Concentrations of oxygen and indole-3-acetic acid in the cambial region during latewood formation and dormancy development in *Picea abies* stems

Leif Eklund1,4, C.H. Anthony Little2 and Richard T. Riding3

1 Department of Engineering and Natural Science, Växjö University, S-351 95 Växjö, Sweden 
2 Canadian Forest Service, PO Box 4000, Fredericton, New Brunswick, E3B 5P7 Canada 
3 Department of Biology, University of New Brunswick, Fredericton, New Brunswick, E3B 6E1 Canada

Received 4 December 1996; Accepted 2 September 1997

Abstract

To manipulate the occurrence of latewood formation and cambial dormancy in *Picea abies* (L.) Karst. stems, potted seedlings were transferred from the natural environment on 9 July, when tracheids early in the transition between earlywood and latewood were being produced, and cultured for up to 5 weeks in a controlled environment chamber having: (1) Warm LD, (25/15 °C during day/night) and long (16 h) photoperiod, (2) Warm SD, (25/15 °C) and short (8 h) photoperiod, or (3) Cold SD, (18/8 °C) and short (8 h) photoperiod. In Warm LD trees, the radial enlargement of primary-walled derivatives on the xylem side of the cambium, as well as xylem production, continued at the same magnitude throughout the experiment. In Warm SD and Cold SD trees, the radial enlargement of primary-walled derivatives declined and the cambium entered dormancy, both developments occurring faster in the Warm SD trees. The concentration of indole-3-acetic acid (IAA) was higher in developing xylem tissue than in cambium + phloem tissues, but did not vary with environmental treatment or decrease during the experimental period. The O2 concentration in the cambial region followed the order of Cold SD > Warm SD > Warm LD trees and was <5%, the threshold for the inhibition of IAA-induced proton secretion, for the first 3 weeks in Warm SD and Warm LD trees. Thus, neither latewood formation nor cambial dormancy can be attributed to decreased IAA in the cambial region. Nor does lower O2 concentration in the cambial region appear to be inhibiting the IAA action that is associated with cambial growth.

Key words: Cambium, dormancy, indole-3-acetic acid, IAA, oxygen, *Picea abies*, tracheid.

Introduction

The vascular cambium of coniferous species native to the northern temperate zone passes through an annual cycle of activity and dormancy. For an extended period following springtime resumption of cambial cell-division activity, the derivatives of cambial fusiform cells enlarge greatly in radial dimension to form a zone of primary-walled radially expanding or expanded cells. Subsequently, the primary-walled derivatives (denoted PD) on the xylem side of the cambium undergo terminal differentiation, involving secondary wall thickening, lignification and protoplast autolysis, to become trachceids. Wide-diameter earlywood tracheids are produced in spring and tracheids having smaller diameters and thicker secondary walls are produced later. A definition for ‘latewood’ remains controversial (Denne, 1989); nevertheless, it is generally recognized that a transition to smaller tracheid radial diameters typically occurs when the photoperiod is declining, in late summer and early autumn. This transition is gradual in some species, such as *Picea abies*, and abrupt in others, and is accompanied or preceded by a reduced rate of cambial mitotic activity that eventually ends in cambial dormancy (Timell, 1986; Creber and Chaloner, 1990; Savidge, 1993).

Short photoperiods have been shown to induce both latewood formation and cambial dormancy in the stem of conifer species (Wareing, 1951; Little and Wareing, 1981; Mellerowicz et al., 1992; Lloyd et al., 1996), but...
the mechanism regulating these events at the molecular level has yet to be explained. One possible explanation is that the concentration of the growth inhibitor abscisic acid (ABA) increases in the cambial region and, in fact, exogenous ABA was observed to decrease tracheid radial diameter and to inhibit tracheid production in *Picea glauca* (Little and Eidt, 1968) and *Pinus radiata* (Jenkins, 1974; Pharis et al., 1981). However, neither the changeover from earlywood to latewood formation nor the onset of cambial dormancy was accompanied by an increase in the cambial region concentration of endogenous ABA in *Picea sitchensis* (Little and Wareing, 1981), *Pinus contorta* (Savidge and Wareing, 1984) or *Pinus densiflora* (Funada et al., 1988). An alternative explanation is that there is a decrease in the cambial region concentration of the growth promoter indole-3-acetic acid (IAA), as strong evidence indicates that exogenous IAA promotes both the production of tracheids and the enlargement of primary-walled cells (Cleland, 1995; Little and Pharis, 1995). Moreover, the concentration of endogenous IAA in the cambial region of various conifers was reported to be higher when the cambium was producing tracheids than when it was dormant (Little and Wareing, 1981; Savidge et al., 1982; Savidge and Wareing, 1984; Sandberg and Ericsson, 1987; Sundberg et al., 1987; Sundberg and Little, 1990), however, not without exception (Sundberg et al., 1990, 1991; Uggla et al., 1996).

There are also conflicting reports as to whether the cambial region IAA concentration decreases during latewood formation. A decline was observed in *Pinus contorta* (Savidge et al., 1982; Savidge and Wareing, 1984) and *Abies balsamea* (Sundberg et al., 1987), but not in *Picea sitchensis* (Little and Wareing, 1981) and *Pinus sylvestris* (Sundberg et al., 1991, 1993). A third possible explanation for the induction of latewood formation and cambial dormancy is that high rates of the growth and maintenance components of respiration (Amthor, 1994; Sprugel et al., 1995) decrease the O$_2$ concentration in the cambial region to the extent that it becomes limiting for IAA action during either the enlargement phase of tracheid differentiation or the division of cambial cells. Evidence for this possibility are the findings that the O$_2$ concentration in holes drilled into the sapwood of field-grown *Picea abies* stems attains a minimum well below 5% late in the cambial growing period (Eklund, 1990; Eklund et al., 1992), and that such low O$_2$ concentrations inhibit IAA-induced proton secretion (Evans and Vesper, 1980; Böttger and Hilgendorf, 1988). There are no reports concerning the relationship between cambial growth and the O$_2$ concentration measured specifically in the cambial region.

The purpose of the present investigation was to determine if the decrease in tracheid radial diameter associated with latewood formation and the entry of the cambium into dormancy in *Picea abies* stems were temporally associated with a reduction in the cambial region O$_2$ concentration to <5%, the presumptive threshold for the inhibition of IAA action (Evans and Vesper, 1980; Böttger and Hilgendorf, 1988). The measurement of the O$_2$ concentration was localized to the cambial region using microdialysis. The cambial region IAA concentration was also determined, because it has not previously been monitored in *Picea abies* stems and whether it changed during the cessation of cambial growth could not be predicted from previous studies.

### Materials and methods

#### Plant material and culture conditions

The experiment was performed with 50, 0.5 m tall *Picea abies* (L.) Karst. trees that were in their fourth growing season, and which had been cultured for the previous 2 years in 5 l pots at the Canadian Forest Service nursery located at Fredericton, New Brunswick, Canada. On 9 July, 1992, about the middle of the cambial growing period, ten matched groups of five trees were selected. One group was harvested, while three groups were placed in each of three controlled environment chambers having either (1) warm temperature (25/15 °C during day/night) and long (16 h) photoperiod (the control environment, denoted Warm LD), (2) warm temperature (25/15 °C) and short (8 h) photoperiod (Warm SD) or (3) cold temperature (18/8 °C) and short (8 h) photoperiod (Cold SD). One group per chamber was harvested after 1, 3 and 5 weeks. During the photoperiod, the chambers were illuminated with a combination of incandescent (40 W) and cool-white fluorescent (FR96T12V110) lamps, giving a photon fluence density of 500 mol m$^{-2}$ s$^{-1}$, measured at the top of the trees. The relative humidity was set at 80%, and the trees were watered and fertilized as required to prevent drought and mineral deficiency. On each harvest occasion, the cambial region concentrations of IAA and oxygen were measured in the third internode of the main stem (i.e. the 3-year-old portion of the stem, initiated through extension growth in 1990). In addition, a stem segment was excised immediately below the place of IAA and O$_2$ measurement and stored in 70% ethanol until used for anatomical investigation.

#### Measurement of IAA

The IAA concentration was measured after dividing the cambial region into two fractions, developing xylem and cambium plus phloem. A 10 cm long segment was excised at 5–15 cm below the apex of the third internode of the main stem. After peeling the bark, the exposed surface on the xylem side was scraped firmly with a scalpel to obtain the developing xylem fraction (400–500 mg FW), which consisted of most of the primary-walled derivatives (PD), cells in the process of secondary-wall deposition and lignification and, in dormant stems, some cambial cells. The exposed surface of the bark peeling was similarly scraped to obtain the cambium plus phloem fraction (50–100 mg FW), which contained some PD in the early stage of expansion, the cambium and phloem. Each fraction was immersed in ice-cold 70% methanol containing 0.01 M butylated hydroxytoluene, and 0.4 µg and 0.1 µg [13C$_6$]-IAA were added to the xylem and cambium plus phloem fractions, respectively, to serve as an internal standard. The scrapings were accumulated on ice, and the interval between excising the segment and placing the scrapings in the methanol solution was about 1 min. The samples were stored at −80 °C.
After warming the samples to room temperature, IAA was purified and measured as described by Savidge (1990), except that the methylation step was omitted. The IAA concentration was determined by combined gas chromatography-mass spectrometry (GC-MS; Hewlett-Packard 5890 and 5970, respectively), performing selected ion monitoring of the trimethylsilyl derivatives of IAA and [13C5]IAA and focusing on the fragments m/z 202 and 208, respectively. The IAA content was calculated using the ratio of the peak areas, after correcting for mass detector response to different fragment ratios. The IAA concentration was expressed on the basis of FW and DW, the latter determined after oven-drying at 60 °C for 24 h.

**Measurement of oxygen**

On every sampling date, the cambial region O2 concentration was measured by microdialysis as described by Ekblad (1991, 1993), using the group of trees in each cabinet harvested on week 5. The probe (CMA/11, CMA Microdialysis AB, Stockholm) was inserted by hand into the wood/bark interface 16 cm below the apex of the third internode of the main stem. The cylindrical membrane covering the probe tip had a length of 4 mm and a diameter of 0.5 mm. The inlet to the probe was connected to a pump (CMA/100, CMA Microdialysis AB, Stockholm) enabling continuous perfusion of the probe with deionized water at a rate of 2 μl min⁻¹. A 100 μl aliquot of dialysate was collected in a 1.6 ml gas-tight vial containing pure nitrogen. After equilibration for at least 1 h, a 5 μl air sample was drawn from the headspace using a gas-tight syringe, and O2 was identified and quantified by selected ion monitoring of the molecular ion m/z 32, using the same GC-MS as for the IAA measurement. GC was done with a 16 m × 0.18 mm (i.d.) methyl-silicone coated column (DB 1, J&W Scientific; Chromatographic Specialties Inc., Brockville, ON, Canada) using helium flowing at 0.6 ml min⁻¹ as carrier. The temperatures in the injector, oven and transfer line were 150 °C, 100 °C and 200 °C, respectively. The mass detector response was linear between 1% and 21% O2.

Oxygen transfer across the membrane was determined as described previously (Ekblad, 1991). Before implantation at week 0, the membrane passed 33.6% of the O2, whereas at the end of the measurement on week 5 this value had declined to 24.6%. The O2 concentration was calculated assuming a linear decrease in membrane efficiency between weeks 0 and 5, and was expressed as the gas phase concentration in equilibrium with the liquid phase outside the microdialysis probe.

**Anatomical investigation**

The progress of cambial growth was monitored by recording tracheid production and radial diameter, as well as the number and radial diameter of cambial derivatives differentiating into tracheids. Stem blocks were dehydrated through a tertiary butyl alcohol series, embedded in Paraplast, sectioned transversely at 10 μm on a rotary microtome, mounted on glass slides and stained in safranin-fast green (Johansen, 1940). Tracheid production, recorded at eight equidistant points around the stem circumference, was measured as the radial width of xylem produced in 1992, starting with the first-formed tracheid in the growing period. The radial latewood tracheid in 1991 (9 μm) was identified and quantified by selected ion monitoring of the molecular ion m/z 32, using the same GC-MS as for the IAA measurement. GC was done with a 16 m × 0.18 mm (i.d.) methyl-silicone coated column (DB 1, J&W Scientific; Chromatographic Specialties Inc., Brockville, ON, Canada) using helium flowing at 0.6 ml min⁻¹ as carrier. The temperatures in the injector, oven and transfer line were 150 °C, 100 °C and 200 °C, respectively. The mass detector response was linear between 1% and 21% O2.

Results

At the start of the experiment on 9 July, when the trees were transferred from the natural environment to the controlled environment chambers, the cambium was mitotically active and there were six primary-walled derivatives (PD) per radial file (Fig. 1A). The radial diameters of the first PD and the last PD were 8 μm and 15.3 μm, respectively (Fig. 2B, C), indicating that PD were expanding radially almost 2-fold at that time. However, the full expansion, from the radial diameter of the fusiform cambial cell (5.5 μm) to that of the first secondary-walled derivative (SD) (18.4 μm), was more than 3-fold (Fig. 2A, D). The radial diameter of the first SD was narrower than that of earlywood tracheids formed at the start of the 1992 growing season (25 μm, range 20–30 μm), but wider than the diameter of the last-formed latewood tracheid in 1991 (9 μm, range 6–12 μm). Moreover, the secondary-wall thickness was greater in the most recently matured tracheids than in tracheids produced earlier in the growing period, however it was less in the most recently matured tracheids than in the last-formed 1991 latewood tracheid (data not shown). Thus the tracheids being formed when the experiment began appeared to be transitional between earlywood and latewood.

In Warm LD (control) trees, tracheids were produced at a consistent rate throughout the experimental period (Fig. 1B) and there were no changes in the radial diameter of the fusiform cambial cells, the first and last PD, and the first secondary-walled derivatives (Fig. 2). In Warm
SD and Cold SD trees, however, the production of tracheids ceased (Fig. 1B). The number of PD had declined by week 3 in these trees, particularly in the Warm SD trees, and no PD were present at week 5 (Fig. 1A). Concomitantly, there was a decrease in the radial diameter of the last PD, when present, and in the first SD (Fig. 2C, D), whereas the radial diameter of the fusiform cambial cells and the first PD, when present, did not vary (Fig. 2A, B). The reduction in the radial diameter of the last PD at week 3 was greater in the Warm SD trees than in the Cold SD trees, however, there were no differences between Warm SD and Cold SD trees in the radial diameter of the first SD at weeks 3 or 5 (Fig. 2C, D). The first SD in the Warm SD and Cold SD trees at week 5 adjoined the cambium, was undergoing or had finished lignification as indicated by safranin staining, and had a radial diameter similar to that of the last latewood tracheid formed in 1991.

When the experiment began, the concentration of IAA, expressed on the basis of both FW and DW, was about 3-fold higher in the developing xylem fraction than in the cambium + phloem fraction (Fig. 3). The IAA concentration in both fractions did not vary with time, method of expression or environmental treatment except on week 5, when the concentration in the developing xylem fraction expressed on a FW basis increased in the Cold SD and Warm SD trees, particularly the latter. This increase reflected the reduction in the water content of the developing xylem from about 80% at weeks 0, 1 and 3 to about 65% and 55% in the Cold SD and Warm SD trees, respectively, at week 5.

The O₂ concentration was lowest in the Warm LD trees and highest in the Cold SD trees throughout the experiment (Fig. 4). The O₂ concentration in Warm LD and

![Fig. 1. Number per radial file of primary-walled derivatives (PD) on the xylem side of the cambium (A), and tracheid production expressed as the ratio of the radial widths of xylem produced in 1992 and 1991 (B), in the third internode of the main stem of trees exposed for up to 5 weeks in a controlled environment chamber having either warm temperature (25/15 °C during day/night) and long (16 h) photoperiod (Warm LD), warm temperature (25/15 °C) and short (8 h) photoperiod (Warm SD) or cold temperature (18/8 °C) and short (8 h) photoperiod (Cold SD). Mean ±SE, n=5. Means accompanied by the same letter are not significantly different at P<0.05.](https://academic.oup.com/jxb/article-abstract/49/319/205/576461)

![Fig. 2. Radial diameter of (A) fusiform cambial cells (FC), (B) the primary-walled derivative on the xylem side nearest to the cambium (first PD), (C) the primary-walled derivative on the xylem side farthest from the cambium (last PD), and (D) the derivative undergoing secondary wall formation on the xylem side nearest to the cambium (first SD) in the third internode of the main stem of trees exposed for up to 5 weeks in a controlled environment chamber having either warm temperature (25/15 °C during day/night) and long (16 h) photoperiod (Warm LD), warm temperature (25/15 °C) and short (8 h) photoperiod (Warm SD) or cold temperature (18/8 °C) and short (8 h) photoperiod (Cold SD). There were no first or last primary-walled derivative in Warm SD and Cold SD trees at week 5. Mean ±SE, n=5. Means accompanied by the same letter are not significantly different at P<0.05.](https://academic.oup.com/jxb/article-abstract/49/319/205/576461)
Warm SD trees was less than 5% for the first 3 weeks, but had increased to about 9% and 16%, respectively, by week 5. The concentration of $O_2$ in Cold SD trees increased steadily during the experiment, being 7% and 22% on weeks 1 and 5, respectively.

**Discussion**

After the trees were transferred from outdoors into the growth chambers, the Warm LD environment not only induced a constant rate of cambial activity, as measured by both the 1992 to 1991 xylem width ratio and the number of PD (Fig. 1), but also maintained the 2-fold PD radial enlargement that was occurring at the time of transfer on 9 July (Fig. 2). Accordingly, the seasonal decline in tracheid production and radial diameter that normally occur outdoors was arrested in the Warm LD trees for the duration of the experiment at a stage comparable to that imposed by the natural environment in our tree nursery during early July. In contrast, and as expected (Wareing, 1951; Little and Wareing, 1981; Mellerowicz _et al._, 1992; Lloyd _et al._, 1996), the Warm SD and Cold SD environments induced the cessation of cambial mitotic activity and decreased PD radial expansion, with the last-formed tracheids having the same radial diameter as the final latewood tracheids produced during the previous year. The relative rate of change in PD number (Fig. 1A) and last PD radial diameter (Fig. 2C) in Warm SD and Cold SD trees suggests that in combination with short photoperiod, cool temperatures inhibited, whereas warm temperatures promoted, tracheid maturation and the cambium’s entry into dormancy.

The reduction in PD radial diameter and tracheid production induced by the Warm SD and Cold SD treatments (Figs 1, 2) was not associated with a decrease in IAA concentration measured in the developing xylem and cambium + phloem fractions (Fig. 3). This suggests that the induction of latewood formation and cambial dormancy by short photoperiod in _Picea abies_ stems cannot be attributed to a decline in the IAA concentration, as also observed in related experiments with _Picea sitchensis_ (Little and Wareing, 1981) and _Pinus sylvestris_ (Sundberg _et al._, 1990, 1991, 1993; Uggla _et al._, 1996), although not with _Abies balsamea_ (Sundberg _et al._, 1987) or _Pinus contorta_ (Savidge _et al._, 1982; Savidge and Wareing, 1984). Similarly, the result from this work that the IAA concentration was greater in the developing xylem fraction than in the cambium + phloem fraction agrees with the results of Savidge _et al._ (1982), but contrasts with those of Sundberg _et al._ (1990). The explanation for the conflicting results concerning the relationships between the IAA concentration in cambial region fractions and the production and radial diameter of tracheids is not known, but conceivably includes differences in such factors as the species investigated, the...
environment in which the experimental trees were cultured, and the procedures used to harvest, store and extract the tissue samples for IAA measurement (Little and Pharis, 1995). Recent findings suggest that the procedure used to obtain the cambial region sample for IAA measurement may be a particularly important variant (Uggla et al., 1996). Using tangential cryosectioning, they showed that a marked radial gradient in IAA concentration exists across the cambial region of *Pinus sylvestris* stems, the peak being in the cambial zone cells. Nix and Wodzicki (1974) demonstrated a similar gradient in radioactivity derived from ^14^C-IAA applied to decapitated *Pinus echinata* stems. Thus, varying the amount and proportion of cambial region cell types in the sample would profoundly perturb the IAA estimate.

The O$_2$ data (Fig. 4) represent the first measurement of respiration specifically in the cambial region. In previous studies the respiration of the entire stem was determined, typically as CO$_2$ efflux (Sprugel et al., 1995), and the values obtained thus included the respiration of tissues situated outside the cambial region. The O$_2$ concentration was about 2% at the start of the experiment (Fig. 4), when both growth respiration and maintenance respiration would have been high and the temperature was warm. A similarly low concentration of O$_2$ was detected towards the end of the cambial growing period in holes bored into the sapwood of large *Picea abies* stems located outdoors (Eklund, 1990; Eklund et al., 1992). The O$_2$ concentration did not change through week 3 in the Warm LD trees, in agreement with tracheid production remaining constant at a rapid rate (Fig. 1). However, at week 5 the concentration of O$_2$ in the Warm LD trees had increased, although no change in PD radial diameter or tracheid production was evident. This increase may reflect a decrease in respiration due to (1) the healing of the wound associated with inserting the microdialysis probe, (2) the tip of the microdialysis probe, the actual site of the oxygen measurement, becoming surrounded by mature tracheids, as cambial growth continued and the zone of differentiating tracheids was displaced laterally, and/or (3) the very recent onset of a decrease in cambial growth. The increase in cambial region O$_2$ concentration that occurred throughout the experimental period in the Cold SD and Warm SD trees can be attributed to a decrease in growth respiration as cambial dormancy developed. The rise in O$_2$ concentration was greater in the Cold SD trees than in the Warm SD trees probably because the relatively cold environment experienced by the Cold SD trees further reduced growth respiration and also inhibited maintenance respiration.

The possibility that the decrease in PD radial enlargement associated with latewood formation, and/or the cessation of cambial cell division connected with the onset of dormancy, is attributable to a low concentration (<5%) of O$_2$ inhibiting IAA-induced hydrogen ion transport (Evans and Vesper, 1980; Böttger and Hilgendorf, 1988), hence IAA action, is not supported by our results. Although the reduction in the radial diameter of the last PD and first SD (Fig. 2C, D) and the cessation of tracheid production (Fig. 1) in Warm SD trees were associated with an O$_2$ concentration below 5% until at least week 3 (Fig. 4), an even lower concentration was measured in the Warm LD trees, in which neither the reduction in PD and SD radial diameters nor cambial dormancy were induced. Moreover, both the decrease in xylem cell radial diameter and the onset of cambial dormancy also occurred in the Cold SD trees although their O$_2$ concentration was above 5% by week 1.

**Acknowledgements**

We thank Dr RA Savidge for critical comments and for the use of a GC-MS. Financial support for L. Eklund was provided by the Swedish Council for Forestry and Agricultural Research, Stockholm, Sweden; Carl Tryggers Stiftelse för Vetenskaplig Forskning, Stockholm, Sweden; and CMA Microdialysis, Stockholm, Sweden.

**References**


Jenkins PA. 1974. Influence of applied indoleacetic acid and...


