 exposure, nutrient loaded seedlings had higher nutrient levels than non-loaded trees (3.2% vs. 1.3% N, 0.4% vs. 0.2% P, 1.2% vs. 0.7% K in nutrient loaded and conventionally fertilized shoots, respectively). Treatments did not differ in height at planting ($p = 0.8$). Over the six years, shoot height was greater in nutrient loaded than non-nutrient loaded seedlings ($p < 0.0001$; Fig. 1a). Carbon and nutrient loading interacted such that NH seedlings were 26% taller than CA trees after 6 years ($p < 0.1$; Fig. 1a). Stem diameter was increased 36% by nutrient loading ($p < 0.001$), but carbon loading had no effect and did not interact with nutrients (Fig. 1b). Nutrient loading increased leader length as of the second year (Fig. 1c). Carbon loading did not affect leader length, but interacted with nutrients ($p < 0.1$) to increase leaders 40% in NH trees compared to CA trees (Fig. 1c). Nutrient loading increased new root growth within four weeks of planting ($p < 0.05$; Fig. 2a). Seven weeks after planting, NH trees had greater new root growth than CA trees (Fig. 2b).

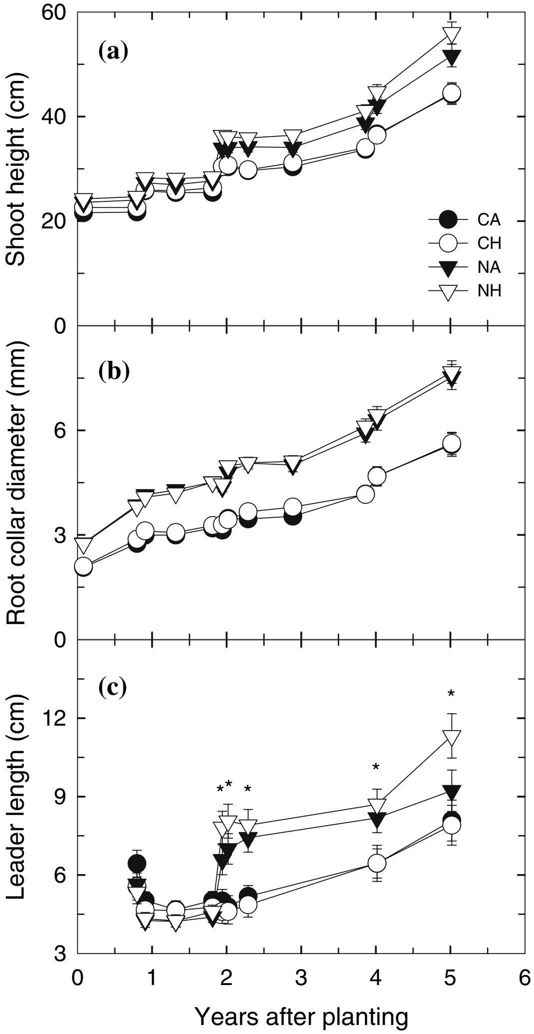

Fig. 1 Effects of nutrient and carbon loading on: (**a**) shoot height; (**b**) stem diameter; (**c**) leader length. Treatments are conventional fertilizer, ambient CO_2 (CA); conventional fertilizer, high CO_2 (CH); nutrient loaded, ambient CO_2 (NA); nutrient loaded, high CO_2 (NH). In (a) and (b), treatment effects were significant on every measurement day ($p < 0.05$); in (c), asterisks indicate a significant nutrient treatment effect at a sampling time ($p < 0.01$). Means ± SE

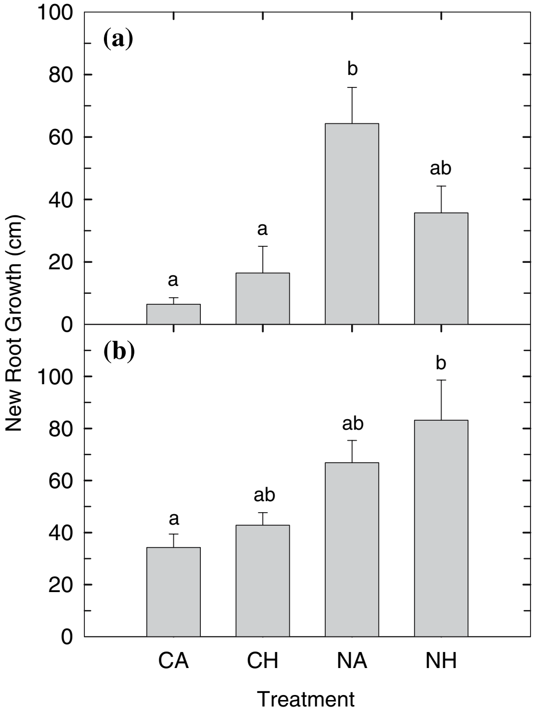

Fig. 2 Effects of nutrient and carbon loading on new root growth: (**a**) 4 weeks and (**b**) 7 weeks after outplanting. Treatments as in Fig. 1. Dissimilar letters denote significant differences within a sampling time ($p < 0.05$). Mean ± SE

Discussion

Exponential nutrient loading increases the growth of conifer seedlings in tubs in a greenhouse (Malik and Timmer, 1995; Malik and Timmer, 1998); similar results were seen in one three-year field study (Malik and Timmer, 1996). Here, we show that exponential nutrient loading during nursery culture increases field performance for at least six years. Shoot height, stem diameter and leader length were greater in nutrient loaded seedlings (NA and NH), demonstrating that the effects of nutrient loading continue well beyond the nursery and immediate establishment phases. Greenhouse and short-term field studies have also shown that nutrient loading increased competitive ability against herbaceous boreal species, mainly through stimulation of post-planting nutrient uptake (Malik and Timmer, 1995; Malik and Timmer, 1998; Imo and Timmer, 2001). In our study plot, an abundance of old field weeds quickly established, shading the trees. Assuming existing trends continue, the nutrient loaded plants will overtop this competing vegetation years earlier than non-loaded seedlings. While the trees in our plot experienced more vigorous competition and warmer temperatures than they would in a more typical, northern planting site, a 5-year companion field study in northern Ontario also found that nutrient loading enhanced black spruce seedling growth (Way, Seegobin and Sage, unpublished).

Carbon loading was only effective when combined with nutrient loading, and although there was no immediate effect on shoot height after the treatment, elevated CO_2 increased shoot height of nutrient loaded seedlings in the field. Black spruce seedlings have a strong CO_2 acclimation response (Johnsen, 1993) that can occur in as little as two weeks (Way and Sage, 2004). Since high nitrogen status suppresses photosynthetic acclimation (Sage, 1994; Moore et al., 1999), nutrient loading may have allowed nutrient and carbohydrate accumulation in NH seedlings. Alternatively, the extra nitrogen may have allowed carbon to be used for growth, resulting in the greater height and root growth, and presumably biomass, seen in the NH seedlings. The effects of carbon loading appear to depend on the timing of CO_2 exposure: Campagna and Margolis (1989) found that black spruce seedlings accumulated biomass in response to a short-term pulse of CO_2 in the spring, but had reduced CO_2 sensitivity in the fall. This is consistent with our study, as our spring carbon loading increased height and root growth, given sufficient nutrients.

In summary, exponential nutrient loading of black spruce seedlings has benefits that accrue for at least six years after planting. Carbon and nutrient loading positively interacted to produce vigorous seedlings that were 25% taller after six years than conventionally fertilized, non-carbon loaded seedlings. These results indicate that short springtime pulses of elevated CO_2 shortly before planting produce superior seedlings without the cost of long-term CO_2 enrichment.

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