



## Tansley review

# Gravitropisms and reaction woods of forest trees – evolution, functions and mechanisms

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## Summary

The woody stems of trees perceive gravity to determine their orientation, and can produce reaction woods to reinforce or change their position. Together, graviperception and reaction woods play fundamental roles in tree architecture, posture control, and reorientation of stems displaced by wind or other environmental forces. Angiosperms and gymnosperms have evolved strikingly different types of reaction wood. Tension wood of angiosperms creates strong tensile force to pull stems upward, while compression wood of gymnosperms creates compressive force to push stems upward. In this review, the general features and evolution of tension wood and compression wood are presented, along with descriptions of how gravitropisms and reaction woods contribute to the survival and morphology of trees. An overview is presented of the molecular and genetic mechanisms underlying graviperception, initial graviresponse and the regulation of tension wood development in the model angiosperm, *Populus*. Critical research questions and new approaches are discussed.

## I. Introduction

The large size and complex architectures of trees were made possible in part by two key evolutionary innovations – the ability of stems to perceive gravity, and the ability to produce specialized woods capable of reinforcing or reorienting woody stems. In many, if not all, forest trees, reinforcement and reorientation are achieved by the production of specialized ‘reaction woods’ capable of generating tremendous force (Timell, 1986; Fournier *et al.*, 2014). Reaction

woods play multiple roles fundamental to the evolution and development of trees, including reorienting displaced stems, replacing lost ‘leaders’ by reorientation of branches, reinforcing stress points, maintaining branch angles, and maintaining posture control as a tree grows. Reaction woods are also central to concepts of biomechanical design and adaptive growth that describe how trees modify their woody bodies in response to being buffeted over time by wind, erosion and other physical environmental forces. Indeed, the presence of reaction wood can be used to date

disturbances (e.g. landslides, windstorms) in forest ecosystems using dendrochronology. Additionally, gravitropisms and reaction woods provide excellent model experimental systems for dissecting the molecular and genetic regulation of wood formation.

Our understanding of the mechanisms underlying gravity perception and gravitropism in woody stems is quickly advancing but still fragmentary. By contrast, gravitropism of the herbaceous, elongating inflorescence stem of the angiosperm, *Arabidopsis*, is increasingly well understood. Gravitropism of the elongating inflorescence stem involves specialized gravity-sensing cells in the endodermis (Fukaki *et al.*, 1998). These cells contain starch-filled amyloplasts, which act as statoliths that sediment in the cell (Toyota *et al.*, 2013). In the leaning inflorescence, amyloplast sedimentation ultimately results in reorientation of plasma membrane-localized PIN auxin efflux carriers within the endodermal cells to point towards the ground (Friml *et al.*, 2002). In the case of an inflorescence placed horizontally, auxin is preferentially transported to the bottom side of the stem. The resulting classical Cholodny–Went growth response (Went, 1974) of increased elongation growth on the bottom of the stem serves to push the stem upwards.

Woody stems must take a different approach to gravitropism, because the lignified wood is no longer capable of elongation growth. Instead, woody stems asymmetrically produce specialized reaction wood in response to gravity. In general, in angiosperms, reaction wood is termed ‘tension wood’ and is produced on the upper side of the leaning stem. Tension wood creates strong contractile force and *pulls* the stem upright. In gymnosperms, reaction wood forms on the bottom side of the leaning stem and is termed ‘compression wood.’ Compression wood *pushes* the stem upright. Thus, the mechanisms responsible for bending woody stems are fundamentally different from those in herbaceous stems. The term ‘opposite wood’ is used to describe wood formed on the stem across from reaction wood (typically on the bottom of an angiosperm stem or the top of a gymnosperm stem), while the term ‘normal wood’ refers to wood formed in upright trees. The adaptive advantages of reaction woods are evident in their prevalence in extant plants, although the evolutionary origins of reaction woods are less certain. Section II of this review will summarize the general features of reaction woods in angiosperms and gymnosperms, as well as evolutionary aspects.

As mentioned earlier, the evolution of reaction wood has been driven by adaptive traits and advantages afforded by the ability to reinforce and reorient woody stems in response to environmental forces such as wind, erosion, soil downhill creep, falling neighbor trees or avalanche. But perhaps more important is the role of reaction woods in building the variety of complex architectures displayed by tree species. Reaction woods have physical properties that can counteract forces resulting from, for example, the junction of a branch with the main stem. Additionally, the evolution and growth of complex tree forms require dynamic ‘posture control’, which is achieved in part through reaction woods (Fournier *et al.*, 2014). For example, reaction wood can help to maintain a set branch angle by counteracting the effects of increasing weight as the branch grows. The adaptive significance of reaction woods,

including their roles in development of tree architectures, will be discussed in Section III.

Recent molecular and genomic studies are beginning to provide fundamental insights into gravity perception in a limited number of tree species, notably in the model angiosperm tree species of the genus *Populus*. In contrast to herbaceous stems, there has been a lack of knowledge about how, or even if, woody stems perceive gravity. Recent results in *Populus* describe the likely gravity-sensing cells in the woody stem, and connect them to changes in auxin transport during initial graviresponse (Gerttula *et al.*, 2015). However, significant knowledge gaps remain, including the relative role of gravity sensing vs mechanical strain in induction of reaction woods, and how branches can undergo plagiotropic growth at a set angle. Section IV of this review will cover some of the classical experiments as well as recent results concerning how woody stems perceive gravity and react to physical stimuli. Additionally, genomic studies have cataloged changes in gene expression, protein profiles, and metabolites during reaction wood formation and are giving first insights into the molecular processes and regulation underlying reaction wood formation. In Section V, mechanisms regulating developmental changes in reaction woods will be discussed, with an emphasis on tension wood in *Populus*.

In addition to the many basic questions of plant biology associated with gravitropisms and reaction woods of trees, reaction wood research has been influenced by the economic importance of reaction woods. Both tension wood and compression wood are major flaws for lumber, associated with warpage and low dimensional stability (Wimmer & Johansson, 2014). Reaction woods also cause warping in veneers, and tension wood causes ‘fuzzy’ grain and substandard finishes. Compression wood has a negative impact on pulping, while tension wood is actually more readily converted to pulp than normal wood but can produce paper with inferior strength (Parham *et al.*, 1977). More recently, reaction woods have garnered interest as a source of biofuels. Tension wood is more easily converted to liquid biofuels than normal wood (Brereton *et al.*, 2012), while the high content of energy-rich lignin in compression wood can be used in cogeneration or other applications. In addition, the emerging concept of trees as feedstock for ‘biorefineries’ (de Jong *et al.*, 2010) could benefit from the unique properties of reaction woods in producing not only biofuels but also bioplastics, unique polymers and valuable coproducts. Reaction woods also affect the broader role of wood in the ecology and adaptation of trees to varied environments (Chave *et al.*, 2009). This review will conclude with thoughts about key research topics, and how integration of experimental treatments and concepts from older literature with current molecular and genomic technologies could lead to significant advances in the near future.

A primary goal of this review is a broad synthesis of current understanding of gravitropisms and reaction wood evolution and development in trees. However, the history of reaction wood research is long and complex, and answers to fundamental questions regarding reaction wood induction and development remain incomplete. A variety of species have been used in experiments, and experimental procedures were typically not standardized across laboratories or species, making comparisons across experiments or integrating results from different

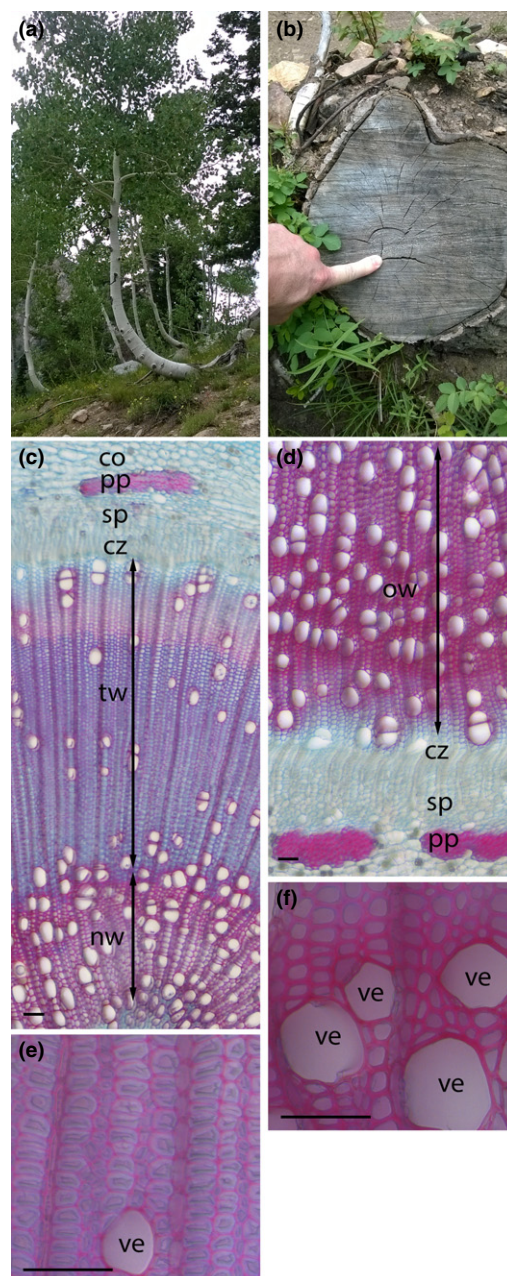
publications problematic. Unfortunately many of the early experiments and concepts regarding the induction and function of reaction wood have not been integrated with more recent molecular and genomic studies, making connections among older and newer literature challenging. For these and related reasons, this review will not be comprehensive in addressing all the literature on the subject of gravitropisms or reaction woods, but will instead highlight selected papers to illustrate primary points. There are excellent, in-depth reviews and books on many topics related to reaction wood, and these will be cited as appropriate to provide the reader access to more detailed literature.

## II. General features and evolution of reaction woods of angiosperms and gymnosperms

Angiosperm and gymnosperm trees produce reaction woods with very different characteristics. Although there are exceptions, generally speaking angiosperm trees produce tension wood, while gymnosperms produce compression wood. General features of tension wood and compression wood are described in the following, but it should be stressed in both cases that the anatomical details of these wood types can vary among species and within individual plants, especially in angiosperms. There is a vast literature describing the anatomy of reaction woods for a large number of species, which has been summarized in two book series (Timell, 1986; Fournier *et al.*, 2014). What follows describes general features of tension wood and compression wood, with indications of some of the common types of variation.

### 1. Anatomical and chemical characteristics of tension wood

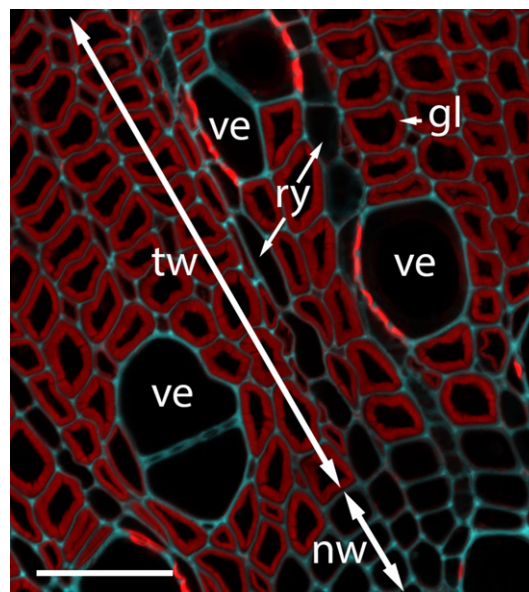
Tension wood is formed on the upper side of leaning angiosperm stems and creates tensile force to pull the stem upward (Fig. 1a,b). In *Populus*, tension wood is characterized by eccentric growth with accelerated cell divisions in the cambial zone, a reduction in the number of vessel elements produced, and the production of specialized tension wood fibers containing a gelatinous cell wall layer (G-layer, Fig. 1c,e) (Jourez *et al.*, 2001; Mellerowicz & Gorshkova, 2012). Opposite wood is similar in anatomy to normal wood and contains numerous vessels and fibers that lack a G-layer (Fig. 1d,f). Tension wood fibers are the cell type responsible for force generation, and the specialized G-layer is thought to be directly involved in force generation (Mellerowicz & Gorshkova, 2012). It is the last cell wall layer formed during fiber development, is highly enriched in cellulose and largely devoid of lignin, and has a cellulose microfibril angle (MFA) approaching zero, which makes the cell resistant to stretching but permissive to swelling (Clair *et al.*, 2011). It has been reported that some angiosperms produce functional tension wood lacking a G-layer, most notably for a number of tropical rainforest species (Fisher & Stevenson, 1981; Clair *et al.*, 2006). However, a recent re-evaluation showed that a G-layer does in fact form for at least some of these species in question, but that it is difficult to identify in mature wood where it is masked by late lignification (Roussel & Clair, 2015). Examination of additional species previously



**Fig. 1** Tension wood form and histology. (a) This *Populus tremula* growing near Salt Lake City, Utah, has been pushed over by avalanche, but has returned orientation of the main stem to near vertical. (b) Cut stump from one of the leaning trees, showing highly eccentric growth with tension wood forming on the top portion of the stem. The pith is indicated. (c) Phloroglucinol and astra blue staining of a *Populus* stem which was grown vertically to produce normal wood, and then placed horizontally to induce tension wood. Normal wood has more vessels and fiber wall stain primarily with phloroglucinol, indicating the presence of lignin. After the transition to tension wood development, fewer vessels are formed and the cellulose-rich G-layer stains strongly with astra blue. (d) Opposite wood of the same stem shown in (c). Similar to normal wood, opposite wood contains numerous vessels. (e) Higher magnification of tension wood fibers, each containing a lignified secondary cell wall and a G-layer, which is the innermost cell wall layer. (f) Higher magnification of fibers in opposite wood, which have a lignified secondary cell wall but no G-layer. cz, cambial zone; co, cortex; nw, normal wood; ow, opposite wood; pp, phloem fibers; sp, secondary phloem; tw, tension wood; ve, vessel element. Bars, 50  $\mu$ m.

reported to lack a G-layer will be required to determine whether the G-layer is universally required to produce functional tension wood.

While the molecular mechanisms responsible for force generation are still uncertain, at least some key elements have been identified (Mellerowicz & Gorshkova, 2012; Fagerstedt *et al.*, 2014), including cellulose MFA (Norberg & Meier, 1996; Bamber, 2001; Clair *et al.*, 2011), xyloglucan endotransglycosylase (XET) (Mellerowicz *et al.*, 2008; Mellerowicz & Gorshkova, 2012), and fasciclin-like arabinogalactan proteins (MacMillan *et al.*, 2010). Different hypotheses have been proposed for mechanisms of force generation, most of which are not necessarily mutually exclusive. The G-layer swelling model stresses the importance of cellulose MFA in force generation (Goswami *et al.*, 2008; Burgert & Fratzl, 2009). In this model, the low MFA of the G-layer renders it highly resistant to extension in the axial dimension, but highly deformable and capable of swelling in the transverse dimension. The swelling of the G-layer could thus produce outward, radial force on the encasing secondary cell wall, resulting in a contraction of the fiber. Mechanisms proposed for driving the swelling of the G-layer have included changes in hydration state, but the simple observation that tension wood contracts upon drying seems to contradict this model (Mellerowicz & Gorshkova, 2012). An alternative mechanism has been proposed, the G-layer longitudinal shrinkage hypothesis, whereby the incorporation of bulky polysaccharide between adjacent microfibrils pushes them apart, resulting in contraction of the G-layer and generation of tensile force (Mellerowicz & Gorshkova, 2012). Xyloglucan linkages between the G-layer and secondary cell wall layers appear to be necessary for force generation, as breaking these linkages in *Populus* using a fungal xyloglucanase inhibits tensile force generation in tension wood (Baba *et al.*, 2009). Such linkages would not seem necessary under the G-layer swelling hypothesis, but would be crucial for force transfer from the G-layer to the secondary cell wall under the G-layer shrinkage hypothesis. Additionally, XET activity that could crosslink the walls has been localized to tension wood fibers (Fig. 2), and XET activity can persist for years after the death of fibers (Nishikubo *et al.*, 2007). Extremely high transcript abundances for fasciclin-like arabinogalactan proteins (AGPs) are a characteristic of tension wood (Lafarguette *et al.*, 2004; Andersson-Gunneras *et al.*, 2006; Azri *et al.*, 2014). AGPs affect stem biomechanics, potentially by directly affecting cell wall structural properties or by affecting cellulose deposition (MacMillan *et al.*, 2010). Lastly, variation in lignin composition have also been noted between fibers and noncontractile vessels, as well as cell wall layers within tension wood fibers (Campbell & Sederoff, 1996; Weng & Chapple, 2010). The decreased lignin content and increased syringyl to guaiacyl subunit ratio characteristic of tension wood fibers has also been noted in Magnoliid species, including *Liriodendron tulipifera*, which do not exhibit detectable G-layers (Yoshizawa *et al.*, 2000; Yoshida *et al.*, 2002), suggesting that lignin plays an important role in the evolution and development of functional tension wood fibers.

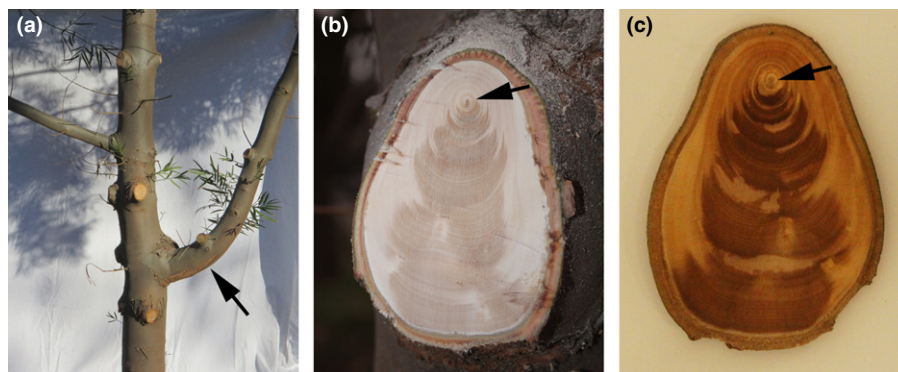


**Fig. 2** Labeling of xyloglucan endotransglycosylase (XET) enzyme activity in *Populus* using a fluorescent XET substrate that becomes incorporated into the developing cell walls. Normal wood fibers do not label (lower right), while G-layers of tension wood fibers strongly incorporate the label. gl, G-layer in tension wood fiber; nw, normal wood; ry, ray; tw, tension wood; ve, vessel element. Bar, 25  $\mu$ m.

## 2. Anatomical and chemical characteristics of compression wood

Almost all aspects of compression wood have been extensively reviewed in the excellent series of books by Tore Timell (Timell, 1986). In many respects, compression wood is defined by features opposite to those of tension wood. Additionally, wood development in gymnosperms is, in general, less variable than in angiosperms. Compression wood is no exception, and is generally more uniform in its characters across species than the much more variable features of tension wood. However, compression wood is apparently absent in gymnosperm lineages of Cycadales and Gnetales (see the Evolution of reaction woods section below).

Compression wood formation is often associated with eccentric growth, with growth rings larger on the lower wide of stems where compression wood forms (Fig. 3). In comparison to normal wood, compression wood tracheids tend to be shorter, have truncated or bent tips, and are rounded in cross-section, resulting in intercellular spaces between the corners of adjoining cells (Timell, 1986; Ruelle, 2014; Wimmer & Johansson, 2014). Notably, secondary cell walls are thicker, have reduced cellulose, and contain higher lignin content with more p-hydroxyphenyl subunits in comparison to normal wood, and show increased resistance to compression. While the MFA of normal wood tracheids is low ( $\sim 10^\circ$ ), the thickened S2 cell wall layer has an increased MFA (30–50°) (Timell, 1986). The effect of MFA is seen in the response to hydration: for normal wood tracheids, swelling results in contraction in length, while swelling in compression wood tracheids results in extension in length (Burgert & Fratzl, 2009). The S3 wall layer is frequently absent in compression wood. In some species, pronounced helical cavities mark the tracheid wall as viewed from the cell lumen. These helical



**Fig. 3** Compression wood formation in a gymnosperm, *Podocarpus*. (a) A lower branch escaped apical control (arrow) and was pushed to a more upright position than other branches by compression wood at the base of the branch. (b) A freshly sawn surface from the base of the branch in (a), showing eccentric growth. The pith of the stem is indicated by the arrow. (c) A section of the base of the branch which has been stained with phloroglucinol to highlight the darkly stained compression wood on the bottom of the branch. The arrow indicates the pith.

thickenings have been suggested to be analogous in function to the coiled springs used in automobile suspension systems (Bamber, 2001).

### 3. Evolution of reaction woods

Unfortunately there are few studies specifically addressing the paleobotany of reaction wood. Although larger fossils from more recent eras can provide good detail (Fig. 4), older fossilized wood is often difficult to interpret because of fragmentation, deformation and degradation. The variation among species and even within individual trees regarding reaction wood anatomy makes it difficult to discern if different fossil specimens came from the same species. In some cases, it is likely that fossilized fragments of wood from a single species have been mistakenly described as different species because of the comparison of specimens with the presence or absence of reaction wood, or root vs stem wood (e.g. see Bailey, 1933, 1934; Patel, 1968).

As discussed by Timell (1983), arborescent Lycophyta were a dominant group during the Carboniferous period, but the unifacial

cambium in this group produced limited secondary xylem. No evidence of compression wood has been seen in fossils of Lycophyta, and the limited secondary xylem was probably more important for water transport than support, which was provided by the extensive cortex and periderm characteristic of these plants. Extinct, arborescent Equisetales had a more extensive secondary xylem, but fossils of stems and branches do not reveal evidence of compression wood. It is possible that compression wood was an innovation arising in the progymnosperms, although the fossil evidence is incomplete (Timell, 1983). The fossil wood of the progymnosperm *Archaeopteris* is similar to extant conifer wood, and can contain rounded tracheids and other features suggestive of compression wood, but other features such as helical cavities are not present and the quality of preservation of these woods precludes establishing the absence of S3 wall layers (Schmid, 1967). However, the extensive secondary xylem of progymnosperms could have been mechanically effective as a righting tissue if compression wood was present.

The fossil record for reaction wood in gymnosperms is also fragmentary and inconclusive, although compression wood is commonly found in gymnosperm fossil woods beginning from at least the late Cretaceous and early Cenozoic (Blanchette *et al.*, 1991; Chapman & Smellie, 1992; Wheeler & Lehman, 2005), and in one report from the early-middle Jurassic (Bodnar *et al.*, 2013). However, extant gymnosperm lineages also provide insights. Although Cycadales do not produce compression wood, an example of eccentric growth on the lower side has been noted for horizontal stem of *Cycas micronesica* (Fisher & Marler, 2006), perhaps suggesting that eccentric growth was a first step toward the evolution of reaction woods. In gymnosperm lineages that do produce compression wood, the anatomy has, in some cases, changed surprisingly little over millions of yr. For example, Ginkgoideae date to c. 300 million yr ago, and the genus *Ginkgo* to c. 210 million yr ago. The wood of the single surviving species, *Ginkgo biloba*, is similar in many respects to modern conifers (Timell, 1960), although its compression wood lacks helical cavities (Timell, 1978), suggesting this feature could be a later evolutionary innovation in other gymnosperm lineages. In summary, one possibility is that compression wood was present in



**Fig. 4** Fossil reaction wood. Tension wood from an unidentified angiosperm from the middle Eocene Clarno Nut Beds, John Day Fossil Beds National Monument (OR, USA). Note the eccentric growth on the upper side of the section. Image courtesy of Elisabeth Wheeler, North Carolina State University (Raleigh, NC, USA).

progymnosperms and was an ancestral trait for some gymnosperms (e.g. Cordaitales, Taxales and Coniferales), but was not acquired or lost in other lineages (e.g. Cycadales and Gnetales).

One obvious question regarding reaction wood evolution is: why did gymnosperms and angiosperms evolve such drastically different reaction woods? In most gymnosperms, tracheids function for both water conduction and mechanical support. In many angiosperms, water transport and support functions have been separated, with vessels (sometimes in addition to tracheids) specialized for water conduction and fibers specialized for mechanical support. Could the evolution of vessels and fibers in angiosperm woods have enabled or perhaps required the innovation of tension wood?

Clues to these questions could be found in extant plants, including basal angiosperms lacking vessels. Importantly, it should be noted that the evolution of vessels is often oversimplified, and should recognize intermediate forms between vessels and tracheids as well as whether vessellessness is an ancestral or derived state for a given taxa (discussed in Carlquist, 1975; <http://www.sherwincarlquist.com/primitive-vessels.html>). Additionally, most features of reaction wood (e.g. eccentric growth or MFA) have not been systematically examined for basal angiosperms. However, a survey of the presence of G-layers (Ruelle *et al.*, 2009) showed that G-layers were generally lacking in most basal angiosperms, including the vesselless *Amborella trichopoda*, which has been placed sister to all extant angiosperms (Soltis *et al.*, 2008; Project, 2013). The first consistent appearance of G-layers is in Lauraceae within the magnoliids (Ruelle *et al.*, 2009). The basal angiosperm *Sarcandra glabra* (Chloranthales, sister to the magnoliids; The Angiosperm Phylogeny, 2009) lacks vessels and has been reported to form reaction wood on the bottom of leaning stems that is analogous to compression wood in gymnosperms (Aiso *et al.*, 2014). Another angiosperm lacking vessels, *Trochodendron aralioides*, can produce tension wood containing G-layers (Hiraiwa *et al.*, 2013), although phylogenetic position (Eudicot) indicates that the lack of vessels is a derived character (The Angiosperm Phylogeny, 2009). Within the gymnosperms, compression wood is not formed in Gnetales, which instead form tension wood (Timell, 1986). Another conspicuous feature of Gnetales is the presence of water-conducting vessel elements, and a carbohydrate composition (Melvin Jean & Stewart Charles, 1969) and guaiacyl-syringyl lignin (Gibbs, 1958) more like angiosperms than other gymnosperms. Examination of lignin in angiosperms lacking vessels that produce tension wood in comparison to those that produce compression-like reaction woods generally shows that species with a high ratio of syringyl to guaiacyl subunits can produce a typical G-layer containing tension wood, while species with a low ratio form compression-like wood (Jin *et al.*, 2007; Aiso *et al.*, 2014). Thus, it seems possible that changes in lignin, MFA and the biochemical makeup of cell walls may have been significant factors in the evolution of tension wood, and not simply the evolution of vessels *per se*. Indeed, it would seem that a tracheid containing an extensive G-layer lacking the hydrophobic properties provided by lignification would not be as effective at water conduction.

### III. Adaptive significance of reaction woods, including relationship to form

Most trees seem capable of producing reaction wood, suggesting that reaction woods are fundamentally important to growth and survival. An obvious role for reaction woods is in reorienting or reinforcing stems displaced by environmental forces, such as wind, bank erosion, snow or avalanche. Perhaps more fundamentally, reaction woods have been evoked as playing an indispensable role in 'posture control' and are one means by which trees balance the advantage of height growth with mechanical safety in challenging physical environments. As discussed in the following, a full understanding of reaction wood induction and development must also include plagiogravitropic (angled) growth of branches and the signaling among leaders and branches in a tree, including apical control.

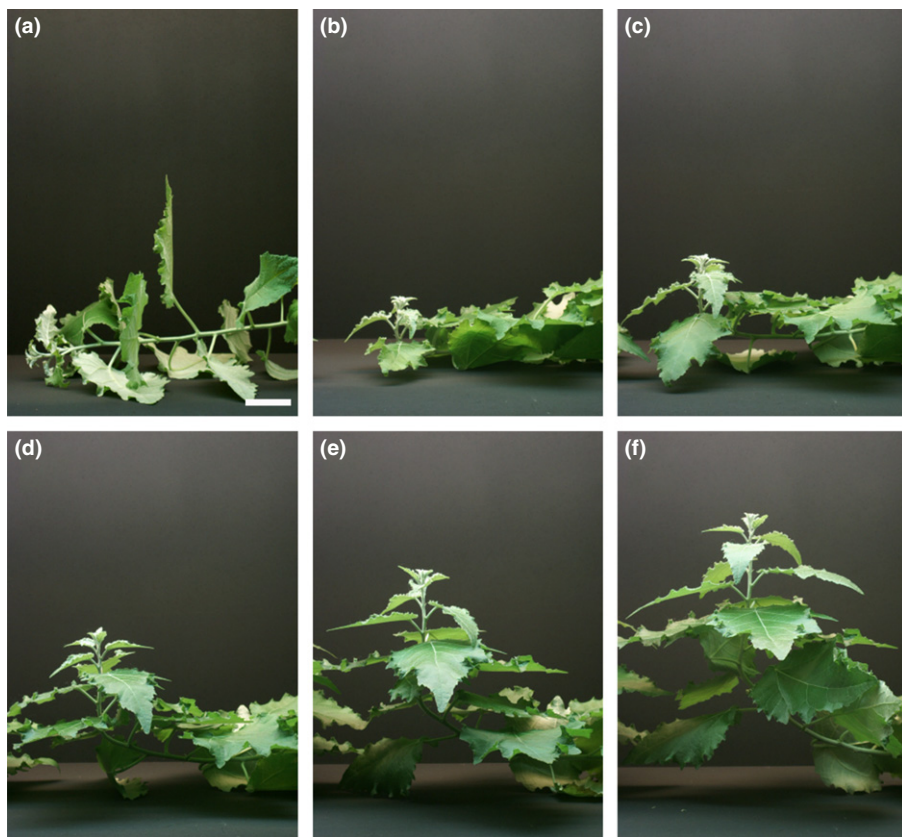
#### 1. Reaction to displacement

Over their life spans, sessile trees are subjected to various environmental stresses that can physically displace them, including wind, snow, erosion and other mechanical forces that push stems and branches from their normal orientation. The ability to reinforce or even correct the orientation of branches or the main stem is thus highly adaptive for trees. Time-lapse videos of a potted *Populus* seedling placed horizontally presents a dramatic example of the ability of reaction wood to change the orientation of a stem (Fig. 5). Within a few hours of being placed horizontally, the apex of the tree still undergoing primary growth will turn upright through elongation growth. Within a few days, the woody portion of the stem undergoing secondary growth begins to lift as well, as a result of tension wood formation. One important observation is that the stem begins to lift relatively uniformly, consistent with gravity perception and response occurring uniformly along the length of the stem, as opposed to signals being propagated from the apex down the stem. Older stems can also respond with reaction wood formation after being displaced (e.g. Fig. 1), although existing wood in larger stems may prevent significant reorientation. Presumably, in these cases, reaction wood is still useful in reinforcing the leaning stem.

#### 2. Roles in architecture including maintenance of branch angle and posture control

Throughout the life of a tree, which in some species may span hundreds or thousands of years, there are ongoing corrections and modifications to the architecture of a tree that are aided by reaction woods. In the process of growing towards light, around obstacles, or away from competitors, trees may adopt complex and often 'unbalanced' postures that nonetheless must withstand the force of gravity. A tree may be further modified by stress or breakage by wind, snow or other physical forces. Add to this the internal stresses created by growth and it seems miraculous that trees can actually remain standing under such challenging circumstances.

Reaction woods provide adaptive changes to wood properties and mechanical forces within stems, to meet mechanical challenges



**Fig. 5** Gravitropism in *Populus*. (a) A *Populus tremula* × *alba* seedling is placed horizontally. (b) Within a few hours, the still elongating apex has turned upward through elongation growth on the bottom of the stem. (c) After c. 3 d, the woody, lignified portion of the stem begins to lift by production of tension wood on the upper side of the stem. (d–f) Over the course of 2 wk of growth, the seedling has significantly reoriented the stem towards the vertical.

associated with architecture in complex, changing physical environments. The distribution of reaction wood around stems and branches indicates a general role for dynamic support of a growing tree (Timell, 1986; Zobel & Van Buijtenen, 1989). Interestingly, reaction wood can form in upright trees, sometimes in response to rapid growth but also in patterns suggesting ongoing adjustments to a stem's orientation relative to gravity, environmental forces, and growth stresses (Wilson & Archer, 1977). Reaction woods also allow a tree to adapt to new environmental conditions. For example, *Fagus* and *Acer* saplings respond to increased light availability resulting from canopy disturbances by reorienting woody branches to more upright positions (Fagerstedt *et al.*, 2014). Tree architecture and reaction woods play key roles in how trees mitigate wind loads (Sellier & Fourcaud, 2009). A seemingly common response to wind in trees is a process termed thigmomorphogenesis, by which trees reduce height growth and increase diameter growth to produce a more squat form (Telewski & Pruyn, 1998; Coutand *et al.*, 2009). This process is accompanied by an increase in reaction wood formation, for example, as seen in willow (Brereton *et al.*, 2012).

Reaction woods are instrumental in reinforcing stress points and maintaining the angle of branches, but typically do not act to pull branches to a vertical position. Rather, each species and genotype has a set branch angle and is thus said to be plagiotropism. As a branch grows, reaction wood can help in maintaining branch angle as the branch is displaced downward by its own weight. Interestingly, different portions of a branch may show significant differences in response to the gravity vector. In some conifers, for

example, the growing tip of the branch may be strongly negatively gravitropic, while the middle and of the branch will be plagiotropism (Timell, 1986). Additionally, branch angle may vary within a tree; for example, in some conifers the topmost branches may be at a significantly higher angle than branches at the base of the main stem. Notably, branches appear to set their equilibrium position relative to the gravity vector and not to the angle of departure of the main axis of the tree, as can be deduced by examining branch angles around the circumference of a leaning tree (Timell, 1986). The importance of reaction woods in maintaining branch angle is illustrated by weeping Japanese cherry (*Prunus spachiana*), which can be converted to a standard nonweeping form by application of GA, which induces tension wood formation (Nakamura *et al.*, 1994). While branch angle is characteristic for individual species and is highly heritable, the mechanisms responsible for the plagiotropism growth of branches are only beginning to be elucidated, as discussed in Section V.

### 3. Reorientation of branches

Many angiosperm and gymnosperm trees have the ability to replace a lost leader (the apex of the main stem) through reorientation of a subtending branch (Timell, 1986). Interestingly, this process clearly shows that there is communication between the leader and the branches below it. The concept of apical control was developed to describe the influence of the leader on branches and is distinct from apical dominance which describes the influence of the apex of a shoot on the outgrowth of subtending lateral buds (Brown *et al.*,

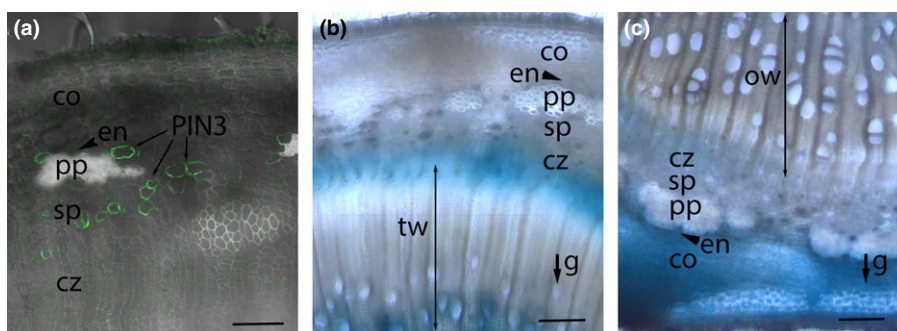
1967). Mechanisms influencing apical dominance and interaction with secondary growth have been recently reviewed (Agusti & Greb, 2013) and are not covered here. The degree of apical control varies among species, but is perhaps best illustrated in conifers with distinctive excurrent forms (e.g. the classical Christmas tree shape). In some such species, if the leader is lost by injury, a subtending branch will become orthogeotropic and be pushed into a vertical position by the formation of reaction wood. The now vertical branch assumes the identity of the leader, and exerts apical control to repress subtending branches from also becoming orthogeotropic. An interesting case is given by *Araucaria heterophylla*, whose branches retain branch identity and grow horizontally, even when removed from the main stem and rooted, suggesting that in some species, branch identity is an irreversible state. Thus, in some species, apical control over branches is dynamic, whereas in others branch identity appears to be a fixed fate. The signals involved in this communication and the mechanisms supporting branch vs leader identities are uncertain.

#### IV. Perception and primary responses to gravity and physical stimuli in woody stems

One general question is whether reaction wood forms in direct response to gravity, or if it forms in response to mechanical forces or other stimuli. This has been a challenging question, and requires experimental approaches that allow separation of effects attributable to gravity and mechanical forces resulting from the bending of stems (Lopez *et al.*, 2014). However, while in fact multiple environmental and physiological signals probably contribute to induction of reaction woods, a number of classical experiments and observations suggest gravity to be the primary factor. To reduce the influence of mechanical (e.g. compressive) forces, a potted tree can be staked as to support the weight of the stem before being inclined. Under such conditions, reaction wood is induced in both angiosperm and gymnosperm trees, suggesting gravity to be a primary signal. A clever experimental approach was used to further dissect the influence of gravity vs mechanical strain, by forcing stems into loops and later assaying their response in terms of reaction wood formation. In the case of a stem forced into a vertical loop, if mechanical strain was the primary inductive signal,

it would be expected that reaction wood should form uniformly around the loop. In crack willow (*Salix fragilis*), vertically looped stems formed extensive tension wood in the upward-facing portions of stems in the top and bottom portions of the stem loop, consistent with reaction wood formation being driven by gravity and not mechanical stresses (Robards, 1965). When this experiment is performed with a conifer, compression wood forms on the bottom side of the stem in both the top and bottom of the loop, again suggesting the stem is responding to gravity and not to mechanical stress (Wilson & Archer, 1977). However, if the stem loop is formed and held in a horizontal orientation, compression wood is formed along the inner circumference of the loop (Wilson & Archer, 1977), suggesting that reaction wood can be induced in the absence of gravistimulation. Indeed, other researchers stress the potential role for the perception of cellular deformation during the flexing of the stem in triggering reaction wood (see Gardiner *et al.*, 2014 for discussion), and mechanosensitive channels and signal transduction mechanisms have been identified in a variety of organisms, including plants (Humphrey *et al.*, 2007; Haswell *et al.*, 2011). One interpretation of these and other results in the literature is that gravity is a primary signal inducing reaction wood formation, but other environmental signals and physiological processes may modify or even induce reaction wood. Additionally, poorly understood physiological relationships among different parts of the tree can modify graviresponse and reaction wood formation, such as in the plagiotropic growth of branches.

One critical question is, how do woody stems perceive gravity? In herbaceous *Arabidopsis* stems, gravity is perceived by endodermal cells containing starch-filled amyloplasts that act as statoliths (Fukaki *et al.*, 1998; Toyota *et al.*, 2013). In trees, the endodermis and cortex are eventually sloughed off during secondary growth, and so cannot be involved in gravity perception by older woody stems. In *Populus*, similar to *Arabidopsis*, young *Populus* stems contain an endodermis containing starch-filled amyloplasts (Leach & Wareing, 1967; Gerttula *et al.*, 2015). An antibody raised against a *Populus* PIN3 auxin efflux carrier labels the plasma membrane of these endodermal cells. In older stems, cells within the secondary phloem acquire statoliths and PIN3 expression (Fig. 6), and thus presumably play the role of gravity sensing in older stems (Gerttula *et al.*, 2015). Importantly, the localization of



**Fig. 6** Gravity sensing and auxin transport in *Populus*. (a) Immunolocalization of PIN3 in a woody *Populus* stem as imaged with confocal microscopy. Green signal corresponds to PIN3 in cells of the endodermis and secondary phloem. (b) DR5 : GUS signal (blue staining) in tension wood of a GA-treated *Populus* stem showing significant staining in the cambial zone. The arrow 'g' indicates the gravity vector. (c) DR5 : GUS signal in opposite wood of the same stem showing strong staining in the cortex. co, cortex; cz, cambial zone; en, endodermis; ow, opposite wood; PIN3, PIN3 immunolocalization signal; pp, phloem fibers; sp, secondary phloem; tw, tension wood. Bars: (a) 50  $\mu$ m; (b, c) 100  $\mu$ m. See Gerttula *et al.* (2015) for additional experimental details and interpretation.

ptPIN3 changes in response to gravity, and when potted trees are placed horizontally, ptPIN3 becomes preferentially localized towards the ground (Gerttula *et al.*, 2015).

To interpret the functional relevance of ptPIN3 relocalization, one must consider the relationship of the endodermis and secondary phloem to the cambium and developing xylem. More specifically, when stems are placed horizontally, ptPIN3 relocalization has different consequences on the top vs bottom of the stem: ptPIN3 orientation towards the ground directs auxin flow towards the cambium and secondary xylem on the top of the stem, while ptPIN3 orientation towards the ground directs auxin flow towards the cortex and epidermis on the bottom of the stem. The consequences of this differential orientation of auxin transport can be visualized by an auxin-responsive DR5 : GUS reporter (Fig. 6). On the top of the stem, DR5 : GUS expression is highest in the cambial zone and developing xylem, while on the bottom of the stem staining is highest in the cortex (Gerttula *et al.*, 2015). This is, in turn, amplified by GA, suggesting GA acts synergistically with auxin. Importantly, this differential transport of auxin also shows how separate and unique fates might be initially determined for tension wood vs opposite wood sides of the stem.

## V. Mechanisms regulating developmental changes in reaction woods

Although still fragmentary, some key molecular aspects of reaction wood development have been identified. In general, it is clear that traditional plant hormones play key roles in reaction wood formation, and that transcription is a major point of regulation of reaction wood induction and development. Increasingly, integration of molecular and genomic and other 'omic' data types is bringing together previously disconnected observations and areas of research. While the following discussion will include mention of compression wood as well as tension wood in other species, it will be primarily focused on tension wood formation in *Populus*, because this is currently the most developed system.

### 1. Role of hormones in induction and regulation of reaction wood

It is clear that hormones including auxin, GAs, and ethylene all play key, interacting roles in the induction and regulation of reaction woods. A primary role of auxin was first described for auxin in experiments where auxin or inhibitors were applied to gravistimulated stems. In general, auxin applied to the top of angiosperm stems inhibits tension wood formation, while auxin applied to the bottom of gymnosperm stems stimulates compression wood formation. The general notion from these experiments is that auxin depletion stimulates tension wood while auxin increase stimulates compression wood. Conflicting results have been obtained by measurement of endogenous auxin concentrations, but at least one report found lower concentrations of auxin on a g<sup>-1</sup> tissue basis in tension wood compared with normal wood after 5–11 d induction by gravistimulation (Hellgren *et al.*, 2004). Interestingly, opposite wood showed a much larger decrease in auxin concentration in this same study,

and the authors rejected the notion that tension wood is formed where auxin concentration is lowest. In studies of the effects of the auxin transport inhibitors on compression wood formation in *Pinus sylvestris*, inhibitors applied in a ring around a shoot resulted in compression wood formation above the ring, consistent with high auxin concentrations triggering compression wood formation (Sundberg *et al.*, 1994). However, measurement of auxin concentrations above the ring of auxin transport inhibitors actually showed lower auxin concentrations than controls.

At least three recent observations must be considered when interpreting these experiments. First, as described previously for *Populus*, the gravity-sensing cells are peripheral to the cambium and respond to gravistimulation by reorientation of PIN auxin transport proteins towards the ground (Gerttula *et al.*, 2015). The exogenous application of growth factors such as auxin to the outside of the stem may thus have unexpected consequences because of the spatial relationships of tissues within the radially organized stem and the active radial transport of auxin. For example, it seems plausible that external auxin application might better mimic the situation on the opposite wood side of a gravistimulated *Populus* stem than the tension wood side. Second, it is now well established that it is not simply the amount of auxin but also differential sensitivity of different cells or tissues that determine auxin response during gravitropisms in roots and herbaceous shoots (Salisbury *et al.*, 1988). Third, the response of the cambium and secondary growth to auxin appears to be highly dependent on interactions with other hormones, including strigolactones (Agusti *et al.*, 2011), as well as CLV3/ESR (CLE) peptide-mediated signaling across secondary vascular tissues that regulates auxin-sensitive cambial stimulation (Suer *et al.*, 2011).

Gibberellin has dramatic effects on tension wood formation. For example, exogenously applied GA was found to induce tension wood in a variety of angiosperm trees (Funada *et al.*, 2008), and GA fed through the transpiration stream induced tension wood formation in weeping cherry, resulting in branches changing from the weeping to the upright form (Nakamura *et al.*, 1994). Direct measurement of bioactive GAs across secondary vascular tissues in *Populus* showed maximum concentrations in the cell expansion zone of secondary xylem (Israelsson *et al.*, 2005). However, assay of gene expression across these same tissues found that expression of the gene encoding the enzyme responsible for the first committed step in GA biosynthesis (ent-copalyl diphosphate synthase) was in the phloem. These results would be consistent with the notion that GA precursors may be synthesized in the phloem and transported to the xylem. This would be another example, along with auxin, of the importance of radial transport of hormones during graviresponse and tension wood formation.

Ethylene has been shown to increase in abundance during both tension wood and compression wood development (Andersson-Gunnerås *et al.*, 2003; Du & Yamamoto, 2003, 2007). Results from exogenous application of compounds affecting ethylene evolution have been inconsistent, making it unclear whether ethylene is sufficient to induce reaction wood formation (Du & Yamamoto, 2007). However, characterization of *Ethylene Response*

*Factors (ERFs)* genes in *Populus* showed that ethylene treatment stimulated cambial activity, changed wood anatomy, and changed the expression of specific ERFs whose expression also change during tension wood formation (Andersson-Gunnerås *et al.*, 2003).

## 2. Regulation of branch orientation and plagiotropic growth

Two questions fundamental to the study of gravitropisms and reaction wood regulation in woody stems are: why do 'leaders' typically display orthogravitropic growth while branches display plagiotropic growth; and how is the plagiotropic growth angle of branches determined? The regulation of branch angle and tree architecture was recently reviewed (Hollender & Dardick, 2015), and will only be briefly summarized here with regard to gravitropism and reaction woods.

Branch angle appears to be set relative to the gravity vector (i.e. displays a gravitropic set point) and not to the relative angle of the branch to the main stem, and varies among species and genotypes (Roychoudhry & Kepinski, 2015). Additionally, branch angle commonly varies within individual trees, frequently with branches higher in the crown having smaller branch angle (Hollender & Dardick, 2015). As previously mentioned, the influence of the leader on branch angle can be seen upon removal of the leader, which induces one or more subtending branches to turn up in many species. *Arabidopsis* grown on a clinostat (to disrupt normal gravistimulation) showed auxin-dependent reorientation of branches to a more upright form (Roychoudhry *et al.*, 2013), suggesting that an active 'antigravitropic offset mechanism' is involved in determining the gravitropic set point of branches. Additionally, it has been speculated that photoassimilates are potential signals in apical control (discussed in Timell, 1986), similar to recent studies implicating photoassimilates in regulating apical dominance (Mason *et al.*, 2014).

Studies of trees with different branch angles and architectures are beginning to reveal the molecular underpinnings of branch angle regulation. A genomics approach was used to clone the gene responsible for peach trees displaying upright, 'pillar' architectures (Dardick *et al.*, 2013). The gene *PpeTAC1* belongs to a gene family found in all sequenced plant genomes, and loss-of-function mutants of the orthologous gene in *Arabidopsis* also show changes in branch angle (Dardick *et al.*, 2013). Auxin concentrations were found to be higher in peach pillar trees than standard trees, with expression of *TAC1* inversely correlated with auxin concentrations (Tworkoski *et al.*, 2015). The sister clade to *TAC1-like* genes is defined by *LAZY1-like* genes, which have been shown to affect plagiotropic growth of lateral organs in maize (Dong *et al.*, 2013), rice (Yoshihara & Iino, 2007) and *Arabidopsis* (Yoshihara *et al.*, 2013) antagonistic to that of *TAC1* (Yoshihara *et al.*, 2013). Auxin gradients associated with graviresponse fail to form in *lazy1* mutants (Godbolé *et al.*, 1999; Li *et al.*, 2007; Yoshihara & Iino, 2007), suggesting that *LAZY-like* genes may play a signaling role linking graviperception and auxin transport. Further study of *TAC1-like* and *LAZY-like* genes may ultimately provide a connection between the fundamental roles of auxin in plagiotropic growth (Roychoudhry & Kepinski, 2015) and reaction wood induction.

## 3. Transcriptional regulation of reaction wood

Previous studies have profiled gene expression in reaction wood formation using expressed sequenced tags (ESTs), microarrays and massively parallel 'next generation' sequencing. Not surprisingly, large numbers of genes have been described as being differentially expressed in reaction wood vs normal wood in various angiosperm species, including *Eucalyptus* (Paux *et al.*, 2005), tulip tree (*L. tulipifera*) (Jin *et al.*, 2011), and poplar (Andersson-Gunnerås *et al.*, 2006; Chen *et al.*, 2015). In pines, large numbers of genes have been identified that are differentially expressed between compression wood and opposite wood, including cell division, cell wall, hormonal, and cytoskeleton-related genes (Ramos *et al.*, 2012; Villalobos *et al.*, 2012; Li *et al.*, 2013). A crucial next step for gene expression studies is to describe how the thousands of genes involved in reaction wood development interact to affect changes in wood anatomy.

Recently, mRNA sequencing combined with computational approaches has further dissected gene expression during tension wood formation in *Populus*. In experiments described by Gerttula *et al.* (2015), misexpression of the Class I KNOX transcription factor ARBORKNOX2 (*ARK2*) (Du *et al.*, 2009) was shown to affect both gravibending and tension wood formation. A linkage was found between transcriptional regulation and the development of functional tension wood fibers capable of producing force: while *ARK2* expression was positively correlated with gravibending, anatomical analysis of the stems revealed that the production of tension wood fibers was actually negatively correlated with *ARK2* expression. Immunolocalization was used to examine two proteins believed to play roles in force generation and tension wood fiber wall development, fasciclin-like arabinogalactan proteins and XET. For both XET activity and immunolocalization of fasciclin-like arabinogalactan proteins, it was found that *ARK2* down-regulation results in fibers that take nearly twice as long to mature a functional G-layer, suggesting that *ARK2* expression influences the timing of production of tension wood fiber walls that are competent to generate force.

In a fully factorial experiment, wild-type, *ARK2*-overexpressing, and *ARK2*-down-regulated genotypes were subjected to treatment with GA or mock control treatment, and either left upright or placed horizontally. After 2 d, RNA was harvested from normal woods of upright trees or tension wood and opposite wood from horizontal trees, and subjected to next-generation sequencing. Differential gene expression analysis showed large numbers of genes differentially expressed in comparisons among wood types, genotypes and GA treatment. Surprisingly, large numbers of genes were differentially expressed in opposite wood when compared with normal wood. While opposite wood has typically been assumed to be similar to normal wood, the large change in gene expression suggests that, in fact, opposite wood may play an active role in graviresponse, although it is not clear what that role might be.

Gene coexpression analysis was used for the same gene expression datasets to assign genes to modules (coexpression clusters) based on expression across the different genotypes, GA treatments, and wood types. This analysis was able to identify gene modules

correlated with both the experimental treatments as well as biochemical phenotypes associated with wood development. For example, a gene module was identified that showed high correlation with MFA and cellulose crystallinity, two characters associated with tension wood. Genes within this module included genes encoding fasciclin-like arabinogalactan proteins that have previously been shown to be highly expressed in tension wood, as well as several key transcription factors previously implicated in regulating wood development or cell wall biosynthesis. This approach thus has the ability to identify gene modules associated with specific tension wood phenotypes, and then identify specific regulatory genes that are candidates for regulating the genes within the module. Extending this approach to additional hormone treatments and additional phenotypes should allow further dissection of the genetic regulation of tension wood formation in the near future, and interconnect gene expression and function with the action of hormones and the development of specific wood developmental phenotypes.

## VI. Research questions and new research approaches

Many fundamental questions remain for gravitropism and reaction wood formation research in trees. How is gravity perception or response modified to produce reaction wood during plagiotropic growth of branches? How does apical control work at a molecular level, and what are the signals? How are eccentric growth, changes in tissue patterning, and induction of modified cell types regulated during reaction wood formation? To what degree are these responses separable? What is the role of radial transport and rays? How is force generated by reaction wood at the molecular level? And what are the regulatory networks ultimately responsible for reaction wood development?

Significant advances in addressing these questions are already being made using 'omics' approaches, as illustrated in the previous examples. Additional technical advances, including gene editing through CRISPR (Pennisi, 2015) and development of functional and comparative genomic approaches directly in tree species, will also play increasingly important roles in advancing research. Advancing forward using new cutting-edge tools should be guided in part by looking back to classical questions and experimental approaches developed before molecular biology or genomics. Importantly, as much as possible, experimental approaches should be standardized across laboratories and species to better allow comparative analyses. A major next challenge is to place the currently disjunct pieces of transcriptional mechanisms, hormonal signaling, and physiological factors into more comprehensive regulatory pathways to describe the control of wood development.

In addition to questions specific to reaction woods, reaction wood research may provide insights into basic processes underlying wood development in a more general sense. For example, the dramatic shift in the number and patterning of vessel elements vs fibers during tension wood formation provides an excellent opportunity to understand how vessel patterning and development are regulated. Eccentric growth during both tension wood and compression wood formation provides an experimental system to

study the regulation of cambial division rate. These and other properties of wood affect not only forest products of high economic importance, but also the health and survival of forests. Wood is, of course, the water-conducting tissue of woody stems, and wood properties such as the number and size of vessels in an angiosperm stem are highly influential in how the tree will respond to drought and climate change.

## VII. Conclusions

Gravistimulation and the downstream consequences on the cambium and wood development make an excellent experimental system for the study of wood formation. Using concepts and experimental treatments defined by previous generations of researchers, current molecular genetic and genomic tools can be used to ultimately dissect the molecular events and regulation underlying gravity perception and response, and the complex developmental changes associated with reaction wood formation. Better knowledge of these processes will provide fundamental insights into the biology of wood formation, as well as information that can ultimately be used to measure, select or manipulate wood traits for application in forest industries, biofuel production, or conservation. I hope that this review has been a useful overview of subjects supportive of these goals, and will perhaps provide you with new concepts to ponder during your next walk in the woods.

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