Wood formation in *Abies balsamea* seedlings subjected to artificial defoliation

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**Summary** We determined the cambial sensitivity and quantified the anatomical differences in xylem of *Abies balsamea* (L.) Mill. seedlings subjected to artificial defoliation to simulate spruce budworm feeding. Defoliation was performed by removing two-thirds of needles of all current-year shoots for up to four consecutive growth cycles to account for inter- and intra-annual xylem formation. In Experiment 1, xylem development was studied from May to October 2005 in seedlings defoliated at the end of June. In Experiment 2, anatomical features of the xylem were measured along the tree rings formed in 2005 and 2006 during the four cycles of growth and defoliation. Control and defoliated seedlings showed similar patterns of cambial activity and timing of xylem differentiation, although fewer enlarging cells were observed in August to September in defoliated seedlings. Tree-ring widths were similar in control and defoliated seedlings, with thinner rings produced in the greenhouse in winter. No effect of defoliation on cell lumen area was observed, and effects on radial cell diameter and wall thickness were found only occasionally. The results indicate that the *A. balsamea* seedlings produced all the resources required to maintain stem growth during the four cycles of defoliation.

**Keywords:** cambium, cell lumen, outbreak, phenology, spruce budworm, stress, wall thickness.

**Introduction**

Foliage removal reduces radial growth during the current and the following year, with the greatest losses observed in response to the highest intensities and frequencies of defoliation (Piene and Little 1990, Anttonen et al. 2002). Removal of 1-year-old needles affects carbohydrate and hormone production in trees, with consequent decreases in tracheid production and stem volume increment (Sundberg and Little 1987, Piene 1989b). Nevertheless, both natural and artificial defoliation, when occurring at low intensities, can have a positive effect on plant growth (cf. Belsky 1986). For example, dry mass of *Pinus resinosa* Ait. and tree-ring width of *Pseudotsuga menziesii* [Mirb.] Franco increased in response to 25% defoliation (Alfaro and Shepherd 1991, Reich et al. 1993). It is difficult to predict tree growth responses to defoliation, however, because the responses vary with species and defoliation intensity.

In *Populus tremuloides* Michx. and *Betula pendula* Roth, artificial defoliations partially affected the wood formation by slightly reducing the vessel size and by inducing an early cessation in radial growth (Anttonen et al. 2002, Jones et al. 2004). Because higher amounts of carbon and nutrients are stored in evergreen foliage, evergreens are known to be less tolerant of foliage losses than deciduous plants (Krause and Raffa 1996). Moreover, the pool of nonstructural carbon available for rebuilding the canopy is three times higher in broadleaves than in evergreens (Hoch et al. 2003). Defoliation should, therefore, more seriously affect the dynamics and the amount of xylem production in conifers than in broadleaves. To our knowledge, few investigations have analyzed the changes in wood features following defoliation.

Weather plays an important role in the geographical distribution and in the behavior of defoliators and their predators, influencing the location and the intensity of infestations (Mattson and Haack 1987, Fleming and Volney 1995). Current projections indicate that the climate is changing and that intense dry periods will be more frequent in the near future (Easterling et al. 2000, Motha and Baier 2005). There is a general agreement that, in North America, these predicted changes in climate will modify the intensity of insect outbreaks and, hence, the amount of damage to forests (Burkett et al. 2005, Gray 2008). Spruce budworm (*Choristoneura fumiferana* Clem.), a native defoliator whose populations periodically reach outbreak densities over wide forested areas of north-eastern North America, causes growth reductions and tree mortality, resulting in considerable losses for the timber industry and the effects on forest dynamics (Blais 1985, MacLean 1985). Although the most severe damage from infestations is observed on mature trees of *Abies balsamea* (L.) Mill., an increase in density of the
insect population could also greatly affect tree regeneration (Blais 1964, MacLean 1980). Older and well-established seedlings of *A. balsamea* are known for their tenacious survival and high resistance to harsh environmental conditions (Johnson et al. 2003, Morin et al. 2008, Rossi et al. 2009), but the effect of severe defoliation on their growth has not been assessed.

The objective of our study was to determine the cambial sensitivity to artificial defoliation to simulate spruce budworm feeding and to quantify the anatomical differences in xylem of control and defoliated seedlings of *A. balsamea*. The defoliation treatment involved removing two-thirds of the needles of all current-year shoots while taking into account inter- and intra-annual xylem formation. Because spruce budworm outbreaks can recur for several years, we simulated the course of a natural outbreak by carrying out several defoliations over four growth cycles.

**Materials and methods**

Two defoliation experiments were conducted on 7-year-old *A. balsamea* seedlings of uniform height (31.1 ± 3.8 cm) and diameter (8.4 ± 1.0 mm) growing in Chicoutimi, Quebec, Canada (48°25′ N and 71°04′ W, 150 m a.s.l.). The seedlings were grown in plastic reversed-conic pots (height 15 cm, diameter 11.5 cm at the base and 15 cm at the top) filled with peat moss and fertilized before each growth cycle with 1 g l⁻¹ of N, P and K (20, 8 and 20) fertilizer. When kept in a greenhouse, a drip irrigation system supplied an amount of water equal in mass to the daily loss of field capacity.

Spruce budworms generally consume only needles produced in the current year, except during the severe outbreaks when they may also feed on 1-year-old and older foliage (Miller 1977). The larvae emerge from over-wintering at the end of May and feed on the new shoots throughout June. In each experiment, the seedlings were split into two groups: control and defoliated. In the defoliation treatment, two-thirds of the current-year needles from all elongating shoots were removed with scissors to imitate spruce budworm feeding. To facilitate needle manipulation and to avoid damage to the remaining needles, the defoliation was performed 3 weeks after bud burst, when current-year shoots in the control seedlings had attained about one-third of their final length. In both experiments, the dates of defoliation corresponded to the last 10 days of June, when spruce budworms consume almost 90% of the total amount of current-year foliage eaten during the entire feeding period (Miller 1977).

**Experiment 1**

Xylem development was studied from May to October 2005 on seedlings arranged in a completely randomized design. The potted seedlings spent early spring in an open field and were transferred to a greenhouse at the end of May. After defoliation on June 28, the seedlings were returned to the open field until November. Stem disks were collected weekly 2 cm above the root collar from 264 plants by randomly selecting six control and six defoliated seedlings per week.

**Experiment 2**

Anatomical features of xylem cells were measured along the tree rings formed in 2005 and 2006 during the four growth cycles, two summers simulated during winter in the greenhouse (growth cycles 1 and 3) and two natural summers in the open field (growth cycles 2 and 4). The simulated summers were initiated by raising the greenhouse temperature to 22 °C and maintaining a nighttime temperature of 17 °C. These temperatures reflect the maximum and the minimum temperatures recorded during the months of June to July in the central part of the geographical distribution of the studied species. To mimic the open conditions of the boreal forest during the growing period, the photoperiod was extended to 18 h at a photosynthetic photon flux of 115 μmol m⁻² s⁻¹ provided by 400-W wide-spectrum high-pressure sodium bulbs (Lucalox LU400, General Electric Co., Cleveland, OH). Before each new growth cycle, the seedlings were maintained at 0 °C for 4–6 weeks. Defoliation was performed at each cycle. Stem disks were collected 2 cm above the root collar from six plants (three control and three defoliated seedlings) in November 2006. During sample collection, all plants were carefully examined and no symptoms of yellowing foliage or presence of knotting roots were detected, indicating that the potted seedlings were healthy. To assess photosynthetic biomass, all needles were collected from the seedlings, oven-dried at 65 °C for 48 h and weighed to the nearest 0.01 g.

In both experiments, the collected stem disks were dehydrated by successive immersions in ethanol and β-limonene, embedded in paraffin, and transverse sections of 8–10-μm thickness were cut with a rotary microtome (Rossi et al. 2006a).

**Anatomical observations and measurements**

In Experiment 1, the sections were stained with aqueous 0.16% cresyl violet acetate and examined within 10–25 min with visible and polarized lights at magnifications of 400–500× to distinguish the developing xylem cells. For each section, the radial number of cambial, enlarging, cell-wall thickening and mature cells were counted along three radial files according to Rossi et al. (2006b). In cross-section, the cambial cells were characterized by thin cell walls and small radial diameters. During cell enlargement, the tracheids still showed thin primary walls but radial diameters were at least twice as those of the cambial cells. Observations under polarized light discriminated between enlarging and cell-wall thickening tracheids. Because of the arrangement of the cellulose microfibrils,
the developing secondary walls glistened when observed under polarized light, whereas no glistening was observed in enlargement zones when the cells were still composed of primary wall only (Abe et al. 1997). The progress of cell-wall lignification was detected by the reaction of cresyl violet acetate with the developing lignin (Deslauriers et al. 2008). Lignification appeared as a color change from violet to blue. A homogeneous blue color over the whole cell wall revealed the end of lignification and the reaching of tracheid maturity (Gričar et al. 2005).

In Experiment 2, the sections were stained with aqueous 1% safranin and fixed on slides with the histological mounting medium Eukitt® (Bio-Optica, Milan, Italy). A camera mounted on an optical microscope was used to record the numerical images and to measure the xylem features with an image analysis system specifically designed for wood cells (WinCELL™, Regent Instruments Inc., Canada). Tree-ring width was measured along eight radial paths around the stem at a magnification of 25× and cell features (cell lumen area, radial diameter and wall thickness) were measured along all the tree rings at a magnification of 400×. At that magnification, measurements included a band of 12–18 rows of cells, for a total of about 250 µm in thickness. Tracheids were classified as belonging to earlywood or to latewood according to Mork’s formula, which classifies all cells with lumen areas of less than twice the thickness of a double cell wall as latewood (Denne 1988).

Statistical analyses

Because the observations for xylem development were made on disks collected from different seedlings, samples were considered independent. Moreover, because the assumption of normality in data distribution was frequently violated, rank comparisons of the number of cambial, differentiating and mature cells between control and defoliated plants were performed with nonparametric Wilcoxon tests. Linear regression and analysis of covariance (ANCOVA) were also used to assess the pattern of cell production during the growing season (14 sampling days) and to compare regression slopes between groups. The normality of residual distribution was verified using the Shapiro–Wilk test. Tree-ring widths and cell features were averaged for each seedling and growth cycle, and treatments were compared by repeated-measures analysis of variance (ANOVA).

Results

Experiment 1

On average, 6–8 closely spaced cells were observed in the cambial zone in May, except in defoliated seedlings on day of the year (DOY) 141 (Figure 1A). From DOY 155 to 246, the number of cambial cells fluctuated, ranging between 5 and 9 cells. Once annual activity ended and the cambium stopped dividing, the number of cells in the cambial zone gradually decreased to 5–6, a lower number than at the beginning of the season. Statistically, significant differences between treatments were observed on several sampling days (Wilcoxon test, \( P < 0.05 \)), with a higher number.
of cambial cells observed in either control or defoliated seedlings but with no particular pattern. Moreover, differences between seedlings were also observed on DOY 141–155, before the seedlings were defoliated (Figure 1A).

Xylem differentiation showed a clear pattern during the year that was associated with the number of tracheids in each developmental phase. At the beginning of cell differentiation (DOY 162), the number of tracheids undergoing enlargement increased (Figure 1B), with wall thickening and lignification beginning the following week (Figure 1C). During the late summer, the number of cells in radial enlargement gradually decreased and the enlargement phase was completed by DOY 260 and 267 for control and defoliated seedlings, respectively. On the last sampling day, 1–2 cells on average were still observed with partially lignified secondary walls. The curves representing the differentiating cells presented an evident depression between DOY 176 and 218 in both treatments (Figure 1B and C). From DOY 176, the number of mature cells increased linearly, representing the accumulation of physiologically active tracheids in the tree ring (Figure 1D). A similar trend in xylem formation was observed in control and defoliated trees with statistical differences detected occasionally, both before and after defoliation (Wilcoxon test, \( P < 0.05 \); Figure 1B–D). A major difference was observed during the cell enlargement phase in August to September (DOY 232–253), with defoliated seedlings having fewer enlarging cells than the control seedlings (Figure 1B).

The linear regressions fit the total number of cells during the year with an \( R^2 \) of 0.90 for both control and defoliated seedlings (Figure 2) and the slopes did not differ significantly (ANCOVA, \( P > 0.05 \)). Although there was significant heteroscedasticity of the residuals (\( \chi^2 = 13.86, P \approx 0.01 \)) for both treatments and the defoliated seedlings had 66.7% of the dry photosynthetic biomass of the control seedlings (34.3 ± 1.6 versus 51.5 ± 10.5 g). Needles on seedlings in the defoliated treatment were derived from new foliage developing on shoots arising from dormant buds at the stem base.

Figure 2. Mean number of xylem cells measured from May to October 2005 in \( A. \ balsamea \) seedlings before (white background) and after (gray background) defoliation. Black and gray regression lines refer to control and defoliated seedlings, respectively.

Figure 3. Tree-ring width in control (solid circles) and defoliated (open circles) \( A. \ balsamea \) seedlings. Values are mean and standard deviation. Growth cycle 0 corresponds to the tree ring produced by the seedlings the year before the defoliation treatment.

\( P < 0.01 \) for control and \( \chi^2 = 11.07, P < 0.01 \) for defoliated seedlings) appearing mainly on the first sampling days, the standardized residuals were normally distributed (Shapiro–Wilk test, \( W = 0.98, P > 0.05 \) for both treatments) and only 15 of the 155 sampling points were located outside the boundaries of 1.72 (confidence limit of 0.05 for the \( t \) distribution), indicating a satisfactory estimation of the regression. Xylem production was estimated at one cell per day (regression slopes of 1.15 and 1.10 for control and defoliated seedlings, respectively), so over the 91 days of activity the cambium produced 104 tracheids along a radial file in both the control and the defoliated seedlings (Figure 2).

**Experiment 2**

Although defoliation removed two-thirds of the current-year needles, at the end of the four growth cycles the defoliated seedlings had 66.7% of the dry photosynthetic biomass of the control seedlings (34.3 ± 1.6 versus 51.5 ± 10.5 g). Needles on seedlings in the defoliated treatment were derived from new foliage developing on shoots arising from dormant buds at the stem base.

Tree-ring width in the stem varied between 0.2 and 1.2 mm (Figure 3), with thinner rings produced when seedlings were grown in the greenhouse in winter (cycles 1 and 3) and wider rings produced in summer, during cycle 2. Although significant differences in tree-ring widths were detected between cycles (repeated-measures ANOVA, \( P < 0.0001 \)), tree-ring widths did not differ significantly between treatments (repeated-measures ANOVA, \( P > 0.05 \)) and no combined cycle × treatment effect was observed (Table 1).

Because of the presence of both narrow and wide tree rings, cell numbers ranged between 1026 and 4628 in the band samples in the anatomical sections. High variability in measured cell features was observed within and between tree rings, leading to high standard deviations (Figure 4). On average, the cell size increased gradually at each cycle in earlywood, whereas cell diameter showed no pattern in latewood although smaller tracheids were produced during cycles 1 and 3. Cell-wall thickness in latewood varied between summer and winter cycles, being thinner in cycles...
Table 1. Summary of F statistics from repeated-measures ANOVA for the tree-ring width and the cell features between- and within-growth cycles for comparisons of treatments (control and defoliated seedlings) and type of wood (earlywood and latewood) in *A. balsamea* seedlings. Asterisks indicate significantly different values at $P < 0.0001$.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Tree-ring width</th>
<th>Lumen area</th>
<th>Radial diameter</th>
<th>Wall thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle</td>
<td>48.80*</td>
<td>31.26*</td>
<td>15.44*</td>
<td>16.51*</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.14</td>
<td>1.99</td>
<td>2.92</td>
<td>0.43</td>
</tr>
<tr>
<td>Wood</td>
<td>–</td>
<td>392.95*</td>
<td>216.32*</td>
<td>71.08*</td>
</tr>
<tr>
<td>Cycle x treatment</td>
<td>2.14</td>
<td>0.70</td>
<td>0.63</td>
<td>1.50</td>
</tr>
<tr>
<td>Cycle x treatment x wood</td>
<td>1.67</td>
<td>2.32</td>
<td>0.53</td>
<td></td>
</tr>
</tbody>
</table>

1 and 3 than in cycle 2. Significant cycle effects were observed for cell lumen area, radial diameter and single wall thickness (repeated-measures ANOVA, $P < 0.0001$) in all cases (Table 1). Although differences in the three measured cell features were detected between earlywood and latewood (repeated-measures ANOVA, $P < 0.0001$), there were no significant differences in lumen area, radial diameter and wall thickness between control and defoliated seedlings (repeated-measures ANOVA, $P > 0.05$; Table 1).

**Discussion**

Removal of two-thirds of the current-year needles on 7-year-old *A. balsamea* seedlings had no effect on either cambial activity or xylem differentiation in the stem. Moreover, repeated defoliation of two-thirds of the current-year needles over each of the four growth cycles had no effect on the anatomy of the xylem produced.

Foliage removal perturbs physiological processes in plants, from photosynthesis to reserve storage, and the subsequent nutrient shortage, particularly in growth substances and photosynthates, inhibits cambial growth (Little 1970a, Honkanen and Haukioja 1994, Krause and Raffa 1996). However, even when carried out at moderate intensities, defoliation does not always have substantial effects in the treated trees. For example, 15 months after defoliation, no difference in the stem radial growth was observed in *P. resinosa* in which 66% of each needle had been removed, and *Pinus sylvestris* L. trees defoliated by 25%, 50% and 75% maintained similar carbon concentrations in all treatments (Krause and Raffa 1996, Lyytikäinen-Saarenmaa 1999). Young trees can respond to defoliation by producing new foliage from dormant and latent buds, as well as by increasing retention of older age-classes of needles (Piene 1989a, 1989b, Piene and Little 1990). We found that the difference in photosynthetic biomass between defoliated and
control seedlings was 33% at the end of the experiment, although two-thirds of the current-year needles had been removed. Removal of current-year shoots at the beginning of the growth period is known to stimulate starch mobilization and to increase net photosynthetic rates of the remaining needles during the same growing season (Sundberg and Little 1987; Reich et al. 1993, Lavigne et al. 2001).

Nonlinear responses in aboveground productivity have been found with increasing severity of defoliation, with no effect at low or moderate defoliation and significant effects at heavy or complete defoliation (Piene and Percy 1984, Piene 1989a, Krause and Raffa 1996, Kolb et al. 1999, Anttonen et al. 2002). Piene and Little (1990) observed that growth losses induced by reductions in foliage mass increased disproportionately with increasing amounts of foliage removed. In evergreens, old needles provide the major source of reserve and currently produced photosynthates during spring growth (Loach and Little 1973, Sundberg and Little 1987, Kozlowsky 1992, Lyytikäinen-Saarenmaa 1999). Moreover, developing shoots and needles act as a sink, changing to a carbon source only at the beginning of summer when the new needles have attained maturity (Clark 1961, Little 1970b). The extent of defoliation in our experiments may not have caused sufficient nutrient stress or metabolite losses to affect cambial activity and wood formation.

Foliation replacement requires carbon reserves for the production of new needles, with considerable energy costs for the whole plant. Gradual weakening of trees is observed following repeated losses of current-year foliage because carbohydrate reserves are gradually consumed and depleted (MacLean and Ostoff 1989, Reich et al. 1993). Carbohydrate in plants is allocated to the different sinks according to preferential partitioning and foliage production, which is the primary sink for assimilates, takes priority over stem growth to ensure an adequate supply of resources to the developing foliage (Piene and Little 1990, Kaitaniemi et al. 1999, Polák et al. 2006, Giovannelli et al. 2007). Although the lack of an effect of our defoliation treatment on cell or on wall production refutes our hypothesis, it demonstrates that _A. balsamea_ seedlings are able to produce the resources necessary to maintain growth of both needles and stem and to efficiently recover the reserves used during the four cycles of defoliation.

Thinner tree rings and latewood with smaller cell sizes and thinner walls were produced in both control and defoliated seedlings during the growth cycles in winter (cycles 1 and 3; Figure 4). Timing mechanisms and temporal adaptations enable plants to alter their physiology and biochemistry in accordance with environmental changes through the perception of light signals by phytochromes (Smith 2000). In tree-ring formation, photoperiod acts as a signal regulating the timing of maximum growth rate and synchronizing radial growth at the annual level (Rossi et al. 2006c). In Experiment 2, although the environment was thermally favorable for seedling growth, the photoperiod was held constant at 18 h. We assumed that the absence of variation in photoperiod would affect cambial activity and cell differentiation during the simulated summers in the greenhouse. On the contrary, lumen area of earlywood showed a pattern unrelated to the summer–winter cycles. In trees, the structure and the size of the xylem is related to plant height (Anfodillo et al. 2006). The observed growth pattern in lumen area corresponded with the increasing height of the seedlings during the successive growth cycles.

Defoliation by spruce budworm is one of the main causes of destruction of _A. balsamea_ mature stands in eastern Canada (Blais 1964). Mortality of _A. balsamea_ caused by the insect outbreak is related to tree size and age, with younger and smaller trees having lower mortality rates (MacLean 1980, Bergeron et al. 1995). Unlike mature trees, seedlings exhibit resistance to the stress occurring following severe defoliations as manifest by their higher metabolism and by their greater capacity for enhancing photosynthesis (Poethig 1990, Reich et al. 1993, Bond 2000, Boege 2005, Mencuccini et al. 2005). Our results demonstrate the high resistance to defoliation of cambial tissues and wood formation in _A. balsamea_ seedlings. The high growth rates of repeatedly defoliated seedlings indicate that there will be continued regeneration and maintenance of _A. balsamea_ stands following spruce budworm outbreaks.

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