Root connections can trigger physiological responses to defoliation in nondefoliated aspen suckers

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Abstract: In species such as aspen (*Populus tremuloides* Michx.), trees are interconnected through their root system owing to their regeneration mode by root suckering. These root connections challenge classic notions of forest dynamics that consider trees as individuals competing for resources, because root connections allow trees to share water, minerals, and carbohydrates. The purpose of this study was to demonstrate that trees can directly influence the physiology of other nearby trees through root connections. In the summers of 2007 and 2008, pairs of aspen suckers (i.e., two suckers connected by a parental root) were selected and divided into three height classes and compared with each other (dominant, codominant, suppressed). Suckers distally positioned on the parental root were manually defoliated, and the effects of defoliation on photosynthesis, stomatal conductance, and specific leaf area (SLA) were measured on connected but nondefoliated suckers. Results showed that defoliation caused physiological responses in the interconnected trees in summer 2007, which was drier than summer 2008. Defoliation of a connected sucker had a greater effect on suppressed suckers, for which mean photosynthesis rate increased by 17% compared with that of controls. The effect was less important for codominant (14% increase) and dominant (12%) trees. SLA of suppressed suckers also increased, while the increase in SLA values was smaller for codominant and dominant suckers. In summer 2008, no defoliation effect was observed, probably owing to high moisture conditions that resulted in much higher stomatal conductance values compared with those in 2007 (+55%). Under high humidity conditions, leaf specific hydraulic conductance does not constrain stomatal conductance, so the rate of CO_2 assimilation was probably at its maximum capacity. This study demonstrated that trees could physiologically interact through root connections, and these interactions should thus be considered in studies of stand dynamics.

Key words: gas exchange, root link, specific leaf area, clonal integration.

Résumé : Chez des espèces comme le tremble (*Populus tremuloides* Michx.), les arbres sont interconnectés par l'intermédiaire de leurs systèmes racinaires, dû à leur mode de régénération par drageonnage. Ces connexions racinaires remettent en question les notions de la dynamique des forêts considérant les arbres comme des individus compétitionnant pour les ressources, puisque les connexions racinaires permettent aux arbres de partager l'eau, les minéraux et les glucides. L'étude visait à démontrer que l'arbre peut influencer directement la physiologie des autres par ses connections racinaires. Pendant les étés de 2007 et 2008, les auteurs ont sélectionné des paires de drageons de peupliers (c.-à-d. deux drageons reliés par une racine mère) et les ont divisés en trois classes pour les comparer les uns aux autres (dominant, codominant, dominé). Ils ont défolié manuellement les drageons en position distale sur la racine mère et ils ont mesuré les effets de la défoliation sur la photosynthèse, la conductance stomatale et la surface foliaire spécifique (SFS) sur les drageons connectés, mais non défoliés. Les résultats montrent que la défoliation entraine des réactions physiologiques chez les arbres interconnectés, à l'été 2007 un été plus sec que celui de 2008. La défoliation d'un drageon connecté a exercé un effet plus marqué sur les drageons dominés, où le taux moyen de photosynthèse a augmenté de 17 % par rapport aux témoins. L'effet fut moins important chez les arbres codominants (14 %) et dominants (12 %). La SFS des drageons des drageons dominés a également augmenté, alors que l'augmentation de la SFS fut plus faible chez les drageons codominants et dominants. À l'été 2008, on a observé aucun effet de la défoliation, probablement parce que les conditions de forte humidité ont engendré des valeurs de conductance stomatale beaucoup plus fortes, comparativement à 2007 (+55 %). Sous des conditions de forte humidité, la conductance hydraulique spécifique ne limite pas la conductance stomatale, de sorte que l'assimilation du CO2 était probablement à sa capacité maximale. Cette étude démontre que les arbres peuvent interagir physiologiquement par leurs connexions racinaires, ce que l'on devrait alors considérer dans les études de la dynamique des peuplements.

Mots-clés : échanges gazeux, lien racinaire, surface foliaire spécifique, intégration clonale.

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Introduction

Trees are traditionally considered discrete entities competing with each other for resources. However, this concept of individuality is challenged by species for which trees are interconnected through their root system, because root connections allow the sharing of resources between trees. Natural root grafts, for example, have been found in more than 150 species (Bormann and Graham 1966) and allow the transfer and sharing of water, minerals, carbohydrates (DeByle 1964; Zahner and DeByle 1965; Fraser et al. 2006) and microorganisms and pathogens (Kuntz and Riker 1955). Interconnection of trees can thus have a significant impact on their ecology and on forest dynamics, since it allows trees to interact with, and be affected by, their interconnected neighbours.

The root system of aspen (*Populus tremuloides* Michx.) has been widely studied, owing to its regeneration mechanism by root suckering (Frey et al. 2003). Following large forest disturbances such as fire or clear-cutting, new shoots (suckers) develop from the residual root system of the previous stand, forming a new forest stand where most trees are interconnected through their parental roots (Shepperd 1993; DesRochers and Lieffers 2001*b*). It is now known that aspen trees can remain interconnected throughout their life span (DesRochers and Lieffers 2001*a*) and that trees from different clones may even form root grafts (Jelínková et al. 2009), increasing the initial level of interconnection between trees. Although most will agree that such a level of interconnection between trees has great ecological significance (Bormann and Graham 1966; Basnet et al. 1993), the fact that tree physiology could be affected by the presence of root connections has never been explicitly demonstrated, and consequently root connections are not considered in forest dynamics and tree physiology concepts.

In contrast to trees, the concept of clonal integration has been well researched in herbaceous plants, and we know that the extent of clonal integration between two or more interconnected ramets depends on the degree of physiological interdependence between them (Pitelka and Ashmun 1985; De Kroon and Van Groenendael 1997). This is likely to be high in aspen suckers because young ramets have few new roots and rely mostly on the parental root system on which they grow (Zahner and DeByle 1965; DesRochers and Lieffers 2001*a*, 2001*b*).

The purpose of this study was to demonstrate that a tree could influence the physiology of another tree through a root connection. With the notion that trees having roots in common are physiologically interdependent, we posed the hypothesis that defoliation of an aspen sucker would trigger a physiological response in a nondefoliated but interconnected sucker. In particular, since defoliation can trigger compensatory photosynthesis (Nowak and Caldwell 1984) in the remaining leaves of a partially defoliated tree (Hart et al. 2000), we expected that it would also be triggered in the leaves of a nondefoliated but interconnected sucker. Suckers establish vascular continuity only with the distal side of the parental root from which they originate (Brown 1935). Aspen suckers are thus largely dependent on the distal side of the parental root, and their survival and growth are greatly reduced if the distal side is cut off, while there is little effect of cutting off the proximal side (Zahner and DeByle 1965).

Hence, physiological responses to defoliation were measured on suckers that had a distally placed neighbouring sucker defoliated.

Our second hypothesis was that the response to defoliation of nondefoliated suckers would vary depending on the comparative size of the defoliated and nondefoliated suckers. Unlike aspen clones invading new territories (Peltzer 2002) or for rhizomatous herb or shrub plants where a "mother plant" produces offspring ramets and supports their initial growth (Noble and Marshall 1983; Zhang et al. 2002), aspen suckers produced after a major forest disturbance are all produced at the same time on a parental root system when the parent tree is dead or removed (Frey et al. 2003). All suckers thus contribute to the maintenance of the parental root system on which they grow, and high sucker density and leaf area are important to support respiration costs of such a large underground biomass (DesRochers and Lieffers 2001*b*). We thus expected that defoliating a larger sucker within a pair would create a greater imbalance between leaf area and root biomass and thereby induce a greater response in the nondefoliated sucker. This would be analogous to root-sprouting herbaceous species (e.g., *Linaria vulgaris* Mill., *Epilobium angustifolium* L.), where clonal integration seemed to be related to the relative value of damaged ramets for the clone (Hellström et al. 2006). To test these hypotheses, a defoliation experiment was conducted in young aspen stands regenerated through root suckering. One member of a pair of interconnected suckers was defoliated and physiological response measured on the nondefoliated sucker.

Materials and methods

Study area

The study sites were located in the boreal forest of northwestern Quebec, Canada. Two sites were sampled in the summers of 2007 (site 1: 48°46′N, 77°48′W; site 2: 48°46′N, 77°47′W) and 2008 (site 3: 48°45′N, 77°50′W; site 4: 48°46′N, 77°49′W). These sites are located on the Quebec–Ontario clay belt resulting from deposits left by proglacial lakes Barlow and Ojibway (Vincent and Hardy 1977). The soil texture was heavy clay grey luvisol (Canada Soil Survey Committee 1998). Mean thickness of the organic matter was about 2 cm. For the past 30 years (1971–2000), mean annual rainfall in this region was 918 mm, with a mean annual temperature of 1.2 °C (Environment Canada 2009). These sites were chosen because they each presented young aspen stands that were 4 years old postharvest. Sucker density on the four sites ranged from 9000 to 25 000 stems·ha^{-1}, with a percent cover ranging from 35% to 80%. Other species had been planted on the sites, mainly white pine (*Pinus strobus* L.) on sites 2 and 4, red pine (*Pinus resinosa* Ait.) on sites 1 and 4, as well as white spruce (*Picea glauca* (Moench) Voss) on site 3.

Selection of suckers

Aspen pairs connected by a parental root were randomly selected at each site. The root connecting two suckers was easily detected by gently brushing off approximately 5 cm of the soil surface between two suckers. Pairs were selected only if both suckers were apparently in good health without any signs of damage on leaves or stems. To avoid confusion

between multiple interconnected suckers, only pairs that were relatively isolated from other suckers (a distance of at least more than the height of the suckers) were chosen. In summer 2007, 30 pairs and 10 control suckers were selected on sites 1 and 2, while 40 pairs were selected at site 3 (10 pairs as control) and 26 pairs at site 4 (8 pairs as control) in 2008. We chose control pairs in 2008 rather than single control trees as in 2007, to have an estimate of the net assimilation rate (A) and stomatal conductance (g_s) within the same replicate control. The size of control trees encompassed the size range of treatment trees.

Distance between connected suckers (root length) was measured, as well as the diameter of the connecting root at mid-distance between suckers. Root morphology between the two suckers was studied to determine which suckers were placed distally or proximally relative to the parental root (Fig. 1); new wood layers laid down by the suckers is mostly produced on the distal side of the parental root, making it larger than the proximal side, and the base of the suckers is usually bent toward the distal side (Brown 1935). Height and basal diameter of each sucker was measured, and sucker pairs were classified according to the size of the proximally placed sucker compared with that of the distally placed sucker (Fig. 1): codominant: the distally and proximally placed suckers were of similar size; dominant: the proximally placed sucker was taller than the distally placed sucker; suppressed: the proximally placed sucker was smaller than the distally placed sucker.

In July 2007 and 2008, when the suckers' leaves were fully expanded, leaves of distally placed suckers were manually removed and collected. Control suckers and pairs were not defoliated.

Physiological measurements

In 2007 (sites 1 and 2), A and g_s were measured on proximally placed and control suckers once before and 13 times after defoliation of the distally placed suckers (up to 36 days after defoliation). Gas exchange measurement days were discontinuous and dependent on weather conditions, as measurements could not be taken on rainy days. In 2008 (sites 3 and 4), three gas exchange measurements were done prior to defoliation of the distally placed suckers (only two were recorded for g_s), and measurements lasted for a 27-day period. A and g_s were measured with an infrared gas analyser equipped with a PLC6 broadleaf cuvette (CIRAS-2, PP Systems, Amesbury, Mass., USA). Measurements were made while supplying a CO_2 concentration of 350 ppm and using natural light with photosynthetically active radiation >1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during measurements. Gas exchange measurements were made between 0900 and 1300 h on sunny days with temperatures typically reaching a maximum of 23–24 °C, from mid-June to mid-July. Measurements were taken on a single leaf from each proximally placed sucker (replicates) in 2007, while three measurements were taken (pseudoreplicates) in 2008 to obtain an estimate of intra-sucker variation. Gas exchange measurements were made on recently matured leaves of approximately the same age and placed on a branch of the main stem in the upper two-thirds of the crown.

Laboratory analysis

At the end of the measurement period, leaves of proximally placed and control suckers were harvested. Together with leaves from distally placed suckers harvested at the beginning of the experiment, total leaf area of suckers was measured using a LI-COR LI- 3100 area meter (LI-COR Biosciences, Lincoln, Nebr., USA). Leaves were then air-dried for 72 h at 70 °C and weighed to obtain dry mass. Specific leaf area (SLA) was calculated by dividing total leaf area by dry mass of leaves for each sucker.

Statistical analysis

Linear mixed models (Pinheiro and Bates 2000) were used to compare the size of distally and proximally placed suckers at the beginning of the experiment (height and diameter of suckers) and to compare SLA of suckers at the end of the experiment. In these models, sites were considered as random effects, to properly account for the structure of the data and estimate within- and between-site variability. We conducted a separate analysis for each year (sites 1 and 2 in 2007, sites 3 and 4 in 2008). Gas exchange values (A and g_s) were also analysed using a linear mixed model, with date and size class of suckers and their interaction as fixed factors, and sites as random factors. This approach allowed the comparison of A and g_s values of suckers from each size class (dominant, codominant, suppressed) with control suckers for each date, taking into account the repeated measures nature of the design. Assumptions of mixed models (normality of residuals, homogeneity of variances, and normality of random effects) were verified (Pinheiro and Bates 2000). Since temperature during measurement of gas exchange had an influence on stomatal conductance, it was included as a covariable in the models. Statistical analyses were performed using R software, version 2.8.1 (R Development Core Team 2007), with results considered significant at $\alpha = 0.05$.

Results

Selected pairs of suckers had been well categorized according to their size at the beginning of the experiment, given that size differences between proximally and distally placed suckers were significant for dominant and suppressed classes, while they were not significant for the codominant class (Table 1). Defoliation of distally placed suckers in summer 2007 increased the A values of proximally placed suckers (Fig. 2A). This increase in A was larger when the smaller the proximally placed suckers were compared with the distally placed suckers: suckers from the suppressed and codominant size classes had A values that were 17% and 14% greater than those of the control suckers, respectively (Fig. 2A). Suckers from the dominant size class also had increased A values compared with those of the controls (+12% on average), but differences were only significant for days 1 ($P = 0.02$), 5 ($P = 0.01$), and 32 ($P = 0.02$; Table S1[1]). There was no difference between the A values of proximally placed and control suckers for most of the measurement period in 2008 (Fig. 2B; Table S2[1]).

Stomatal conductance (g_s) of suckers from the three size classes and that of the control suckers was 55% greater in

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[1]Supplementary data for this article are available on the journal Web site (http://www.nrcresearchpress.com/doi/suppl/10.1139/B11-062).

Fig. 1. Diagram showing the three size classes of aspen suckers, in relation to the defoliated sucker, used in the experiment.
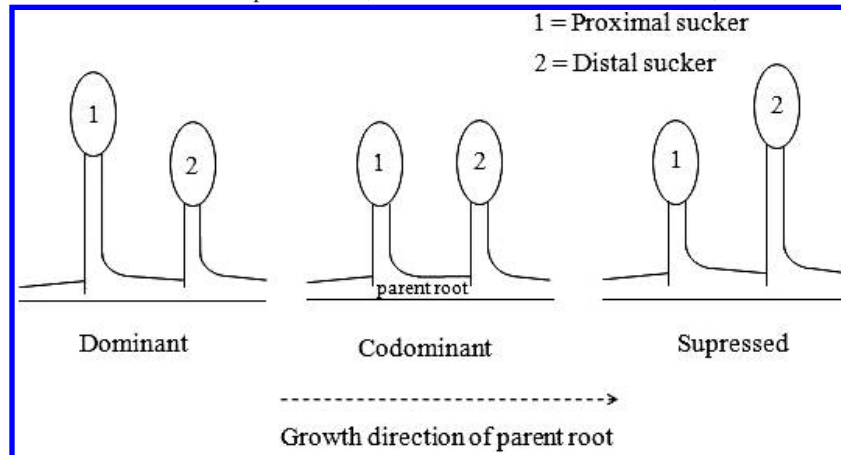

Table 1. Mean initial height and diameter of proximally and distally placed suckers for sites 1 and 2 and sites 3 and 4 and for each size class before distally placed suckers were defoliated.

	Height (cm)		Diameter (mm)	
Class	Proximally placed suckers	Distally placed suckers	Proximally placed suckers	Distally placed suckers
Sites 1 and 2				
Dominant	155.63*	118.26*	14.52*	11.01*
Codominant	147.70	144.53	13.94	12.78
Suppressed	105.19*	145.94*	9.63*	13.62*
Sites 3 and 4				
Dominant	161.11*	128.89*	10.78*	8.67*
Codominant	187.14	188.28	13.86	13.29
Suppressed	130.00*	162.91*	6.36*	9.36*

Note: *, $P < 0.05$: significant difference between proximally and distally placed suckers.

2008 compared with that in 2007 (155–381 mmol·m^{-2}·s^{-1} in 2007 vs. 358–685 mmol·m^{-2}·s^{-1} in 2008; Fig. 3). There was no difference between g_s of proximally placed dominant and control suckers throughout the measurement period in 2007 or 2008 (Tables S3, S4[1]).

Although they were initially similar in size, suckers in the codominant size class in 2007 had greater leaf area at the end of the measurement period compared with total leaf area of the distally placed suckers defoliated at the beginning of the measurement period ($P = 0.005$, data not shown). This indicates that nondefoliated suckers had produced new leaves during this period, which was indeed observed in the field. Results were similar in 2008 for suckers in the suppressed and dominant size classes, while total leaf area of the proximally and distally placed codominant suckers (measured at the end and at the beginning of the measurement period, respectively) was similar (data not shown). Defoliated suckers had also produced a new set of leaves by the end of the experiment.

Mean SLA values of proximal suckers from the suppressed size class were greater than those of the control and dominant suckers at the end of the measurement period in 2007 (Fig. 4A). Differences in SLA compared to controls were less important for suckers in the codominant and dominant size classes (Fig. 4A). In 2008, only suckers from the codominant size class had mean SLA values lower than mean SLA of controls (Fig. 4B).

Discussion

This study showed that compensatory photosynthesis (Nowak and Caldwell 1984) could be rapidly induced in a nondefoliated aspen sucker through a root connection with a defoliated tree. This constitutes one of the first examples of how the physiology of trees can be directly affected by root connections. The effect of defoliation in 2007 was greater the smaller the nondefoliated sucker was compared with the defoliated sucker (Fig. 2). Since they are interconnected, photosynthesis of both suckers contributes to the photosynthate supply of the communal root system. This compensatory photosynthesis would be necessary to compensate for the loss of the photosynthetic activity of the defoliated suckers from a common root/suckers system. Classic notions of forest dynamics in relation to competition for resources between neighbouring trees are challenged by these root connections, which allow the transfer and sharing of substances between connected trees. For example, when a certain proportion of interconnected trees dies, there may be little enhancement of availability of resources for the remaining trees if roots of dead trees are still alive and continue to occupy the soil (DesRochers and Lieffers 2001a). This could explain the contradictory results obtained from thinning experiments in aspen forests (Penner et al. 2001) or in stands where trees are interconnected through root grafts (Tarroux et al. 2010). The ecological significance of root connections should thus be considered in the dynamics of such stands, because if trees

Fig. 2. Net photosynthesis (A) of proximally placed and control suckers after defoliation of distally placed suckers in 2007 (A) and in 2008 (B). Significant differences compared with the control at each measurement date are represented with one asterisk ($P < 0.05$). Error bars represent standard errors of the mean.
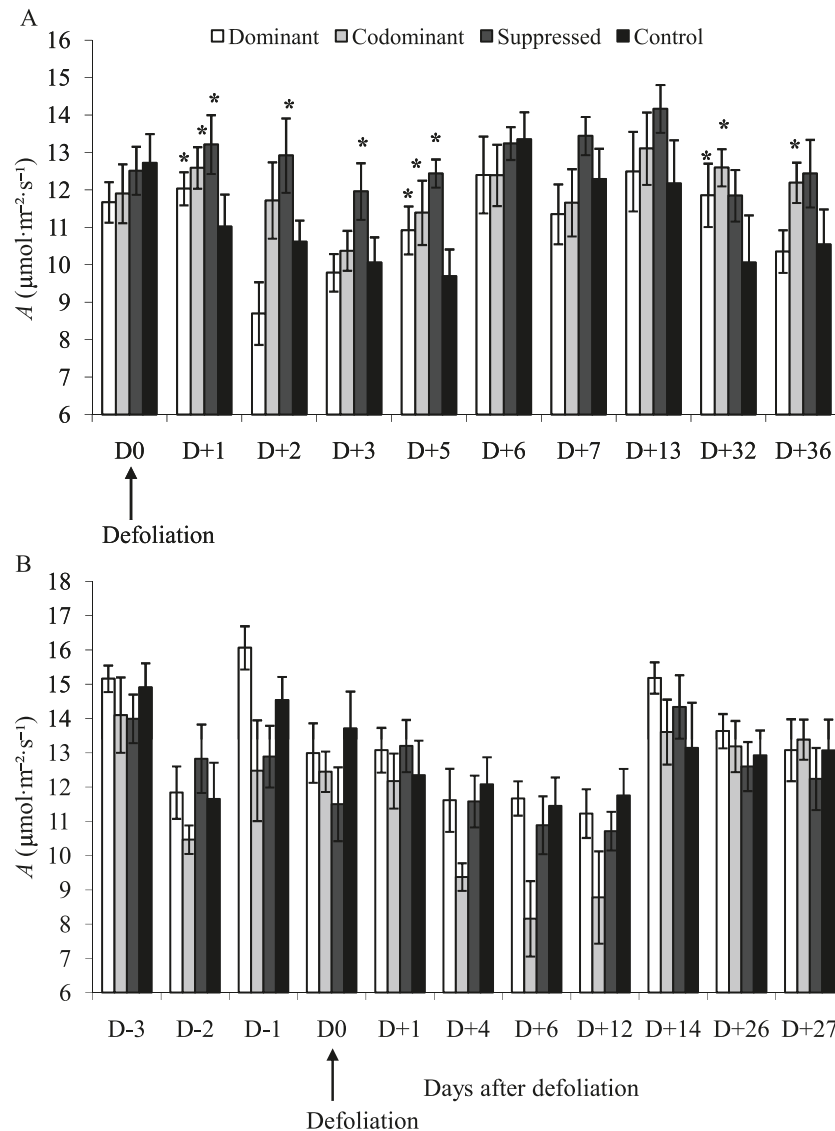

share a common root system, they have the potential to interact with each other.

Clonal integration has been widely studied in herbaceous plants, and resource translocation was mostly found in the distal direction (acropetally), towards the developing apex of the clone (Noble and Marshall 1983; Pitelka and Ashmun 1985). In aspen, although expansion of a clone from a mother tree is possible (Barnes 1966, Peltzer 2002), suckers are usually produced from an existing root system after apical dominance has been eliminated by the death of the mother tree (Frey et al. 2003). The suckers establish vascular continuity with the distal portion of the parent root (Brown 1935) and are thus highly dependent on resource translocation coming from the proximal (or basipetal) direction (Zahner and DeByle 1965). Moreover, at first, interconnected suckers all grow on the same root system and are thus highly integrated until they have developed their own roots. It was believed that trees became independent after their own roots developed (Brown and DeByle 1989); however, we now

know that they remain interconnected throughout their life span (DesRochers and Lieffers 2001a). These root connections (mother roots) remain seemingly functional, because they include the root systems of otherwise dead trees, suggesting translocation of assimilates in phloem tissues (De-Byle 1964; DesRochers and Lieffers 2001a).

Although aspen suckers benefit from an already established root system from which they originate, this large underground biomass (Shepperd and Smith 1993) is nonphotosynthetic and can constitute a very large sink for photosynthates, requiring high total leaf area to be maintained alive (DesRochers and Lieffers 2001b). Hence when large suckers are defoliated, their leaf area represents a larger proportion of the total leaf area of a common root system, i.e., a greater imbalance between leaf area and root biomass. Therefore, photosynthetic rates of the remaining suckers have to increase even more to compensate for the growth and maintenance respiration of the system, in comparison to when smaller suckers (less leaf area) are defoliated. For white pine trees

Fig. 3. Stomatal conductance (g_s) of proximally placed and control suckers after the defoliation of distally placed suckers in the summers of 2007 (A) and 2008 (B). Significant differences compared with controls are represented with one asterisk ($P < 0.05$). Error bars represent standard errors of the mean.
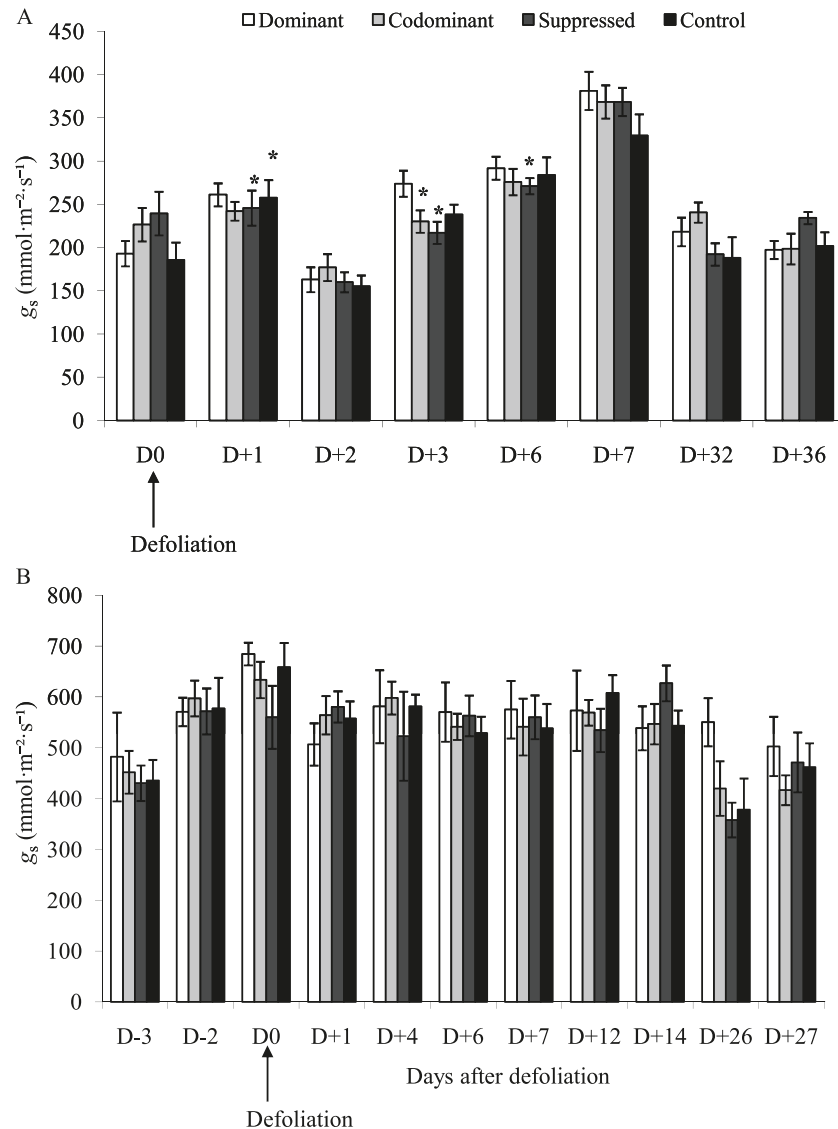

connected through natural root grafts, Eis (1972) had also shown that if a dominant tree from a dominant/suppressed union was cut, the suppressed tree could hardly offset the loss of foliage of its larger neighbour, while cutting the suppressed tree had little effect on wood production of the dominant tree. It was also found in *Lolium multiflorum* Lam. (a grass species) that defoliated suckers could become strong sinks for photoassimilates from other nondefoliated shoots (Marshall and Sagar 1965), revealing a high level of integration between ramets (Pitelka and Ashmun 1985). For *Linaria vulgaris*, a root-sprouting perennial herb, it was found that even if ramets of a clone were physiologically integrated and interdependent, support through a net transfer of carbon to a damaged ramet did not occur (Hellström et al. 2006). Klimešová et al. (2009) also found that intraclonal communication between ramets of root-sprouting *Epilobium angustifolium* was restricted. Perhaps the main difference between herb species and aspen is the cost and perenniality of shoots, which is much lower in herbs than in trees, and thus

the sacrifice of a defoliated ramet seems more likely to occur. However ,we question this notion of "sacrificing" less valuable (or damaged) ramets of a clone (Hellström et al. 2006) for aspen, because ramets are morphologically integrated and clones cannot "decide" to severe the root connection to the communal parental root system (DesRochers and Lieffers 2001a). More likely, ramets and parts of the communal root system will die off when the amount of total leaf area of the clone becomes too small to compensate for respiration costs of the parental root system and leafless ramets (DesRochers and Lieffers 2001b; DesRochers et al. 2002).

In 2008, however, results showed no effect of defoliation on the nondefoliated suckers (Fig. 2B). The difference between 2007 and 2008 could be explained by the very high g_s values in 2008 compared with those in 2007 (Fig. 3). Moisture conditions were very different between 2007 and 2008: total precipitation over the measurement periods amounted to 80.5 mm in 2007 versus 211.5 mm in 2008 (Environment Canada 2009). Under normal weather conditions, g_s in aspen

Fig. 4. Mean specific leaf area (SLA) of proximally placed suckers from each size class and for controls in 2007 (A) and 2008 (B). Bars represent standard errors of the mean, and bars with the same letter are not significantly different ($P = 0.05$).
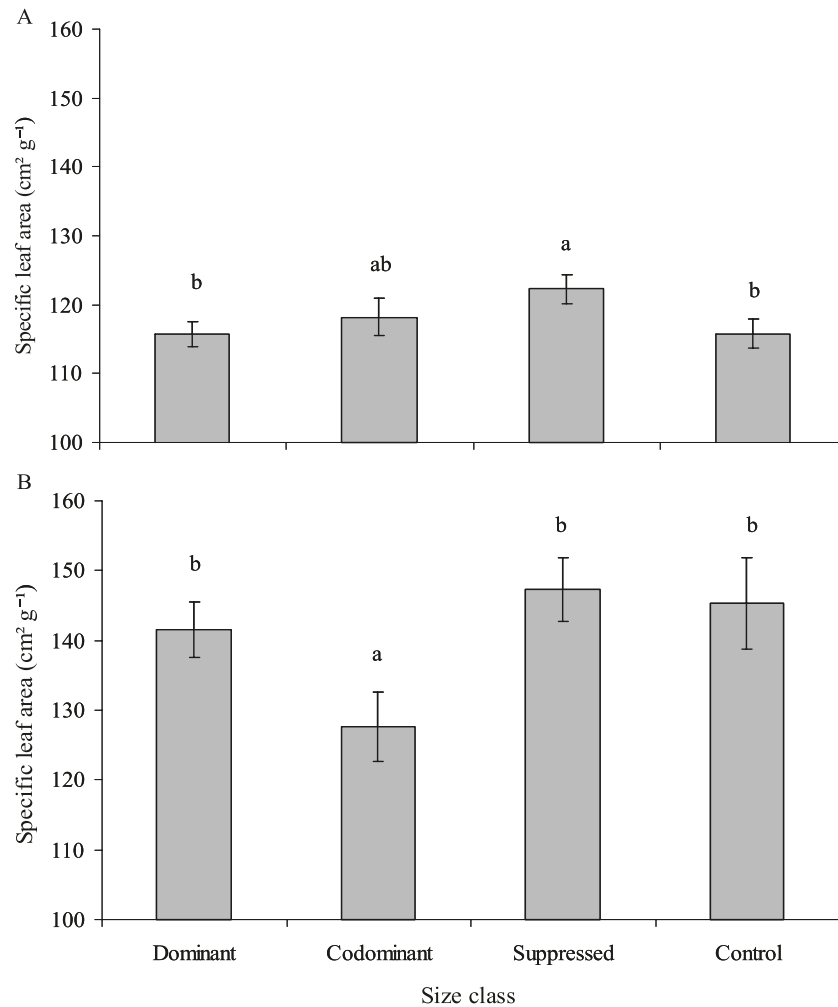
is about 300–350 mmol·m^{-2}·s^{-1} (Hart et al. 2000; DesRochers et al. 2003), whereas it ranged from 350 to 700 mmol·m^{-2}·s^{-1} in 2008 (Fig. 3B). In a partial defoliation study, Hart et al. (2000) also found that compensatory photosynthesis was difficult to observe on residual leaves under low vapour pressure deficit (i.e., good moisture conditions), because specific hydraulic conductance of leaves did not limit g_s of trees. Thus, A, which is largely controlled by g_s (Pessarakli 2005), was probably already maximal in 2008, somewhat concealing the response to defoliation. It was probably the dry conditions of 2007 during the measurement period that allowed us to measure a compensatory photosynthesis effect of defoliating connected suckers. However, if humidity conditions had been too low as to completely limit g_s to maintain leaf water potential above the wilting point under high vapour pressure deficit (Hart et al. 2000), it is probable that no increase in A would have been observed. This may have limited survival of defoliated suckers and reduced overall productivity of the interconnected suckers, illustrating a good example of the ecological significance of root connections.

Mechanisms involved in the increase of photosynthesis according to size class of the suckers do not appear to be related to an increase in leaf hydraulic properties of the nondefoliated suckers; even if suckers in the suppressed and codominant size classes had higher A values than the controls following defoliation in 2007 (Fig. 2), their g_s values were lower (Fig. 3A). Thus, unlike many studies that have shown that increased A was often accompanied by an increase in g_s in residual leaves following partial defoliation (Heichel and Turner 1983; Ovaska et al. 1992; Reich et al. 1993; Pataki et al. 1998), the results of g_s in 2007 showed an opposite trend (Fig. 3A). The observed increased in A coupled with a decrease in g_s could be explained by the increase in the SLA of nondefoliated suckers, which also increased proportionally to the imbalance between the size of defoliated versus nondefoliated suckers (Fig. 4A).

SLA is recognized as one of the most important functional attributes in plants (Lambers et al. 1998). It is jointly determined by the density and thickness of the leaf (Witkowski and Lamont 1991). Thus, an increase in SLA indicates an increased leaf area per unit dry mass, which reduces light attenuation in the leaf (Shipley 2006). Increased SLA is also a sign of decreased density of leaf tissues and increased water content, water content being the most important parameter that determines SLA (Shipley 1995). Water in leaves is mainly localized in the cytoplasm of cells, which contains nutrients and enzymes responsible for photosynthesis. Thus, an increase in SLA often leads to increased cytoplasmic com-

ponents such as nitrogen, which is usually well correlated with SLA (Garnier et al. 1999; Shipley and Lechowicz 2000). This can in turn lead to increased chlorophyll synthesis (Liu and Dickmann 1996) and A (Field and Mooney 1986; Reich et al. 1995; Feng et al. 2008). Again, the SLA of nondefoliated suckers measured in 2008 did not respond to defoliation (Fig. 4B), which could again be explained by high humidity conditions and high g_s values (Fig. 3B). SLA values were also greater in 2008 than in 2007 (Fig. 4). High SLA values could also reflect low sugar content in leaves (Meziane and Shipley 2001), which may have been caused by the defoliation. Leaves of aspen suckers, however, do not constitute important storage organs for carbohydrates (Landhäusser and Lieffers 2003).

Conclusion

Trees sharing a root system can physiologically interact with one another. This study showed that a defoliation stress could impact an interconnected sucker and cause compensatory photosynthesis. This was likely due to an increase in SLA, allowing nondefoliated suckers to compensate for the loss of leaves of the defoliated sucker. The root connection thus allows neighbouring interconnected suckers to respond to environmental stresses as a unit rather than individually. Up to a certain degree of severity, root connections could temper physiological impacts of stressful events. On the other hand, more severe stresses affecting the majority of trees in a communal root system could potentially accelerate the death of a stand, if residual trees were unable to compensate for the loss of photosynthetic capacity, thus causing stands to decline as a whole (DesRochers and Lieffers 2001a).

Acknowledgements

References

Barnes, B.V. 1966. The clonal growth habit of American aspens. Ecology, **47**(3): 439–447. doi:10.2307/1932983.

Basnet, K., Scatena, F.N., Likens, G.E., and Lugo, A.E. 1993. Ecological consequences of root grafting in Tabonuco (*Dacryodes exelsa*) trees in the Luquillo experimental forest, Puerto Rico. Biotropica, **25**(1): 28–35. doi:10.2307/2388976.

Bormann, F.H., and Graham, B.F., Jr. 1966. Natural root grafts. Bot. Rev. **32**(3): 255–292. doi:10.1007/BF02858662.

Brown, A.B. 1935. Cambial activity root habit and sucker shoot development in two species of poplar. New Phytol. **34**(3): 163–179. doi:10.1111/j.1469-8137.1935.tb06839.x.

Brown, J.K., and DeByle, N.V. 1989. Effects of prescribed fire on biomass and plant succession in western aspen. USDA For. Serv. Res. Pap. INT-412.

Canada Soil Survey Committee. 1998. The Canadian system of soil classification. NRC Research Press, Ottawa.

De Kroon, H., and Van Groenendael, J. 1997. The ecology and evolution of clonal plants. Backhuys Publishers, Leiden.

DeByle, N.V. 1964. Detection of functional intraclonal aspen root connections by tracers and excavation. For. Sci. **10**: 386–396.

DesRochers, A., and Lieffers, V.J. 2001a. The coarse-root system of mature *Populus tremuloides* in declining stands in Alberta, Canada. J. Veg. Sci. **12**(3): 355–360. doi:10.2307/3236849.

DesRochers, A., and Lieffers, V.J. 2001b. Root biomass of regenerating aspen (*Populus tremuloides*) stands of different densities in Alberta. Can. J. For. Res. **31**: 1012–1018.

DesRochers, A., Landhäusser, S.M., and Lieffers, V.J. 2002. Coarse and fine root respiration in aspen (*Populus tremuloides*). Tree Physiol. **22**: 725–732.

DesRochers, A., Van Den Driessche, R., and Thomas, B.R. 2003. Nitrogen fertilization of trembling aspen seedlings grown on soils of different pH. Can. J. For. Res. **33**(4): 552–560. doi:10.1139/x02-191.

Eis, S. 1972. Root grafts and their silvicultural implications. Can. J. For. Res. **2**(2): 111–120. doi:10.1139/x72-022.

Environment Canada. 2009. Climate normals in Canada. Environment Canada, Service of Atmospheric Environment, Ottawa, Ont.

Feng, Y.U., Fu, G.L., and Zheng, Y.U. 2008. Specific leaf area relates to differences in leaf construction cost, photosynthesis, nitrogen allocation, and use efficiencies between invasive and noninvasive alien congeners. Planta, **228**(3): 383–390. doi:10.1007/s00425-008-0732-2.

Field, C., and Mooney, H.A. 1986. The photosynthesis–nitrogen relationship in wild plants. *In* On the economy of plant form and function. *Edited by* T.J. Givnish. University Press, Cambridge.

Fraser, E.C., Lieffers, V.J., and Landhäusser, S.M. 2006. Carbohydrate transfer through root grafts to support shaded trees. Tree Physiol. **26**(8): 1019–1023.

Frey, B.R., Lieffers, V.J., Landhäusser, S.M., Comeau, P.G., and Greenway, K.J. 2003. An analysis of sucker regeneration of trembling aspen. Can. J. For. Res. **33**(7): 1169–1179. doi:10.1139/x03-053.

Garnier, E., Salager, J.L., Laurent, G., and Sonié, L. 1999. Relationships between photosynthesis, nitrogen and leaf structure in 14 grass species and their dependence on the basis of expression. New Phytol. **143**(1): 119–129. doi:10.1046/j.1469-8137.1999.00426.x.

Hart, M., Hogg, E.H., and Lieffers, V.J. 2000. Enhanced water relations of residual foliage following defoliation in *Populus tremuloides*. Can. J. Bot. **78**: 583–590.

Heichel, G.H., and Turner, N.C. 1983. CO_2 assimilation of primary and regrowth foliage of red maple (*Acer rubrum* L.) and red oak (*Quercus rubra* L.): response to defoliation. Oecologia, **57**: 14–19. doi:10.1007/BF00379555.

Hellström, K., Kytöviita, M.-M., Tuomi, J., and Rautio, P. 2006. Plasticity of clonal integration in the perennial herb *Linaria vulgaris* after damage. Funct. Ecol. **20**(3): 413–420. doi:10.1111/j.1365-2435.2006.01115.x.

Jelínková, H., Tremblay, M.F., and DesRochers, A. 2009. Molecular and dendrochronological analysis of natural root grafting in *Populus tremuloides* (salicaceae). Am. J. Bot. **96**(8): 1500–1505. doi:10.3732/ajb.0800177.

Klimešová, J., Pokorná, A., and Klimeš, L. 2009. Establishment growth and bud-bank formation in *Epilobium angustifolium*: the effects of nutrient availability, plant injury, and environmental heterogeneity. Can. J. Bot. **87**(2): 195–201. doi:10.1139/B08-128.

Kuntz, J.E., and Riker, A.J. 1955. The use of radioactive isotope to ascertain the role of root grafting in the translocation of water, nutrients and disease inducing-organisms organisms among forest trees. Proc. U.N. Int. Conf. Peaceful Uses At. Energy, **12**: 144–148.

Lambers, H., and Chapin, F.S., III, and Pons, T. 1998. Plant physiological ecology. Springer-Verlag, Berlin.

Landhäusser, S.M., and Lieffers, V.J. 2003. Seasonal changes in carbohydrate reserves in mature northern *Populus tremuloides* clones. Trees (Berlin), **17**: 471–476.

Liu, Z., and Dickmann, D.I. 1996. Effects of water and nitrogen interaction on net photosynthesis, stomatal conductance, and water-use efficiency in two hybrid poplar clones. Can. J. Bot. **97**: 507–512.

Marshall, C., and Sagar, G.R. 1965. The influence of defoliation on the distribution of assimilates in *Lolium multiflorum* Lain. Ann. Bot. **29**: 365–370.

Meziane, D., and Shipley, B. 2001. Direct and indirect relationships between specific leaf area, leaf nitrogen and leaf gas exchange. Effects of irradiance and nutrient supply. Ann. Bot. **88**(5): 915–927. doi:10.1006/anbo.2001.1536.

Noble, J.C., and Marshall, C. 1983. The population biology of plants with clonal growth. J. Ecol. **71**(3): 865–877. doi:10.2307/2259598.

Nowak, R.S., and Caldwell, M.M. 1984. A test of compensatory photosynthesis in the field: implications for herbivory tolerance. Oecologia, **61**(3): 311–318. doi:10.1007/BF00379627.

Ovaska, J., Walls, M., and Mutikainen, P. 1992. Changes in leaf gas exchange properties of cloned *Betula pendula* saplings after partial defoliation. J. Exp. Bot. **43**(10): 1301–1307. doi:10.1093/jxb/43.10.1301.

Pataki, D.E., Oren, R., and Phillips, N. 1998. Responses of sapflux and stomatal conductance of *Pinus taeda* L. trees to stepwise reductions in leaf area. J. Exp. Bot. **49**(322): 871–878. doi:10.1093/jexbot/49.322.871.

Peltzer, D.A. 2002. Does clonal integration improve competitive ability? A test using aspen (*Populus tremuloides* [Salicaceae]) invasion into prairie. Am. J. Bot. **89**(3): 494–499. doi:10.3732/ajb.89.3.494.

Penner, M., Robinson, C., and Woods, M. 2001. The response of good and poor aspen clones to thinning. For. Chron. **77**(5): 874–884.

Pessarakli, M. 2005. Handbook of photosynthesis. CRC Press, Boca Raton, Fla.

Pinheiro, J.C., and Bates, D.M. 2000. Mixed-effects models in S and S-PLUS. Springer-Verlag, Berlin.

Pitelka, L.F., and Ashmun, J.W. 1985. Physiology and integration of ramets in clonal plants. *In* The population biology and evolution of clonal organisms. *Edited by* J.B.G. Jackson, L.W. Buss, and R.E. Cook. Yale University Press, New Haven, Conn. pp. 399–435.

R Development Core Team. 2007. R: a language and environment for statistical computing, reference index version 2.8.1. R Foundation for Statistical Computing, Vienna, Austria.

Reich, P.B., Walters, M.B., Krause, S.C., Vanderklein, D.W., Raffs, K.F., and Tabone, T. 1993. Growth, nutrition and gas exchange of *Pinus resinosa* following artificial defoliation. Trees (Berlin), **7**(2): 67–77. doi:10.1007/BF00225472.

Reich, P.B., Kloeppel, B.D., Ellsworth, D.S., and Walters, M.B. 1995. Different photosynthesis–nitrogen relations in deciduous hardwood and evergreen coniferous tree species. Oecologia, **104**(1): 24–30. doi:10.1007/BF00365558.

Shepperd, W.D. 1993. The effect of harvesting activities on soil compaction, root damage, and suckering in Colorado aspen. West. J. Appl. For. **8**(2): 62–66.

Shepperd, W.D., and Smith, F.W. 1993. The role of near surface lateral roots in the life cycle of aspen in the central Rocky Mountains. For. Ecol. Manage. **61**(1–2): 157–170. doi:10.1016/0378-1127(93)90196-T.

Shipley, B. 1995. Structured interspecific determinants of specific leaf area in 34 species of herbaceous angiosperms. Funct. Ecol. **9**(2): 312–319. doi:10.2307/2390579.

Shipley, B. 2006. Net assimilation rate, specific leaf area and leaf mass ratio: which is most closely correlated with relative growth rate? A meta-analysis. Funct. Ecol. **20**(4): 565–574. doi:10.1111/j.1365-2435.2006.01135.x.

Shipley, B., and Lechowicz, M.J. 2000. The functional coordination of leaf morphology and gas exchange in 40 wetland plant species. Ecoscience, **7**: 183–194.

Tarroux, E., DesRochers, A., and Krause, C. 2010. Effect of natural root grafting on growth response of jack pine (*Pinus banksiana*) after commercial thinning. For. Ecol. Manage. **260**(4): 526–535. doi:10.1016/j.foreco.2010.05.008.

Vincent, J.S., and Hardy, L. 1977. L'évolution et l'extension des lacs glaciaires Barlow et Ojibway en territoire québecois. Geogr. Phys. Quat. **31**: 357–372.

Witkowski, E.T.F., and Lamont, B.B. 1991. Leaf specific mass confounds leaf density and thickness. Oecologia, **88**: 486–493.

Zahner, R., and DeByle, N.V. 1965. Effect of pruning the parent root on growth of aspen suckers. Ecology, **46**(3): 373–375. doi:10.2307/1936349.

Zhang, C., Yang, C., and Dong, M. 2002. Clonal integration and its ecological significance in Hedysarum laeve, a rhizomatous shrub in Mu Us Sandland. J. Plant Res. **115**(2): 113–118. doi:10.1007/s102650200016.