

Carbohydrate transfer through root grafts to support shaded trees

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Summary We investigated whether root grafts between lodgepole pine (*Pinus contorta* var. *latifolia* Dougl. ex. Loud.) trees can transfer sufficient carbohydrate reserves from a source tree to a grafted sink tree to affect the vigor of trees growing in a light-limited environment. Eleven plots were established in early spring and two grafted tree pairs and two independent non-grafted trees were selected at each plot. One tree in a grafted pair and one non-grafted tree were shaded at each plot, whereas the remaining trees were non-shaded during the experimental period. Shaded trees had significantly lower carbohydrate reserves and smaller crowns than non-shaded trees following one growing season. Grafted shaded trees had significantly higher root total nonstructural carbohydrate concentrations than non-grafted shaded trees, indicating that root grafts partially offset the effects of shading. Also, large root grafts transferred proportionately more carbohydrates to the shaded trees than small root grafts. Carbohydrates transferred through root grafts could allow grafted trees to persist under conditions where non-grafted trees would be removed by competition.

Keywords: competition, forest ecology, lodgepole pine, *Pinus contorta*, stand dynamics.

Introduction

Following stand-replacing disturbances, some tree species establish at high densities and there is initially little competition among seedlings (Cannell et al. 1984, Kenkel et al. 1997). After establishment, individual growth rates diverge and trees begin to differentiate into crown classes (Knox et al. 1989, Nilsson et al. 2002). It is generally assumed that competition for critical resources (i.e., light, water, nutrients) controls the rate and pattern of tree mortality in forest stands (e.g., Mohler et al. 1978, Knox et al. 1989, Nilsson et al. 2002) and that mortality is concentrated among the smallest individuals (Mohler et al. 1978, Cannell et al. 1984, Kenkel et al. 1997). Competition for light is usually considered to be asymmetric because taller trees are able to capture a disproportionate amount of the resource (e.g., Knox et al. 1989, Bernston and Wayne 2000). As a result, smaller trees in dense stands generally perish because of lack of light.

It has been suggested that the presence of root grafts may alter normal competitive relationships among trees and influence forest stand dynamics (Kuntz and Riker 1956, Bormann 1962, Eis 1972). Previous research has indicated that water (Schultz and Woods 1967, Stone and Stone 1975) and carbohydrates (Bormann 1961, Bormann 1966) can be transferred across root grafts. The quantity of water or carbohydrates or both, that can be moved through root grafts is not known, but it has been demonstrated that intact trees can transport enough photosynthate to keep the root systems of girdled trees (Bormann 1966, Stone 1974) and stumps (e.g., Bormann 1961, Schultz and Woods 1967, Eis 1972) alive for years following removal of the photosynthesizing tops. It is unclear, however, whether these resources can be transferred to trees in inferior light environments such as might occur under conditions of asymmetric competition for light (Knox et al. 1989, Bernston and Wayne 2000).

We chose lodgepole pine (*Pinus contorta* var. *latifolia* Dougl. ex. Loud.) to study root grafting because it commonly regenerates at extremely high densities following wildfire (Lotan and Critchfield 1990, Blackwell et al. 1992) and it can form extensive root grafts in dense stands more than 15 years of age (Fraser et al. 2005). In the juvenile stages, these dense stands often have poor crown differentiation and, as a result, suppressed trees that normally would be removed through competition often persist and so there may be little change in density over time (Blackwell et al. 1992, Reid et al. 2003). We hypothesized that the transfer of resources through root grafts in lodgepole pine helps to support the continued existence of trees in inferior positions in dense stands. To test this hypothesis, we determined whether root grafts can transfer carbohydrate reserves from a source tree to a grafted sink tree that grows in a light-limited environment. We also assessed whether this relationship affects the vigor of grafted trees relative to non-grafted trees growing in similar environments.

Materials and methods

Site description

All study sites are located in three pure fire-origin lodgepole pine stands near Swan Hills, Alberta, Canada (54°45'12" N, 115°42'14" W). At the start of the study, the trees were

3.0–6.1 m tall and 2.2–9.6 cm in stem diameter at 10 cm height and 14–19 years old. All sites are flat or slightly inclined and soils are Gray Luvisols.

Field assessments

Eleven plots, each containing six trees were established in May 4–6, 2004. Each plot contained two grafted pairs of trees and two independent non-grafted trees. Based on earlier results (Fraser et al. 2005), trees growing within 20 cm of each other have a high probability of being root grafted, whereas trees at least 80 cm apart were likely not grafted. The grafting status was verified at the end of the experiment (see below). The overall density of the plots was > 8000 trees ha⁻¹, but tree pairs or single trees were at least 80 cm from their neighbors. All of the study trees were in a dominant or co-dominant canopy position and both trees in a given grafted pair were of similar height and stem diameter. Plot areas varied in size, but trees within the same plot were generally within 10–15 m of each other (depending on the distribution of grafted tree pairs). Plots were located at least 100 m apart and there were 3–4 plots in each of the forest stands.

At each plot, the lowest intact live whorl of branches (defined as having green needles on all branches of the whorl) was identified and marked on each tree. A branch (with needles) was removed from the mid-crown of each tree for carbohydrate analysis. One non-grafted tree and one grafted pair of trees were randomly selected in each plot and left undisturbed during the experiment. The remaining trees were assigned to a shading treatment, with one tree in the grafted pair being shaded, the other tree remaining non-shaded. Therefore, at each plot there was one non-grafted non-shaded tree (NN), one non-grafted shaded tree (NS), one grafted pair of non-shaded trees (GNP) and one grafted pair of trees comprising one shaded tree (GS) and one non-shaded tree (GN).

For the shading treatment, black fiberglass insect screen (7 strands cm⁻¹) that blocked about 75% of the photosynthetically active radiation (measured with a Sunfleck ceptometer, ITC International, Armidale Australia) was used. To shade the trees evenly, the screen was sewn into two different sized cylinders: one about 4 m long and 1.5–2 m in diameter and the other about 1 m long and 30 cm in diameter. The larger cylinder covered the entire tree crown up to the base of the leader. The smaller cylinder loosely covered the leader, leaving about 30–40 cm of extra screen on top of the leader to allow for growth during the experiment. The insect screen was securely wrapped around each shaded tree with twine and the top of the small cylinder covering the leader was closed to ensure each tree would remain fully covered during the experiment. Two Hobo H8 (Onset Computer Corporation, Bourne, MA) temperature data loggers were located at each of three randomly selected plots (one data logger at mid-crown under the shading screen and one at mid-crown of a non-shaded tree) to record crown temperatures during the experimental period.

On August 30–31, 2004, following one growing season, all study trees in each plot were cut and the grafted or non-grafted

status of each tree determined following root excavation. At each plot, current leader increment and crown recession distance (distance between locations of lowest whorl of live branches in May versus August) were measured. Further, a branch (with needles) at mid-crown, a non-grafted coarse root 3 cm long and 1–2 cm in diameter and a stem section (10 cm above ground level) were collected from each tree for carbohydrate, nutrient and stem analyses. The grafted stumps and large roots of each grafted tree pair was removed intact from the ground and transported to the laboratory for analysis.

Laboratory assessments

Stem diameter, tree age, rate of stem diameter growth during the experiment (2004 growth) and total stem diameter growth during the previous four years (2000–2003 growth) were determined for each tree from the stem sections. Relative stem growth (RSG) of all trees was calculated as:

$$\text{RSG} = \left(\frac{2004 \text{ growth}}{(2000-2003 \text{ growth})/4} \right) \times 100\% \quad (1)$$

All grafts were removed from the grafted root system with a reciprocating saw and were serial sectioned with a band saw into 0.25–0.50 cm thick sections. Graft sections were then examined and the age of the graft, the xylem area of the graft and the phloem circumference of the graft were measured and recorded.

Samples of branches collected from mid-crown (spring and fall collections), needles on branches collected at mid-crown (spring and fall collections) and coarse roots (fall collection only) were collected for total nonstructural carbohydrate (TNC) analysis. Spring and fall tissue samples were gathered during the afternoons of May 6 and August 31, 2004, respectively. All tissue samples were stored on ice, transported to the laboratory, oven dried at 68 °C for 2–3 days and then ground with a Wiley mill. Total nonstructural carbohydrate concentrations were measured as described by Chow and Landhäusser (2004). Total nitrogen (N) and phosphorus (P) contents in new needles formed at mid-crown during the experiment were determined by Kjeldahl digestion (Bremner and Mulvaney 1979).

Statistical analysis

The relationship between shading and crown air temperature was tested as a completely randomized design. The relationships between treatments (GS, GN, GNP, NS, NN) and leader increment, crown recession distance, relative stem growth, root TNC concentrations and new needle total N and P amounts were analyzed as a randomized block 2 × 2 factorial design with two grafting treatments (grafted, non-grafted) and two shading treatments (shaded, non-shaded). The relationships between treatments and changes in needle TNC concentrations and branch TNC concentrations were analyzed as a repeated measures 2 × 2 factorial analysis of variance. Because

there were two trees in the GNP treatment at each plot and both trees received the same treatment, the data for these trees were averaged at the plot level. The GNP trees were included at each plot to determine whether there were any differences between non-grafted non-shaded trees and grafted non-shaded trees. Because there were no statistical differences detected between GNP and NN trees for any response variables ($P > 0.100$), the GNP trees were removed from the final analysis of variance. All data conformed to the assumptions of normality and equality of variance. The root excavations revealed that three “grafted” pairs of trees (two GS–GN pairs and one GNP–GNP pair) were not actually grafted together, so the number of replicates within each treatment was: 9 GS, 9 GN, 13 NS and 15 NN. In both the randomized block and repeated measures factorial analyses, each plot was designated as a “block,” so there were 11 blocks in this study.

The relationships between root, branch and needle TNC concentrations and graft xylem area, graft phloem circumference, age of graft or the number of grafts connecting the two trees was analyzed by simple linear regression. If a tree pair was connected by more than one graft, the phloem circumferences/xylem areas of all grafts were summed so that the total phloem circumference/xylem area connecting two trees was analyzed. The linear regression analysis incorporated all grafted trees (GS, GN and GNP). Release 8.1 of SAS (SAS Institute, Cary, NC) was used for all analyses, multiple comparisons were done with linear contrasts and a significance level of $\alpha = 0.05$ was used for all response variables.

Results

The shading treatments did not significantly affect either daily maximum ($P = 0.133$) or minimum ($P = 0.783$) temperature within the crown. Crown temperatures in shaded and non-shaded trees were within 1.1 °C during the day and within 0.1 °C at night from May–August.

There were no significant interactions between shading and grafting for any response variables tested ($P > 0.122$). At the end of the study, root TNC concentrations differed significantly between treatments ($P < 0.001$; Figure 1). Root TNC concentrations was significantly affected by both the shade treatment ($P < 0.001$) and the presence of root grafts ($P = 0.032$); non-grafted shaded (NS) trees had significantly lower root TNC concentrations than grafted shaded (GS), grafted non-shaded (GN) and non-grafted non-shaded (NN) trees. On average, NS trees had root TNC concentrations of 3.5% compared to 5.7% in GS trees, 7.1% in GN trees and 6.9% in NN trees (Figure 1).

Neither branch nor needle TNC concentration differed significantly between treatments (branch $P = 0.085$, needle $P = 0.119$). At the end of the study, branch TNC concentrations were 7.2% and needle TNC concentrations were 10.1%, regardless of shading or the presence of root grafts.

The amount of crown recession, leader increments and RSG rates differed significantly between treatments (all $P < 0.001$). For these three variables, the shading treatment significantly

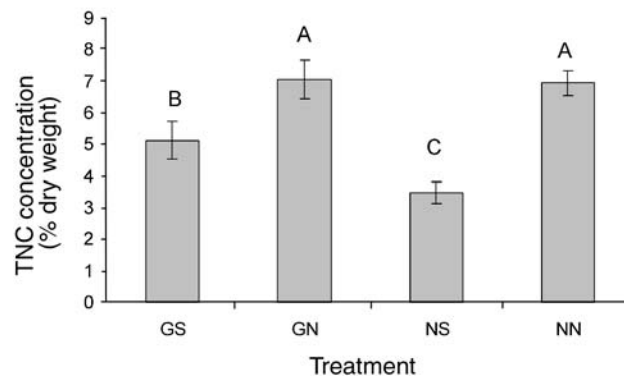


Figure 1. Effects of shading and root grafting treatments on total nonstructural carbohydrate (TNC; sugar + starch) concentrations in coarse root tissues of the trees in August 2004. The treatments were: grafted shaded (GS), grafted non-shaded (GN), non-grafted shaded (NS) and non-grafted non-shaded (NN) trees. Bars represent the standard error of the mean and bars with different letters are significantly different at the 95% confidence level.

reduced crown and stem growth (crown recession $P < 0.001$, leader increment $P < 0.001$, RSG $P = 0.007$; Table 1), but the presence of root grafts had no effect (crown recession $P = 0.298$, leader increment $P = 0.703$, RSG $P = 0.936$; Table 1).

Total nitrogen (N) and phosphorus (P) contents in the new needles did not differ significantly between treatments (nitrogen $P = 0.060$, phosphorus $P = 0.723$). Total N averaged 1.35% and total P averaged 0.17% across all treatments.

Both graft xylem area ($P < 0.001$, $R^2 = 0.850$) and graft phloem circumference ($P = 0.005$, $R^2 = 0.291$) increased with increasing graft age (Figures 2A and 2B). The differences in root TNC concentrations between GS and GN trees decreased with increasing ratios of graft phloem circumference:mean tree volume ($P = 0.017$, $R^2 = 0.584$; Figure 3). This relationship was observed only when GS and GN trees were analyzed; in GNP trees, root TNC concentrations were unaffected by the ratio of graft phloem circumference to mean tree volume of the grafted pair ($P = 0.974$). Also, no relationships were observed between the number of root grafts connecting two trees, graft xylem area or graft phloem circumference and branch or needle TNC concentrations.

Table 1. Leader increment, crown recession distance and relative stem growth rates of trees after 4 months of treatment. The treatments were: grafted shaded (GS), grafted non-shaded (GN), non-grafted shaded (NS) and non-grafted non-shaded (NN) trees. Numbers in parentheses represent the standard error of the mean. Values followed by different letters are significantly different at the 95% confidence level.

Treatment	Leader increment (cm)	Crown recession distance (cm)	Relative stem growth rate (%)
GS	14.0 (± 1.5) a	15.7 (± 7.9) c	76.6 (± 11.2) f
GN	36.8 (± 2.2) b	0.0 (± 0.0) d	98.6 (± 13.1) ef
NS	12.3 (± 2.3) a	36.4 (± 11.1) c	70.4 (± 10.52) f
NN	33.2 (± 2.5) b	0.0 (± 0.0) d	112.6 (± 11.3) e

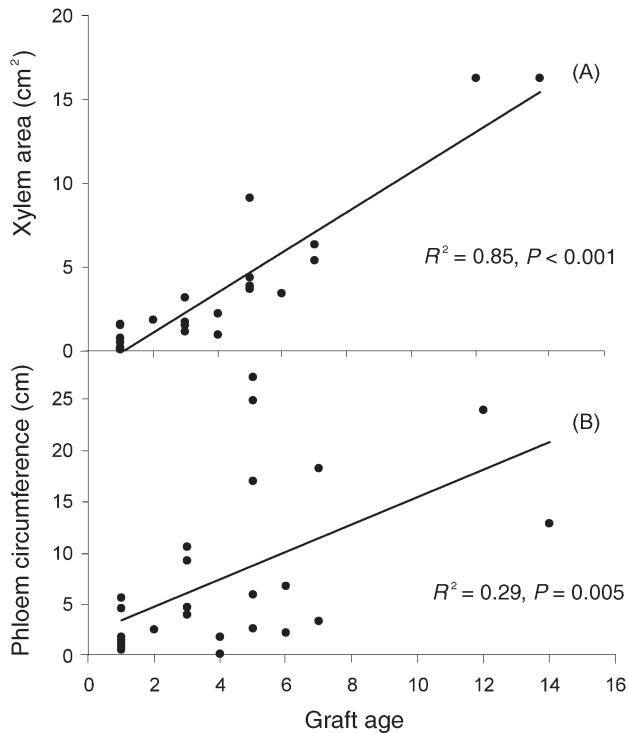


Figure 2. Relationships between graft age and xylem area (A) and between graft age and phloem circumference (B).

Discussion

Our results indicate that trees in inferior positions gained resources from adjacent trees through root grafts. Total non-structural carbohydrate (TNC) concentrations in coarse roots were about 40% greater in grafted shaded (GS) trees than in non-grafted shaded (NS) trees (Figure 1). The largest impact of the grafts was manifest in the maintenance of higher TNC concentrations in the roots of shaded trees with grafts compared with shaded trees without root grafts (Figure 1). The ef-

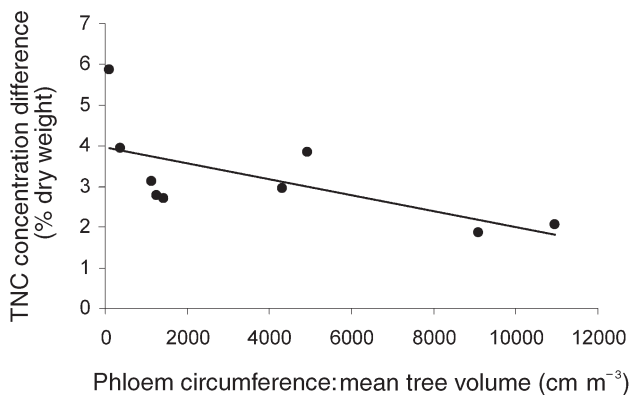


Figure 3. Relationship between the ratio of phloem circumference to mean tree volume of the grafted pair (cm m^{-3}) and the difference in total nonstructural carbohydrate (TNC) concentrations between grafted pairs of trees where one tree was shaded (GS trees) and one tree was non-shaded (GN trees).

fects of root grafts on crown growth and foliar carbohydrate concentrations were less clear. There was a strong trend for less crown recession ($P = 0.076$) and for greater relative stem growth and greater leader increments in grafted shaded trees compared with non-grafted shaded trees (Table 1). However, the overall impacts of grafts were less evident on aboveground parts than at the root level. Given that the root system is the first resource sink to be in contact with the carbohydrates obtained from grafted neighbors, it is possible that few carbohydrates were passed on to the more distal organs above ground. A similar relationship has been observed in herbaceous plants grown in nutrient-poor soils where the root system sequestered the majority of available nutrients and passed few resources on to the rest of the plant (reviewed by Clarkson 1985).

Because there were declines in root carbohydrates and crown growth in shaded trees, especially in the non-grafted shaded trees, it is clear that the deep-shading treatment allowed relatively little photosynthesis to occur (see photosynthetic light response curves by Landhäusser and Lieffers 2001) and it is unlikely that our shaded trees would have survived a second growing season. Consequently, the size of the phloem connection joining many of the grafted tree pairs may have been insufficient to conduct enough reserves to satisfy the demands of the root system as well as the more distal organs at the top of shaded trees. The size of graft appeared to have been important in determining how much photosynthate was passed between trees (Figure 3) and in most cases, it seemed that the grafts were too small to deliver sufficient carbohydrates to maintain these large and heavily shaded neighbors. If the shaded neighbor was proportionately smaller than the non-shaded tree, as would be the case in asymmetric competition, the grafts may have been better able to support the shaded tree.

We predicted a parasitic relationship between the grafted shaded (GS) and grafted non-shaded (GN) tree pairs, with the shaded neighbor negatively affecting the growth and root carbohydrate supply of the GN trees relative to the non-grafted non-shaded (NN) trees. However, we detected insignificant growth reductions in the GN trees over one growing season (Table 1), suggesting that the GN trees were unaffected by the apparent transfer of TNC to the GS trees (Figure 1). It is possible that the increased absorptive surface area of the grafted root system improved the water supply to the foliage of non-shaded trees because the shaded trees likely had low stomatal conductance as a result of the artificial boundary layer and low irradiances. Also, the photosynthetic efficiency of GN trees may have been increased as a result of the high sink strength of the grafted shaded neighbor and grafted root system (e.g., Neales and Incoll 1968, Herold 1980, Myers et al. 1999, Pieters et al. 2001).

In conclusion, grafted trees growing in light-limited environments were partially supported by carbohydrates transferred across root grafts from their more vigorous partners (Figure 1). Even a moderate transfer of resources to a subordinate tree, however, could reduce competitive asymmetry (Knox et al. 1989, Kenkel et al. 1997, Nilsson et al. 2002), especially when the transfer occurs year after year. Generally,

mortality is greatest among the most suppressed individuals; however, suppressed trees that are grafted to a more vigorous partner may persist longer, especially if connected with a large graft. Thus, root grafting may partially explain the slow self-thinning of high-density lodgepole pine stands.

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