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Indole-3-Acetic Acid Controls Cambial Growth in Scots Pine by Positional Signaling[1]

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The vascular cambium produces secondary xylem and phloem in plants and is responsible for wood formation in forest trees. In this study we used a microscale mass-spectrometry technique coupled with cryosectioning to visualize the radial concentration gradient of endogenous indole-3-acetic acid (IAA) across the cambial meristem and the differentiating derivatives in Scots pine (*Pinus sylvestris* L.) trees that had different rates of cambial growth. This approach allowed us to investigate the relationship between growth rate and the concentration of endogenous IAA in the dividing cells. We also tested the hypothesis that IAA is a positional signal in xylem development (C. Uggla, T. Moritz, G. Sandberg, B. Sundberg [1996] Proc Natl Acad Sci USA 93: 9282–9286). This idea postulates that the width of the radial concentration gradient of IAA regulates the radial number of dividing cells in the cambial meristem, which is an important component for determining cambial growth rate. The relationship between IAA concentration in the dividing cells and growth rate was poor, although the highest IAA concentration was observed in the fastest-growing cambia. The radial width of the IAA concentration gradient showed a strong correlation with cambial growth rate. The results indicate that IAA gives positional information in plants.

Cambial growth involves the production of secondary xylem and phloem elements. The lateral meristem responsible for this growth, the vascular cambium, normally consists of 5 to 15 dividing cells in a radial direction (Larson, 1994), the so-called cambial zone. The rate of cambial growth is the major determinant for the production of wood in forest trees, and it is determined by both the radial number of dividing cells in the cambial zone and the rate of cell division for each of the cambial zone cells (Gregory and Wilson, 1968; Wilson and Howard, 1968; Gregory, 1971). Cambial growth is adjusted to the demands of water transport required by the leaf biomass and to provide the mechanical strength necessary to support the crown and to withstand wind forces (Zimmermann and Brown, 1971). It is also well established that stem-diameter growth is often found to be greatest within the young crown and to decrease gradually down the stem. As a consequence, the amount and pattern of growth along the stem are determined by the size and arrangement of the crown (Larson, 1963; Hall, 1965). Such coordination requires a signaling system that integrates apical with cambial growth.

The plant hormone IAA appears to fulfill the requirements for such a signal. Developing buds and young shoots are major sources of IAA, which is transported in a basipetal polar fashion (Kaldeway, 1984; Little and Savidge, 1987). Experiments with exogenous IAA have clearly demonstrated that polarly transported IAA induces formation of primary vascular tissues (Sachs, 1981; Jacobs, 1984; Aloni, 1995) and maintains the structure and cell-division activity of the vascular cambium (Savidge, 1983). IAA also affects the rate of cambial growth, as measured by tracheary element production in both 1-year-old shoots and mature stems in a dose-dependent manner (Little and Savidge, 1987; Little and Sundberg, 1991). These findings strongly suggest a function for IAA as a signal that integrates apical growth with production of vascular tissues. Accordingly, variations in cambial growth patterns along the stem have been explained by the postulated existence of longitudinal concentration gradients of IAA (Larson, 1969; Aloni and Zimmermann, 1983).

With the development of accurate techniques for measuring IAA in plant tissues, it has become possible to test these ideas. The physiological relevance of IAA in regulating cambial growth was demonstrated by measuring the resulting internal concentrations of IAA after experiments with exogenous IAA, or IAA transport inhibitors, in shoots of Scots pine (*Pinus sylvestris* L.; Sundberg and Little, 1990; Sundberg et al., 1994). Applying IAA transport inhibitors resulted in both a depletion of IAA and an inhibition of cambial growth below the point of application (Sundberg et al., 1994). This observation demonstrates that the IAA needed for cambial growth is mainly supplied through the polar transport system. Replacing the bud with an exogenous IAA source supplied the subjacent stem tissues with an amount of IAA comparable to that found in intact control shoots (Sundberg and Little, 1990). When different amounts of exogenous IAA were supplied, the resulting internal IAA levels correlated well with the cambial growth response. In the same study, it was also demonstrated that the endogenous supply of IAA to the tissue of the 1-year-old shoot could be supplemented with laterally applied IAA to induce a physiological relevant increase in internal

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Abbreviations: IAA_{max}, maximum IAA level in the cambial zone; IAA_{tot}, amount of IAA per tangential square-centimeter area.

IAA. The increase in IAA was associated with a stimulation of cambial growth. This finding demonstrates that the supply of endogenous IAA to the vascular cambium in shoots is suboptimal for cambial growth and that IAA can act as a growth regulator in intact plants. In spite of these findings, numerous investigations of endogenous IAA in intact trees have failed to demonstrate a consistent relationship between IAA concentration and variations of cambial growth rate in time and space (for review, see Little and Pharis, 1995).

By using a novel MS technique with increased sensitivity, it was recently demonstrated that endogenous IAA is distributed as a steep concentration gradient across the cambial meristem and the differentiating derivatives in both Scots pine (Uggla et al., 1996) and hybrid aspen (Tuominen et al., 1997). From the visualization of this gradient it was suggested that IAA could function as a morphogen and act as a positional signal that controls cambial growth rate by regulating the number of dividing cells. This view is contrary to the idea that IAA stimulates the rate of cell cycling through variations in concentration within the cells of the cambial zone. In this study the radial IAA gradient was visualized in trees of Scots pine that had different cambial growth rates. Thus, it was possible to evaluate the significance of the IAA concentration, specifically in the dividing tissue, and the role of IAA as a positional signal for controlling cambial growth.

MATERIALS AND METHODS

The plant material consisted of 43-year-old Scots pine (*Pinus sylvestris* L.) trees that were grown in an experimental forest site at Norrliden, northern Sweden (64° 21′ N/19° 46′ E). The stand was a part of a field nutritional experiment that was set up in 1973 (Holmen et al., 1976). For the present investigation, five fast-growing trees were selected from a fertilized and thinned parcel, and five slow-growing trees were selected from a control parcel.

On June 28, 1994, during the most active period of cambial growth, the trees were felled, sampled, and measured. Each tree was sampled at three positions along the stem: at breast height (1.3 m above ground), just below the lowermost living branch (approximately mid-stem), and at the ninth internode from the top of the tree (i.e. close to the center of the crown). Stem discs were removed and dried at room temperature for measurements of the width of the five last-completed annual rings. For IAA analysis, blocks about 2 × 10 cm in tangential area, consisting of extraxylary tissues and a few annual rings of xylem, were collected. The blocks were immediately frozen in liquid N_2, transported to the laboratory on dry ice, and stored at −80°C. Three trees of each kind with typical growth patterns were selected and further analyzed.

Preparation and Anatomical Characterization of Samples

IAA was measured in 30-μm tangential, longitudinal sections from the cambial zone and developing and mature xylem and phloem tissues as described by Uggla et al. (1996). A sampling series that consisted of consecutive sections from the phloem to the xylem was removed from a 3- by 15-mm specimen using a cryomicrotome (HM 505 E, Microm Laborgeräte GmbH, Walldorf, Germany) equipped with a steel knife cooled to −20°C. The area of each tangential section was measured before it was transferred to an Eppendorf tube and stored at −80°C. For radial localization of the tangential sections, cross-sections were hand cut with a razor blade at both ends of the specimen after every third tangential section. These cross-sections were mounted in glycerol and examined under a light microscope (Axioplan, Zeiss) using Nomarski optics. Different tissues and developmental stages are defined according to anatomical and histochemical criteria. Functional phloem was the part of the phloem that was arranged in orderly radial files. Cells to the outside of the functional phloem, consisting of compressed cells, were considered to be nonfunctional phloem. Thin-walled cells with a narrow radial diameter, not exceeding the combined radial diameter of a pair of recently divided cells, were defined as cambial-zone cells. The radially expanding, thin-walled cells between the cambial zone and the functional phloem were defined as differentiating phloem.

The transition from radially expanding tracheids to tracheids forming a secondary wall was determined by the presence of birefringence within the cell walls under Nomarski optics. To detect the transition from tracheids under secondary-wall formation to mature tracheids, two methods were used. First, secondary-wall formation was considered to be complete when an S_3 layer was detectable under polarized light (Bailey, 1954). Second, autolysis was considered to be incomplete when RNA could be detected by its fluorescence after staining with Acridine orange (Gahan, 1984). The zone where both an S_3 layer and RNA were present was defined as the zone of transition from differentiating to mature, autolysed tracheids. The number of tracheary derivatives formed after cambial reactivation is defined as the sum of tracheary derivatives under radial expansion and secondary-wall formation and mature, current-year tracheids. The number of cells in each zone from the cambial zone to the zone of mature tracheids, the radial width of the cambial zone and the zone of radially expanding tracheids, and the radial diameter of the three latest-formed mature tracheids were determined for nine radial files of the hand-cut cross-sections obtained from each end of the specimen before tangential sectioning.

IAA Quantification

Quantitative measurements of endogenous IAA in each 30-μm tangential section was performed using an isotope-dilution MS technique according to the method of Edlund et al. (1995). One to six nanograms of [$^{13}C_6$]IAA (Cambridge Isotope Laboratories, Woburn, MA) was added to each sample as an internal standard. Analysis was done by GC-selected reaction monitoring MS using a JMS-SX/SX102A instrument (Jeol).

For each tree and position, IAA is expressed as IAA_{tot} and IAA_{max}. IAA_{tot} is calculated by summing the amounts of IAA per square-centimeter section for each section of the sampling series from each position (missing values inter-

polated). IAA_{max} is calculated as the mean of the three highest values within the sampling series. Thus, IAA_{tot} expresses the total amount of IAA per tangential square-centimeter area, and IAA_{max} expresses the maximum amount of IAA per square-centimeter section that is reached within each sampling series.

Statistics

The Spearman's rank-correlation procedure was used for the statistical analysis (Zar, 1984). This method is preferable when data are not normally distributed. Because of interdependence among the three positions within a tree, correlations were calculated separately for each position.

RESULTS

Patterns of Cambial Growth

Cambial growth was measured by counting the radial number of tracheary derivatives produced since the start of the growing season. A large variation in growth was evident between trees and positions, and for all trees greatest growth was within the crown and decreased down the stem (Fig. 1A). These growth patterns were also reflected in the accumulated stem-diameter growth during the last 5 years at each sample position (Fig. 2), indicating that the documented growth patterns were stable in the selected trees. The number of the current-year tracheary derivatives produced by the time of sampling is assumed to reflect differences in cambial growth rate between the samples. This assumption relies on a contemporaneous reactivation of cambial growth for trees with different growth rates, which has been demonstrated in a similar stand of Scots pine (Valinger, 1992). It also relies on the fact that reactivation of cambial growth in mature pine trees is simultaneous throughout the stem (Savidge and Wareing, 1981; Sundberg et al., 1991). Cambial growth rate showed a good correlation with the number of cambial-zone cells (Fig. 3A), as well as with the radial width of the cambial zone (Fig. 3B).

The radial diameter of tracheids was greater in the fast-growing trees than in the slow-growing trees at comparable positions, but the difference was small (Fig. 2). Furthermore, a tendency toward a basipetal increase in tracheid diameter was seen in the fast-growing trees but not in the slow-growing ones.

Distribution Pattern of IAA

In all trees the radial distribution pattern of IAA described a steep concentration gradient, with a maximum within the cambial zone (Fig. 2). This observation confirms previous results from much older Scots pine trees (Uggla et al., 1996). The gradient tended to be steeper on the phloem side compared with the xylem side. In most cases, the radial width of the entire IAA gradient approximated the combined width of the dividing and expanding cells. However, two of the trees (Fig. 2, D and E) exhibited a divergent appearance of the gradient at the xylem side at some positions. Although the visualized gradient represents only the IAA distribution pattern at a limited sample area at one time, we believe that this pattern is relatively stable in time and space. This assumption is based on earlier results from the same species, in which the IAA amount in individual trees did not show any great fluctuations during the grow-

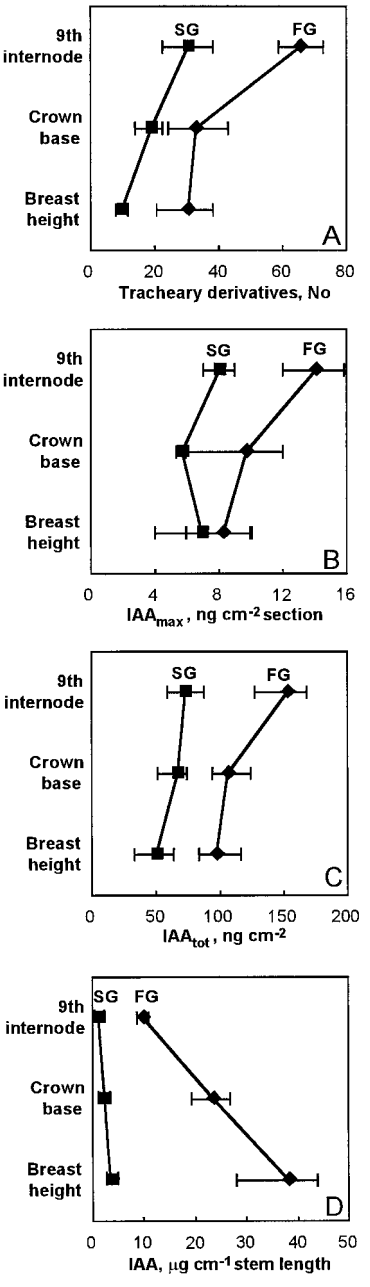

Figure 1. Longitudinal pattern of cambial growth and IAA levels in the cambial meristem and its differentiating derivatives in fast-growing (FG) and slow-growing (SG) trees. A, Number of current-year tracheary derivatives. B, IAA_{max} in the cambial-zone cells. C, Amount of IAA per tangential square-centimeter area (IAA_{tot}). D, Amount of IAA per centimeter stem length. Values represent the means of three trees. Horizontal bars indicate maximum and minimum values.

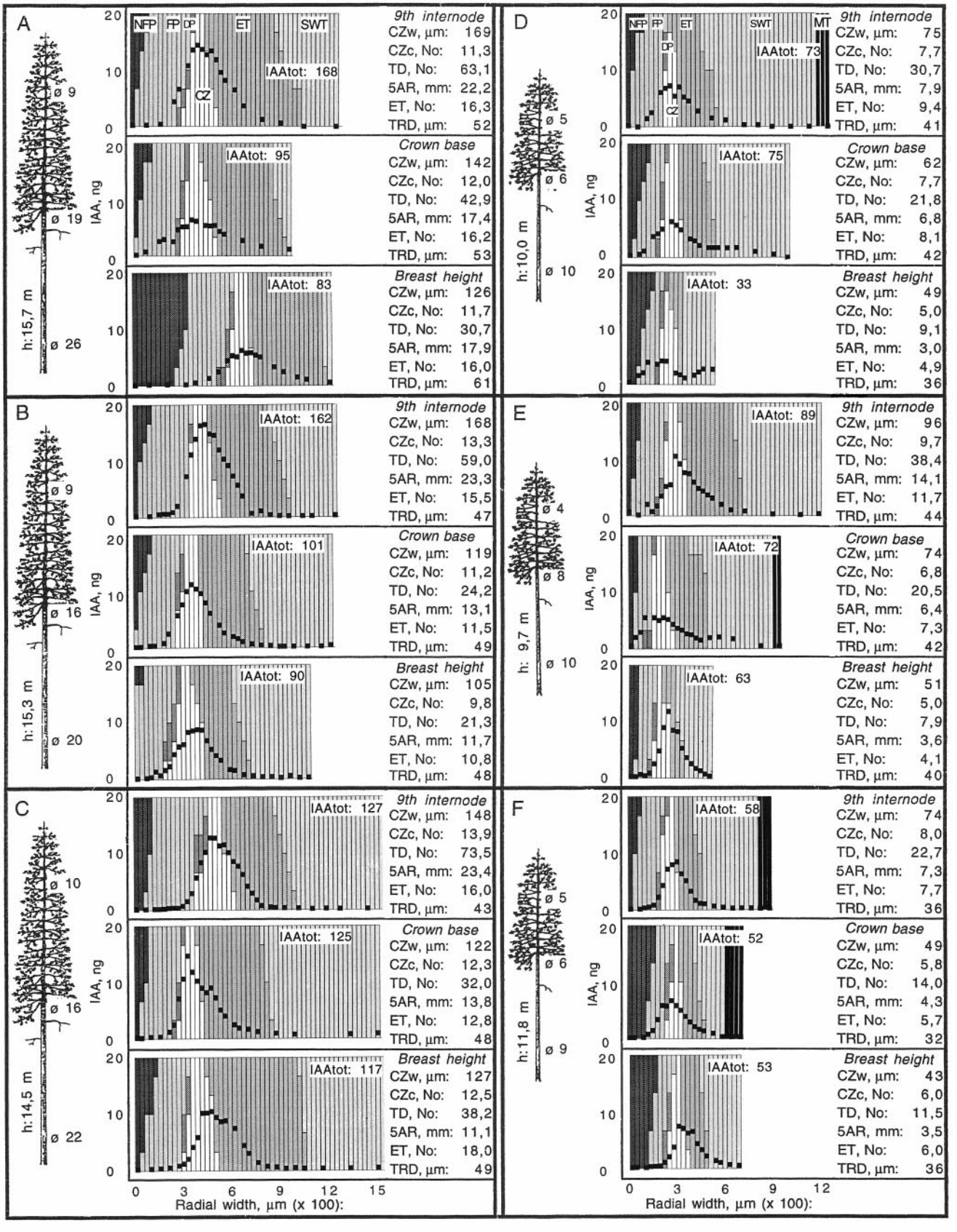

Figure 2. (Legend appears on facing page.)

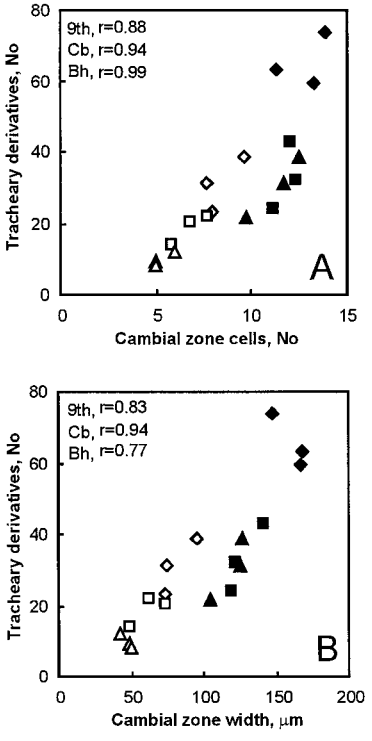

Figure 3. Correlation between cambial growth rate (number of tracheary derivatives) and number of cambial-zone cells (A) and radial width of the cambial zone (B). Correlation coefficients are indicated for each position of all trees. ◆, ◇, Ninth internode from top (9th); ■, □, crown base (Cb); and ▲, △, breast height (Bh). Open symbols, Slow-growing trees; closed symbols, fast-growing trees.

ing period (Sundberg et al., 1991). Moreover, the IAA amount in adjacent 6- × 6-mm stem samples from Scots pine, covering a total area of 30 × 18 mm, did not vary much (C. Uggla and B. Sundberg, unpublished data).

IAA_{max}

IAA_{max} was calculated as the mean of the three highest values within each gradient. Considering all trees and all positions, a 4-fold difference in IAA_{max} was found between the highest (15.9 ng cm^{-2} section) and the lowest (4.0 ng cm^{-2} section) value. Because weight and water content do not vary much between tangential sections from the cambial zone in different trees or positions, IAA_{max} will reflect the molar concentration of IAA. The molar concentration would be in the range of 10 to 40 μM using a weight of 2.5 mg cm^{-2} tangential section and a water content of 90%, which has been estimated for the dividing and expanding tissues, where IAA_{max} was found in an earlier study (Uggla et al., 1996). The fast-growing trees had higher IAA_{max} values than the slow-growing trees in the top position but not at the base of the tree (Figs. 1B and 2). A clear decrease in IAA_{max} between the top and the base of the crown was found in two of the fast-growing trees, but apart from that, longitudinal concentration gradients were not evident (Figs. 1B and 2).

IAA_{tot}

The total amount of IAA per area unit is represented by the integrated area under the gradient. The total amount was higher in the fast-growing trees than in the slow-growing trees (Fig. 1C). Considering all trees and all positions, a 5-fold difference was found between the lowest (33 ng cm^{-2}) and the highest (168 ng cm^{-2}) value. In all trees IAA_{tot} decreased in a basipetal manner, but the pattern and extent of this decrease varied considerably between trees (Fig. 2).

By multiplying IAA_{tot} by the corresponding stem circumference at each position, an estimate of the total amount of IAA within a 1-cm strip around the stem was obtained. This gives information about the variation in the total pool of IAA along the stem, which was found to increase from the top position to the base of the stem (Fig. 1D).

Radial Width of the IAA Gradients

Because IAA induces cell division and cell expansion we previously hypothesized that the radial IAA gradient is a part of the mechanism that provides positional information to the developing cambial derivatives (Uggla et al., 1996). According to this idea the meristematic zone is maintained within a concentration window along the gradient. Thus, the width of the cambial zone should relate to the width of the IAA gradient. However, it is clear from the data in this investigation and from a previous study in hybrid aspen (Tuominen et al., 1997) that no obvious threshold value of IAA defines the cambial zone. For example, expansion of the cambial derivatives on the xylem side is taking place under IAA levels similar to those found within the cambial zone toward the phloem side. Also, the concentration window in which cell division occurs is very different between trees with different growth rates. It is therefore not obvious how to evaluate the role of the IAA gradient width in

Figure 2. (Continued from facing page.)
Radial distribution pattern of IAA across the cambial meristem and its differentiating and mature derivatives at different positions along the stem of three fast-growing (A–C) and three slow-growing (D–F) trees. Each column represents a 30-μm tangential section and its relative composition of different tissues. Endogenous IAA content for each section is indicated with a solid square. IAA was measured at three positions on each tree: 9th internode (top position), crown base, and breast height. Growth characteristics for each tree and for mature and developing vascular tissues are indicated. The IAA_{tot} per square-centimeter stem area (i.e. the integrated area under the gradient) is indicated at the upper-right corner for each position. h, Tree height; ∅, stem diameter; CZw, cambial-zone width; CZc, cambial-zone cells; TD, tracheary derivatives in the current-year annual ring; 5AR, radial width of the annual rings formed during the last 5 years; ET, expanding tracheids; TRD, radial diameter of mature tracheids; NFP, nonfunctional phloem; FP, functional phloem; DP, developing phloem; CZ, cambial zone; SWT, tracheids undergoing secondary wall thickening; and MT, mature tracheids.

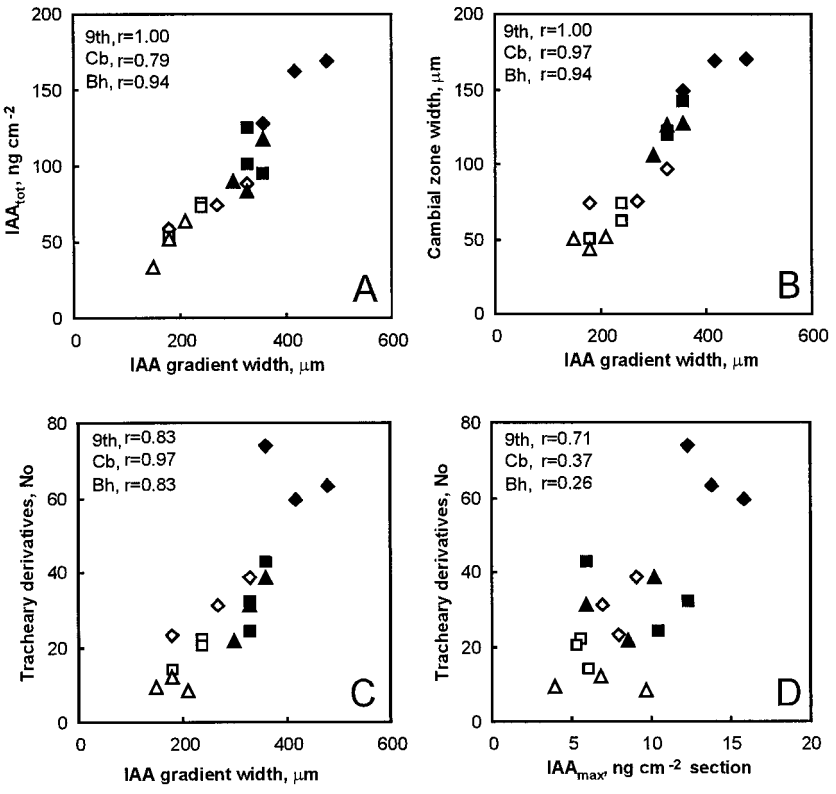

Figure 4. Correlation between IAA_{tot} and IAA gradient width (A), cambial zone width and IAA gradient width (B), cambial growth rate (number of tracheary derivatives) and IAA gradient width (C), and cambial growth rate (number of tracheary derivatives) and IAA concentration in the cambial zone (IAA_{max}; D). Correlation coefficients are indicated for each position in all trees. ♦, ◇, Ninth internode from top (9th); ■, □, crown base (Cb); ▲, △, breast height (Bh). Open symbols, Slow-growing trees; closed symbols, fast-growing trees. IAA gradient width was calculated at a threshold value of 3.5 ng.

positional signaling. But it is noted that the supply of IAA to the vascular cambium, as reflected in IAA_{tot}, is closely correlated with the gradient width at a threshold value of 3.5 ng (Fig. 4A), which therefore was used for correlative studies.

Correlative Studies

A strong correlation was found between the widths of the IAA gradient and the cambial zone (Fig. 4B). Because the width of the cambial zone is correlated with tracheid production rate, a correlation between gradient width and tracheid production was also evident (Fig. 4C). However, the IAA concentration in the cambial zone (IAA_{max}) was poorly correlated with cambial growth rate (Fig. 4D), although it is noted that the high rate of cambial growth in the top position of the fast-growing trees is associated with high IAA_{max} values.

DISCUSSION

Formation of secondary xylem requires positional information that coordinates the radial pattern of the developmental zones of division (the cambial zone), expansion, and wall formation, which can be observed in cross-sections of the stem (Uggla et al., 1996). Positional information is also involved in the regulation of cambial growth rate by defining the width of the cambial zone and therefore also the radial number of dividing cells and, hence, tracheid production (Gregory, 1971; Fig. 3). Positional information can be conveyed by morphogens, which originate in specific organizing centers and create a concentration gradient in the surrounding tissues by diffusion (Wolpert, 1996). Such a gradient will establish a morphogenetic field, and cells will develop according to their position in this field. In animals much evidence has been obtained for morphogenetic fields in the control of the patterned differentiation during embryo development (Gurdon et al., 1995; Lecuit et al., 1996). In the present study IAA was demonstrated to be consistently distributed as a concentration gradient across the cambial meristem and its developing derivatives along the main trunk of mature Scots pine trees (Fig. 2), confirming earlier results from this species (Uggla et al., 1996) and from hybrid aspen (Tuominen et al., 1997). This concentration gradient shares features of morphogenetic fields that are found in animal systems (Wolpert, 1981). For example, it has a typical width of less than 1 mm and the source of the morphogen (IAA) is well defined by its polar transport in the cambial zone. These features, together with the well-established morphogenetic role for IAA in cell division and cell expansion, imply that the radial IAA gradient has a function as a morphogenetic field with the potential to give positional signaling in cambial growth.

The close correlation between the widths of the IAA gradient and the cambial zone supports the idea that IAA is a positional signal in plants, organizing the pattern of secondary growth. Its control of cambial growth rate can be explained from the following observations. An altered supply of polarly transported IAA to the cambial zone is reflected in the width of the radial IAA gradient (Fig. 4A). The radial gradient defines the width of the cambial zone

(Fig. 4B), which in turn is closely correlated with the radial number of dividing cells and, therefore, also with the rate of cambial growth (Fig. 3). Not surprisingly, a direct correlation between the radial width of the IAA gradient and rate of cambial growth was also evident (Fig. 4C). However, it is clear that the cambial zone is not positioned within a certain concentration window along the radial IAA gradient. Therefore, other as-yet-unknown positional signals are also involved in the control of the cambial-zone width.

The entire width of the radial IAA gradient approximates the radial width of expanding cells, i.e. the combined zones of cell division and cell expansion. A similar observation has been made in hybrid aspen, in which transgenic trees with ectopic expression of bacterial IAA biosynthetic genes exhibited a wider IAA gradient compared with wild-type trees, which was related to a wider zone of expanding cells (Tuominen et al., 1997). This suggests that cambial derivatives will continue to expand as long as they are positioned within the field of a significant IAA concentration, i.e. within the radial IAA gradient. It therefore follows that the width of the IAA gradient modulates the radial width of xylem elements by regulating the duration of their expansion. This notion is supported by the wider zone of expansion and the wider radial diameter of tracheids in the fast-growing trees compared with the slow-growing trees (Fig. 2). Furthermore, significant levels of IAA are not present in the differentiating cells forming a secondary wall, which suggests that endogenous IAA is not needed to maintain cell wall thickening.

A role for IAA as a positional signal controlling developmental patterns in cambial growth need not preclude its having a role in the control of rates of division and expansion of individual cambial derivatives. The high-sensitivity GC-MS technique for IAA measurements used in this investigation provides a unique opportunity to study the correlation between IAA concentration, specifically in the dividing cells, and cambial growth rate. It was found that the differences in cambial growth rate between trees and positions in most cases could not be explained by differences in IAA concentration in the cambial zone (Fig. 4D). But the cambia with the highest growth rates (i.e. the top position in the fast-growing trees) contained a high IAA concentration in the cambial-zone cells. In agreement with Gregory (1971), it is also noted that the number of cambial-zone cells in the fast-growing cambia was not increased in proportion to their growth rate (Fig. 3A). Therefore, their high growth rate is partly attributed to a higher rate of cell division, possibly induced by the increase in IAA concentration.

To understand the role of IAA in regulating cambial growth patterns it is essential to elucidate the underlying mechanisms that control the axial and radial distribution of IAA in the secondary body of the plant. Unfortunately, we have only limited knowledge about IAA homeostasis at the whole-plant level. The presence of longitudinal concentration gradients has often been assumed and implicated in explanations of cambial growth patterns (Larson, 1969; Aloni and Zimmermann, 1983). However, this assumption does not consider that polarly transported IAA has a radial

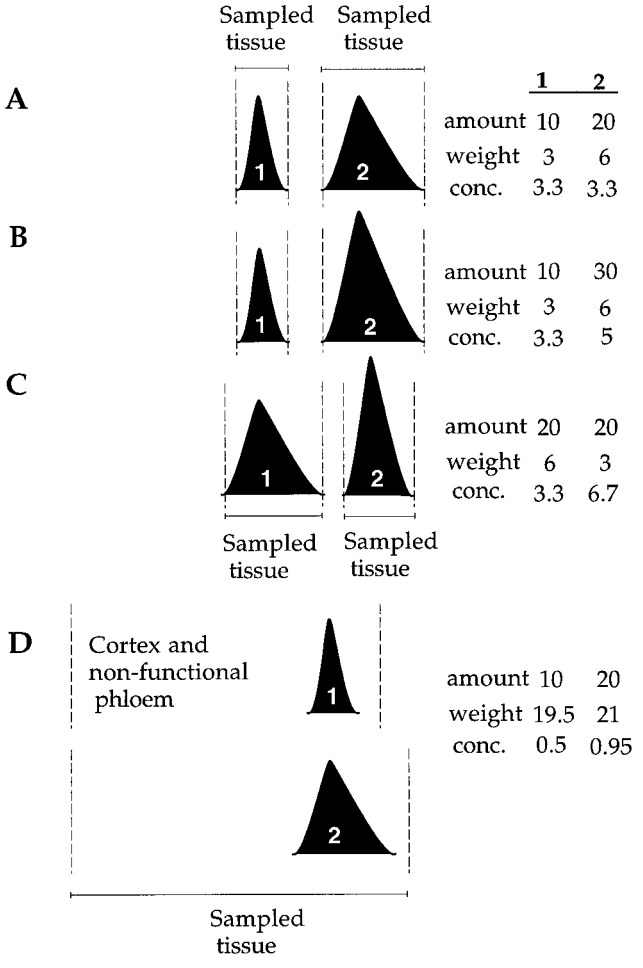

Figure 5. Schematic drawing showing the effect of different shapes of radial IAA distributions and sample sizes on calculated IAA concentrations in samples from extraxylary stem tissues. A, Difference in gradient width, but not in peak concentration, will be reflected in IAA amount but give similar IAA concentrations. B and C, Differences in both peak concentration and gradient width can result in differences in IAA amount and IAA concentration (B), but it can also result in differences only in IAA concentration (C). D, When nonfunctional phloem and cortex tissues low in IAA content are included, the calculated IAA concentration will mirror the IAA amount. Although the radial IAA gradients illustrated are fictitious, all cases illustrated have actually been found in Scots pine trees (Uggla et al., 1996; Fig. 2; C. Uggla and B. Sundberg, unpublished results). amount, The integrated area under the gradient; weight, weight of the sampled tissues; and conc., calculated amount of IAA per weight unit.

distribution pattern, resulting in widely different concentrations in the cambium and its developing derivatives. The existence of longitudinal concentration gradients can therefore be evaluated only by visualizing the radial IAA distribution. The data obtained here show that the idea of longitudinal IAA concentration gradients is not generally applicable when considering the peak IAA concentration (IAA_{max}), although a clear decrease in IAA_{max} is observed between the top and the crown base of the fast-growing trees (Fig. 1B). The absence of consistent longitudinal concentration gradients is particularly intriguing, considering the long

distance between sampling positions. Our data suggest that a change in IAA supply to the vascular cambium results in a change in radial gradient width (Fig. 4A), but only when IAA supply is large will the peak concentration be affected. It is also noteworthy that IAA_{tot} does not change much along the branchless part of the stem (Fig. 1C). However, the amount of IAA per unit stem length increases between crown base and breast height (Fig. 1D), implying either that transport capacity is decreasing basipetally or that there is de novo biosynthesis of IAA in the stem.

Another point of great importance is the mechanism(s) that control the radial distribution pattern of IAA across the cambial meristem and the differentiating derivatives. The shape of the gradient is determined by the supply of polarly transported IAA, the amount of IAA that is laterally transported, and the catabolism and/or removal of laterally transported IAA. The latter is a prerequisite for the creation of the concentration gradient. IAA removal may be a result of IAA reaching the stream of mass flow in xylem and phloem, which is supported by findings of endogenous IAA in xylem and phloem sap (Allen et al., 1979; Hoad, 1995). However, a more controlled catabolism of IAA also seems likely. The mechanism of IAA removal/catabolism will affect the width of the radial gradient and, therefore, be indirectly involved in the control of xylem development and cambial growth.

Earlier studies of seasonal and spatial variations of IAA concentrations in samples containing a mixture of dividing, developing, and mature vascular tissues have given inconsistent results (for review, see Little and Pharis, 1995). Also when using amount and concentration as a basis of expression, different patterns of IAA variation have been obtained (Sundberg et al., 1990, 1991, 1993). This can be explained in light of the large differences in IAA concentration across these tissues. From hypothetical cases it can be seen that differences in IAA amount need not be reflected in differences in calculated IAA concentrations per sample weight, and vice versa (Fig. 5). This is because the calculated IAA concentration in the sample will depend on the radial distribution pattern of IAA and the amount of sampled tissue. Thus, IAA concentration estimates in samples from the cambium and its neighboring tissues are of limited use in evaluating the variation of IAA supply to these tissues. In those cases, however, when all extraxylary tissues have been sampled (e.g. Sandberg and Ericsson, 1987; Sundberg and Little, 1990), estimates of IAA concentration will mirror the amount of IAA (Fig. 5D).

Taken together, our results support the idea that IAA has a role as a positional signal and that it regulates cambial growth rate by determining the radial population of dividing cambial-zone cells. However, it is not unlikely that the concentration of IAA in the cambial meristem has an additional role in controlling rates of cell divisions. We also conclude that the assumption of a decreasing concentration gradient of IAA down the stem is not always valid.

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