

proposal

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1 Introduction to wood structure and formation

As trees grow they produce wood in order to become taller and wider. Becoming taller and increasing canopy size is an effective way to out compete the other trees and plants for light. With increasing height and width comes increasing weight, wind drag and internal pressures (for water transport), which requires either enough redundant strength in the existing structure (such as young monocotyledons) or for the tree to strengthen its structure as it increases its size. In dicotyledons and gymnosperms this occurs in two ways, apical and cambial growth on branches, roots and the stem(s).

Softwoods have a simpler micro structure than hardwoods, consisting mainly of axially elongated pointed cells named tracheids which serve as both mechanical support structures and water conduits. Although varying with species, softwoods may also contain radially orientated tracheids, radially or axially orientated parenchyma cells and other cell types. Tracheids are the dominant form of cells within the stems and branches.

Hardwoods contain a more complex micro structure with a number of different cell types. Fibres provide structural support as their primary function, while similar to softwood tracheids they differ in some key aspects, being shorter in the longitudinal direction, more rounded in the transverse outline, tend to have smaller lumens and have little role in sap ascent. However the ends do taper to points as in softwood tracheids. Libriform fibres tend to be longer than fibre tracheids, have thicker walls and are solely for support. Fibre tracheids function in both conduction and support, as in softwoods, however their appearance in wood with vessels suggests that they function primarily for support, and perhaps are an intermediate evolutionary feature between the softwood tracheid and the libriform fibre. Septate fibres divide their cell lumens into chambers without crossing the primary cell wall. Septate fibres are produced in the late stages of division just prior to the death of the cytoplasm, and appear to resemble axial parenchyma cells, and have been hypothesised to store starches, oils and resins.

Vessels are the main conduits for sap ascent. Vessels are comprised of multiple vessel elements being joined at the ends to form long conduits, which can

extend short distances (often less than 200mm) or can be as long as the height of the tree. These elements are connected through pores or perforations in perforation plates at the end walls of the cells. The arrangement of vessels into groups is species dependent and usually described as ring porous (the vessels congregate in early wood) or diffuse porous (vessels are distributed throughout both early and late wood).

Rays are formed from radially orientated cells often tracheids or parenchyma. Hardwoods typically contain multiseriate parenchyma rays, but there are a number of species with uniseriate or a combination of ray sizes, comparatively softwoods rarely contain multiseriate rays. Parenchyma ray cells are living within sap wood, however during the transition to heartwood die and are used for storage of extractives. Rays also provide a mechanical advantage by diverting the axial force flow reducing buckling and shear stresses between fibres.

Further cell types also exist, such as vasicentric tracheids which have profuse side wall pitting exhibiting deformation from the expansion of the surrounding vessels. Axial parenchyma cells are generally abundant and tend to exist in vertical files and are expected to play a role in the development of heartwood. More detailed wood anatomy and has little bearing on this project and is discussed in a number of wood anatomy texts.

In order to reorient stems and branches of (most) trees produce reaction wood which provides a force in order to reorient the tissue. Typically this reorientation is toward the light or upwards as is defined by the negative gravitropism hypotheses. Other reasons for reorientation such as reducing wind drag have also been suggested. In softwoods this reorientation is caused by the production of compression wood. Compression wood forms on the outside of the stem or branch and (expands? so that it is under compression? causing a restoring force). Hardwoods on the other hand produce tension wood on the inside of the desired curve which (contracts?) resulting in a curve forming. Traditionally the G-layer (G-layer), a layer primarily consisting of low angle cellulose fibrils on the inside of the fibre tracheids, is credited with forming growth stresses within the tension wood. However some hardwoods produce tension wood without producing a G-layer such as *E. Nitens*.

Primarily, at different resolutions this work focuses on the fibre tracheids as they are the structural cells expected to be responsible for growth stresses in normal and reaction wood within hardwoods. The fibre tracheids consist of a number of cell wall layers depending on the species, the particular cell and its primary function. Normal wood fibres within *Eucalyptus* species (CHECK THIS) consist of a middle lamina (connecting the fibre to the surrounding cells) a primary cell wall and a secondary cell wall consisting of S1, S2 and S3 layers (produced in chronological order so the exact composition will change depending on the cells developmental stage). The S2 layer is the largest layer and consists of cellulose microfibrils wrapped helically around the cells longitudinal axis. This cellulose is contained within a matrix of hemicelluloses (examples) and lignin giving the cell wall properties of a fibre reinforced matrix. –how does this provide structure–

In order for the living cambial cells to produce wood, each cell must go

through division from its parent cell, growth and death. Because the cambium (and apical meristem) are continually dividing it allows for the tree to be a dynamic structure changing its form to become better adapted to its current environmental setting even though large portions (ie the wood) are dead. The transition from division through elongation and development to death is expected to play a role in the development of growth stresses within the stem.

1.1 Cell division, formation, elongation and death

Dicotyledons and gymnosperms grow in two main ways, upward apical growth and outward cambial growth. Note monocotyledons (for example palms) do not produce secondary growth and instead diameter forms as part of primary growth.

As the cambium is forming, fusiform and ray initials are created from the apical shoot cells. Fusiform initials are short radially and tangentially with tapered ends. From the cambial initials, cells to the inside create the vertical elements of xylem (tracheids, vessels, fibers, parenchyma, etc.), while cells outside become phloem. Ray initials produce horizontal elements (rays).

Cambial cells divide in two ways, periclinal and anticlinal. Periclinal cell division occurs to the inner and outer of the cambial layers. As the cell division to the inside occurs the volume of secondary xylem that is being formed increases the tangential stress on vascular cambium resulting in an extension of the cambial circumference. Although over time many plants show an increase in the longitudinal and tangential dimensions of the cambial initials it is likely that this expansion is mainly facilitated by anticlinal division followed by the expansion of the daughter cells next to the parent.

During primary wall formation rapid elongation occurs. When the cells divide from their parents they remain fixed to their neighbours via the middle lamina. The internal hydrostatic (turgor) pressure causes cell expansion. The osmotic flow of water from the outside the cell to the inside (due to a lower solute concentration outside the cell than in) which is constrained by the primary cell wall, the primary cell wall becomes under increasing tension as more water flows into the cell. Because the centre of the cell has restricted movement, in order for elongation (to dissipate the increasing tensile forces generated from the inflow of water) to occur the cell turns the biosynthesis of cell wall constituents to produce tip growth. Growth at the tips of the cells allows for the cells to remain a constant thickness, so no stretching is needed during the elongation phase, as has been suggested previously. The expansion of the cells is suspected to be controlled via modulation of the primary cell wall rather than via turgor pressure. – note that primary wall has randomly orientated MFs embedded in hemicellulose and pectic compounds and becomes lignified after S layer added, ML is non lignified, note often compound middle lamina is used to describe the ML and P at once as are hard to distinguish— Once the cell has reached its full size biosynthesis of the S1 starts. Typically the S1 layer is thin and comprises of very high angle microfibrils, within the layer many laminates are found. Within each laminate the MFs are closely aligned, however between each laminate they

can (but do not necessarily) differ greatly, or even reverse the direction of the helix the MFs form around the cell, although lower right to upper left orientation tends to be favored. Close to the S2 layer the MFA decreases rapidly. The S2 layer bound to the inside of S1 is typically much thicker and has more vertically orientated microfibrils compared to the primary, S1 and S3 layers, these MFs circle the cell axis from lower left to upper right. S2 contains the majority of the lignin within the cell. In some cases, most commonly in late wood a thin S3 layer is also produced with high MFA, reversing the direction of the MF helixes to lower right to upper left.

Finally if tension wood is being produced a Gelatinous layer may be produced on the inside of the inner most wall (S2 or S3). The G-layer has near vertically orientated microfibrils and very little lignification. It is suspected that the G-layer plays an important role in the generation of reorientation stresses.

At some point during the formation of the secondary cell wall, or soon after the cell shrinks vertically and expands tangentially. Because of the connectivity between cells this results in growth stresses forming within the stem, this phenomenon is discussed in greater detail in —. After the secondary wall formation cell ‘death’ occurs as part of the transition from sap wood into heartwood. While the hollow, dead cells play an important role in water transport and mechanical support of the tree, over time any residual nutrient that can be used by living cells— heartwood stuff—

What is the deal with Rays—

1.2 Cells and wood in the context of a whole tree

Wood as a material within the tree has three major functions to achieve; water transport, nutrient transport and mechanical structure. Softwoods achieve water transport and mechanical structure within tracheids, while parenchyma cells are used for nutrient transport. Hardwoods have evolved a more complicated internal structure of vessels and fibre tracheids in order to separate out the functions of water transport and mechanical support respectively.

—advantages and disadvantages of this—

The growth stresses that form as part of cell formation are thought to provide a superior mechanical structure. Because of the continual formation of new cells providing growth stresses on the periphery of the stem the older wood which has completed its formation and cell death must be contracted further with each new layer of cells attempting to contract. The result of this is the older wood near the centre of the stem becomes compressed while the newer cells can not contract to the extent that would leave them in their lowest energy state remain in tension, until the bond between the old wood and new is separated releasing the forces restricting this contraction (and extension in the centre)

Reaction wood as described above provides the ability for the stem to reorientate in order to be best adapted to its environment at any given time.

These properties of wood allow for an adaptive organism to survive..

2 History of work on growth stresses

It is suspected that growth stresses develop within tracheids during the formation of the secondary cell wall, although the exact timing and mechanism for developing growth stresses is still of much debate. The most current theory is a hybrid of the older cellulose contraction and lignin swelling hypothesis.

A brief discussion of work relating to growth stresses prior to 1965 is given below, Archer (growth stresses book intro) provides a full review of suggested theories up until 1965.

Wood workers have unintentionally known of growth stresses within trees for centuries. Usually referred to as ‘a pull towards the sap’ when cutting boards good craftsmen would section the log in such a way as to get a straight board once it is removed from the log (and the growth stresses released). Most work early on in the study of growth stresses surrounded investigating how/why boards changed shape when cut from an intact stem.

Martley (1928) was possibly the first to study growth stresses in a scientific manner. Initially he argued that the curvature of planks sawn from logs was due to the current growth not being able to support the dead weight of the tree until lignification was complete. As a result the centre is under compression while the periphery had zero stress. However calculations showed that the self weight was not sufficient to cause the observed longitudinal dimension changes of the timber.

After Martley’s work a small number of authors investigated growth stresses through the 30’s and 40’s. Jacobs, although testing 34 hardwood species, focused mainly on Eucalyptus and in 1938 argued that (longitudinal) tension successively develops in the outer layers of the stem as it grows, and as a consequence of the tension, compression must form in the centre of the stem. Jacobs later used *E. gigantea* to describe a strain gradient developing during growth. Experimentally Jacobs made use of strip planking, measuring the deflection of the board after removal from the log, and the length change when the planks were forced back straight. He showed that wood tends to shrink in the longitudinal direction at the periphery while extend near the pith (indicating in the log the planks are under compression in the centre and tension at the extremities).

Further Jacobs put forward a number of hypothesis to explain how the growth stresses were forming. First arguing that it is very unlikely that dead cells (wood) could extend within the core in order to create the observed stress gradient. Instead suggesting the causes of; weight of the tree, surface tension and sap stream forces, cellulose and colloidal complexes, lignin intercellular substances and the primary or secondary cell wall. Although without any evidence did not claim any of these to be the major cause.

Stresses relating to reaction wood received more attention through the 30s and 40s for both soft and hardwoods. Jacobs 1945 stated that the reorientation of stems is caused by a modification to the already existing stress gradient throughout the stem. One option he presented was simply that the eccentric growth causes larger number of cell sheaves to be added to the upper side of

the curve each providing the same amount of contraction force, this results in an angle correction even with identical cells. Sap tension is also considered, but more importantly Jacobs notes the possibility of tensions being formed within the cell walls of tension wood. Munch 1938 speculated that the addition of matter into the cell wall could cause compression wood. .. Jacobs 1945 also found that it was commonly the case that the amount of compression wood developed and the stem angle recovery had a poor relationship. He suggested maybe it was in fact the normal strain pattern in tension which corrects the lean, the compression wood merely acted as a pivot, not contributing a tensile force on the lower side of the stem.

Boyd 1950 Developed a new experimental technique in order to investigate the stress profile further. By cutting a slit longitudinally in the centre of the log, attaching strain gauges onto the wood inside the slit and successively shortening the log from both ends he obtained direct extension measurements from inside the stem. -found that the crossover point is about $1/3$ rad of the log from the periphery-

Most commonly growth stresses were investigated from the longitudinal direction, however cells also change dimension in the transverse direction, this leads to a more complicated three dimensional stress field developing within even a straight stem. Koehler 1933 showed that a saw cut radially through a disk has a tendency to close near the periphery suggesting that the peripheral cells are under tangential compression with the inner cells under radial tension. He suggested this was the cause of shakes in standing timber. Jacobs 1945 removed inner circles from disks of a number of species and found when an inner portion is removed the disks circumference increases. Jacobs again argued that strain in the sap stream along with cells being wider tangentially than radially led to the observed lateral stresses. Although he also mentions the possibility of secondary thickening from the deposition of lignin as a possible contributing factor. Boyd 1950a developed an experiment whereby he removed a wedge from a disk and measured the radial expansion, showing the disks were in fact under radial tension. Further additional species were found to be in agreement with the results of Jacobs 1945 when the inner circles were removed from disks. Boyd also shows that the longitudinal stresses manifesting as transverse stresses via Poisson ratios are only approximately one tenth that of the measured stresses.

Boyd 1950c provides an in-depth rebuttal of the available theories at the time, arriving at the conclusion that the cell wall development must control the shape change which results in growth stresses. Further he postulates that cellulose is primarily responsible with lignin and carbohydrates also playing important roles when stresses are formed in normal, compression and tension wood.

Wardrop 1965 commented that a tensile stress generated in the cellulose transitioning into a crystalline state could be the explanation for cells contracting during the formation of the secondary wall. Cellulose contraction aligned well with the observation of the G-layer (which has a very low MFA) being common in a number of tension wood producing species, and also gave the ability for low MFA normal wood to contract. Bamber 1978 further argued cellulose contraction claiming turgor pressure in normal wood cells remained high enough that

the cells did not contract before the lignin was deposited, once/during lignin deposition the cellulose became crystalline and shrunk, causing the cell to become shorter, the mechanism for tension wood is essentially the same. Compression wood on the other hand was explained by the cellulose being laid down and then the turgor pressure decreasing, causing the cell to contract before lignin was deposited. In turn the cellulose was under compression, resulting in the tendency for the compression wood cells to expand.

Boyd (1972) presented (or rather popularised) the alternative (more widely accepted) hypothesis of lignin swelling (first conceived by Munch 1938). Tensile stress is gained in cells of low MFA by lignin deposition into the cell wall, pushing the cellulose fibrils apart, which in turn shrinks the longitudinal length of the cell and increases the tangential width. When MFA is high, the opposite occurs, lengthening the cell and reducing its tangential width. This shape change is not readily apparent in compression wood (characterised as short fat tracheids) until the release of the stress acting on the CW, where by the cells become longer and skinnier.

Around the same time two other lesser known hypotheses were presented, strains due to changes in water content Hejnowicz 1967, argued that the stresses in compression wood are related to the inhibition of water by the cell walls, which results in swelling, because the expansion of compression wood is equal to the shrinkage due to drying. —paper disproving this—

brodzki 1972 hypothesised strains due to 1,3-linked glucan (laricinan) deposition within the helical checks of the S2 cell wall layer could be the most significant factor in longitudinal growth stress generation. Boyd 1978 refuted this idea arguing (along with other issues) that the laricinan would expand into the cell lumen not causing any stresses in the cell wall, unless a (non-observed) constraining median restricted the expansion.

— Gills 1973

Through the late 70's and 80's Archer produced a number of papers in two series, 'on the distribution of growth stresses' —refs— mainly concerning the mathematical treatment of the stress fields within trees. — and 'on the origin of growth stresses' —refs— primarily concerned with the underlying mechanisms generating growth stresses.

The 'on the distribution of growth stresses' series presented a comprehensive mathematical framework for the treatment of the stress field within living trees. Advancing on Kubler's work Archer introduced orthotropic solutions which allowed for each new growth increment to alter the stress distribution within the stem in a self equilibrating fashion. The other advancement made was the increased accuracy from the crossover point from compression to tension now being governed by the moduli in both the radial and circumferential directions. Archer went on to develop a numerical approximation to the stress fields generated by asymmetric growth strains and inclined grains, allowing for variation within growth stresses. Finally he used the developed methods to present solutions for a number of hardwood species.

'on the origin of growth stresses' Archer investigated the mechanisms behind growth stress generation.

More recently theories regarding the nature of hemicelluloses and their bonding have been used in an attempt to remove some of the issues associated with the cellulose contraction hypothesis. One major issue of cellulose contraction is that in its initial form it was argued that the crystallisation process of cellulose shortened its length. —ref— showed that when cellulose crystallised it became longer as the chains increased order. Two theories have been advanced to combat the issue of lengthening during crystallisation in order to retain an updated version of the cellulose contraction hypothesis.

— argues that at the edge of the cellulose fibrils the cellulose becomes disordered and is consequently able to bond with hemicelluloses, which have a slightly shorter repeat length than the cellulose crystal. These hemicelluloses bonded to the outside of the fibril cause the fibril to be compressed in the crystalline centre, while under tension on the surface. An interesting consequence is the contraction of the cellulose due to the hemicellulose bonding should be dependent on the area/volume to circumference/surface area ratio. A potential way to test this hypothesis is discussed in section —

The second theory put forward in an attempt to correct the issues surrounding cellulose lengthening during crystallisation is from — who argues that hemicelluloses form within the fibrils and push them apart causing the cellulose fibrils to contract. Interestingly mechanistically this is very similar to the lignin swelling hypothesis. By in causing the MFs to no longer run straight, instead they have to use some of their length to deviate past a cluster of hemicelluloses consequently shortening the overall distance the fibril can cover. One side effect of having these deviations is fibrils should not have a consistent cross sectional area over their whole length, where the hemicelluloses have been deposited should result in an increased cross section. potential way to test this?—

The most recent attempts made to describe the formation of growth stresses have been made by Yamamoto and his team. They argue a combination of both lignin swelling and cellulose contraction is needed, called the unified hypothesis. It should be noted that — and others suggested that both theories were likely to contribute to growth stress generation. By unifying the hypotheses they follow the current thinking of a number of authors in other areas of wood science that both tension and compression wood are not distinctly different types of wood and are instead extreme versions of normal wood. —more from Yamamoto—

— lots more in here from 80s-now — Note Muller et al 2006 found low hemicellulose content in G-layer timell 1969 higher conc of lignin in S2 layer when G-fibres present

The generation of longitudinal maturation stress in wood is not dependent on diurnal changes in diameter of trunk — new info on water pressure hyp

There are currently a number of outstanding issues associated with all of the current hypotheses/theories. When and how do the stresses get generated is still of much debate, over the last couple of decades it has become fairly widely accepted that the generation of the stresses occurs during or immediately after the deposition of the secondary cell wall. Most commonly either the G-Layer or the S2 layer are considered responsible. What the mechanism(s) is within the cell wall has been hypothesised about at great length (as discussed above),

however no theory presented so far is without country experimental evidence.

Another outstanding issue, common to many biological problems is why do particular traits vary so much between individual and species? One of the more debated topics around growth stress generation is whether the generation mechanisms for stress in reaction wood are extreme versions of the same mechanisms in normal wood. The G-layer is not found in normal wood, however not all tension wood producing species produce G-layers. Lignin swelling could potentially fit this criteria for normal and compression wood, however modification of Boyds theory would be needed address the dependence of a MFA as some wood with lower than 40 degree MFA still produces compressive forces, and there has been reported to be little lignin within the G-layer, which is suspected to be responsible or at least partly responsible for tension generation. Boyds theory combined with excessive mild compression wood formation in core wood still allows for the same tensile generation mechanisms to be used by older cambiums, as long as the MFA is suited to the task.

It is fairly well accepted (although almost by default) that growth stresses exist because they provide a mechanical advantage for survival. However to quantify the mechanical advantage with so much variability between individuals, and no known way of controlling growth stress generation this is very difficult.

Growth stresses studies have been largely confined to model, or common species however there are a number of species which appear to form intermediates or ‘strange’ forms of reaction wood. For example *Hebe* is a angiosperm which appears to form compression wood rather than tension wood.

2.1 Why growth stresses exist

Hardwoods typical have much larger growth stress magnitudes than softwoods. —why— is this true ‘xylem cell development’?— Some young conifers have been reported to have larger compressive stress at the stem than at the pith, this may be attributed to the abundance of compression wood in juvenile conifers observed by some. Once older they follow the same radial stress profile as hardwoods.

The commonly accepted argument for the reason of growth stresses existence is the mechanical hypothesis. The mechanical hypothesis argues that a number of wood properties, including the development of growth stresses evolved in order to provide increase mechanical stability of trees in order to increase their survival. The mechanical hypothesis as applied to growth stresses argues that because wood is stronger in tension than compression by preloading the outer edge of the stem in tension it increases the non-destructive bending radius on the inside of the curve when a force is applied causing the stem to bend. The growth stresses produced by reaction wood allow for the tree to correct its center of gravity, orientate in such a way as to minimise external loads such as wind and position it’s self for optimum light interception. All of these increase competitiveness.

—speculation from various authors Typically when attempting to determine

the reasons for why wood properties exist one of four hypothesis are used; mechanical, hydraulic, time dependent and a combination of the previous three. Initial speculation as the the reason for growth stresses existence came from Martley (1928) who briefly entertained the mechanical hypothesis based on self weight. Jacobs (1945) suggested they were a byproduct of sap tension, which he later retracted Jacobs (196?) when sap pressures were recalculated at a much lower value than the generally believed values at the time. .. Growth stresses indubitable have an effect on the mechanical stability of trees, although it is conceivable that the effect may be byproduct of another driver.

2.2 Issues growth stresses cause

At harvesting growth stresses are released by the saw cut (and crosscutting etc) and can ruin structural and veneer logs due to the resulting splitting and warping. Growth stresses, particularly reaction growth stresses increase the danger for the faller by effects such as saws binding and ‘barber chairing’.

When the stem is felled or cross cut, growth stresses are released around the saw cuts causing shortening at the periphery and extension in the centre. The dimension change is maximum at the saw cut, reducing as distance from the cut increases. When the contraction/extension force exceeds the plastic limit of the stem splitting occurs. The cracks tend to propagate in the radial direction by cell wall peeling, although the cracks tend to only be a few centimeters deep they significantly reduce the value recovery for both structural and peeler logs.

Prolonged compression at the centre of the stem during growth can exceed the elastic limit of the wood, resulting in internal defects such as brittle heart. When the stem is felled these defects have already occurred and hence there is no way to prevent them during felling, however selection for low growth stress producing families should significantly reduce the occurrence of internal defects.

Within mills during processing growth stresses cause a number of issues leading to reductions in value recovery. Because growth stresses are released when the stem is sectioned via sawing (plain, quarter etc.) the resulting shape change can cause the saws to jam. The main value loss at this stage of processing comes from the need to saw boards multiple times in order to release the stresses while still allowing for the final board dimensions to be retrieved. Increasing the number of times the boards are sawn to get their end dimensions gives not only poor saw use efficiency but the major economic loss comes from the final yield being as low as 30

3 Proposed theoretical and experimental work

3.1 A proposed modification to the lignin swelling hypotheses

The lignin swelling hypotheses (Boyd 1950) argues the deposition of lignin into the secondary cell wall forces the cellulose fibrils away from each other, because

cellulose is very stiff when it bows because of the lignin pushing the fibrils apart the cell changes shape based on the MFA. One of the main arguments for the use of the cellulose contraction hypothesis is that the G-layer is mainly crystalline cellulose and hence is not effected by lignin swelling. However if the outer of the cell is constrained transversely by high MFA fibrils (as in the P and S1 layers) and surrounding cells, when lignin is deposited in the S1, S2 and/or S3 layers causing swelling, cell expansion will occur toward the cell lumen (as long as the MFA is conducive to transverse swelling). If the G-layer is already constructed when this swelling into the lumen takes place it will cause a bowing of the cellulose fibrils within the G-layer and consequently contraction of the cell.

To create the maximum amount of contraction from the G-layer there will be an optimum MFA within the secondary wall (most commonly the S2 layer) dependent on cell geometries, around 40 degrees. Further because the G-layer is contracting the cell, it requires the rest of the cell to be as flexible as possible (without compromising the cells other properties). Flexible cells commonly exhibit higher MFAs than stiff cells within the S2 layer. By having a non-stiff structure the G-layer can cause more contraction on the individual and surrounding cells while under less bowing from lignin swelling. Therefore the optimum MFA of the secondary wall (excluding the G-layer) will provide the G-layer with the maximum ability to contract the cell when MFA is in the mid range, i.e. non-stiff and maximum transverse swelling for minimal longitudinal dimension change.

The qualitative basis of the lignin swelling hypothesis in its current form remains unmodified and can still account for normal and compression wood growth stresses.

3.2 Theoretical work

Over the years there have been a number of attempts to mathematically model cells (usually fibres or tracheids) from cell wall constituents (Mark 1967, Koponen et al. 1989, Harrington et al. 1998, Yamamoto and Kojima 2002, Kojima and Yamamoto 2004 are a few example) however very few efforts have used these techniques to investigate the formation of growth stresses (archer 1987, Yamamoto 1998, Guitard et al. 1999).

Currently the most advanced model for how growth stresses develop within the cell wall was presented by Almeras et. al. (2005) using the unified hypothesis (Okuyama et. al. 1986, Okuyama et. al. 1994, Yammamoto et. al. 1991, Yammamoto et. al. 1992 and Yammamoto 1998) utilising both the lignin swelling and cellulose contraction hypotheses. For details see section —.

Proposed model of the cell: Modelling of a generic single cell with variable cell wall properties to investigate the required geometry and constituents to create maximum longitudinal and tangential extension and contraction via the lignin swelling hypothesis. The single cell model should have the capacity to put limits on what stress generation the lignin swelling hypothesis is theoretically capable of.

Because the proposed experiments (see section —) induce tension wood in

species both with and without G-layers an experimental upper limit of the lignin swelling hypothesis should be reached and compared to the theoretical one derived above. By including the G-layer (assuming the experimental work shows the G-layer is a contributing factor to the production of growth stresses in tension wood) within the model (by implementing the hypothesis above), and comparing the required chemical make up and cell geometries with the tension wood experimentally investigated, light should be shed on the likelihood of the lignin swelling hypothesis being extendable to include G-layer type tension wood.

It is expected that the base model and parameters will be similar to those utilised to describe lignin swelling by Almeras et. al. (2005) and Yammamoto (1998). Cell wall layer radii, thickness, S2 layer MFA, moduli of the CMF bundles and matrix will all be included. Additional variables will be included as necessary. It is intended to add the standard deviation of the MFA within the S2 layer, as in Harrington (1998), MFA for the G-layer when present will also be included with S2 interaction. Because the maturation process is time dependent, time dependent functions will be considered allowing for partial and concurrent cellular development. Boundary conditions will be initially derived from those presented by Almeras et. al. (2005) and further modified for increased realism and/or usability of later models.

Time permitting if experiments find that the lignin swelling models don't account for the strains present a proviso for cellulose contraction could be included. Between the experimental results and the cellular models, calculations as to how much contraction is needed should be possible, giving an initial starting point to look more in depth into cellulose contraction mechanisms.

Proposed model of stem: Because of the nature of the experimental work it is required to be undertaken at a macroscopic scale, while the proposed theoretical model is at a cellular (nano to micro meter) scale. The scale difference between the two methods causes an issue in that they are not directly comparable (as a sample of wood is not homogeneous). In order to overcome the scale dependency it is proposed a second theoretical model be produced which will operate at a macroscopic scale with the purpose of simulating the experiments undertaken. By parametrised with the single cell model (which has been parametrised with the experimentally derived cell anatomy and geometry) approximations to the actual sample being tested should be able to be made and compared to the experimental outcomes. This proofing is required to make sure that the results of the single cell model is providing are realistic.

3.3 Experimental work

Currently neither lignin swelling or cellulose contraction have any direct experimental evidence. The tension which cellulose is under on the stem periphery has been directly measured using x-ray diffraction showing the repeat length of 1.035nm in tension wood decreasing to 1.033nm when the stress is released (Clair 2006).

Experimental evidence of the G-layer providing contraction within tension

wood has been presented by Goswami (2008). Longitudinal extension and tangential contraction was observed when the G-layer was enzymatically removed from tension wood poplar samples. The S2 layer was reported to have a high MFA (36 degrees) as has been reported previously and for other G-layer producing species –refs–. Goswami (2008) suggested lateral swelling of the G-layer caused the contraction.

The primary goal of the set of experiments which will be presented within this chapter (when used to parametrize the mathematical modeling work) is to attempt to identify which cell wall constituents are controlling stress generation and how they are controlling stress generation under different conditions. In order to evaluate stress generation mechanisms a number of experimental techniques have been identified.

Basic cell wall anatomy and geometry needs to be investigated for the NZDFI species involved in this project. Where possible literature values will be used to approximate these values for model parametrization. The following properties are required, however will only be sort from experimental techniques when it is deemed there is a significant advantage over available literature values.

The cell wall anatomy under different wood types (tension, normal and opposite) needs to be investigated for the various NZDFI species (principally *E. bosistoana*). The anatomy study will consist of investigating which species produce a G-layer (microscopy with staining) and what cell wall structure is associated with its production (Electron Microscopy). The cellulose, lignin and other constituent contents will be determined for tension, normal and opposite wood (Wood nitration combined with NMR studies) along with the MFA and the MFA standard deviation in all three wood types (x-ray diffraction). Fibre diameter, length and lumen size will also be obtained (microscopy). Within tension wood the removal of the G-layer (in G-layer producing species) will be needed in order to determine the secondary cell wall properties of tension wood (enzymatic removal).

The cell wall constituent study results will be used to make comparisons between the growth stresses produced by the stems and the different properties within the cell walls. With the anatomy results collected from tension, normal and opposite wood comparisons can be made not only within trees but also between trees. By comparing tension wood with the G-layer removed with normal wood with similar properties some insight into the role of the G-layer should be gained. Note that growth stresses for a large number of samples will be collected during the breeding work, however because of the time consuming nature of the experimental works presented here only a small number of specimens will be tested as needed.

In order to produce the three types of wood required two different growth manipulation techniques are suggested:

Technique one; Young stems (less than three month old growth from coppice) will be restrained to a loop, similar to Jacobs loops (Jacobs 1945) and allowed to grow for approximately one year, with regular adjustments of the restraints to make sure the cambium is not damaged. From the same plants a second leader will be selected and restrained to a straight pole to provide normal wood

of the same genetics.

Technique two; Straight one year old stems (from coppice, and seedlings of a mixture of *camadilencia*, *tricarpa* and *quadrangularta*) will be bent and restrained and allowed to growth for a further 6-12 months, with regular adjusting of the restraints to avoid cambium damage. Normal wood samples can be collected from these stems from wood produced before manipulation and away from the bend site. These plants will be selected from *camaldulensis* (reported to produce S1-G tension wood (Baba 1996)), *quadrangulata* and *tricarpa* depending on the suitability of the plants available.

The other set of experiments proposed is to investigate the extent of an effect the G-layer has on tension generation within tension wood, and how the G-layer generates these tensions.

Proposed experiment one: Taking samples with G-layers and applying a vacuum pump to suck an enzyme treatment into the fibres and vessels to degrade the G-layer releasing the strain which the G-layer is applying to the samples will cause a shape change. By comparing the initial and final shape change the strain the G-layer was imposing on the samples can be obtained. Further the rest of the growth stresses can be released using more traditional techniques such as split tests or planking, by releasing the remaining strain the proportion of stress associated with the G-layer and other cell wall components (assumed to be the S2 layer) can be determined.

Proposed experiment two: Release the stress with a split or planking test, then remove the G layer using the same method as above. When the tension caused by the G-layer is released relaxation back towards the the initial state should be observed. The proportion of G-layer induced strain and S2 induced strain will then be evident.

Proposed experiment three: During growth induce tension wood production by forcing curvature into the living stem. By introducing an enzyme treatment to the plant while it is still transpiring should degrade the G-layer and reverse any straightening that was caused by the G-layer.

Any of the three proposed experiments, if they work will provide the proportions of the strain in tension wood which can be attributed to the G-layer.

3.4 Breeding

Because growth stresses cause a number of issues for harvesting and milling timber, tree breeding programs can and have be used in order to select for genetics which reduce these effects. –previous breeding for GS– There is no reason to expect breeding for growth stresses differs significantly from (conventionally) breeding trees for any other trait, which is process which has been developed over centuries. Over the last few decades many advances have been made in experimental and statistical techniques which rapidly improve the time and accuracy of conventional breeding.

It is suspected that the most efficient way to minimise the issues growth stresses cause during the production of timber is through appropriate genetic selection. *Eucalyptus* species, in particular *bosistona* are showing promise within

the NZDFI trials to produce high value naturally durable structural timber. In order to see the yield efficiency required to make this product profitable growth stresses need to be reduced to minimise the effects discussed in section —. While within the NZDFI project there are a number of other concerns for breeders (such as durability, form and growth rate) growth stresses also need to be considered. Using conventional breeding methods discussed below growth stresses will be minimised within the NZDFI genetics. Currently a number of trials have been established or will soon be established, these include:

Permanent sample plots (whole forests) are located throughout New Zealand (primarily in the Marlborough Nelson area). These plots are for profit forestry plantations ranging in ages up to 8 years old. Because the plots are not specifically research plots limited testing can be undertaken on the trees. These trials consist of the species — set up as alpha latause trials. Some of the genetic material is duplicated in other research specific experiments described below.

The Harewood trial: All trials at Harewood are set out as randomised individual trials. Principally this work will be concerned with *E. bosistoana* of which there are two trials. One with xx replicates of xx families, planted in (when?) and coppiced in (when), due to be harvested in spring/summer 2015. The other *E. bosistoana* trial has xx replicates of xx families, was planted in (when?) and harvested for the first time in 2012, the plants were then coppiced and harvested again in December 2014. Four families representing the highest and lowest growth stress generating genetics were coppiced for a second time and will be due for harvest in 2016. Preliminary results from the 2012 and 2014 harvests show reasonably high heritability of growth strains, particularly within family rankings. The same data was collected from — *E. argophloia* plants planted in — measured and coppiced in 2012, with final measurements completed in 2014. In ground plantings of *bosistana*, — have been planted in 2014 and will continue in 2015 with *bosistana*. Note most of the plantings required to get the material for the studies outlined in section — are also grown at this site.

Woodvile trial: Due to the success of the previous trials NZDFI is currently setting up a xxx plant trial with xxx replicates of xxx families of *bosistana*, xxx replicates of *argophloia* and possibly *globoidea*. The trials are set up as alpha lattices and harvest is expected in late 2016 or 2017. The intention is to have as much overlap as possible with the existing genetics within the current and previous trials.

Note the term family is used here to mean the same mother, but not necessarily the same father. If seeds are collected at different times even from the same tree variability exists due to possibly of different set of fathers. Also some *Eucalyptus* self propagate, but it is unknown which ones or what proportion of seeds are self propagated within the NZDFI genetic material. Self propagation is ignored.

Within the structure of the breeding program there is the ability to statistically check within family genetics results from young stems and mature stems. Growth stress measurements are a good candidate for this type of selection as the expected biological processes underlying growth stress generation are age

independent (bar the dependence of some influencing factors associated with the typical radial pattern of density, MFA etc).

Experimental tests for breeding: In order to select for low growth stress producing families experimental tests need to be undertaken on each plant. The main test to be used to determine the extent of growth stresses within the breeding population is the split test (also known as the Pairing Test) as described in Chauhan (2010). The test involves taking a significantly long section of stem and using a saw cutting along the pith to create a radial split. The diameter of the stem is taken before testing and the width of the opening measured immediately after splitting. Once the opening is measured the stem is cut to across the grain to give two samples (one from each side of the split). Density is measured by taking measuring the mass (using balances) and volume using the displacement method on each of the pieces. Acoustics are also taken using wood-spec to calculate the dynamic modulus, and hence the stress can be derived. Other properties such as bark thickness are also recorded for other purposes. Due to the size of the Woodville trial it may be the case that less tests are carried out. Decisions on the essential tests will be made near the time of harvesting. Note throughout testing the samples are kept in a green state.

Statistical methods: Although the particular statistical techniques best suited to the data set obtained from the trial results can't be known before the data is collected, it is expected that typical breeding statistical techniques will be applicable. The two objectives to be achieved for the breeding trials are to estimate genetic parameters, (in particular heritability of growth stresses) and to predict breeding values for families. Mixed model methods are commonly used within tree and animal breeding.

Potential for the use of modern technologies such as portable NIR to investigate older trees (in the Marlborough and Nelson plots) without destructively testing them may be of use. NIR has shown promise in predicting longitudinal surface strain in Sugi green logs. Non-destructive evaluation of surface longitudinal growth strain on Sugi (*Cryptomeria japonica*) green logs using near-infrared-spectroscopy. Surface tests such as described by –ref– will be required to either calibrate an NIR predictive model, or if NIR is unfeasible to acquire surface strain values on older specimens.

Partially destructive testing may be appropriate on a limited number of older trees. Core samples will be taken for other works, however at this stage there are no methods for the measurement of growth stresses (directly or indirectly) from core samples. Surface strain tests using strain gauges as have been used by a number of authors –refs– could be undertaken if necessary.

- 4 Intentions**
- 5 Objectives**
- 6 Costs**
- 7 Timeline**