Maximizing Nutrient Storage in Nursery Culture to Promote Retranslocation and Growth of Outplanted Seedlings

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Introduction

Newly outplanted seedlings depend highly on remobilization of internal nutrient reserves to support new growth because initial poor root soil contact, root restriction and slow development limit uptake from the soil (Burdett et al. 1984, Burdett 1990, Nambiar and Sands 1993). Although exponential nutrient loading promotes nutrient storage and remobilization to facilitate early establishment success in tree seedlings, mechanisms to explain an increased or decreased retranslocation with pre-plant nutrient reserves (van den Driessche 1991, Millard and Proe 1993) has yet to be elucidated. Improved field response of exponentially loaded over non-loaded seedlings on a variety of site types (Timmer and Munson 1991, Quoreshi and Timmer 2000) and on simulated soil fertility gradients (Xu and Timmer 1999, Salifu and Timmer 2001) have generated interest in exponential nutrient loading, but more insight into optimizing fertilizer prescription for this practice are needed to refine intensive tree seedling culture. These concepts have been successfully examined in conifers (Quoreshi and Timmer 2000, Salifu and Timmer 2003b) and in tropical species (Imo and Timmer 1992, Xu and Timmer 1999). However, little is known about how exponential nutrient loading might influence nutrient storage and remobilization processes in deciduous tree species. Exponential nutrient loading may benefit deciduous species since significant quantities of nutrients are not lost from foliage but are resorbed into root and shoot tissues (Aerts 1996, Tagliavini et al. 1998, Duchesne et al. 2001, Yuan et al. 2005) prior to senescence. For example, foliar nutrient resorption can account for about 50-90% of the nutrients in stem and root tissues (Aerts 1996, Tagliavini et al. 1998, Yuan et al. 2005). Consequently, roots and shoots serve as important sinks for N storage during senescence and sources of N for new growth in spring (Dickson 1989, Tagliavini et al. 1998).

A proposed model of exponential nutrient loading (Fig. 1) suggests that plant growth and nutritional response to increased fertilization conforms to a curvilinear pattern depicting phases of nutritional states in plants ranging from deficiency to toxicity which aid in rationalizing fertilizer prescriptions to improve nutrient diagnosis (Timmer 1997).

Traditionally based on biomass alone (van den Driessche 1974, Grossnickle 2000), this model has been configured to include plant nutrient status as well in order to improve diagnostic capacity. Its application has been demonstrated in black spruce (*Picea mariana* [Mill.] BSP) container production systems (Salifu and Timmer 2003b), but has yet to be extended to deciduous species. We tested application of this model across a broad spectrum of soil fertility ranging from nutrient deficiency to toxicity in container and bareroot production systems to quantify optimum fertilizer prescriptions for northern red oak (*Quercus rubra* L) seedlings. We hypothesized that (1) plant growth and nutritional response will conform to a curvilinear pattern

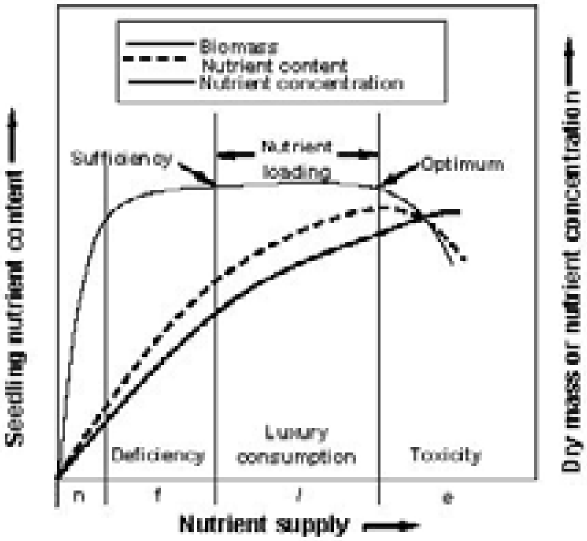

Figure. 1. *Plant growth and nutrient status conforms to a curvilinear pattern with increased fertilization. Fertilizer f supplements native fertility (n) to maximize growth at sufficiency. Extra high fertilization or nutrient loading (l) induces luxury uptake in excess of growth demand, which are stored as reserves for later utilization. Excess fertilization (e) may induce toxicity signified by diminished plant growth and N content at increasing tissue N concentration (Adapted from Salifu and Timmer 2003b).*

with increased fertility consistent with trends depicted in the proposed model, (2) exponential nutrient loading will induce luxury uptake to increase internal nutrient reserves in cultured plants, and (3) higher pre-plant nutrient reserves and increased sink strength will explain greater retranslocation in outplanted seedlings. We focused on N and P in the present study because these elements most commonly limit plant production (Chapin 1980), and because their important role in controlling plant growth and metabolism is well understood (Epstein 1972).

Materials and Methods

Greenhouse trials

Black spruce seedlings were reared for 18 weeks in styro-block trays filled with uniformly screened peat and vermiculite (3:1 v/v) mixture at the University of Toronto greenhouses using the same seed source and peat substrate. Each styro-block tray, representing an experimental unit contained 40 mL cavities (209 cavities per tray). Seasonal dose rates (Fig. 2) ranged from 0-80 mg N plant^{-1}, applied conventionally (10 mg seedling^{-1}) or exponentially (30-80 mg plant^{-1}). These fertility treatments were randomly assigned to trays and arranged in a randomized complete block design (RCBD) with four replications, which were then placed on raised benches in a heated and ventilated greenhouse at temperature 18-25 ^{o}C, humidity 65-85%, and an extended 20 h photoperiod supplemented with sodium vapor lamps at a light intensity of 250 µmol m^{-2} s^{-1}. A commercial water-soluble fertilizer 20N-20P_2O_5-20K_2O plus microelements (Plant Products Co Ltd., Brampton, Ont.) was applied in solution. Further details can be found in Salifu and Timmer (2003b).

Northern red oak container plants were germinated using an equivalent seed source and seedlings grown for 16 wk in 2.8 l Treepots™ (Stuewe and Sons, Corvallis, OR, USA). An experimental unit was represented by 18 pots. Seasonal dose rates ranged from 0-50 mg N plant^{-1}, applied conventionally (25 mg N plant^{-1}) or exponentially (25-150 mg N plant^{-1}). The conventional treatment was chosen to represent the average rate generally used for production of container red oak seedlings (Beckjord et al. 1980, Struve 1995). The eight fertilizer treatments (Fig. 2 and 3, left) were installed as a RCBD and arranged on a greenhouse bench (mean day/night temperature of 24/20 °C) under ambient light conditions in the Department of Horticulture and Landscape Architecture Plant Growth Facility at Purdue University, West Lafayette IN, USA (40°25'N, 86°55'W). A commercial water-soluble fertilizer (Miracle Gro® Excel® 15N-5P_2O_5-15K_2O plus other macro- and micro-elements [The Scotts Company, Marysville, OH, USA]) was applied in solution. For all container trials, each pot was irrigated to container capacity determined gravimetrically at planting (White and Mastalerz 1966). Supplemental irrigation was supplied twice weekly by periodic weighing of pots to determine amount of water to be added to bring pots back to container capacity.

Bareroot northern red oak seedlings were grown under operational conditions (Jacobs 2003) for 18 wk at the Vallonia State Nursery (38°85'N, 86°10'W) south of Indianapolis, Indiana, USA (Jacobs 2003). Seeds were mechanically sown in the fall of 2003 to obtain about 54 seedlings m^{-2} after germination. Fertilizer rates ranged from 0-3.2 g N plant^{-1} season^{-1}. The ten treatments (Fig. 3, right) were laid-out as a RCBD with 4 replicates. The standard practice at this nursery is to supply a total of 0.84 g N seedling^{-1}season^{-1} at seven equal amounts (bi-weekly), which was chosen as the conventional (C) treatment in this study. Ammonium nitrate (34-0-0) in crystal form was broadcast manually on treatment plots. For all trials, weekly applications for exponential treatments followed exponential functions (Timmer and Aidelbaum 1996, Timmer 1997) designed to synchronize fertilizer supply with exponential growth and nutrient uptake of seedlings (Ingestad and Lund 1986, Ingestad and Agren1995). Seedlings were harvested at the end of nursery culture and processed according to standard protocols detailed in Salifu and Timmer (2003b).

Outplanting trials

Nursery reared black spruce seedlings were outplanted in the field or grown under controlled greenhouse environments to examine importance of prior nursery culture in promoting retanslocation and seedling growth. Transplanted seedlings received either 0 [control] or N ranging from 200-400 kg N ha^{-1} simulating a range of soil fertility from poor to rich (Salifu and Timmer 2001, 2003a). N was supplied as 20N-20P_2O_5-20K_2O plus micro-elements (Plant Products Co Ltd., Brampton Ont.) to field seedlings. For the greenhouse trial, N was supplied with the irrigation as $^{15}NH_4$ $^{15}NO_3$ enriched to 5 at. % ^{15}N (34-0-0, ISOTEC Inc. USA) in sand culture. Thus, current uptake was labeled with ^{15}N which could be distinguished from retranslocation (unlabeled N) in new growth. Chelated (EDTA 42% and DTPA 13%) micronutrients were applied at the rate of 0.03 g L^{-1} and phosphorus (P) supplemented by KH_2 P_2O_5 (0-52-34, Plant Products Co Ltd., Brampton Ont.) at the rate of 60 kg P ha^{-1} to avert deficiency of other nutrients. Net N retranslocation was estimated for field seedlings as detailed in Salifu and Timmer (2001). The greenhouse experiment was conducted to directly quantify retranslocation to confirm higher retranslocation by loaded seedlings under field conditions (Salifu and Timmer 2003a). Further details on experimental protocols and statistical procedures can be found in Salifu and Timmer (2001, 2003a, 2003b).

Results and Discussion

Greenhouse response

Plant growth and P response increased with nutrient supply in the deficiency range, remained stable during luxury uptake, but declined at higher N addition (Fig. 2), which appears consistent with trends shown in the conceptual model (Fig. 1) for container black spruce. Similar trends were observed with N for black spruce (Salifu and Timmer 2003b) and for container (Fig. 3, left) and bareroot (Fig. 3, right) red oak seedlings. Fertilization increased (p = 0.0001) red oak seedling dry mass by 113-260% in bareroot culture and by 34-65% in containers.

Seedling dry mass production was maximized at sufficiency, which corresponded to 30 and 25 mg N plant^{-1} season^{-1} for container black spruce and red oak, respectively, and 0.84 g N plant^{-1} season^{-1} for bareroot red oak seedlings. Red oak seedling N content increased (p = 0.0001) by 184-397% in bareroot culture and by 14-77% in containers in response to increased N supply. Nutrient loading induced luxury nutrient uptake, which increased N and P storage by 175 and 48% in black spruce, 27 and 45% for container red oak and 39 and 32% for bareroot red oak, demonstrating capacity of this practice to build internal nutrient reserves in plants. Optimum loading occurred at 64 and 100 mg N plant^{-1} season^{-1} for container black spruce (Fig. 2) and red oak (Fig. 3, left), respectively, and at 1.62 plant^{-1} season^{-1} for bareroot red oak seedlings (Fig. 3, right). Toxicity associated with reduced growth and N content at higher fertility (Haynes 1986, Salifu and Timmer 2003b) occurred beyond 80 mg N seedling^{-1} season^{-1} dose rate for container black spruce, and beyond 1.62 g N plant^{-1} season^{-1} for bareroot red oak (Fig. 3, right).

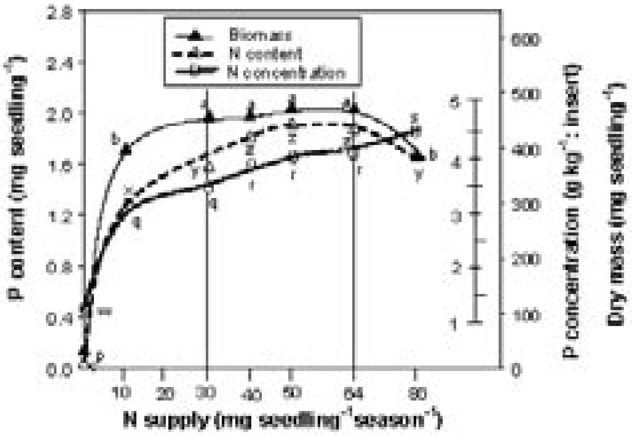

__Figure. 2.__ Growth and P response to increasing nutrient supply for black spruce seedlings grown for 18 wk in a greenhouse environment. Points marked with different letters within each parameter differ significantly according to Tukey's highly significant difference test α = 0.05.

Nutrient toxicity decreased growth and N content by 69 and 44%, respectively, for bareroot red oak and by 81 and 56%, respectively, for container red oak. Toxicity reduced growth (17%) and P content (13%) in black spruce seedlings. Exponential delivery schedules (0.84E or 25E) were more effective in promoting nutrient acquisition, which increased N uptake by 16% for container (Fig. 3, left) and bareroot (Fig. 3, right) red oak seedlings than when applied conventionally (0.84C or 25C). These results are in general agreement with published information (Xu and Timmer 1999; Salifu and Timmer 2003b). The general similarity of experimental data with trends depicted in the conceptual model demonstrate model suitability for rationalizing and quantifying optimum fertilizer prescriptions for raising high quality forest tree seedlings for field planting. Induced luxury uptake in red oak seedlings should not be lost through leaf fall because of resorption. This important nutrient conservation mechanism by deciduous tree species can recover about 50-90% of the nutrients from senescing leaves, which are conserved as stored reserves in stem and root tissues for later utilization (Aerts 1996, Yuan et al. 2005). Thus, outplanted red oak seedlings with higher internal nutrient reserves as conditioned by loading may readily draw on these stored resources for new growth (Aerts 1996, Tagliavini et al 1998, Yuan et al. 2005) to facilitate early establishment success.

Field response

Although similar in dry mass at outplanting, loaded black spruce seedlings contained 22 mg N compared with 8 mg N in non-loaded plants. Nutrient loading increased growth on the poor and rich soils by 100 and 35%, respectively, in the field (Fig. 4, top left) and by 100 and 23%, respectively, in the greenhouse (Fig. 4, top right) when compared with non-loaded plants. Nutrient loading also increased net N retranslocation 7 and 4 fold on the poor and rich soils, respectively (Fig. 4, bottom left). Increased sink strength (Fig. 4, top) and higher pre-plant nutrient reserves (22 vs. 8 mg N) explained greater retranslocation in loaded seedlings as previously noted elsewhere (Salifu and Timmer 2001, Nambiar and Fife 1991). It was difficulty to discriminate between tree and soil derived N in plant

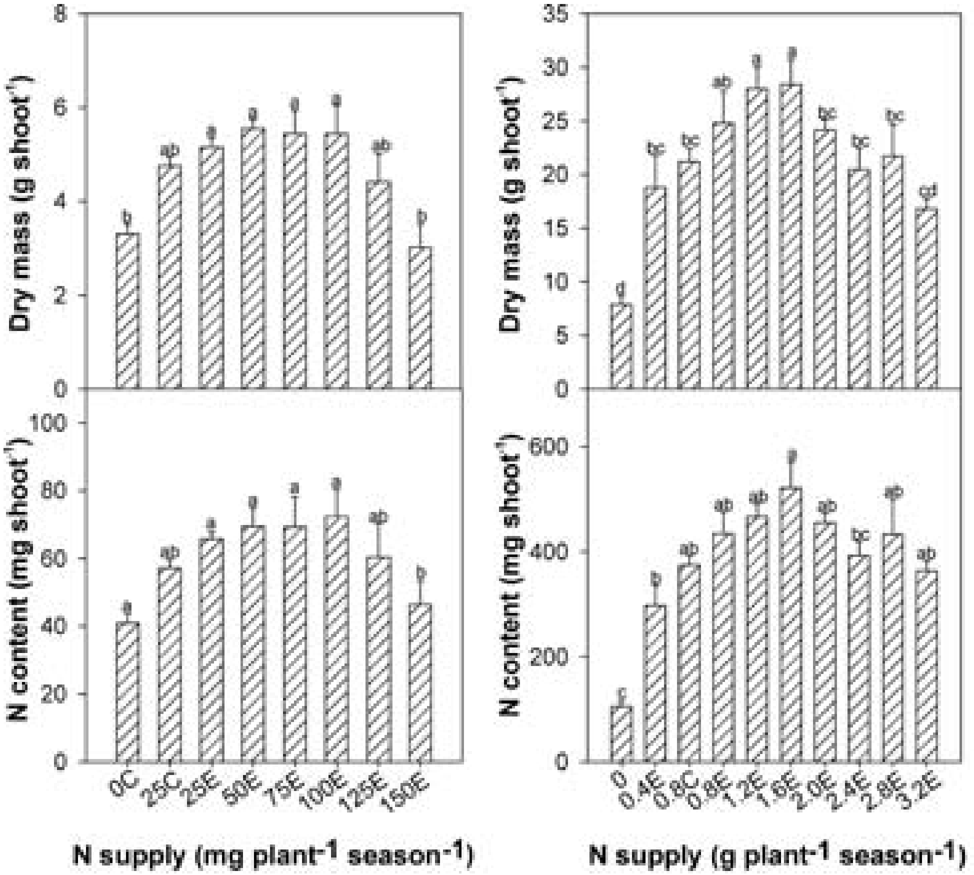

Fig. 3. *G□*
Bars marked with different letters differ significantly according to Tukey's highly significant difference test α = 0.05.

tissues using net estimates, which resulted in erroneous conclusions that rates of retranslocation diminishes with soil fertility (Salifu and Timmer 2003a). Consequently, the stable isotopic technique was used to discriminate between tree (unlabeled N) and soil (labeled N) derived N in new growth, which addressed limitations associated with the net approach (Salifu and Timmer 2003a). Direct retranslocation estimates (unlabeled N) in new growth (Fig. 4, bottom right) confirmed improved retranslocation after nutrient loading previously observed with net estimates (Salifu and Timmer 2001).

Nutrient loading also promoted nutrient acquisition. For example, non-loaded seedlings accumulated 17 and 34 mg N in new growth on the poor and rich soils, respectively, compared with 36 and 74 mg N for loaded plants under field conditions. For the greenhouse study, similar comparisons results in 3 and 19 mg N acquired by non-loaded compared with 8 and 28 by loaded seedlings on the poor and rich soils, respectively. Internal cycling accounted for 6% of the N in new growth of non-loaded plants and 20% of the N demand for new growth of loaded seedlings in the field study. Similarly, retranslocation met 16 and 32% of the N demand for new growth of non-loaded and loaded plants, respectively, in the greenhouse trial. However, when no N was supplied on the poor soil, plants relied entirely on internal cycling to meet seasonal growth demand (100%). This suggests plants can rely entirely on retranslocation for growth, a capacity enhanced by nutrient loading. Other studies have also shown that internal nutrient cycling can meet up to 40-60% of the annual N demand for new growth in plants (Miller 1984, Lim and Cousens 1986, Cheng and Fuchigami 2002). The loading response persist in time as exemplified by 62% increase in dry mass by day 120 (Salifu and Timmer 2001) and by 50% four years after outplanting (Malik 1998). Study results suggest exponential nutrient loading could be more successful in promoting early seedling establishment success than conventional silvicultural methods such as broadcast fertilization at outplanting (Burdett et al. 1984), which may increase brush competition (van den Driessche 1991, Staples et al. 1999).

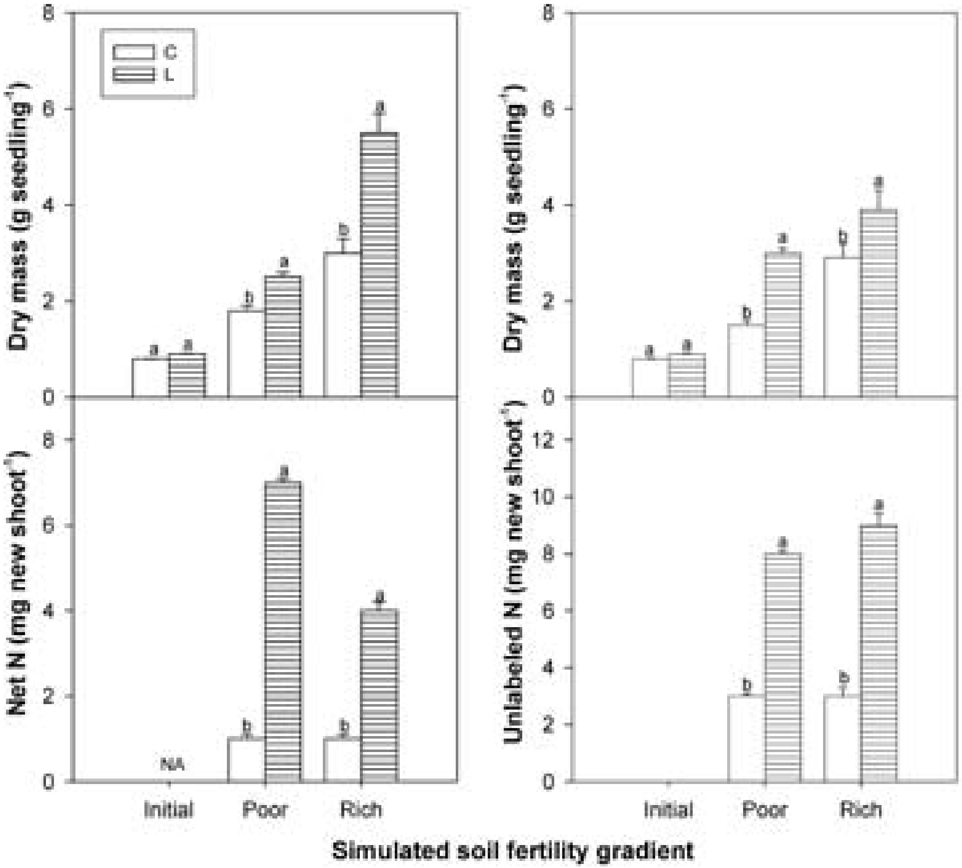

Fig. 4.
seedlings planted on simulated poor and rich soils in the field (left) or under greenhouse environments (right) for 120 days. Paired bars marked with different letters differ significantly according to Tukey's highly significant difference test α = 0.05.

Conclusions

For each species and growing system, plant growth and nutritional response to increased fertilization appeared consistent with trends shown in the conceptual model. These findings demonstrate suitability of the exponential nutrient loading model for rationalizing and quantifying optimum fertilizer prescriptions for forest tree seedlings. Black spruce seedling biomass was maximized at sufficiency (30 mg N plant^{-1} season^{-1}) while N and P content of tissues peaked at the optimum loading rate (64 mg N plant^{-1} season^{-1}). Nutrient loading induced luxury uptake that raised plant N content (175%) and P content (48%) in black spruce seedlings. Optimum plant dry mass production and nutrient toxicity for red oak seedlings occurred at 100 and 125 mg N plant^{-1} season^{-1}, respectively, in containers and at 1.6 and 2.0 g N plant^{-1} season^{-1}, respectively, for bareroot culture. Higher pre-plant N reserves increase net retranslocation 4-7 fold in loaded black spruce relative to conventional plants. Direct retranslocation estimates using tracers in a controlled greenhouse confirmed nutrient loading promotes retranslocation as observed with net estimates under field conditions. Isotopic determinations also addressed limitations associated with net estimates (Salifu and Timmer 2003a). Higher pre-plant nutrient reserves and increased sink strength explained greater retranslocation in loaded seedlings. Exponential nutrient loading demonstrates potential to improve plant nutrient diagnosis and can be applied to other species or cultural systems. This new approach will help refine and optimize fertilizer recommendations to produce high quality seedlings for field planting.

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