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 unisteriate 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 reorientate stems and branches of (most) trees, reaction wood is produced which provides a force in order to reorientate the tissue. Typically this reorientation is toward the light or upwards as is defined by the negative gravitropism hypothesises. Other reasons for reorientation such as reducing wind drag have also be suggested. In softwoods this reorientation is caused by the production of compression wood. Compression wood forms on the out side of the stem or branch and expands longitudinally causing a restoring force). Hardwoods on the other hand produce tension wood on the inside of the desired curve which contracts longitudinally resulting in a curve forming. Traditionally the gelatinous 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 *Eucalyptus 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 consist of a middle laminar (connecting the fibre to the sounding 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 macrofibrils 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 form division through elongation and development to death is expected to play a role in the development of growth stresses within the stem.

–maybe put johnathans pic of wood sturture in here some where—

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 pedant.

During primary wall formation rapid elongation occurs. When the cells divide from their parents they remain fixed to their neighbors 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. 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 laminar 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 necessary)

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 helices to lower right to upper left.

Finally if tension wood is being produced a Gelatinous layer (G-layer) may be produced on the inside of the inner most wall (S1, 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 connectedness between cells this results in growth stresses forming within the stem, this phenomenon is discussed in greater detail in section —-. 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 separates 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 provides the ability for the stem to reorient 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 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.

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 sawed 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 decipher the 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 a angle correction even with identical cells. Sap tension was 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 the normal strain pattern in tension which correct the lean, the compression wood mealy acted as a pivot, not contributing a tensile force on the lower side of the stem.

Boyed (1950) developed a new experimental technique in order to investigate the stress profile further. By cutting a slit longitudinal 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 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 contributing factor.

Boyed (1950a) developed and experiment whereby he removed a wedge from a disk and measured the radial expansion, showing the disks were 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.

The Poisson effect is the revelation that a change of dimension of a material in one direction will result in a change of dimension in the other directions, this relationship is characterised by the Poisson ratio (within the elastic region of deformation). Within growth stress literature there has been some investigation of this effect as it appears the redistribution of stress through the Poisson ratio from the longitudinal to tangential direction is not sufficient to account for the

observed tangential strains, which can vary even for a given longitudinal strain.

Boyd (1950c) provides an in depth rebuttal of the available theories at the time, arriving at the conclusion the 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 rolls 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 had 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 tern 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) un-

til 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 hypothesis were presented, Hejnowicz 1967 and brodzki 1972. 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 Kublers work Archer introduced orthotropic solution 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 tangential 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. Finely he used the developed methods to present solutions for a number of hardwood species.

Archer followed up his series on growth stress distribution with 'on the origin of growth stresses' where he attempted to mathematically investigate individual cells, presenting an explicit relationship between strains and growth increment of the cell wall. The relationship relates MFA and swelling strain, he argues that these results are consistent with the lignin swelling hypothesis for compression wood. In tension wood, by increasing the ratio of area of cell wall to total cell cross sectional area by adding a G layer could theoretically (with the parameters Archer used) produce a tensile stress of 36MPa.

–boyd 1977 Basic cause of differentiation of tension and compression wood— Boyd (1977) reinvestigated most of the hypothesis available at the time regarding the reason for the development of reaction wood. He argued that (as had been previously rejected) the stress hypothesis, claiming that reaction wood is a response to imposed stresses is the only available hypothesis which fits the data, and that previous efforts to reject it had resulted from misinterpreted results. In doing this he provides reasoning to reject the hypothesis regarding gravitational responses, intrinsic growth direction, auxin distribution and turgor pressure.

A common argument that is made for the cellulose contraction hypothesis is the correlation between cellulose content and strain. Higher proportions of cellulose compared to lignin correlate to tensile strains, while high lignin content correlates well to compressive strains –refs–. It has been well reported that compression wood is partly characterised by an increase in lignin content –ref–, which has been used as an argument for the lignin swelling hypothesis. Tension wood however is often but not necessarily correlated with and increased

proportion of cellulose. –tests on tension wood with no G-layer– Within tension wood of G-layer producing species tensile strain and whole cell cellulose content correlate well due to the G-layer having a very low lignin content –ref– The proportions of cellulose and lignin within the cell after the G-layer has been removed do not share this correlation –ref–.

— timell 1969 higher conc of lignin in s2 layer when G-fibres present

–timell compression wood in geynosperms–

After Bamber 1979 disputed the reliability of Boyd 1972, Boyd 1985 and Bamber 1987 disputed each others analysis's however no new information was presented, rather a number of issues around interpreting biological data were highlighted.

Kubler 1987 provided an in depth review of the hypothesis, evidence and experimental methods at the time, much of which has been discussed above. He presents a table summarising the literature reporting strains for different species, highlighting the large intra and inter tree variation even within a single species.

Yamamoto et al. produced a number of papers entitled 'Generation Process of Growth Stresses in Cell walls' –refs– where both the lignin swelling hypothesis and the cellulose contraction hypothesis are considered in detail, including new experimental evidence for each. – more on these results— some must mention the critical MFA (the point of no longitudinal change) —

After the experimentation of –refs— finding the critical MFA to be between 25 and 30 degrees for a number of species –refs–, Okuyama et al 1993 (growth stresses in tree, in Japanese) and yamamoto et al 1995 (series num 6) suggested the unified hypothesis. Although the idea of both lignin swelling and cellulose contraction being responcable for growth stress development had been suggested before –refs– it was formalised here. In an attempt to solve the critical MFA descrepency –yam et al 1995– augmented the barber and meylan –ref– cell wall

model to include a S1 layer. The resulting model was the first to be able to acount for production of both tensile and compressive stresses over a wide range of MFAs, however this was only achievable using unnatural parameter values, (in particualar –what were they–) The S1 layer introduced utalises a constant MFA of 90 degrees, with the S2 layer varying from 0 to 60. Cell wall maturation ocoured in two descrete steps, first the cellulose framework is constructed then the lignin depositions ocour. From the model they showed that with an incresing S1 layer thickness the critical MFA reduces. Unfortunatly they found the model was unable to produce realistic tangentail strains unless unnatural parameters were used.

—maybe include the cell wall model picture in here some where—

Yamamoto 1998 further refined the idea by introducing a much more rigorus frame work, incorporating time dependence into the cell wall maturation model. The work presented shows the failings of each lignin swelling and cellulose contraction, even when time dependence is include. Time dependence does allow for good agreement between the modeled unified hypothesis and experimental values from sugi –ref–. The poor agreement with tangential stresses is explained as being easy to decrease through stress relaxation in comparison to the longitudinal stress when inside the trunk.

–Guitard 1999 growths tress generation a new mechanical model of the dimensional change of wood cells during maturation— Guitard et al 1999 used a S2 layer model which took the transmition of shear between fibres into account, resulting in non-zero shear moduli. Previously intergral conditions had been used to goven the longitudinal stresses, presumably as they satasfy the nessassery condtion implicet within stress feild equlibram conditions, Guitard et al 1999 however introduced a local condition on every elementory volume. They argue that although this approch does not satasy the nessassery equlibram condtions

it provides better agreement with experimental results when combined with dimentional chenages within the microfibril bundel. In particular this model provides a much better prediction of transverse strains while being less complex than previous attmepts.

Yamamoto et al (origon of the biomechanical properties of wood related to fine structuer of the multi layer cell wall) 1999 further advance his 1998 model to include drying stresses and moisture depened youngs modulus, however little changes were made with regard to the growth stress model. This paper was followed by——

–something to think about, yamamoto 1998 — during the thick secodary wall lignification lignin precursors are accumulated among the gaps of the CMF framework and the hemicellulose-lignin matrix which have have already been depositied and they are polymerized immediately therefore the amount of substance increases irreversibly inside the matrix skeleton whos volume is spartually limited. — Can we work out a volume change during polymerisation, then use this to get impose a force within the pores? — –Once transported to the cell wall, monolignols are oxidized to phenolic radicals (molecules in the phenol group that have free valance electrons?) that undergo polymerization by chemical coupling–

– the monolignols are deposited and form/add to the lignin macromolecule, the bonding its self causes whole to be greater than the sum of its parts causing the force on the FAs. –NOTE not nessasserly/probably not a simetric volume increase. control over the lignin assembly exerting force on the cellulose may even play a role in the critical MFA and the tangential/longitudinal stress ratio.— –can work out the required force from the required deformation to fit exp evidence, and the stiffness of cellulose. After this get a computational chemest to confirm using molecular models.

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.

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 mechanically this is very similar to the lignin swelling hypothesis. By causing the MFs to no longer run straight, instead they have to use some of their length to deviate passed a cluster of hemicelluloses consequently shortening the over all 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.

Both of these hypothesis would likely (although not necessarily) result in a positive correlation between strain and hemicellulose content within the G-layer,

however Muller et al 2006 found low hemicellulose content in the G-layer – compared to what –

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

— lots more in here from 80s-now —

It is worth noting that because bark is also formed by cambial cells differentiating, when the cambium divides to the outside, the cells (typically) phloem –check– become bark. The bark is therefore under transverse tension. Some of this is alevated via the bark peeling as it ages and is forced further from the cambium, however the remaining ring can still be providing a significant stress on the stem as it tries to contract. Bark rings contracting when removed from disks has been observed by kraus 1867 Krabbe 1882. Okuyama et al 1981 measured 750 micro strains in Japanese cedar. Bark is often observed to split, indicating the maximum strain that the bark can withstand is often reached before it can be shed, resulting in a limited amount of stress in the outer regions of the bark layer.

Currently there are three commonly used experimental methods for measuring surface strains. The Nicholson (1971) method, the 'French' method and the strain gauge method.

After the developments of Boyd and Jacobs in testing for growth stresses it became apparent there was a need for a rapid testing procedure. Nicholson (1971) developed the first of these measuring the released strain between two metal pins on the surface of the sample, cut from the surface of logs. While considered a rapid method in 1971, updated versions of this test are still used for measuring surface strains but not practical for (or considered rapid) for testing larger numbers of stems such as in breeding trials. The 'French' method (current

iteration Gerard et al 1995) involves drilling a hole between two reference points, with a dial measuring the distance change between the two points.

Okuyama et al (1981) adopted the use of strain gauges to measure stem surface stresses of particular layers of wood. Other methods were also derived around the same time, Guenau and chardin 1973, Guenau and Kikata 1973, and kikata and kiwa 1977 investigated drilling holes near strain gauges to release strains. Saurat and Gueneau 1974, 1976 introduced an apparatus which utilised two knife blades at a set distance, one knife blade bent as the strain was released via drilling the strain release was measured on the blade.

Measuring strains inside the stem proved to be more difficult. Kikata (1972) adopted Jacobs planking method and electric strain gauges for improved accuracy. Kubler (1959) and Wilhelmy and Kubler (1973) drilled holes of known diameters into stems and attempted to measure the change in shape of the hole as the log was successively crosscut closer to the test site, as Boyd (1950) had done. Ploge and Thiercelin 1979 attempted to measure the effect of growth stresses on increment cores, although they found that the stresses had an effect on the corer its self squashing it into an oval shape. Ferrand 1982 found a correlation between longitudinal strain and tangential core diameter between -0.67 and -0.77, showing they can be used for near non destructive growth stress testing.

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.

Unfortunately most literature has investigated very few samples and reports high variability within individuals and species,—

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 lest 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 Other noteworthy literature

–Experimental papers on cell properties at macro/molecular scales– –the molecular dynamics modeling papers–

A number of complex models mathematical models have been presented from the molecular to the cellular (and whole organ) level –refs–, unfortunately as growth stress was not a primary concern to these researches they are neglected. Luckily these works have made significant advancements in other areas of understanding which need to be incorporated into growth stress research. –maybe a little on these advances, —Ref a review paper—– Breifly discuss the math tecneques used—Has any one used descrete or hybrid systems?—

2.2 Why growth stresses exist

Hardwoods typically 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.

Tangential stresses have been suggested to resist mechanical failure in times of frost (when water inside the cells freezes and expands) and drought (when water tension is very high) Kubler 1983.

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.

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.

2.3 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'.

End splits, heart checks, and ring shakes all reduce the value of the timber in a stem. 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.

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 sawed 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 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 parameters 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 the magnitude of stress generation the lignin swelling hypothesis is theoretically capable of under different constituent and geometric makeups.

Because the proposed experiments (see section –) induce tension wood in species both with and without G-layers an experimental upper limit of the stress generation the lignin swelling hypothesis is capable of should be reached and compared to the theoretical one derived above.

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 cell wall layers, as in Harrington (1998), pore size (or conversely fibril aggregate size) (Fahlen 2005, Chang 2014, Salmen 2012, Kim 2012) and cell wall constituents (Baba 2009, Donaldson et al. 2001) and layer properties/geometries (Bergander 2002, Grozdits 1982, Almeras 2005, Yammamoto 1995, 1998, Chang 2014, salmen 2002) to form a model, conceptually similar to the qualitative architec-

ture presented by Mellerowicz et al. 2012, salmen 2009 and others –try to find original–. 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.

One of the major differences between the model presented here and in previous literature is the inclusion of fibres intertwining macrofibrils. Recently Chang et al. (2014) measured the pore size and shape within tension wood and oppressed wood of poplar during cell wall maturation. With this recent advancement reasonable assumptions around how regularly fibrils interact with other fibrils outside of their host macrofibril can be made. It is thought that these pores occur between joining fibrils connecting the macrofibrils into the larger structure that is the forming cell wall. If the deposition of lignin into the pores forcing the fibrils apart is the mechanism by which growth stresses develop the quantity of pores and pore sizes are important parameters to investigate as they will largely effect the ability of the mechanism to cause stress.

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 (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 parameterizing with the single cell model (which has been parameterized 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 will make sure that the results the single cell model is providing are realistic.

3.2 Experimental work

Currently neither lignin swelling or cellulose contraction (described in section —) have any direct experimental evidence. The tension which cellulose is under on the stem periphery has been directly measured using x-ray diffraction showing a strain reduction of 0.2% in cellulose 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 were 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 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 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 of different wood types (tension, normal and opposite) needs to be investigated for the various NZDFI species (principally E. *E. bosis-*

toana). The anatomy study will consist of investigating which species produce a G-layer (microscopy with staining) and the cell wall structure associated with its production (Electron Microscopy). The cellulose, lignin and other constituents will be determined for tension, normal and opposite wood (Acid hydrolysis 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).

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 camadulensis, tricarpa and quadrangulata) 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 away from the bend site. These

plants will be selected from camaldulensis (reported to produce S1-G tension wood (Baba 1996)), quadrangualata and tricarpa depending on the suitability of the plants available, and there ability to produce a G-layer will be investigated with microscopy with staining –stain ref–.

The following experiment is proposed in order to investigate the proportion of the stem reorientation that is due to the G-layer. During growth tension wood production is induced by forcing curvature into the living stem, as described above. By introducing an enzyme treatment to the plant while it is still transpiring to degrade the G-layer and reverse any straightening that was caused by the G-layer. With the G-layer removed the remaining stress can be released via planking or splitting.

3.3 Breading

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. There is no reason to expect breading for growth stresses differs significantly from (conventionally) breeding trees for any other trait. 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 *E. bosistoana* 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) 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 solely research plots, limited testing can be undertaken on the trees. These trials consist of the species –need list– set up as alpha latause trials. Some of the genetic material is duplicated in other research specific experiments described below.

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 2011 and coppiced in 2013 (check these), due to be harvested in spring/summer 2015. The other E. *E. bosistoana* trial has 10 replicates of 20 families, was planted in (2010?) 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 strain generated family rankings. The same data was collected from *E. argophloia* plants planted in 2010? measured and coppiced in 2012, with final measurements completed in 2014. Note most of the plantings required to get the material for the studies outlined in section — are also grown at this site.

Due to the success of the previous trials NZDFI is currently setting up a 4168 plant trial with 81 families of *E. bosistoana* (with an increase to 160 expected in

spring 2015), 336 seedlings over 13 families of *E. argophloia*. 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. Any effects of self propagation are 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, although the trees are grown under different environmental conditions.

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 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 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 Chauhan (2010). 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.

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 for these purposes.

4 Objectives

The proposed research has two main objectives; add information to the debate on how growth stresses form within trees and to increase the quality of the breading stock within the NZDFI project for the production of high value timber.

5 Costs

Privet repositories for GIT and Authorea

Travel expenses to 8th Plant Biomechanics Conference - Japan, November 30-December 4th 2015

Travel expenses for use of equipment which cant be sourced at UC, potently for use of NMR, SEM, AFM.

Travel expenses to Woodville and Marlborough as required.

Bluefern - generally free, unless a very high workload is needed. Unlikely to be the case. Potently the use of other HPC facilities if they are better suited to the task.

Charges associated with the use of eh AFM.

Sundry items including lab materials and chemicals. Operational expenses for loop and bending trials at Harewood.

6 Timeline

March 2015	Set up loop and bending trials
	Proposal due 1 April 2015
April 2015	Harewood analysis
	Write up preliminary results
May-July 2015	Harvesting loops and bending trials
	Anatomy study
	Enzyme experiment
August 2015	One year report due 1st September 2015
September-October 2015	Write up anatomy and enzyme studies
	Cellular modeling
November 2015	Marlborough strain tests
December 2015	Japan
	Re-establish loop trials
	Harewood harvest
January 2016	Harewood analysis
	Marlborough analysis
	Update preliminary results
February-Apr 2016	Modelling
May-July 2016	Harvesting loops
	Further anatomy study from loops
	Update experimental paper
August-November 2016	Finish Modeling
	Write up modelling
December 2016	Woodville harvest
	Harewood harvest
January-February 2017	Woodville analysis
	Harewood analysis
	Analyse Marlborough, Harewood and Woodville together
	Write up breading paper
March-October 2017	Complete remaining tasks and papers
	Submit thesis

7 Projected publications

On heritability of growth stresses in *Eucalyptus bosistoana*. Using data from Woodville, Harewood and Marlborough. –Is this a paper or report to NZDFI?–

Anatomy study from loops, reporting G-layer, MFAs etc –probably letter or tech note –

On G-layer enzyme removal effect on looped samples

Presenting modeling work

8 Preliminary results

2012/2014 Harewood analysis

anatomy study

enzyme experiment