

RONI ALONI

## PHYTOHORMONAL MECHANISMS THAT CONTROL WOOD QUALITY FORMATION IN YOUNG AND MATURE TREES

**Abstract.** The secondary xylem, namely, the wood, in trees is induced and controlled by streams of inductive hormonal signals which shape wood quality and quantity. Auxin is the primary hormonal signal which controls wood formation; it is mainly produced in young leaves, moves downward through the cambium and induces the wood. Cytokinin, from root tips moves upward, increases the sensitivity of the cambium to the auxin signal and stimulates cambial cell divisions. Gibberellin promotes shoot elongation and induces long fibres and tracheids. Centrifugal movement of ethylene from differentiating-xylem cells outwards to the bark induces the radial vascular rays. In conifer trees, jasmonate-induced defence response, which is mediated by ethylene, induces the traumatic resin ducts. Large traumatic resin cavities that damage the wood in response to wounding and stress can be prevented by lowering the sensitivity to ethylene. Along the tree axis, gradients of decreasing auxin concentrations from leaves to roots induce gradients of cell width, wall composition and density in the wood. The juvenile wood in trees is induced by young leaves, while the adult wood can be produced further away from these leaves. Therefore, to swift the transition from juvenile wood to the high quality adult wood at the base of the trunk, fast stem elongation of young trees should be promoted. This can be achieved by growing young trees in high densities and by minimizing competition with annuals and grass species. Likewise, as root tips provoke juvenile effects in the shoot, fast root growth should be endorsed to minimize their effects. Rapid stem elongation can also be achieved by manipulating endogenous hormonal concentrations in transgenic trees. Elevating endogenous bioactive gibberellin concentrations in transgenic trees promotes stem elongation, increases long fibre and tracheid production, and modifies lignin biosynthesis.

### 1. INTRODUCTION

The aim of this presentation is to elucidate the hormonal mechanisms that control wood formation in trees, and to provide the required hormonal understanding for upgrading tree breeding and improving wood quality and production in forest trees. Plant hormones are organic molecules that at low concentrations influence plant growth and development. The hormones are synthesized in various locations, move through specific transport pathways to sites where they regulate growth and differentiation. The hormonal signals that induce the wood move in the vascular tissues; they regulate multiple organized processes of cell divisions, growth and differentiation building complex xylem cell patterns typical to each plant species. This chapter summarizes the current knowledge on the subject and provides information and new concepts on the development of the secondary xylem in trees and herbaceous model plants. In particular, I focus on the hormonal signals and how they regulate the differentiation of various types of xylem cells in the axial and radial directions, including the induction of traumatic resin ducts, the control of cell size and density, and the regulation of juvenile-adult phase transition. I also clarify how the cambium and the xylem cells have been specialized during evolution from conifers to ring-porous trees.

RONI ALONI

## 2. HORMONAL SIGNALS THAT REGULATE XYLOGENESIS

The four major hormonal signals that induce and control wood formation in trees are: auxin, cytokinin, gibberellin and ethylene (Aloni 2001). Their roles in regulating cambium activity and secondary xylem differentiation will be clarified below. The hormonal control of wood formation is mediated by the expression of specific genes and numerous transcription factors which regulate various aspects of cell differentiation and secondary growth (Groover 2005; Groover and Robischon 2006; Nagawa et al. 2006; Demura and Fukuda 2007; Persson et al. 2007).

The vascular system of the plant is composed of xylem (water-conducting tissue) and phloem (food-conducting tissue) which are complex, being composed of several types of cells, which are induced by a number of developmental signals (Roberts et al. 1988; Evert 2006). Along the plant axis, the vascular cells are induced and controlled by longitudinal streams of inductive hormonal signals (Sachs 1981; Aloni 1987, 2001), while the vascular rays are induced and regulated by radial hormonal flows (Lev-Yadun and Aloni 1995; Aloni et al. 2000).

### 2.1. Auxin (*IAA*)

The auxin hormone, namely, indole-3-acetic acid (IAA) is the most common naturally occurring auxin. IAA is the major shoot signal which regulates all aspects of vascular differentiation in plants (Aloni 2001). The polar transport of IAA from young shoot organs (Aloni 2004) downward via the cambium to the root tips (Aloni et al. 2006a) induces and controls wood formation. The continuity of the vascular tissues along the plant axis is a result of the steady polar flow of IAA from leaves to roots (Aloni 1987).

The pioneering study of Jacobs (1952) demonstrated that the auxin produced in young leaves is the limiting and controlling factor in xylem regeneration around a wound. The percentage of the bioactive auxin hormone, namely, the free IAA is very low in a given tissue and might range from 1% to 5% of the total auxin (Ljung et al. 2002). Most of the auxin in cells is covalently bound to other molecules and these conjugated molecules are inactive as hormonal signals, and serve as a reservoir from which the free auxin can be released. Free IAA is not detected (by *DR5::GUS* expression) in the apical bud and the youngest leaf primordia (Aloni et al. 2003) or youngest flower primordia (Aloni et al. 2006b), which are all loaded with conjugated auxin, detected by antibodies (Aloni et al. 2003; Aloni et al. 2006b). Bound auxin is accumulated in the shoot apex, youngest leaves and flowers likely due to the local upward polar auxin flow from the free-auxin-producing leaves (Reinhardt et al. 2003). The youngest leaf primordia, start as sinks for IAA (Reinhardt et al. 2003; Benková et al. 2003; Scheres and Xu 2006) and become sources of free IAA during leaf development (Aloni et al. 2003; Aloni 2004; Teale et al. 2006). This developmental auxin pattern stimulates me to put forward the following new concept, proposing that due to the importance and requirement for constant IAA supply from the shoot organs to the rest of the plant, the shoot establishes a large reservoir of conjugated auxin molecules at the shoot apex and

## PHYTOHORMONAL MECHANISMS THAT CONTROL WOOD QUALITY

youngest shoot organs, from which the free IAA will later be hydrolysed continuously in young growing leaves. Thus, the massive bound auxin pool in the young shoot organs guarantees the constant supply of free auxin, which induces and regulates wood formation along the tree axis.

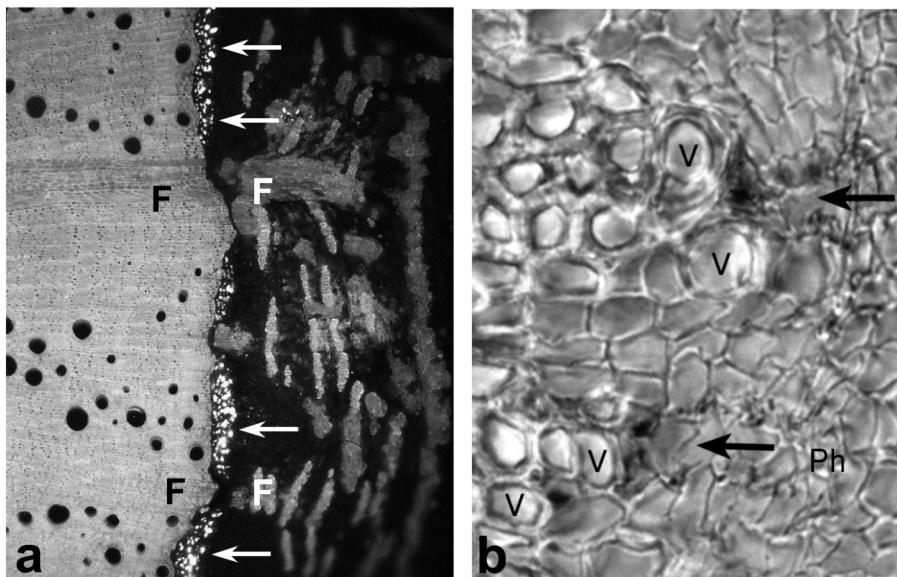
Free-auxin production during leaf development was explained by the *leaf-venation hypothesis* (Aloni 2001). The hypothesis was confirmed (Aloni et al. 2003) showing that the primary sites of free-IAA production are the developing hydathodes (the water secreting glands which develop in the tip and later in the lobes). During leaf-primordium development, there are gradual shifts in the sites and concentrations of IAA production, progressing from the hydathode of the elongating tip, continuing downward along the expanding blade margins, and ending in the central regions of the lamina (Aloni 2001, 2004; Aloni et al. 2003). This pattern of sites of IAA concentration was also confirmed by auxin analysis by GC-MS/MS techniques (Müller et al. 2002). Likewise, flowers, fruits and seeds produce auxin in a gradual shifting pattern during their development (Aloni et al. 2006b).

The orderly pattern of vascular tissues from leaves to roots was explained by the *canalization hypothesis* (Sachs 1981). According to this hypothesis, IAA flow, which starts by diffusion, induces a polar IAA transport system which promotes IAA movement and leads to canalization of the IAA flow along a narrow file of cells. The continuous polar transport of IAA through these cells induces a further complex sequence of events which terminates in the formation of a vessel (Sachs 1981). Recent molecular evidence supports the canalization hypothesis demonstrating that rearrangement of polar IAA flow changes tissue polarity through modification of the site of the PIN protein (an essential component involved in IAA efflux) on the plasma membrane (Sauer et al. 2006) as has been predicted by the canalization hypothesis.

A steep gradient of IAA occurs in *Pinus* trees across the vascular cambium and its derivatives, with a peak in the cambium (Uggla et al. 1996). It has been suggested that positional information triggering the differentiation of different types of vascular cells may be derived from this IAA gradient rather than from its concentration (Uggla et al. 1996, 1998).

The polar movement of free IAA from leaves to roots occurs via a few specific transport pathways (Aloni 2004). The movement of IAA via the cambium regulates cambial activity, and induces xylem (Uggla et al. 1996; Aloni 2001) and phloem (Aloni 2001, 2004) differentiation. The formation of a radial pattern of vessels and sieve tubes (Fig. 1a) indicates specific polar IAA streams which move in the cambium in preferred locations (between the vessels and sieve tubes which are induced by these IAA streams). Indeed, analysis of free-IAA transport with *DR5::GUS* expression confirmed that the transport of IAA streams through the cambium occurs preferably between vessels and sieve tubes (Fig. 1b), but not adjacent to vascular rays (Aloni 2004).

Auxin from adult leaves moves into the phloem, where the IAA moves rapidly in a non-polar fashion, up and down through intact sieve tubes (Morris et al. 1973; Goldsmith et al. 1974). This non-polar IAA transport in the sieve tubes is not involved in cambium activity and therefore does not induce wood formation in intact



*Figure 1. Patterns of cell differentiation and free-auxin distribution in the cambium. (a)* Cross section (XS) in the stem of *Quercus calliprinos* (stained with 0.01% aniline blue and observed with epifluorescence microscope, following Aloni and Peterson 1997) showing radial distribution of vessels and sieve tubes (marked by arrows) among fibres (F) in the xylem and phloem, indicating that IAA which induces the sieve tubes (white spots) and the vessels (dark rounded spots in the wood, which is located on left side) moves in the cambium preferably between these vascular conduits, x 20. *(b)* XS in the same orientation, in *Arabidopsis thaliana* showing free-IAA distribution (marked by DR5::GUS expression, which forms the dark spots, indicated by arrows) between the phloem (Ph) sieve tubes and the xylem vessels (v), x 750.

trees. The non-polar IAA is considered a house-keeping signal which regulates callose levels in sieve tubes (Aloni 2004).

It has long been known that hormones influence each other's biosynthesis and effects induced by one hormone may be mediated by others. Thus, high IAA concentrations can enhance gibberellin biosynthesis; likewise, elevated IAA levels can induce ethylene biosynthesis (Taiz and Zeiger 2006).

## 2.2. Cytokinins (CKs)

Cytokinins are adenine derivatives and the most common CK is zeatin. CKs produced in the root cap, are major hormonal signals of the root (Miyawaki et al. 2004; Aloni et al. 2005, 2006a). The CKs exist as free and bound molecules. The free forms promote cell divisions in meristematic tissues including the vascular cambium. CKs increase the sensitivity of the cambium to the auxin signal, which

## PHYTOHORMONAL MECHANISMS THAT CONTROL WOOD QUALITY

has promoted the development of ring-porous wood during evolution of trees (Aloni 1991, 2001). CKs regulate many developmental activities in plants, such as promoting shoot development, stimulating lateral bud growth (breaking shoot apical dominance), and the breaking of bud dormancy in deciduous trees during spring. Furthermore, CKs promote leaf development and delay leaf senescence.

CKs produced in the root tips are transported upward from roots to shoots in the xylem, via vessels (Aloni et al. 2005; Sakakibara et al. 2006; Kuroha and Satoh 2007) and tracheids. The upward CK transport is regulated by the transpiration stream, where the hormone moves mainly to developing shoot organs with high transpiration rates (Aloni et al. 2005). Nitrate (not  $\text{NH}_4^+$ ) supply to nitrogen-depleted roots causes a rapid up-regulation of *IPT* genes resulting in an increase of CKs content in the root, which is transported via the xylem upward into the shoot (Gessler et al. 2004; Miyawaki et al. 2004; Rahayu et al. 2005; Sakakibara et al. 2006). CKs rapidly down-regulate the expression of *IPT1,3,5,7* (Miyawaki et al., 2004), which may emphasize the regulatory role of root-to-shoot CK mass transport on shoot CK synthesis. This suggests that synthesis of CK in the shoot could guarantee the CK availability in an emergency under conditions of insufficient CK supply from the root, e.g., under nitrogen deficiency (Miyawaki et al. 2004; Takei et al. 2004) and in young shoot organs of trees (Gessler et al. 2004) which are further away from the roots. Under conditions of insufficient CK supply from the root, nitrate-responsive *IPT3* is expressed in the phloem (Takei et al. 2004; Miyawaki et al. 2004) and produces CK in the shoot, which could regulate cell divisions in the cambium.

### 2.3. Gibberellins (GAs)

Gibberellins are a large family of more than 125 tetracyclic diterpenes. Some of them are essential endogenous regulators which promote cell and stem elongation and many other developmental functions in plants, while many of the GAs are inactive and may serve as precursors of the bioactive  $\text{GA}_1$ , which promotes stem elongation (Taiz and Zeiger 2006). Gibberellins regulate the transition from juvenile to adult phases; thus in conifers, exogenous GA application can induce the reproductive phase and cone production in young trees; conversely, in woody angiosperms including many fruit trees, gibberellins promote vegetative growth by the inhibition of flowering (Goldschmidt and Samach 2004) and when bioactive GAs are applied to mature woody plants they may induce rejuvenation (Taiz and Zeiger 2006). GA from mature leaves stimulate cell divisions in the cambium and induce fiber formation (Hess and Sachs 1972; Aloni 1979, 1985, 2001). Bioactive  $\text{GA}_1$  and  $\text{GA}_4$  were predominantly found in the expansion zone of differentiating-xylem cells in *Populus* suggesting a main role for GA during early stages of wood formation, including cell elongation (Israelsson et al. 2005). Stems of transgenic plants with elevated GA concentrations grow rapidly and produce longer fibres (Eriksson et al. 2000; Biemelt et al. 2004). Furthermore, GA also regulates lignin biosynthesis (Aloni et al. 1990).

#### *2.4. Ethylene*

The gas ethylene ( $C_2H_4$ ) is a plant hormone which is synthesized in many tissues in response to stress. Wounding, flooding, wind, bending, high auxin levels, elevated cytokinin concentrations and methyl jasmonate promote ethylene synthesis in trees. Elevated  $C_2H_4$  concentrations can inhibit stem elongation and may promote leaf and fruit abscission. Ethylene stimulates defence responses to injury or disease and reduces vessel width (Aloni et al. 1998). The ethylene produced in the differentiating-wood cells diffuses in the centrifugal direction and this radial ethylene flow through the cambium initials induces vascular ray (by promoting cell divisions in the cambial fusiform initials) and the enlargement of existing rays (Levyadun and Aloni 1995; Aloni et al. 2000). When the centrifugal transport of ethylene outward to the plant environment is blocked by flooding water, the ethylene accumulates in the cortex, or the bark, and the resulting local high  $C_2H_4$  concentrations can induce aerenchyma (Li et al. 2006), which enables aeration of flooded stems and roots. High ethylene concentrations also promote lateral and adventitious root formation (Aloni et al. 2006a; Kuroha and Satoh 2007). Asymmetric patterns of elevated ethylene concentrations may be induced in stems and branches in response to gravistimulation, bending and wind, which could probably be promoted by asymmetric distribution of elevated IAA concentrations. However, no clear modifications in IAA distribution pattern could be detected following gravistimulation (Hellgren et al. 2004), which might indicate a technical difficulty in tissue sampling. Asymmetric hormonal distributions can promote reaction wood formation: the formation of compression wood in conifers and tension wood in hardwoods (Timell 1986; Zobel and van Buijtenen 1989).

In conifers, the ethylene hormone induces chemical defences against insects and pathogens. Wounding and ethylene can promote traumatic resin duct formation in conifer woods (Hudgins and Franceschi 2004). The resin duct epithelial cells produce oleoresin terpenoids, which protect the tree from insects and their associated pathogens (Keeling and Bohlmann 2006; Ralph et al. 2007).

#### *2.5. Jasmonates (JAs)*

The phytohormone jasmonic acid (JA) and its volatile methyl ester (MeJA) are fatty acid derived cyclopentanones. In plants, they activate defence-related genes against insects and pathogens (Howe 2004). MeJA moves in both the phloem and xylem pathways. The MeJA enters into the phloem and moves in the sieve tube sap along with photoassimilates. MeJA promotes its own transport; whole plant experiments suggest that enhanced transport of both MeJA and sugar may be due to MeJA enhancing the energy of the plasma membrane (Thorpe et al. 2007).

In Pinaceae, application of methyl jasmonate (MeJA) can induce traumatic resin duct formation (Hudgins et al. 2003; Hudgins and Franceschi 2004; Huber et al. 2005). This MeJA-induced defence response is mediated by ethylene, which

## PHYTOHORMONAL MECHANISMS THAT CONTROL WOOD QUALITY

promotes the formation of traumatic resin-secreting epithelial cells (Hudgins and Franceschi 2004).

### 2.6. Brassinosteroids (BRs)

Besides the well-known roles of auxin and cytokinin in vascular differentiation, there is evidence for the role of brassinosteroids in this process (Carlsbecker and Helariutta 2005). Nanomolar levels of BRs stimulate tracheary element formation in isolated mesophyll cells of *Zinnia elegans* (Iwasaki and Shibaoka 1991) and regulate expression of genes associated with xylem formation (Fukuda 1997). BRs from the epidermis might influence the differentiation of vascular tissues (Savaldi-Goldstein et al. 2007) and possibly control tissue-type-specificity of vascular cell proliferation (Carlsbecker and Helariutta 2005). However, although it is likely that BRs are involved in xylem differentiation, their role in wood formation in trees requires further clarification.

### 2.7 Abscisic acid (ABA)

Abscisic acid is the long-distance stress signal produced in roots as soil is drying and is transported through the xylem from roots to the shoot to regulate the closure of stomata and retard meristematic activity (Taiz and Zeiger 2006). The flow rate of water through tracheids and vessels is crucially affected by stomata opening and closing; ABA closes the stomata and could retard shoot development, while cytokinin from the root tips which is transported upward through the xylem (Aloni et al. 2005) has a positive effects on stomata opening (Dodd 2003), on cambium activity (Aloni 2001), and shoot development (Taiz and Zeiger 2006). ABA, which is the universal stress hormone of higher plants, has a central role in plant developmental plasticity; it is likely involved in slowing down and stopping wood formation in trees towards their winter dormancy by retarding and ending their cambium activity. Evidently, in intact *Eucommia ulmoides* trees, the expression of the auxin binding protein1 (ABP1), one of the putative receptors of auxin, is promoted by auxin during the period of cambium activity and is inhibited by ABA during dormancy (Hou et al. 2006).

## 3. REGULATION OF XYLEM CELL DIFFERENTIATION

In the xylem, the conducting elements are the tracheary cells. They function in long-distance water transport, as nonliving cells after autolysis of their cytoplasm. Tracheary cells are characterized by secondary wall thickenings which enable them to retain their shape when dead, despite the pressure of the surrounding cells. The two fundamental types of xylem conduits are: the tracheid (typical to gymnosperms) and the vessel (of angiosperms) which is built of vessel elements. Among the vessels

## RONI ALONI

are the specialized supporting cells, the fibres. In the radial direction, the wood contains the vascular rays which are usually built of parenchyma cells (Evert 2006).

### 3.1. Tracheid differentiation

A tracheid is a non-perforated long cell with bordered pits. Tracheids are both the conductive and supportive cells that build the ‘softwood’ of gymnosperms. Auxin movement through the cambium of pine trees induces the differentiation of tracheids from cambium initials (Larson 1969; Uggla et al. 1996, Sundberg et al. 2000). In young pine seedlings, tracheids can also be produced from parenchyma cells by application of auxin and gibberellin (Kalev and Aloni 1998; Aloni et al. 2000). Auxin by itself induced very short tracheids, while the gibberellin, in the presence of auxin, promoted tracheid elongation by stimulating intrusive growth of both the upper and lower ends of the differentiating tracheids. Tracheid differentiation could also be promoted by ethylene (Aloni et al. 2000).

### 3.2. Vessel differentiation

A vessel is a long continuous tube made up of numerous vessel elements connected end-to-end by perforation plates and limited in length by imperforated walls at both extremities. Vessels, and not vessel elements, are the operating units in the ‘hardwood’ of angiosperms. Their dimensions are important parameters for understanding long-distance water transport, wood quality, xylem pathology, wood adaptation and evolution. Vessels do not end randomly in the stem of young trees; e.g., in *Populus* and *Olea*, vessel endings are significantly higher at the nodes (Salleo et al. 1984). Therefore, the nodes are considered “safety zones”, because gaseous emboli and fungal spores fail to pass through the endings. In diffuse-porous trees the longest vessels are about 1 m long, whereas in ring-porous trees the largest earlywood vessels are extremely long and reach the length of the tree itself (Zimmermann and Jeje 1981). An increase in vessel diameter markedly increases the efficiency of water transport, owing to decrease in resistance to flow, whereas increase in both diameter and length decreases safety of water conduction, in terms of cavitation (Tyree and Zimmermann 2002; Hacke et al. 2006).

The vessels are induced by the polar flow of auxin originating in young leaves (Jacobs 1952; Sachs 1981; Aloni 2004). High IAA concentrations stimulate rapid cell differentiation resulting in narrow vessels; while low IAA levels induce slow differentiation, which permits more time for cell expansion until secondary wall deposition, therefore resulting in wide vessels. Along the tree axis, the IAA induces gradual gradients of increasing vessel diameter and decreasing vessel density from leaves to roots. The hormonal mechanism that regulates these vessel gradients (Aloni and Zimmermann 1983) as well as the mechanism which regulates the formation of large earlywood vessels in ring-porous trees (Aloni 1991) will be clarified below.

## PHYTOHORMONAL MECHANISMS THAT CONTROL WOOD QUALITY

### 3.3. Fibre differentiation

Fibres are long and narrow cells with thick secondary walls that are usually heavily lignified. Differentiation of fibres in the ‘hardwood’ of angiosperms and in the phloem is induced by gibberellin in the presence of auxin, and the GA which induces fibres originates in mature leaves (Hess and Sachs 1972; Aloni 1979, 1987). The numerous fibres in the latewood of ring-porous trees are induced during the summer and autumn by mature leaves that produce GAs (Aloni 1991, 2001; Aloni et al. 1997), while their IAA moves into the sieve tubes (Morris et al. 1973; Goldsmith et al. 1974). When both gibberellin and auxin were applied exogenously to stems from which the leaves had been excised (to remove endogenous hormone production by these leaves), high IAA concentrations stimulated rapid differentiation of short fibres with thick secondary walls, while high levels of GA resulted in long fibres with thin secondary walls (Aloni 1979; Roberts et al. 1988). Both gibberellin and auxin also regulate lignin biosynthesis (Aloni et al. 1990). Applications of gibberellin and auxin to various industrial plants increase long fibre production (Aloni 1985). In addition, cytokinin promotes fibre differentiation (Aloni 1982; Saks et al. 1984), whereas ethylene can retard fibre formation (Aloni et al. 1998).

Understanding the gibberellin biosynthesis pathway (Taiz and Zeiger 2006) enables molecular manipulation of GA production. Over-expression of GA 20-oxidase, a gene encoding the enzyme responsible for the rate limiting step involved GA synthesis, enhances fibre yield (Eriksson et al. 2000). The transgenic tobacco plants and poplar trees showed higher levels of GAs in their shoots and an increase in fibre length (Eriksson et al. 2000; Eriksson and Moritz 2002). This genetic manipulation also increased the inactive GAs, due to GA 2-oxidase catalysis. We have recently used another approach to elevate gibberellin concentrations by silencing GA 2-oxidase (i.e., preventing deactivation of the bioactive gibberellin), which elevates the bioactive GA concentrations in transgenic model plants (*Arabidopsis*, tobacco and Kenaf) and promoted rapid shoot elongation, increased fibre production, increased fibre size, and decreased fibre lignification (Aloni R., Avni A., and Dayan J., Provisional USA Patent 60/802,516 filed 23/05/2006). Low lignification can reduce the cost of paper and other fibrous materials produced from Kenaf. Endogenous bioactive gibberellin concentrations could be boosted up by inducing both the over-expression of GA 20-oxidase and silencing the GA 2-oxidase genes, which could result in synergistic effects. These manipulations could also modify lignin metabolism and change lignin structure and content.

### 3.4. Ray differentiation

The radial component of the secondary vascular tissues is the vascular rays. The rays serve as radial transport pathways between the xylem and phloem. The rays are induced and controlled by radial moving signals and they are shaped (their longitudinally elongated structure) by axial signal flows. Ethylene, synthesized in the differentiating-wood cells and moving centrifugally through the cambium, seems

## RONI ALONI

to be the major hormonal signal which controls the initiation and regulation of ray size and ray spacing in the cambium (Lev-Yadun and Aloni 1995; Aloni et al. 2000).

In young trees, there is a natural gradual increase in ray size with increasing distance from the pith and with growing distance from the young leaves. These developmental patterns might result from a gradual decrease of IAA concentrations and a gradual increase in ethylene synthesis with increasing distance from the young leaves. In conifers, the ray initials occupy about 10% of the cambium surface, whereas in woody angiosperms the ratios are more variable ranging from 0% rays (in rayless wood) to about 25% ray volume. A substantial increase in ray dimensions occurs in response to wounding. This wound effect on ray size can be induced by ethylene application. The proliferation of ray parenchyma cells on the cut surface following wounding enables the recovery of the injury and regeneration of wood after wounding (Lev-Yadun and Aloni 1995; Aloni et al. 2000).

### 3.5. Resin duct differentiation

Resin ducts lined with resin-secreting epithelial cells are a common feature in conifers (Evert 2006). They protect the tree from insects and their associated pathogens (Keeling and Bohlmann 2006; Ralph et al. 2007). In the Pinaceae there are genera (e.g., *Pinus*, *Picea*, *Larix* and *Pseudotsuga*) which produce resin ducts as a natural feature and in response to injury will also produce traumatic resin ducts, other genera (e.g., *Abies*, *Tsuga*, *Cederus* and *Pseudolarix*) produce only traumatic resin ducts in response to wounding, and there are genera (e.g., *Cupressus* and *Juniperus*) which never produce any resin ducts (Fahn 1990; Evert 2006). The natural occurring resin duct system is built of longitudinal and radial (occurring inside large vascular rays) ducts. The largest number of resin ducts is produced when the cambium of an injured branch is intensively active (Fahn and Zamski 1970; Fahn 1990). In *Pinus halepensis*, auxin which enhances radial growth of wood also promoted resin-duct formation (Fahn and Zamski 1970), however this resin-duct formation is not a direct auxin effect because the resin ducts developed only about one month after the auxin application. The application of the ethylene-releasing agent, ethrel (2-chloroethylphosphonic acid) to *P. halepensis* seedlings promoted the production of longitudinal resin ducts in their wood (Yamamoto and Kozlowski 1987). Jasmonate, more specifically, methyl jasmonate (MeJA), which activates defence-related genes, seems to be the primary signal which induces traumatic-resin-duct formation in conifers (Hudgins et al. 2003; Hudgins and Franceschi 2004; Huber et al. 2005), and this jasmonate-induced defence response is mediated by ethylene (Hudgins and Franceschi 2004). This was evident in studies on *Pseudotsuga menziesii* which showed that the MeJA induced ethylene production earlier and 77-fold higher than wounding. Pre-treatment of *P. menziesii* stems with an ethylene response inhibitor (1-methylcyclopropene) inhibited the MeJA and wound responses (Hudgins and Franceschi 2004).

Severe stress and wounding might induce large resin cavities which damage the wood for technological use. This damage could be prevented in trees with low ethylene sensitivity. Similarly, we demonstrated that crown-gall tumour

## PHYTOHORMONAL MECHANISMS THAT CONTROL WOOD QUALITY

development which is also regulated by ethylene was inhibited on the ethylene-insensitive tomato, the *Never ripe* mutant (Aloni et al. 1998). *In vitro* production of pine trees from shoots is well established (Hargreaves et al. 2005). Regularly, to increase survival of adventitious originated plants, the culture jars are aerated to reduce the build-up of ethylene concentrations during the culture process. I propose that conifer trees with lowered sensitivity to ethylene would be selected during the tissue-culture process by keeping the jars closed. Only tissues of ethylene-insensitive trees will survive. Various lines with different sensitivities to ethylene can be selected and will be analysed for traumatic-resin-duct formation and afterwards for their resistance under field conditions. It is expected that selected tree lines with lowered ethylene sensitivity will show decreased response to wounding and consequently limited traumatic-resin-duct formation.

Lowering ethylene sensitivity in conifers may reduce their insect resistance. This issue can be solved genetically by introducing into the selected trees toxin genes against insects (Gordon et al. 2007; Gurevitz et al. 2007). For example, transgenic *Pinus radiata* trees containing a *Bacillus thuringiensis* toxic gene displayed variable levels of resistance to insect damage, with one transgenic line being highly resistant to feeding damage (Grace et al. 2005). Anti-attractant treatments against insects may also be used (Erbilgin et al. 2007).

## 4. VASCULAR DIFFERENTIATION IN BRANCH JUNCTION

The *segmentation hypothesis* (Zimmermann 1983) suggests that the xylem at a branch junction consists of narrow vessel elements or tracheids that form a bottleneck for water transport. The hydraulic segmentation of lateral branches from the main stem gives priority of water supply to the main stem over the branches (Zimmermann 1983; Aloni et al. 1997). Spiral vascular tissues and circular vessels are induced by the movement of auxin in circular patterns (Sachs and Cohen 1982). Circular vessels are often produced in the upper side of branch junctions and their size and frequency increase continuously with age and branch width (Lev-Yadun and Aloni 1990). The circular vessels do not function in water transport and they actually interrupt and reduce water flow in the upper side of the branch junction. Therefore, the long-distance water transport into branches occurs preferably through the branch sides and its lower region (Aloni et al. 1997). The young-leaf biomass, which produces IAA, regulates the development of a branch and its success in competition with other branches. Greater IAA production induces more longitudinal vessels or tracheids parallel to the wood grain which gives the branch improved access to the water resources of the tree. Thus IAA export from a branch regulates branch vigour (Kramer and Borkowski 2004; Kramer 2006).

Pruning young trees improves the growth of their main stem and their wood quality. Pruning branch-stem junctions stops their circular pattern formation, resulting in the production of uniform trunk wood without knots in the post-pruning annual rings and optimization of mechanical wood properties at the junction sites.

RONI ALONI

## 5. WOOD GRADIENTS

### 5.1. Regulation of vessel size and density along the tree axis

The auxin hormone descending from young leaves to root tips acts as a morphogenetic signal which forms polar concentration gradients along the plant axis. Such IAA gradients in the vascular cambium provide directional and location information to differentiating cells along the morphogenetic fields. Therefore, a decreasing gradient of IAA concentrations along the tree axis from leaves to roots may result in a general and gradual increase in tracheid dimensions, or vessel diameter which is associated with decrease in vessel density, with increasing distance from the young leaves (Aloni and Zimmermann 1983; Zimmermann 1983; Aloni 1987; Leitch 2001). Accordingly, the narrow tracheids or vessels differentiate near the young leaves, where the highest auxin concentrations are expected, while the widest tracheids and vessels are formed in the roots, at the greatest distance from the auxin sources. The gradual increase in vessel diameter from leaves to roots is associated with a gradual decrease in vessel density. Hence, vessel density is generally greater in branches, where the vessels are narrow, than in roots, where they are wide (Aloni and Zimmermann 1983; Leitch 2001).

We proposed that the general increase in vessel size and decrease in vessel density along the plant axis is regulated by a gradient of decreasing auxin concentrations from leaves to roots (Aloni and Zimmermann 1983). This is based upon the assumption that the steady polar flow of IAA from leaves to roots controls these polar changes in the vascular system. High auxin concentrations near the young leaves induce narrow vessels because of their rapid differentiation, allowing only limited time for cell growth. Conversely, low IAA concentrations further down result in slow differentiation, which permits more cell expansion before secondary wall deposition, and thereby results in wide vessels. Vessel density is controlled by auxin concentration; accordingly, high auxin concentrations (near the sites of IAA production) induce greater density, while low concentrations (further down, towards the roots) diminish density. Consequently, vessel density decreases from leaves to roots (Aloni and Zimmermann 1983; Leitch 2001). This hypothesis was experimentally confirmed by showing that various auxin concentrations applied to decapitated stems induce substantial gradients of increasing vessel diameter and decreasing vessel density from the auxin source towards the roots (Aloni and Zimmermann 1983). High auxin concentration yielded numerous vessels that remained small because of their rapid differentiation; low auxin concentration resulted in slow differentiation and therefore in fewer and larger vessels.

Studies on transgenic plants with altered levels of IAA confirmed the general relations between IAA concentration and vessel size and density. Thus, auxin-overproducing plants (i.e., ones overexpressing the *iaaM* gene) contained many more vessel elements than did control plants, and their vessels were narrow (Klee et al. 1987); conversely, plants with lowered IAA levels (i.e., expressing the *iaaL* gene as an anti-auxin gene) contained fewer vessels of generally larger size (Romano et al. 1991).

## PHYTOHORMONAL MECHANISMS THAT CONTROL WOOD QUALITY

### 5.2. Regulation of juvenile-adult transition

Woody plants have two distinct growth phases: juvenile and adult. In the juvenile period, trees grow vigorously during their early years. Gradually their vegetative growth declines and fruit production begins. The transition from juvenile to mature wood occurs gradually during tree growth and might be regulated by hormonal signals from leaves and root tips. Juvenile wood, which occupies the center of the stem and is produced during the first 5 to 20 annual growth rings (depends on tree species) is characterized by short cells, thin cell walls and low specific gravity, and the outcome is low strength (Zobel and van Buijtenen 1989), which may result in the development of reaction wood. The maturation process of cambium and wood could occur in different rates along the tree axis and various adult wood features might mature independently.

Little is known about the hormonal mechanisms that control the juvenile-adult transition in trees. Near the young leaves, the high auxin concentrations induce short and narrow tracheids, fibres, or vessels, which become gradually larger with the increasing distance from the IAA sources. Gibberellin from mature leaves promotes vascular cell elongation and, therefore, contributes to the gradual increase in xylem cell dimensions. The mature leaves are also a major source of photosynthetic products needed for increasing secondary cell wall deposition typical to mature wood. The distance from young leaves to a given site in the cambium and differentiating wood increases gradually during stem growth and this gradual increasing distance (which decreases the effects of young leaves) likely promotes the gradual phase change from juvenile to mature wood. Thus, the secondary xylem in the recent annual ring(s) at the base of the trunk becomes gradually adult, while the wood produced in the upper twigs of the crown remains juvenile. I suggest that the cambial age of juvenile-mature wood transition and pith-to-bark-profile of wood density (Mutz et al. 2004) are controlled by the downward decreasing gradients of IAA concentrations from the young leaves (Aloni and Zimmermann 1983; Aloni 2001) which regulate the gradual transition to mature wood. In addition, this phase transition is likely promoted by the gradually increasing effects of mature leaves on wood quality. This new hormonal concept should be analysed experimentally.

Roots of the English ivy (*Hedera helix*) produce gibberellin-like substances which promote juvenile shoot growth (Frydman and Wareing 1973). When the stems are young and short, the 'root factors' retard the juvenile-adult phase change. During the years, the stem elongates substantially, consequently the concentrations of the root signals decrease, which promotes apical bud maturation and the development of the adult shoot. Application of various bioactive gibberellins to adult *Hedera helix* shoots induce rejuvenation (Frydman and Wareing 1974), namely, a transition of adult shoots to the juvenile phase with typical juvenile features.

To uncover the possible role of root tips on the juvenile-adult transition in forest trees, grafting experiments were conducted on *Eucalyptus globulus*. By grafting adult branches on a juvenile rootstock we substantially reduce the distances between the root tips and the adult buds, thus increasing the root-tips effects on the adult

RONI ALONI

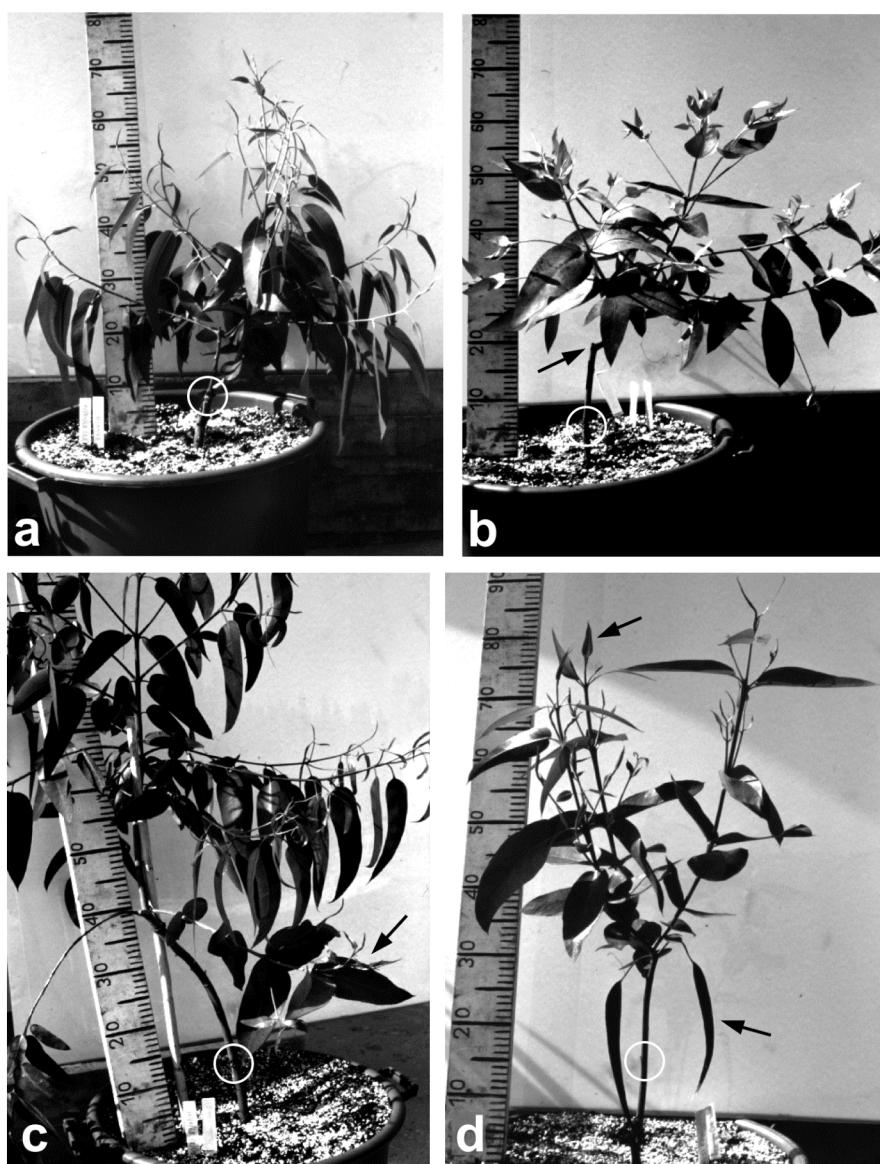


Figure 2. Photographs showing the effects of a 6-month-old juvenile rootstock on 20-year-old adult grafted branch of *Eucalyptus globulus*, 4 months after grafting. The graft union is marked with a white ring. (a) Well developed grafted shoot with adult leaves only. (b) Only juvenile looking leaves developed from a lateral adult bud after the upper internodes (above arrow) died. (c) The lowest adult bud developed to a juvenile branch (arrow). (d) Gradual transition of leaves on three branches: from mature (lower arrow) to juvenile leaves, and a gradual transition to adult leaves (upper arrow).

## PHYTOHORMONAL MECHANISMS THAT CONTROL WOOD QUALITY

buds. In these experiments, well developed 20-year-old adult branches were grafted on 6-month-old juvenile rootstocks. The results demonstrated that the juvenile roots induced rejuvenation, in which some of the adult buds in the grafted branches started to show juvenile characteristics in leaf structure and branch phyllotaxis. On well developed graft unions, on which the grafted branches grow vigorously, all the new leaves showed adult morphology (Fig. 2a) and produced flowers. Conversely, when the apical bud and the youngest internodes of the grafted branch degenerated (because of slow graft-union establishment), the remaining lateral adult buds developed juvenile looking leaves and typical juvenile phyllotaxis (Fig. 2b) with no flowers. In addition, various in-between shoots were found. Usually, only the lowest branch developed as a juvenile branch (marked by arrow in Fig. 2c) although it originated from an adult bud, demonstrating the strongest root influence on the lowest bud, which is the closest to the juvenile root. Rarely, a few apical buds developed and they started to produce juvenile looking leaves for a few weeks, and with time have gradually returned to produce adult leaves (marked by the upper arrow in Fig. 2d). These results indicate that the roots produce a signal, or signals, which can be transported through a graft union. This root signaling promotes juvenile features in the shoot, which likely retard the juvenile-adult transition in intact trees. Therefore, to improve wood quality by promoting early development of adult wood, rapid root growth should be encouraged, which will increase the distance of the root tips from the stem and thus reduce the juvenile-promoting-effects of the root. Gibberellins (Frydman and Wareing 1973, 1974) and cytokinins (Aloni et al. 2005, 2006a) are the potential root-tip hormonal candidates that could influence the juvenile-adult wood transition.

## 6. HORMONAL WOOD EVOLUTION

### 6.1. From tracheids to vessels and fibres

Vessel elements are more efficient conductors of water than tracheids, since the water flows through vessel elements occurs via perforations, rather than diffusion through the cell walls of tracheids (Tyree and Zimmermann 2002). Tracheids appeared in ancient land plants about 430 million years ago, while vessel elements were recorded much later, about 140 million years ago, and became dominant in angiosperms (Aloni et al. 2000; Raven et al. 2005). Vessel elements have evolved independently from tracheids in several diverse groups of plants, making them an excellent example of parallel evolution (Bailey 1944). The naturally occurring perforated tracheids are very rare in conifer trees (Bannan 1958). The suggestion that these perforations have been induced by polar auxin movement was supported experimentally by the formation of perforations in tracheids following the application of high auxin concentration to hypocotyls of young pine seedlings (Aloni et al. 2000). Therefore, these tracheids with perforations support the general view about the evolutionary origin of vessels from tracheids.

Fibres, like vessels, have originated from tracheids of more primitive plants (Aloni et al. 2000). IAA movement through the cambium of conifer trees induces

## RONI ALONI

the differentiation of tracheids from cambium initials (Savidge 1996; Uggla et al. 1996); the possible role of gibberellin has not been studied. Auxin induced very short tracheids which differentiated from parenchyma cells in the hypocotyls of young pine seedlings, while there was need for both auxin and gibberellin for inducing the differentiation of long tracheids (Kalev and Aloni 1998; Aloni et al. 2000). Therefore, we suggested that during xylem evolution, the original hormonal mechanism for the differentiation of tracheids in primitive plants has become more specific in higher plants. From the ancient inducing mechanism for typically elongated tracheids (a combination of auxin and gibberellin) the signal for each xylem element in higher plants has become specific: auxin by itself induces short vessel elements (Jacobs 1952; Sachs 1981; Aloni 2004), whereas gibberellin, in the presence of auxin, has become the specific signal which induces long fibres (Aloni 1979, 1987). This means, that the well known evolutionary transition from tracheids to fibres and vessel elements reflects the hormonal specialization which has occurred during evolution (Aloni et al. 2000).

### 6.2. *From diffuse-porous to ring-porous wood*

The vascular cambium is the lateral meristem that actively divides and produces the secondary xylem and phloem (Larson 1994). Polar transport of IAA from leaves to roots through the vascular cambium, in the presence of cytokinin from the root cap [possibly also CK from the sieve tubes (Sakakibara et al. 2006)], keeps the cambium active during growing seasons. Deciduous trees lose their leaves during periods of extreme environmental conditions and then their cambium becomes dormant.

In temperate deciduous hardwood trees, the size differences of vessels in the earlywood and latewood are quite marked and two main xylem categories can be distinguished: diffuse-porous wood and ring-porous wood. In diffuse-porous wood the vessels are more or less uniform in size, whereas in ring-porous wood the vessels produced at the beginning of the growth season are significantly wider than those produced at the end of the season (Evert 2006). Earlywood vessels in ring-porous trees can be huge (width of up to 500 µm and length of the tree itself) and therefore are very efficient in water conductance, even though they usually function for only one season (Tyree and Zimmermann 2002; Evert 2006). The challenge to understand the mechanisms that have shaped these vessel patterns during the evolution of temperate deciduous hardwood trees requires elucidation of the roles of tissue sensitivity (Trewavas 1983; Bradford and Trewavas 1994) and specific hormonal signalling in these trees (Aloni 1991, 2001).

Aloni (1991) and Wheeler and Baas (1991) suggested that ring-porous trees have originated from diffuse-porous species. The development of ring porosity has probably arisen independently multiple times during the diversification of angiosperms, and different lineages might therefore have modified mechanisms in different families. The *limited-growth hypothesis* (Aloni 1991) proposes that during the evolution of temperate deciduous hardwood trees, the ring-porous species have developed from diffuse-porous species under the selective pressures of limiting

## PHYTOHORMONAL MECHANISMS THAT CONTROL WOOD QUALITY

environments which resulted in limited vegetative growth. It was further postulated that the natural selection for ring-porous wood has led to a decrease in the intensity of vegetative growth, accompanied by reduced auxin levels. The latter was followed by an increase in the sensitivity of the cambium to relatively low auxin stimulation; these changes created the conditions which enable wide-earlywood-vessel differentiation (Aloni 1991), as will be clarify below. Evidence that supports the hypothesis comes from observations that a diffuse-porous tree (*Populus euphratica*) and a ring-porous tree (*Quercus ithaburensis*) can change their porosity under opposite environmental conditions. Thus, under stress conditions when extension growth is suppressed both tree species produced narrow annual rings characterized by ring-porous wood (as predicted by the hypothesis), whereas under favourable conditions when extensive growth is intensive both species produce wide annual rings with diffuse-porous wood (Liphschitz 1995).

Cytokinins from the root caps (Aloni et al. 2005, 2006a) increase the sensitivity of the cambium to developmental signals originating in leaves (Aloni 2004; Aloni et al. 2003, 2006b). Cytokinin prevents the usually rapid occurring IAA conjugation (Coenen and Lomax 1997); therefore, elevated CK concentration allows low-concentration-IAA movement in the cambium, which may explain the increased sensitivity of the cambium to auxin. Experimental evidence from transformed plants (Zhang et al. 1995; Eklöf et al. 1997) supports the idea that reduced auxin concentrations can elevate cytokinin concentration, which would enhance tissue sensitivity (Trewavas 1983; Aloni 1991). The experiments demonstrate that auxin or cytokinin modify the content of the other hormone by affecting its rate of synthesis. Reduced auxin concentration increases free cytokinin level (Palni et al. 1988; Zhang et al. 1995; Eklöf et al. 1997). This, in turn, enhances cambium sensitivity to low-level IAA streams originating in swelling buds and creates the special conditions that enable the differentiation of very wide earlywood vessels during a limited period of time in spring (Aloni 1991, 2001).

The increased cambium sensitivity in ring-porous trees enables early cambium reactivation at the beginning of the growth season before bud break. This adaptation of ring-porous trees created the special internal conditions that enable them to respond to initial flows of extremely low IAA concentrations originating from dormant looking (before swelling) buds a few weeks before bud break (Aloni 1991, Aloni and Peterson 1997, Aloni et al. 1997), stimulating slow vessel differentiation which permits more cell expansion before secondary wall deposition, resulting in the formation of very wide earlywood vessels. Therefore, their first wide earlywood vessels are initiated six to two weeks before the onset of leaf expansion (Suzuki et al. 1996). Conversely, in diffuse-porous species, the first earlywood vessels are initiated two to seven weeks after the onset of leaf expansion (Suzuki et al. 1996), and because of its low sensitivity, their cambium requires high auxin levels (from fast growing young leaves) for reactivation.

Diffuse-porous species start the growth season a few weeks earlier than ring-porous trees and have a longer growth season which is characterized by continuous production of young leaves during a few months. Conversely, ring-porous trees which are late leafing trees (Lechowicz 1984) produce young leaves for only a short period of a few weeks and later they have mainly mature leaves (Aloni et al. 1997).

## RONI ALONI

Because young diffuse-porous trees possess greater growth intensity they might produce more xylem per year than young ring-porous trees (Aloni et al. 1997). The continuous production of young leaves on diffuse-porous trees stimulates continuous production of vessels along the entire growth season with relatively thin-wall fibres. Whereas the mature leaves on the ring-porous trees induce the development of numerous well-developed hard lignified fibres during most of the growth season. These diverse earlywood and latewood properties in ring-porous wood, namely, the soft wide earlywood vessels *versus* the numerous hard latewood fibres affect lumber stability and can have major effects on wood and fibre utilization.

### 7. CONCLUDING REMARKS

Understanding the phytohormonal mechanisms that control wood formation enables one to improve wood production and quality by modifying tree growth, wood development and response to stress. The hormonal regulation of trees can be controlled with physiological and molecular methods and the selected trees should produce superior wood for industry. To improve forest trees, their endogenous hormonal concentrations and the sensitivity to hormones can be modified. For instance, increasing the endogenous bioactive gibberellin concentrations in transgenic forest trees improves stem elongation, increases longer fibre and tracheid production and modifies lignin biosynthesis. Likewise, decreasing the sensitivity to ethylene (to minimize traumatic-resin-duct formation) in conifer trees is expected to promote their growth and prevent the development of large traumatic resin cavities that damage the wood in response to wounding and stress.

### 8. REFERENCES

- Aloni R (1979) Role of auxin and gibberellin in differentiation of primary phloem fibers. *Plant Physiol* 63: 609-614.
- Aloni R (1982) Role of cytokinin in differentiation of secondary xylem fibers. *Plant Physiol* 70: 1631-1633.
- Aloni R (1985) Plant growth method and composition. United States of America, Patent No 4507144.
- Aloni R (1987) Differentiation of vascular tissues. *Annu Rev Plant Physiol* 38: 179-204.
- Aloni R (1991) Wood formation in deciduous hardwood trees. In: *Physiology of Trees*. AS Raghavendra (ed), Wiley & Sons, New York, pp. 175-197.
- Aloni R (2001) Foliar and axial aspects of vascular differentiation - hypotheses and evidence. *J Plant Growth Regul* 20: 22-34.
- Aloni R (2004) The induction of vascular tissue by auxin. In: *Plant Hormones: Biosynthesis, Signal Transduction, Action!* PJ Davies (ed), Kluwer Academic Publishers, Dordrecht, pp. 471-492.
- Aloni R, Alexander JD, Tyree MT (1997) Natural and experimentally altered hydraulic architecture of branch junctions in *Acer saccharum* Marsh. and *Quercus velutina* Lam. trees. *Trees* 11: 255-264.
- Aloni, R, Aloni E, Langhans M, Ullrich CI (2006a) Role of cytokinin and auxin in shaping root architecture: regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. *Ann Bot* 97: 883-893.
- Aloni R, Aloni E, Langhans M, Ullrich CI (2006b) Role of auxin in regulating *Arabidopsis* flower development. *Planta* 223: 315-328.
- Aloni R, Feigenbaum P, Kalev N, Rozovsky S (2000) Hormonal control of vascular differentiation in plants: the physiological basis of cambium ontogeny and xylem evolution. In: *Cell and Molecular*

## PHYTOHORMONAL MECHANISMS THAT CONTROL WOOD QUALITY

- Biology of Wood Formation.* RA Savidge, JR Barnett, R Napier (eds), BIOS Scientific Publishers, Oxford, pp 223-236.
- Aloni R, Langhans M, Aloni E, Dreieicher E, Ullrich CI (2005) Root-synthesized cytokinin in *Arabidopsis* is distributed in the shoot by the transpiration stream. *J Exp Bot* 56: 1535-1544.
- Aloni R, Peterson CA (1997) Auxin promotes dormancy callose removal from the phloem of *Magnolia kobus* and callose accumulation and earlywood vessel differentiation in *Quercus robur*. *J Plant Res* 110: 37-44.
- Aloni R, Schwalm K, Langhans M, Ullrich CI (2003) Gradual shifts in sites of free-auxin production during leaf-primordium development and their role in vascular differentiation and leaf morphogenesis in *Arabidopsis*. *Planta* 216: 841-853.
- Aloni R, Tollier T, Monties B (1990) The role of auxin and gibberellin in controlling lignin formation in primary phloem fibers and in xylem of *Coleus blumei* stems. *Plant Physiol* 94: 1743-1747.
- Aloni R, Wolf A, Feigenbaum P, Avni A, Klee HJ (1998) The *Never ripe* mutant provides evidence that tumor-induced ethylene controls the morphogenesis of *Agrobacterium tumefaciens*-induced crown galls on tomato stems. *Plant Physiol* 117: 841-847.
- Aloni R, Zimmermann MH (1983) The control of vessel size and density along the plant axis - a new hypothesis. *Differentiation* 24: 203-208.
- Bailey IW (1944) The development of vessels in angiosperms in morphological research. *Amer J Bot* 31: 421-428.
- Bannan MW (1958) An occurrence of perforated tracheids in *Thuja occidentalis* L. *New Phytol* 57: 132-134.
- Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, Jürgens G, Friml J (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115: 591-602.
- Biemelt S, Tschiersch H, Sonnewald U (2004) Impact of altered gibberellin metabolism on biomass accumulation, lignin biosynthesis, and photosynthesis in transgenic tobacco plants. *Plant Physiol* 135: 254-265.
- Bradford KJ, Trewavas AJ (1994) Sensitivity thresholds and variable time scales in plant hormone action. *Plant Physiol* 105: 1029-1036.
- Carlsbecker A, Helariutta Y (2005) Phloem and xylem specifications: pieces of the puzzle emerge. *Curr Opin Plant Biol* 8: 512-517.
- Coenen C, Lomax TL (1997) Auxin-cytokinin interactions in higher plants: old problems and new tools. *Trends in Plant Sci* 2: 351-356.
- Demura T, Fukuda H (2007) Transcriptional regulation in wood formation. *Trends Plant Sci* 12: 64-70.
- Dodd IC (2003) Hormonal interactions and stomatal responses. *J Plant Growth Regul* 22: 32-46.
- Eklöf S, Åstot C, Blackwell J, Moritz T, Olsson O, Sandberg G (1997) Auxin-cytokinin interactions in wild-type and transgenic tobacco. *Plant Cell Physiol* 38: 225-235.
- Erbilgin N, Gillette NE, Mori SR, Stein JD, Owen DR, Wood DL (2007) Acetophenone as an Anti-attractant for the Western Pine Beetle, *Dendroctonus Brevicomis* LeConte (Coleoptera: Scolytidae). *J Chem Ecol* 33: 817-823.
- Eriksson ME, Israelsson M, Olsson O, Moritz T (2000) Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. *Nat Biotechnol* 18: 784-788.
- Eriksson ME, Moritz T (2002) Daylength and seasonal expression of gibberellin 20-oxidase isolated from hybrid aspen (*Populus tremulata* × *P. tremuloides* Michx.). *Planta* 214: 920-930.
- Evert RF (2006) *Esau's Plant Anatomy, Meristems, Cells, and Tissues of the Plant Body - their Structure, Function, and Development*. Wiley & Sons, Hoboken, NJ.
- Fahn A (1990) *Plant Anatomy*, 4<sup>th</sup> edn. Pergamon Press, Oxford.
- Fahn A, Zamzki E (1970) The influence of pressure, wind, wounding and growth substances on the rate of resin duct formation in *Pinus halepensis* wood. *Israel J Bot* 19: 429-446.
- Frydman VM, Wareing PF (1973) Phase change in *Hedera helix* L. II. The possible role of roots as a source of shoot gibberellin-like substances. *J Exp Bot* 24: 1139-1148.
- Frydman VM, Wareing PF (1974) Phase change in *Hedera helix* L. III. The effects of gibberellins, abscisic acid and growth retardants on juvenile and adult ivy. *J Exp Bot* 25: 420-429.
- Fukuda H (1997) Tracheary element differentiation. *Plant Cell* 9: 1147-1156.
- Gessler A, Kopriva S, Rennenberg H (2004) Regulation of nitrate uptake at the whole-tree level: interaction between nitrogen compounds, cytokinins and carbon metabolism. *Tree Physiol* 24: 1313-1321.

## RONI ALONI

- Goldschmidt EE, Samach A (2004) Aspects of flowering in fruit trees. Proc 9<sup>th</sup> IS on Plant Bioregulators, SM Kang et al. (eds.), Acta Hort 653, pp. 23-27.
- Goldsmith MHM, Catealdo DA, Karn J, Brenneman T, Trip P (1974) The nonpolar transport of auxin in the phloem of intact *Coleus* plants. *Planta* 116: 301-317.
- Gordon D, Karbat I, Ilan N, Cohen L, Kahn R, Gilles N, Dong K, Stuhmer W, Tytgat J, Gurevitz M (2007) The differential preference of scorpion alpha-toxins for insect or mammalian sodium channels: Implications for improved insect control. *Toxicon* 49: 452-472.
- Grace LJ, Charity JA, Gresham B, Kay N, Walter C (2005) Insect-resistant transgenic *Pinus radiata*. *Plant Cell Rep* 24: 103-111.
- Groover AT (2005) What genes make a tree? *Trends Plant Sci* 10: 210-214.
- Groover A, Robischon M (2006) Developmental mechanisms regulating secondary growth in woody plants. *Curr Opin Plant Biol* 9: 55-58.
- Gurevitz M, Karbat I, Cohen L, Ilan N, Kahn R, Turkov M, Stankiewicz M, Stuhmer W, Dong K, Gordon D. (2007) The insecticidal potential of scorpion beta-toxins. *Toxicon* 49: 473-489.
- Hacke UG, Sperry JS, Wheeler JK, Castro L (2006) Scaling of angiosperm xylem structure with safety and efficiency. *Tree Physiol* 26: 689-701.
- Hargreaves CL, Grace LJ, van der Maas SA, Menzies MI, Kumar S, Holden DG, Foggo MN, Low CB, Dibley MJ (2005) Comparative *in vitro* and early nursery performance of adventitious and axillary shoots from epicotyls of same zygotic embryo of control-pollinated *Pinus radiata*. *Can J For Res* 35: 2629-2641.
- Hellgren JM, Olofsson K, Sundberg B (2004) Patterns of auxin distribution during gravitational induction of reaction wood in poplar and pine. *Plant Physiol* 135: 212-220.
- Hess T, Sachs T (1972) The influence of a mature leaf on xylem differentiation. *New Phytol* 71: 903-914.
- Hou H-W, Zhou Y-T, Mwange K-N, Li W-F, He X-Q, Cui K-M (2006) ABP1 expression regulated by IAA and ABA is associated with the cambium periodicity in *Eucommia ulmoides* Oliv. *J Exp Bot* 57: 3857-3867.
- Howe GA (2004) Jasmonates. In: *Plant Hormones: Biosynthesis, Signal Transduction, Action!* PJ Davies (ed), Kluwer Academic Publishers, Dordrecht, pp. 610-634.
- Huber DP, Philippe RN, Madilao LL, Sturrock RN, Bohlmann J (2005) Changes in anatomy and terpene chemistry in roots of Douglas-fir seedlings following treatment with methyl jasmonate. *Tree Physiol* 25: 1075-1083.
- Hudgins JW, Christiansen E, Franceschi VR (2003) Methyl jasmonate induces changes mimicking anatomical defenses in diverse members of the Pinaceae. *Tree Physiol* 23: 361-371.
- Hudgins JW, Franceschi VR (2004) Methyl jasmonate-induced ethylene production is responsible for conifer phloem defense responses and reprogramming of stem cambial zone for traumatic resin duct formation. *Plant Physiol* 135: 2134-2149.
- Israelsson M, Sundberg B, Moritz T (2005) Tissue-specific localization of gibberellins and expression of gibberellin-biosynthetic and signaling genes in wood-forming tissues in aspen. *Plant J* 44: 494-504.
- Iwasaki, T, Shibaoka, H (1991). Brassinosteroids act as regulators of tracheary-element differentiation in isolated *Zinnia* mesophyll cells. *Plant Cell Physiol.* 32: 1007-1014.
- Jacobs WP (1952) The role of auxin in differentiation of xylem around a wound. *Amer J Bot* 39: 301-309.
- Kalev N, Aloni R (1998) Role of auxin and gibberellin in regenerative differentiation of tracheids in *Pinus pinea* L. seedlings. *New Phytol* 138: 461-468.
- Keeling CI, Bohlmann J. (2006) Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defence of conifers against insects and pathogens. *New Phytol* 170: 657-75.
- Klee HJ, Horsch RB, Hinchee MA, Hein MB, Hoffmann MB (1987) The effects of overproduction of two *Agrobacterium tumefaciens* T-DNA auxin biosynthetic gene products in transgenic petunia plants. *Gene Dev* 1: 86-96.
- Kramer EM (2006) Wood grain pattern formation: a brief review. *J Plant Growth Regul* 25: 290-301.
- Kramer EM, Borkowski MH (2004) Wood grain patterns at branch junctions: modeling and implications. *Trees* 18: 493-500.
- Kuroha T, Satoh S (2007) Involvement of cytokinins in adventitious and lateral root formation. *Plant Root* 1: 27-33.
- Larson PR (1969) Wood formation and the concept of wood quality. *Yale Univ School For Bull* 74, New Haven.
- Larson PR (1994) *The Vascular Cambium: Development and Structure*. Springer-Velag, Berlin.

## PHYTOHORMONAL MECHANISMS THAT CONTROL WOOD QUALITY

- Lechowicz MJ (1984) Why do temperate deciduous trees leaf out at different times? Adaptation and ecology of forest communities. Amer Natur 124: 821-842.
- Leitch MA (2001) Vessel-element dimensions and frequency within the most current growth increment along the length of *Eucalyptus globulus* stems. Trees 15: 353-357.
- Lev-Yadun S, Aloni R (1990) Vascular differentiation in branch junctions of trees: circular patterns and functional significance. Trees 4: 49-54.
- Lev-Yadun S, Aloni R (1995) Differentiation of the ray system in woody plants. Bot Rev 61: 45-84.
- Li S, Pezeshki SR, Shields FD (2006) Partial flooding enhances aeration in adventitious roots of black willow (*Salix nigra*) cuttings. J Plant Physiol 163: 619-628.
- Liphschitz N (1995) Ecological wood anatomy: changes in xylem structure in Israeli trees. In: *Wood Anatomy Research 1995*. Proc Inter Symp Tree Anatomy and Wood Formation. W Shuming (ed), Tianjin, China. International Academic Publishers. Beijing, pp 12-15.
- Ljung K, Hull AK, Kowalczyk M, Merchant A, Celenza J, Cohen JD, Sandberg G (2002) Biosynthesis, conjugation, catabolism and homeostasis of indol-3-acetic acid in *Arabidopsis*. Plant Mol Biol 50: 309-332.
- Miyawaki K, Matsumoto-Kitano M, Kakimoto T (2004) Expression of cytokinin biosynthetic isopentenyltransferase genes in *Arabidopsis*: tissue specificity and regulation by auxin, cytokinin, and nitrate. Plant J 37: 128-138.
- Morris DA, Kadir GO, Barry AJ (1973) Auxin transport in intact pea seedlings (*Pisum sativum* L.): the inhibition of transport by 2,3,5-triodobenzoic acid. Planta 110: 173-182.
- Müller A, Dückting P, Weiler EW (2002) A multiplex GC-MS/MS technique for the sensitivity and qualitative single-run analysis of acidic phytohormones and related compounds, and its application to *Arabidopsis thaliana*. Planta 216: 44-56.
- Mutz R, Guille E, Sauter UH, Nepveu G (2004) Modelling juvenile-mature wood transition in Scots pine (*Pinus sylvestris* L.) using nonlinear mixed-effects models. Ann For Sci 61: 831-841.
- Nagawa S, Sawa S, Sato S, Kato T, Tabata S, Fukuda H (2006) Gene trapping in *Arabidopsis* reveals genes involved in vascular development. Plant Cell Physiol 47: 1394-1405.
- Palni LMS, Burch L, Horgan R (1988) The effect of auxin concentration on cytokinin stability and metabolism. Planta 174: 231-234.
- Persson S, Caffall KH, Freshour G, Hileya MT, Bauer S, Poindexter P, Hahn MG, Mohnen D, Somerville C (2007) The *Arabidopsis* irregular xylem8 mutant is deficient in glucuronoxylan and homogalacturonan, which are essential for secondary cell wall integrity. Plant Cell 19: 237-55.
- Rahayu YS, Walch-Liu P, Neumann G, Römhild V, von Wirén N, Bangerth F (2005) Root-derived cytokinins as long-distance signals for  $\text{NO}_3^-$ -induced stimulation of leaf growth. J Exp Bot 56: 1143-1152.
- Ralph SG, Hudgins JW, Jancsik S, Franceschi VR, Bohlmann J (2007) Aminocyclopropane carboxylic acid synthase is a regulated step in ethylene-dependent induced conifer defense. Full-length cDNA cloning of a multigene family, differential constitutive, and wound- and insect-induced expression, and cellular and subcellular localization in spruce and Douglas fir. Plant Physiol 143: 410-424.
- Raven PH, Evert RF, Eichhorn SE (2005) *Biology of Plants*. 7<sup>th</sup> edn, Freeman, New York.
- Reinhardt D, Pesce E-R, Steiger P, Mandel T, Baltensperger K, Bennett M, Traas J, Friml J, Kuhlemeier C (2003) Regulation of phyllotaxis by polar auxin transport. Nature 426: 255-260.
- Roberts LW, Gahan BP, Aloni R (1988) *Vascular Differentiation and Plant Growth Regulators*. Springer-Verlag, Berlin.
- Romano CP, Hein MB, Klee HJ (1991) Inactivation of auxin in tobacco transformed with the indoleacetic acid-lysine synthetase gene of *Pseudomonas savastanoi*. Genes Dev 5: 438-446.
- Sachs T (1981) The control of patterned differentiation of vascular tissues. Adv Bot Res 9: 151-262.
- Sachs T, Cohen D (1982) Circular vessels and the control of vascular differentiation in plants. Differentiation 21: 22-26.
- Sakakibara H, Takei K, Hirose N (2006) Interactions between nitrogen and cytokinin in the regulation of metabolism and development. Trends in Plant Sci 11: 440-448.
- Saks Y, Feigenbaum P, Aloni R (1984) Regulatory effect of cytokinin on secondary xylem fiber formation in an *in vivo* system. Plant Physiol 76: 638-642.
- Salleo S, LoGullo MA, Siracusano L (1984) Distribution of vessel ends in stems of some diffuse and ring-porous trees: the nodal region as 'safety zones' of the water conducting system. Ann Bot 54: 543-552.

## RONI ALONI

- Sauer M, Balla J, Luschnig C, Wisniewska J, Reinöhl V, Friml J, Benková E (2006) Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. *Genes Dev* 20: 2902-2911.
- Savaldi-Goldstein S, Peto C, Chory J (2007) The epidermis both drives and restricts plant shoot growth. *Nature* 446: 199-202.
- Savidge RA (1996) Xylogenesis, genetic and environmental regulation. *IAWA J* 17: 269-310.
- Scheres B, Xu J (2006) Polar auxin transport and patterning: grow with the flow. *Genes Dev* 20: 922-926.
- Sundberg B, Uggla C, Tuominen H (2000) Cambial growth and auxin gradients. In: *Cell and Molecular Biology of Wood Formation*. RA Savidge, JR Barnett, R Napier, (eds), BIOS Scientific Publishers, Oxford, pp 169-188.
- Suzuki M, Yoda K, Suzuki H (1996) Phenological comparison of the onset of vessel formation between ring-porous and diffuse-porous deciduous trees in a Japanese temperate forest. *IAWA J* 17: 431-444.
- Taiz L, Zeiger E (2006) *Plant Physiology*, 4<sup>th</sup> edn. Sinauer, Sunderland MA.
- Takei K, Ueda N, Aoki K, Kuromori T, Hirayama T, Shinozaki K, Yamaya T, Sakakibara H (2004) *AtPT3* is a key determinant of nitrate-dependent cytokinin biosynthesis in *Arabidopsis*. *Plant Cell Physiol* 45: 1053-1062.
- Teale WD, Paponov IA, Palme K (2006) Auxin in action: signalling, transport and the control of plant growth and development. *Nature Rev Mol Cell Biol* 7: 847-859.
- Thorpe MR, Ferrieri AP, Herth MM, Ferrieri RA (2007) <sup>11</sup>C-imaging: methyl jasmonate moves in both phloem and xylem, promotes transport of jasmonate, and of photoassimilate even after proton transport is decoupled. *Planta* (in press).
- Timell TE (1986) *Compression Wood in Gymnosperms*. Vol 2, Springer-Verlag, Berlin.
- Trewavas AJ (1983) Is plant development regulated by changes in concentration of growth substances or by changes in the sensitivity to growth substances? *TIBS* 8: 354-357.
- Tyre MT, Zimmermann MH (2002) *Xylem Structure and the Ascent of Sap*, 2<sup>nd</sup> edn. Springer-Verlag, Berlin.
- Uggla C, Mellerowicz EJ, Sundberg B (1998) Indole-3-acetic acid controls cambial growth in Scots pine by positional signaling. *Plant Physiol* 117: 113-121.
- Uggla C, Moritz T, Sandberg G, Sundberg B (1996) Auxin as a positional signal in pattern formation in plants. *Proc Nat Acad Sci USA* 93: 9282-9286.
- Wheeler EA, Baas P (1991) A survey of the fossil record for dicotyledonous wood and its significance for evolutionary and ecological wood anatomy. *IAWA Bull n.s.* 12: 275-332.
- Yamamoto F, Kozlowski TT (1987) Effect of ethrel on growth and stem anatomy of *Pinus halepensis* seedlings. *IAWA Bull n.s.* 8: 11-19.
- Zhang R, Zhang X, Wang J, Letham DS, McKinney SA, Higgins TJV (1995) The effect of auxin on cytokinin levels and metabolism in transgenic tobacco tissue expressing an *ipt* gene. *Planta* 196: 84-94.
- Zimmermann MH (1983) *Xylem Structure and the Ascent of Sap*. Springer-Verlag, Berlin.
- Zimmermann MH, Jeje AA (1981) Vessel-length distribution in stems of some American woody plants. *Can J Bot* 59: 1882-1892.
- Zobel BJ, van Buijtenen JP (1989) *Wood Variation, its Causes and Control*. Springer-Verlag, Berlin.

## 9. AFFILIATION

*Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel*

## 10. CITATION

Aloni R. (2007) Phytohormonal mechanisms that control wood quality formation in young and mature trees. In: *The Compromised Wood Workshop 2007*. K. Entwistle, P. Harris, J. Walker (eds). The Wood Technology Research Centre, University of Canterbury, Christchurch, New Zealand, pp 1-22.