UNIVERSITY OF CAPE TOWN

How do trees die following low intensity fires: Exploring the hydraulic death hypothesis.

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Abstract:

The mechanism by which trees die following a low intensity fire is poorly understood. Traditionally, cambial necrosis (Carbon starvation hypothesis) has been used to explain post-fire tree mortality, however, this does not explain why some trees die within days following a fire. To address this, the hydraulic death hypothesis argues that post-fire tree mortality is a result of a reduction in hydraulic conductance rather than the necrosis of cambium. There are a number of proposed mechanisms by which hydraulic failure can occur during a fire: firstly, plume-induced cavitation as a result of high vapor pressure deficit (VPD) in a fire-plume has been shown to reduce hydraulic conductance. Secondly, changing surface tension as water is heated has also been shown to increase the chance of cavitation. The final mechanism is a reduction in conductance as a result of direct xylem vessel deformation due to the visco-elastic properties of xylem walls (lignin). In order to determine the relative importance of each proposed mechanism, stems of *Kiggelaria africana* and *Eucalyptus cladocalyx* were exposed to 70 and 100°C in two treatments designed to isolate the effect of each mechanism. An oven treatment was used as a surrogate for a fire-plume in order to demonstrate VPD-induced cavitation and a water bath treatment (transpiration inhibited) was used to demonstrate xylem deformation (along with microscopy). This was possible because post-treatment flushing was indicative of the initial cause of the reduction as cavitation is reversible while deformation is permanent. The data was then explored using a Hydraulic Death model we created based on a xylem conductance model from literature. The results showed that VPD-induced cavitation as well as deformation are able to reduce hydraulic conductance in trees exposed to fire, however, *E. cladocalyx* showed higher loss of conductance at 65°C than *K. africana* and deformation was only seen to occur in water bath treatments and only in *K. africana*. Here we propose that a chain of events provides a mechanism for slowing the rate of heating in branches exposed to a fire-plume and that cavitation plays a protective role. Model exploration implied that vulnerability segmentation is responsible for preventing fire-plume induced runaway cavitation. This is in agreement with the “safety valve hypothesis”, however, rather than drought stress, it is a fire-plume which causes the cavitation. It was also found that *E. cladocalyx* was able to prevent deformation because of xylem vessel characteristics (thick vessel walls) and not bark properties. We propose that the necrosis of cambium and phloem leading to the inability to refill cavitated vessels is the actual cause of mortality in trees exposed to low intensity fires. The ability to refill is dependent on water availability and carbohydrate content, which is highly sensitive to drought. As resprouters store water and carbohydrates in lignotubers and stems, they are less sensitive to pre-fire conditions. However, the survival of cambium and phloem is essential to the refilling process and thus the mechanism for reducing heat transfer, bark properties as well as xylem characteristics work in combination to determine persistence after a fire.

Introduction:

The mechanism by which trees die following a low intensity fire is poorly understood. There have been a number of direct and indirect explanations proposed, but still the questions regarding the physiological mechanism remain, to a large extent, unanswered (Dickinson & Johnson, 2001; Michaletz & Johnson, 2007; Kavanagh *et al.*, 2010; Midgley *et al.*, 2010). The conventional explanation for post-fire tree mortality has focused on the necrosis of living cambium and phloem cells and is referred to as the Carbon starvation hypothesis (Pinard & Huffman, 1997; Dickinson & Johnson, 2004). Necrosis of cambium occurs at about 60°C, with the degree of necrosis often scaled with variables such as bark thickness and tree height (Michaletz & Johnson, 2006; Sevanto *et al.*, 2013). As phloem occurs above the vascular cambium layer closer to the bark, it can be assumed that phloem reaches the same temperature as vascular cambium during a fire (Dickinson & Johnson, 2001) and it has been shown that these tissues can reach up to 100°C in simulated forest fires (tissue temperature will not rise above 100°C until all moisture has been lost from the stem, Michaletz *et al.*, 2012). According to the Carbon Starvation Hypothesis, the mechanism believed to be responsible for inducing mortality is phloem necrosis which leads to root starvation, similarly to ring-barking. However, it can take months or even years for individuals to die through carbon starvation of the roots (Dickinson & Johnson, 2004), whereas it is often observed that post-fire mortality occurs within days or weeks following a low intensity fire (i.e. chlorotic foliage appearing rapidly) (Paysen & Narog, 1993; Balfour & Midgley, 2006; Midgley *et al.*, 2011). It has been suggested that such a rapid mortality following a low-intensity fire is more likely due to hydraulic failure in the plant rather than necrosis of vascular cambium and phloem, and is referred to as the "Hydraulic death hypothesis" (Balfour & Midgley, 2006; Kavanagh *et al.*, 2010; Michaletz *et al.*, 2012).

For example, Midgley *et al.* (2011) showed that 62.2% of the fire-resistor *Leucospermum conocarpodendron* individuals that were exposed to a low intensity fire and subsequently died, all passed within 10 days after the fire event. In order to demonstrate that the cause of death was hydraulic failure rather than cambium necrosis, they ring-barked live terminal branches of some of the individuals that survived and returned 3 months later. They found that none of the ring-barked individuals had died during the 3 months after the fire, and concluded that the cause of death was clearly not loss of the cambium but rather due to progressive dehydration as a consequence of damage to the plant hydraulics. Interestingly, several 15-20m tall *Pinus pinea* individuals that co-occurred with the *L. conocarpodendron* on the study site also died rapidly following the fire event, even though their canopies were not directly exposed to any flames at all but rather the fire-plume only.

It has been proposed that hydraulic death occurs due to a reduction in xylem conductance, which can be as a consequence of both drought and fire-induced stress. A reduction in xylem conductance limits the amount of water that the photosynthetic organs receive and if the reduction is large enough, it can lead to mortality due to water stress (Sperry & Hacke, 2004; Paysen *et al.*, 2006). This suggests that water-stress would be the ultimate cause of death for plants exposed to fire, however, the mechanisms driving mortality are speculative with little experimental evidence to support them. Recently, there have been a number of proposed mechanisms that can explain how the hydraulic conductance of trees can be both directly and indirectly reduced following a fire, via cavitation or physical damage to the vessels. These mechanisms provide further evidence in support of the hydraulic death hypothesis, however, their relative importance and contribution to tree mortality is poorly understood. By testing the effect and discussing the relevance of these mechanisms, it might help explain why some trees die in low intensity fires, even when not directly exposed to flames.

Indirect reduction in hydraulic conductance: Plume-induced cavitation.

Using a hydraulic conductance model, Kavanagh *et al.* (2010) demonstrated theoretically that the hot plume generated above a surface fire can cause significant strain on xylem vessels through localized changes in the atmosphere water composition and thus demand. As the fire-plume consists of hot dry air, it creates conditions of high vapor pressure deficit (VPD) in the canopy, which leads to rapid water loss through the leaves and petioles. This in turn causes increased tension in the xylem water column, resulting in cavitation (abrupt phase change to vapor) or embolism through air seeding (Cochard, 1992). This would indirectly result in reduced xylem conductance, as embolised vessels would no longer conduct sap (Sperry *et al.*, 2003).

The hydraulic conductance model uses a number of variables (soil water potential, stomatal conductance, vapor pressure deficit and specific leaf xylem conductance) to predict the xylem water potential, which can then be related to their relevant vulnerability curves in order to estimate the degree of xylem cavitation. Their results showed that high intensity fires cause foliar necrosis through cavitation up to 30m high in lodge pole pine and western larch when wind was absent. At lower intensities, cavitation varied with species and with wind speed but was still significantly high. It was also noted that while stomata are known to close in compensation to water stressed conditions in order to prevent increased water column tension (Barnard & Ryan, 2003; Brodribb &

Holbrook, 2006), the response is slow and with the hot plume being introduced to the canopy rapidly in wildfires, it is unlikely that stomata would be able to respond in time to prevent cavitation of vessels . However, even if stomata were able to compensate with a rapid response, it is very likely that a significant amount of water would still be lost through leaf cuticles under these high VPD conditions (Scoffoni *et al.*, 2014).

While one might expect this overwhelming loss of control over leaf water-loss to cause rapid runaway cavitation throughout the plant, this is not necessarily the case and there is strong evidence in support of the vulnerability segmentation hypothesis as a mechanism for preventing this (Tsuda & Tyree, 1997). The theory of vulnerability segmentation argues that the separation of xylem-damaged tissue may protect the main bole from irreversible loss and relates to the concept that leaves and petioles function as safety valves (Chen *et al.*, 2009; 2010). Previous studies have demonstrated support for the 'safety valve' hypothesis and it has been shown that plants preferentially cavitate xylem in more expendable branches, thus improving the likelihood of maintaining functioning xylem in the main bole (Tyree & Sperry, 1989). This would imply that a high level of vulnerability segmentation would be beneficial to trees that have been exposed to fire and are likely to suffered significant cavitation.

While it has been shown theoretically that vessel cavitation induced by the high VPD condition in a wildfire plume can indirectly reduce xylem conductance, this has not been shown experimentally and so its biological relevance is not fully understood.

Direct reduction in hydraulic conductance: Xylem deformation and surface tension.

In a set of experiments, Michaletz *et al.* (2012) demonstrated that by heating stems to 65°C and 95°C there was a significant reduction in xylem conductivity compared to controls. They suggested that the reason behind the reduction was due to changes in sap surface tension as well as xylem deformation. Sap surface tension is inversely proportional to temperature and decreases *c.*22% between 0 and 100°C (Vargaftik *et al.*, 1983). Therefore, vulnerability to cavitation would increase in fires as temperatures rise. This was demonstrated by perfusing branch segments with an ethanol-water mixture with a surface tension equal to that of water at 95°C. When branches were treated this way, there was an 11-21% increase in cavitated conduits, due to changes in surface tension alone, compared to control branches treated with water only.

They were also able to demonstrate xylem deformation, which is when the conduit walls and diameters are altered due to heat, making them less efficient at water transport. This was done by placing air-pressurized branch segments in water baths at 65°C and 95°C and subsequently measuring xylem conductance. They found that heat treated branches had significantly lower xylem conductance than controls and also provided qualitative evidence of xylem deformation using microscopy, where vessels were photographed in deformed states.

Xylem deformation can be attributed to the visco-elastic nature of the polymers contained within the cell walls, with lignin being the most relevant and abundant (Michaletz *et al.,* 2012). These lignin polymers have both the properties of viscous fluids and elastic solids, depending on the temperature, with the viscous state occurring between 60°C and 90°C (Michaletz *et al.*, 2012). Heating causes the glass-plastic transition state to be reached and lignin becomes plastic (above 60°C) but when cooled again the conduit returns to its glass state and remains in a deformed condition. This can lead to a reduced xylem conductance due to increased vulnerability to air-seeding as a result of the inconsistent surface (Plavcová *et al.*, 2013) as well as smaller conduit diameter. It has also been shown that some vessels may even collapse (Lens *et al.*, 2013). For example, upon severe dehydration tracheid walls in pine needles can completely collapse, however, this is rapidly reversed upon rehydration (Cochard, 2004). It has been suggested that high sap tension due to high transpiration rates during fire could possibly enhance the degree of deformation of vessels due to the increased forces acting on the cell walls. This suggests that pre-fire water stress could possibly increase the deformation effect as the water column is already under high tension.

Recovery following direct and indirect reduction in hydraulic conductance:

In order to maintain overall hydraulic capacity, plants need to either reduce the chance of cavitation or they need to reverse its effect. It is well known that plants are able to recover from reduced hydraulic function (as a result of embolism) through the process of vessel refilling and there is evidence that the process can even occur on a daily basis in some plants (Johnson *et al.*, 2012). While refilling has generally been related to conditions where the entire vascular system of the plant can be pressurised using root pressure, it has also been shown that refilling is also possible during transpiration, when the xylem remains under tension (Zwieniecki, 2013). The precise mechanism behind vessel refilling under tension is a heavily debated topic, however, there is a general agreement that active transport of carbohydrates is involved (Zwieniecki, 2013). What needs to be mentioned is that while refilling allows recovery from drought, it is not known whether it is possible following fire disturbance and this needs to be addressed.

Assuming refilling is possible, while one might expect cavitation due to a fire plume (indirect reduction) to be reversible via active refilling, it is highly unlikely that deformed and damaged vessels (direct reduction) would be able to recover due to the permanent physical damaged sustained. Pre-fire soil water potential has also been hypothesized to play a noteworthy role in fire-induced tree mortality. In a recent paper by van Mantgem *et al.* (2013), they showed that pre-fire climatic water deficit (drought) was related to an increase in post-fire mortality in a number of conifer species in the Western USA. This is because in drought stressed trees, not only is refilling of vessels limited, but the water column is already under tension increasing the probability of cavitation (van Mantgem *et al.*, 2003; Kavanagh *et al.*, 2010).

While models predicting tree mortality based on hydraulic failure have been created, they only include the effects of indirect mechanisms on hydraulic conductance and do not include any direct effects (i.e. changes in surface tension and deformation) nor consider vulnerability segmentation. By combining both mechanisms in a model, it is possible to predict the relative contribution of each in causing hydraulic failure, which can then be compared to real data. This would be useful in helping to determine the physiological mechanisms involved in rapid post-fire tree mortality thus improving models as they are based on the understanding of underlying biophysical processes rather than relying on observable parameters (i.e. bole and crown scorch) that are rather indirect observations of the underlying mechanisms.

The goal of the investigation was to experimentally test for the effects of both indirect and direct mechanisms of reducing hydraulic conductance and then to explored them with a model. This allows us to identifying the causational mechanisms of post-fire mortality, as well as recovery, in an attempt to answer the question; how do trees die following a low intensity fire? It was predicted that hydraulic death is a consequence of failing recovery as well as anatomical traits and that *K. africana* would be more susceptible to hydraulic death than *E. cladocalyx*.

Methods:

Field collection:

Samples of *Eucalyptus cladocalyx* and *K. africana* were collected adjacent to the Department of Biological Sciences, University of Cape Town. Prior to sampling, stem water potential (Scholander Pressure Chamber) and stomatal conductance (Delta-T type AP4 porometer) was measured to ensure that the trees were not overly water-stressed at time of sampling. For each species, branches roughly 2m in length were removed from individual trees as this ensured that the segments were much longer than the maximum vessel length of each species. Branches were cut under water, in order to prevent accidental embolism, and then transported to the lab with the cut end submerged in distilled water.

Samples were kept in a phytotron chamber mimicking late summer conditions (14 hour day, 25°C day and 15°C night) where they remained for 24h in order to acclimatize to the same initial conditions. After the 24h elapsed, water potential and stomatal conductance was re-measured in order to confirm whether the phytotron acclimatization routine was effective. Branches that were actively transpiring with minimal water stress were used for further experimentation.

Xylem conductance:

Whole shoot conductance measurements were made following the methods of (Kolb *et al.*, 1996). Briefly, shoots were defoliated underwater by removing the lamina at its junction with the petiole leaving as much of the petiole intact as possible. This ensured that even the most distal portions of the xylem vessels, which may be most prone to hydraulic failure, were incorporated into the measurement. The base of the shoot was then fitted via tubing to a beaker containing a supply of 0.01M KCl in reverse osmosis (RO) water, which had been filtered to 0.22μm. The whole shoot was then inserted into a cylindrical vacuum chamber with only the base of the stem with the tubing protruding out while ensuring an airtight seal by using a diameter-adjustable rubber connector. Tubing was connected to a reservoir of KCl on an electronic balance that was linked to a computer which automatically logged balance values every ten seconds. Once the shoot was inserted into the chamber, a partial vacuum was pulled on the chamber that pulled water from the reservoir on the balance through the xylem, all while logging the flow rate by dividing the change in the reservoir mass by time. By doing this, flow rate at different vacuum pressures can be recorded and a linear trend line's gradient will equal the xylem conductance (pressure-flow gradient). By dividing by leaf area, xylem conductance can be standardized to a leaf specific xylem conductance (K_{leaf}).

After initial xylem conductance was measured, air-emboli were removed by flushing branches with 0.22μm filtered, 0.01M KCl at 175 KPa for 1h. Cut ends were re-shaved before flushing to ensure a clean edge free from any possible debris or damage gained during the initial measurements. Stems were stored in distilled water in a 4°C cool room overnight between initial xylem conductance measurements and flushing procedures.

Maximum vessel length:

The maximum vessel length for both species was determined by perfusing pressurized air (0.1 MPa) into the proximal end of branch segments while placing the distal end into a bucket of water. The distal end was then progressively cut back by 1cm until bubbles could be seen streaming from the cut surface. Maximum vessel length was then assumed to be roughly the same length as the stem segment remaining.

Vulnerability curves:

Vulnerability curves for both *E. cladocalyx* and *K. africana* were determined using the pressure-sleeve method developed by. Briefly, 6 branch segments of each species were collected while ensuring that the segment length was longer than the maximum vessel length already determined for each species. Due to the growth form of *E. cladocalyx* as well as the limitations on segment sizes that are suitable for use in a pressure sleeve, it was not possible to obtain long enough stem segments that completely lacked foliage. This meant that there were at least some open vessels present in the samples, which possibly lead to a slight overestimation of the stems vulnerability to cavitation in *E. cladocalyx*. Segments collected were flushed for 1h using 0.01 M KCl at 175 KPa in order to remove any native emboli and therefore represent maximum conductance for the samples. Segments were cut at both ends and inserted into a pressure-sleeve attached to a standard Scholander pressure chamber. Bark was removed around the entire circumference of the stem portion inserted into the pressure sleeve to facilitate the entry of air into the xylem. The pressure sleeve was pressurized to a desired applied pressure for 10 minutes before allowing a period of relaxation. The purpose for the relaxation period was to ensure that air would not be outgassing from the cut end during water flow measurements as this could lead to an overestimation of the vulnerability, as the pressure gradient across the length of the stem is unequal. *K. africana* required roughly 3 minutes of relaxation time before measurements could be taken, however, *E. cladocalyx* required up to 3h of relaxation time in order for air to stop outgassing from the cut ends, especially at higher applied pressures (>3MPa). Once the appropriate relaxation time was allowed, the water flow through the sample was measured (by applying a pressure head) before applying the next pressure increment to the sample.

To test whether the vulnerability curves produced from the two species were significantly different from one another, curves were fitted to an exponential sigmoidal function (equation 1) in order to produce comparable slope parameters:

$$(1)\quad \mathrm{PLC} = 100/(1+\exp(a(\Psi-b)))$$

where Ψ is the water potential, PLC is the corresponding percent loss of conductivity and a and b are constants describing slope parameters (Pammenter and Van der Willigen, 1998). A student t-test was then used to determine whether the slope parameters (a and b) produced by the two species were significantly different.

Temperature-time curves:

In order to ensure that the treatments were reaching an effective temperature range (60°C to 90°C) within the given treatment duration, shoots were placed into an oven at 70°C and 100°C for 6 min and their internal sap wood temperatures was continuously measured using imbedded Type-T thermocouples connected to a data logger (Campbell Scientific CR10x). The stem diameter of the shoots were measured and shoots grouped into either large diameter (0.6-0.9 cm diameter) or small diameter (<0.6 cm diameter) classes.

Treatments:

Three treatments were designed in order to test for direct heating effects and VPD effects separately:

Control: No manipulation. After acclimation in the phytotron, shoots were defoliated and initial conductance was determined. The shoots were then flushed and their xylem conductance re-determined.

Oven: This treatment tested for the combined effects of both VPD and direct heating. After acclimation, shoots had their cut end wrapped with Parafilm™ and inserted into a beaker filled with polystyrene to ensure an airtight seal and no direct conductive heating of the stem through contact with the glass beaker. Shoots were then placed into a convection oven at either 70°C or 100°C for 6 minutes with all foliage still intact on the shoot. The oven was used as surrogate for hot, dry plume generated above a fire during combustion. Temperatures were determined from Michaletz *et al.* (2012). After the oven treatment conductance was measured then stems were flushed and re-measured, as for the controls.

Water bath: originally the treatment was attempted by painting leaves and petioles with clear nail polish before heat was applied, however, there was still a significant loss of water from the leaves and thus the treatment was unsuccessful. The solution was to place whole intact shoots into a hot water bath that was at either 70°C or 100°C. The water bath treatment ensured that no water could be lost from the stem segment during the 6 minute heating period and thus there would be no increase in the sap tension in the xylem during the treatment duration. This was done to ensure that only direct heating of the stem could contribute to a loss of conductance as emboli are impossible to form when submerged in water. After the oven treatment the shoots were defoliated and measured before stems were flushed and re-measured, as for the controls.

Statistical analysis: Significance between treatments was determined in R using a two-way ANOVA followed by a Tukey HSD post-hoc analysis.

Microscopy:

Two methods of microscopy were used to determine whether any physical damage occurred in the xylem vessels of both species when subjected to heating treatments.

Light microscope: Samples from each species as well as each treatment were sectioned into 6μm cross-sections using a microtome and then stained with toluidine blue O. Sections were examined at 40X and 100X (oil) using a Nikon Eclipse 50i Compound Microscope. A Nikon DS Camera Control Unit DS-U2 and DS-5M Camera head was used to capture images which were then processed using NIS Elements Documentation software.

SEM: Again, samples from each species and treatment were sectioned using a microtome, however, sections were not stained. Fresh samples could be used as there was no significant difference in resolution when samples were freeze-dried overnight compared to the fresh cuts. Sections were examined using a Phenom proX desktop scanning electron microscope and processed using an Elemental Identification (EID) software package.

Images captured using the compound microscope and SEM were chosen based on the maximum degree of deformation observed.

Hydraulic death model:

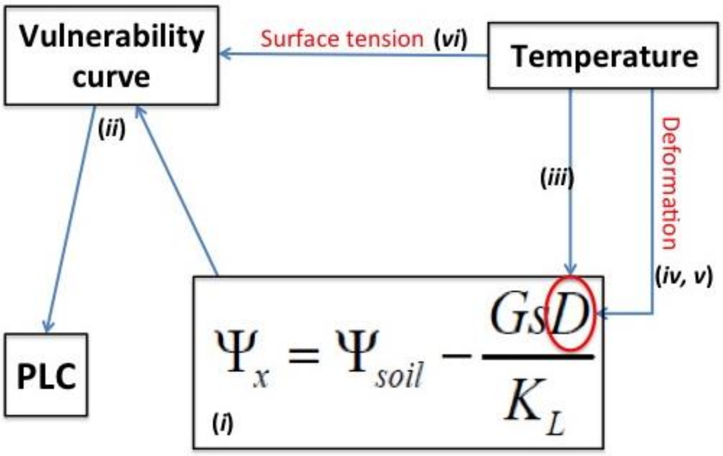

Figure 1: Schematic representation of model variables and effect interactions. Roman numerals in brackets refer to corresponding equations.

A model was developed in order to test the relative contributions of both direct and indirect mechanisms of reducing xylem conductance. The model was based on the hydraulic conductance model develop by (Bond & Kavanagh, 1999) (Figure 1*i*). The hydraulic conductance model was chosen as it can be used to predict the degree of sapwood cavitation in tree canopies based on the relationship between *D*, soil water potential, Gs and specific leaf conductance. This relationship can be described mathematically as:

(*i*) $\Psi_X = \Psi_S - GsD/K_{leaf}$

where Ψ_S is soil water potential (KPa), Gs is canopy stomatal conductance (mmol m^{-2} s^{-1}), *D* is the vapor pressure deficit (KPa) and K_{leaf} is leaf specific conductance (mmol m^{-2} s^{-1} MPa^{-1}).

Table 1: Values for variables used in model simulations for *E. cladocalyx* and *K. africana*. Units in text.

	Average non-stressed value					Vulnerability curve parameters	
Species	**Gs**	**K_{leaf}**	**Ψ_S**	***D***	**RH**	***a***	***b***
E. cladocalyx	0.3	6	-0.5	1	5	1.09	-1.90
K. africana	0.04	2	-0.5	1	5	1.33	-1.31

In order to predict the percentage loss of conductivity (PLC) for a stem at a given Ψ_X, the values produced in the model were related to the relevant vulnerability curves produced for each species (Figure 1*ii).* This was done by fitting the data to an exponential sigmoidal curve, based on the slope characteristics of vulnerability curves, using equation 1.

The variables in the model were assigned values based on what would be expected in a favorable non-stressed environment (Table 1), however, Gs was estimated by solving for the best fit in which the model predicted Ψ_X, and corresponding PLC, matches with the actual measured PLC at that Ψ_X (Figure 9). In order to create a temperature profile with which the model responds to, *D* values were generated based on the relationship between relative humidity and temperature (Figure 1*iii*):

(*iii*) $D = e_S - e_a$

where e_S is the saturation water vapour pressure and e_a is the actual water vapour pressure at a particular temperature and relative humidity. Relative humidity was based on data obtained from (Kremens *et al.*, 2003) where they measured temperatures up to 100°C with 5% relative humidity in a fire plume. By increasing temperature from 10°C to 100°C, a vapor pressure deficit profile as a function of temperature was created.

The model was then adjusted to include the direct phenomenon of xylem wall deformation and decreased surface tension as a function of temperature. Xylem wall deformation has been shown to affect the xylem conductance of a given stem and therefore, it was included in the model by adjusting K_{leaf} as critical temperature ranges were reached (Figure 1*iv*). These critical temperatures are between 60-90°C and reflect the thermal softening of lignin due to the transition between glass-like and gel-like states at these temperatures. Deformation effect for each species was included as a scalar variable which was directly derived from the experimental data in which deformation was quantified (i.e. water bath treatment). *E. cladocalyx* showed no evidence for xylem deformation and was excluded, however, *K. africana* did show evidence for deformation and PLC was measured to increase by ±43% between 65°C and 95°C. This was expressed logically in the model as:

(*iv*) Deformation scalar = IF temp<60 then K_{leaf} = 1, else IF temp > 95 then 0.43, ELSE (1- (m*t)+c)/100

where m and c are the slope and intercept of the linear regression, and t is the temperature.

(*v*) K_{leaf} = original K_{leaf} * ((100-PLC)/100) * deformation scalar

The effect of changing surface tension due to temperature was also included in the model (Figure 1*vi*). Surface tension is expected to influence the vulnerability curve as it increases the probability of cavitation at higher temperatures. Michaletz *et al.* (2012) have shown an 11-21% increase in cavitation due to changes in water surface tension alone, at 95°C. This was done by perfusing *Populus balsamifera* branch segments with 3.75% (w / w) ethanol (approximately the same surface tension as water at 95°C) and measuring hydraulic conductance. To incorporate this effect into our model, a linear regression was derived based on the results of Michaletz *et al.* (2012) and PLC was expressed as a function of a surface tension scalar. This was included in the model as a logical argument:

(*vi*) Surface tension scalar: IF temp<=20 then 1, ELSE if temp>95 then 1.16, ELSE 1+(m(t-20)+C)/100

where m and c are the slope and intercept of the linear regression, and t is the temperature in °C.

The model parameters were then adjusted to represent common curve parameters for leaves and petioles, which were derived from literature (Table 2). *a* and *b* were set at 1.5 and -1 respectively for both species. Data obtained from oven treatments at 70 and 100°C was then added as reference points against which the model could be compared. This was done by calculating the average oven treatment PLC using the equation:

(*vii*) PLC = $((K_{max} - K_{oven})/K_{max})$*100

where K_{max} is K_{leaf} of flushed controls and K_{oven} is K_{leaf} of oven treated stems prior to flushing.

Although samples were placed in an oven at both 70 and 100°C for 6 minutes, the maximum temperature of *K. africana* and *E. cladocalyx* stems only reached 65 and 80°C when exposed to the respective treatments (Figure 3). Taking this into consideration, the measured data reference points were adjusted to reflect actual temperatures reached rather than treatment temperatures.

Table 2: Common slope parameters of leaves and petioles obtained from literature.

Species	Type	*a*	*B*	Source
Austrocedrus chilensis	Leaves	1.15	-0.91	(Scholz *et al.*, 2014)
Nothofagus dombeyi	Leaves	4.05	-1.42	(Scholz *et al.*, 2014)
Acer saccharinum	Petioles	1.99	-0.50	(Tyree, 1997)
Vitis vinifera	Petioles	3.24	-0.95	(Zufferey *et al.*, 2011)

Results:

Vulnerability curves:

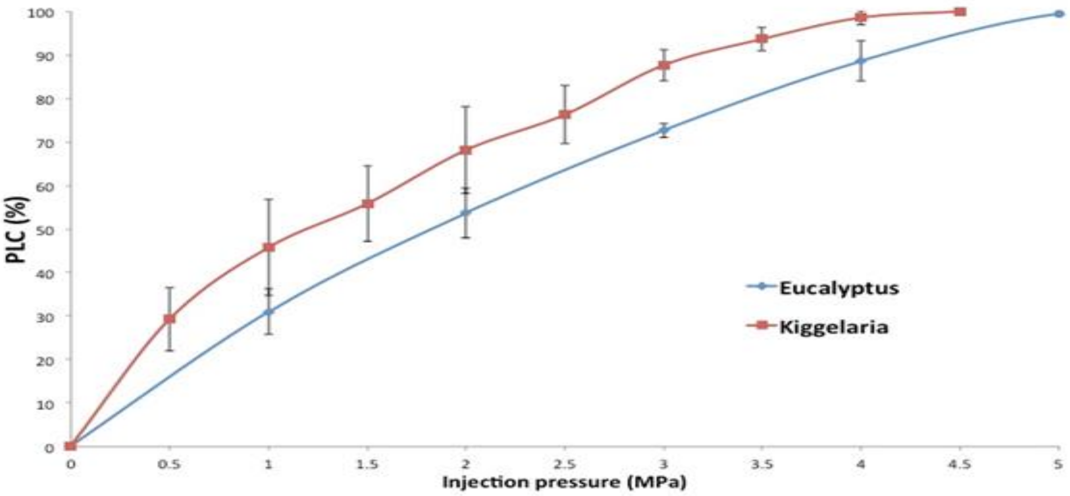

Figure 2: Mean (± 1 SE) percentage loss in hydraulic conductivity (PLC) as a function of injection pressure for stems of Kiggelaria and Eucalyptus.

Table 3: Measured vulnerability curve slope parameters (sigmoidal function) and maximum xylem vessel length for *E. cladocalyx* and *K. africana*.

Species	*a* (slope)	*b* (*P50*)*	Vessel length (cm)
K. africana	1.28	-1.19	56
E. cladocalyx	1.49	-1.92	61.5

*students t-test, p=0.055 at 95% confidence interval.

E. cladocalyx had a maximum vessel length of ±61.5cm while *K. africana* had a maximum vessel length of ±56cm. The vulnerability curves produced for both *E. cladocalyx* and *K. africana* showed no significant difference in both the slope (a) and P50 (b) of the curves when fitted to an exponential sigmoidal function (Figure 2, Table 3).

Temp-time curves:

Heating treatments resulted in xylem temperatures that exceeded the minimum target of 60°C for all species (Figure 3). At 70°C, xylem temperatures ranged between 61.2 and 64.4°C). At 100°C xylem temperatures ranged between 76.2 and 82.1°C. This confirmed that variations in bark thickness and related stem properties did not result in significantly different heating of the xylem.

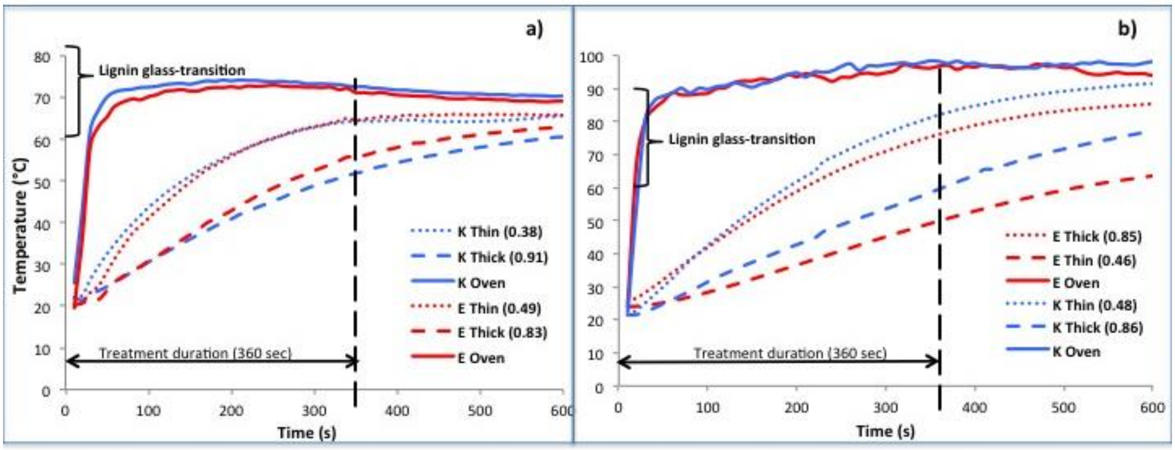

Figure 3: Temperature vs. time for both *E. cladocalyx* and *K. africana* stems of various thickness placed in an oven at a) 70°C and b) 100°C for 6 minutes. Treatment duration as well as lignin glass-transition temperature range (60°C-90°C) is included. Legend: "K" is *K. africana* stem temperature, "E" is *E. cladocalyx* stem temperature, "Oven" is the oven temperature, "Thin" is smaller diameter stem temperature and "Thick" is larger diameter stems temperature. Number in bracket indicates average stem diameter in cm.

Treatments:

E. cladocalyx:

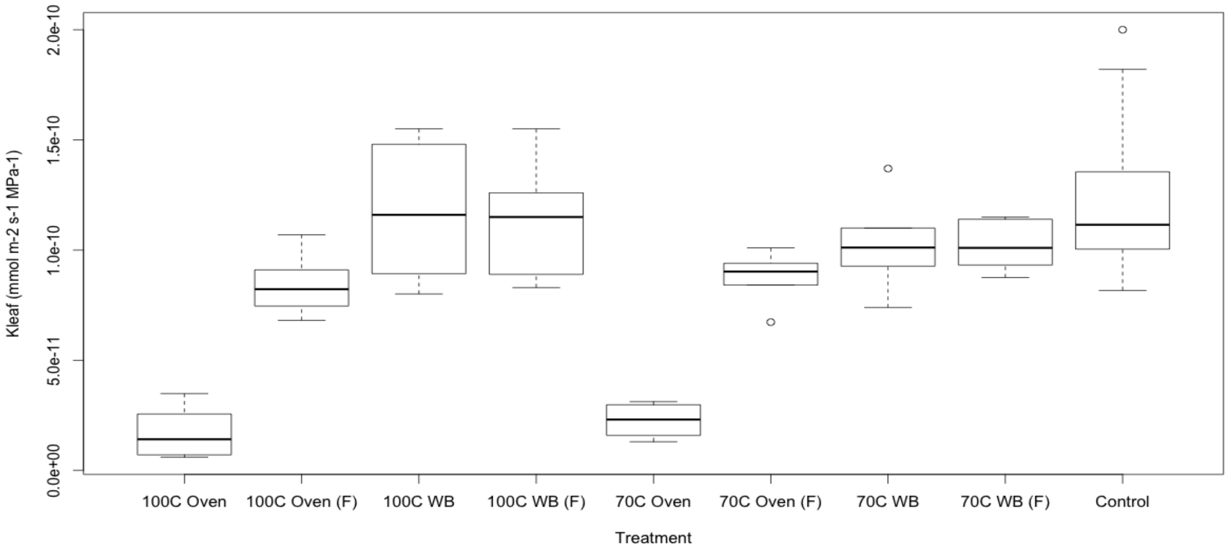

Figure 4: Box-plot representation of K_{leaf} for all treatments for *E. cladocalyx* stems. WB, water bath treatment.

There was no significant difference between *E. cladocalyx* stems treated as controls in both the 70°C and 100°C, nor was there a difference between control stems and control stems that were flushed (Figure 4). This allowed all control stems for the 70°C and 100°C as well as the flushed stems to be combined into one treatment called "control", thus improving the power of statistical analyses. For *E. cladocalyx*, the only statistically significant difference in treatment responses came from samples placed in oven treatments (Figure 4). Stems placed in both the 70°C and 100°C oven treatments showed a significant difference in K_{leaf} compared to controls ($p<0.05$ for both), with an oven treated stems having a much lower K_{leaf} than controls. The reduction in K_{leaf} for both 70°C and 100°C were similar in magnitude and were not statistically different ($p=0.99$). When 70°C and 100°C oven treated stems were flushed, the K_{leaf} returned back to values significantly similar to controls ($p=0.077$ and 0.03 respectively) indicating a full recovery to maximum conductance. Stems treated in water baths at 70°C and 100°C showed no significant reduction in K_{leaf} ($p=0.798$ and 1 respectively) and values remained similar to those of controls, including treated stems that were flushed after post-treatment.

K. africana:

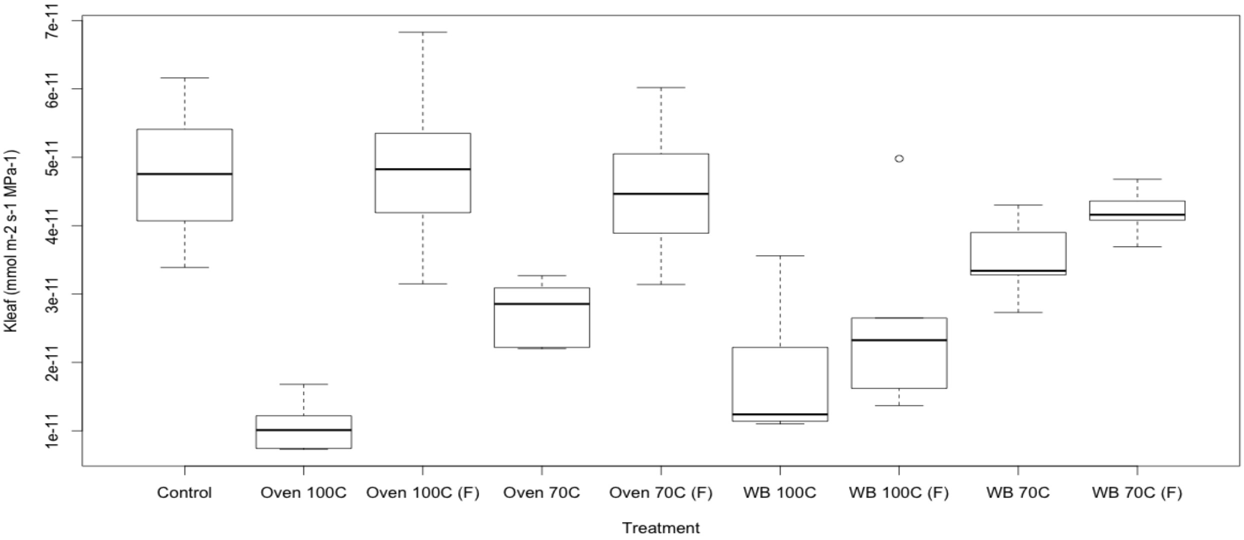

Figure 5: Box-plot representation of K_{leaf} for all treatments for *K. africana* stems. WB, water bath treatment.

Similar to *E. cladocalyx*, there was no significant difference between *K. africana* stems treated as controls in both the 70°C and 100°C, nor was there a difference between control stems and control stems that were flushed (Figure 5). This again allowed all control stems for the 70°C and 100°C as well as the flushed stems to be combined into one treatment called "control". Stems treated in the oven at 70°C showed a significant difference in K_{leaf} to that of the controls ($p<0.05$ for both treatments), however, the magnitude of K_{leaf} reduction was significantly greater in the 100°C treatment than the 70°C treatment ($p=0.03$, Figure 5). When these oven treated stems were flushed, the K_{leaf} returned to statistically similar values to that of the control (p=0.99 for both), indicating a full recovery to maximum conductance. Stems that were subjected to the water bath treatments at 70°C showed a slight reduction in K_{leaf} compared to controls, however, there was no real significant difference between the two treatments at 95% family-wise confidence level (p=0.094). When these stems were flushed there was no significant difference between unflushed and control stems (p=0.94 and p=0.99 respectively). Stems subjected to the 100°C water bath treatment showed a significant difference in K_{leaf} compared to controls ($p<0.05$) with a reduction in K_{leaf} statistically similar in magnitude to that of stems treated in the oven at 100°C (p=0.9). When these stems were subsequently flushed, there was no significant difference between the unflushed and flushed stems (p=0.8) and the post flush K_{leaf} remained significantly reduced compared to controls ($p<0.05$).

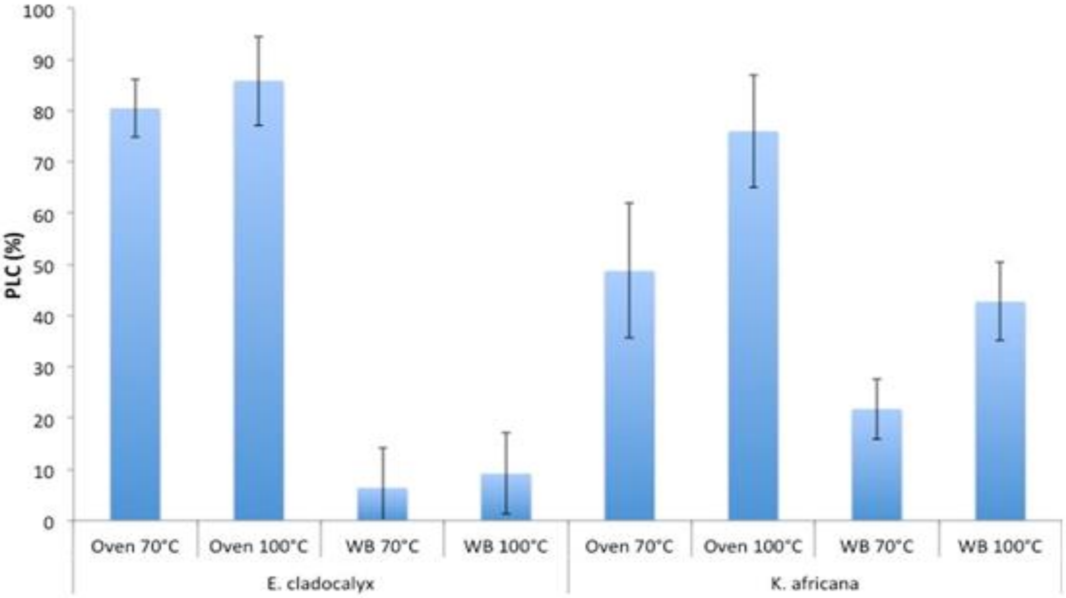

Figure 6: Average PLC (as a function of flushed controls) for *E. cladocalyx* and *K. africana* stems exposed to 70°C and 100°C in both the oven and water bath (WB) treatments. Error bars indicate standard error.

Microscopy:

Conduit wall deformation was visually determined in branches of *K. africana* and *E. cladocalyx* exposed to all treatments. *E. cladocalyx* showed no visual signs of deformation or conduit collapse and xylem of heat-treated stems did not look different to controls with well-defined walls in both cases. Stems of *K. africana* that were treated in the oven also showed no significant xylem deformation, however, stems treated in the water bath showed significant damage and deformation compared to controls, especially in the 100°C treatment. This can be seen as the vessel walls are not as well defined as controls and vessel lumens seem to be irregular in shape.

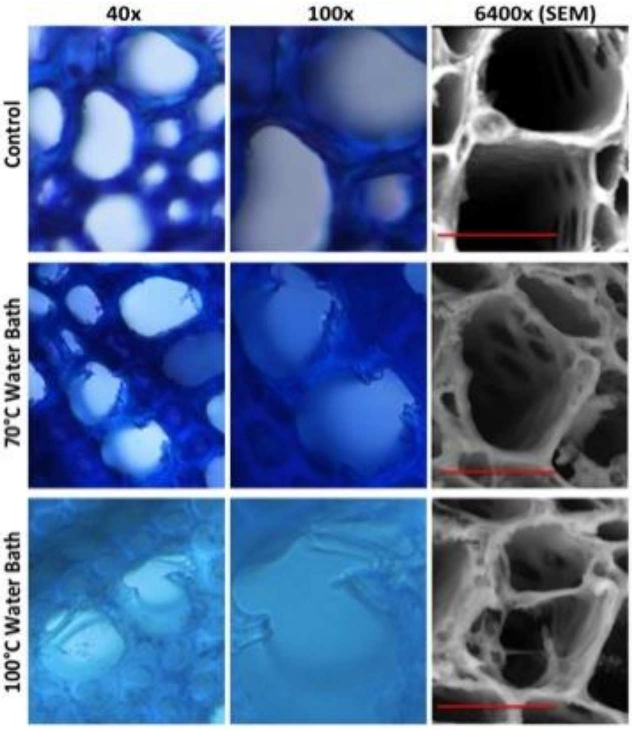

Figure 7: Xylem cross-sections of *K. africana* stems for control, 70°C and 100°C water bath treatments. Bars, 20µm for SEM only.

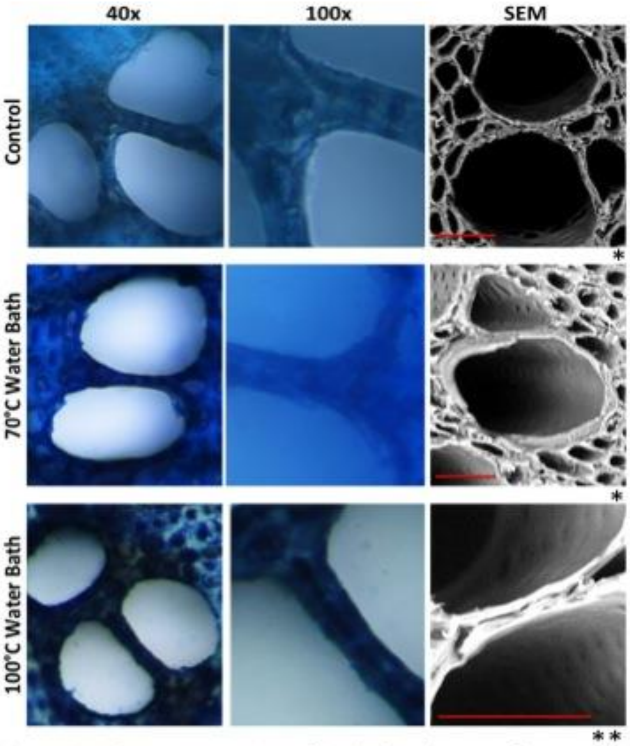

Figure 8: Xylem cross-sections of *E. cladocalyx* stems for control, 70°C and 100°C water bath treatments. Bars, 20µm for SEM only. * 1100x, bar = 30µm **2600x, bar = 25µm

Hydraulic Death Model:

The model was run using curve parameters derived from the measured stem vulnerability curve as well as leaf and petiole vulnerability curve parameters estimated from literature.

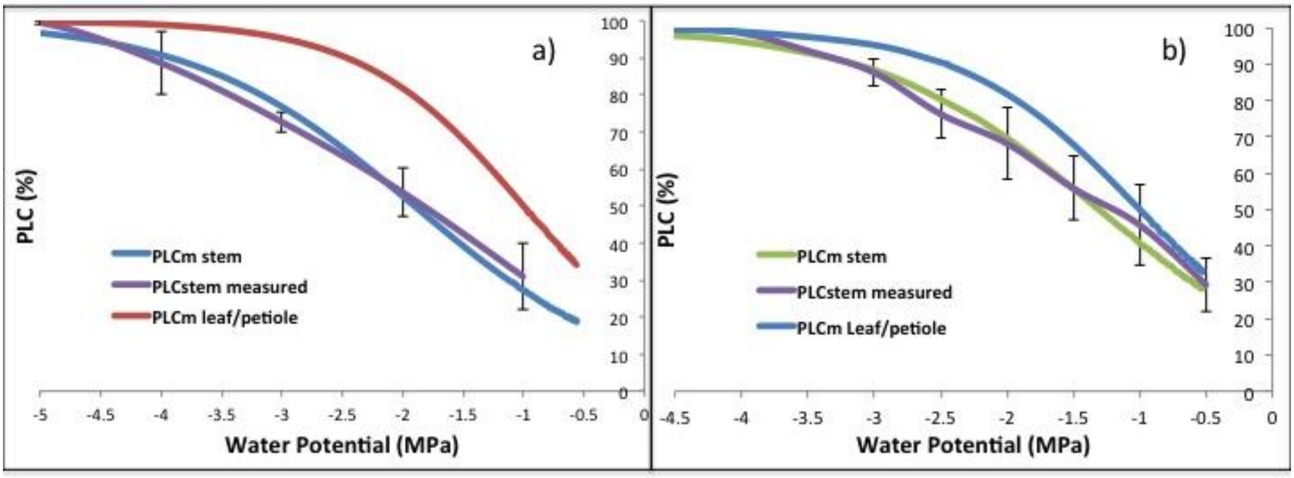

Figure 9: Measured stem vulnerability curves (PLCstem measured ±SE) as well as model fit based on stem (PLCm stem) and leaf/petiole (PLC leaf/petiole) estimated vulnerability curves for; a) *E. cladocalyx* and b) *K. africana*.

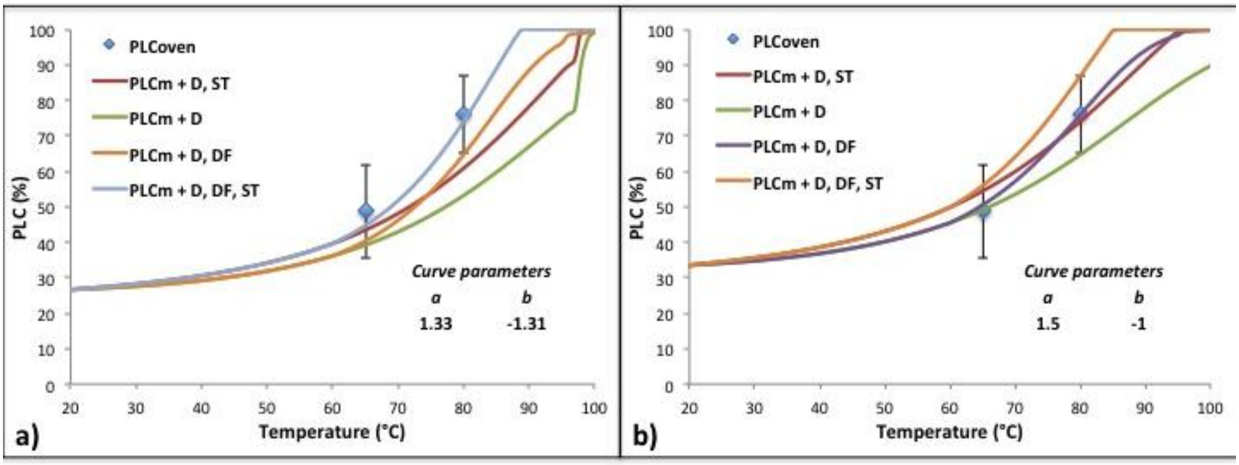

Figure 10: Multiple effects model simulations of temperature vs. PLC for *K. africana* a) stem and b) leaf/petiole curve parameters. Legend: "PLCoven" represents actual measured PLC ±SE and "PLCm" represents model predicted PLC as a function of temperature. "D" is VPD effect, "ST" is surface tension effect and "DF" is xylem deformation effect.

K. africana:

Measured vulnerability curve and fitted model curves were similar and showed a good fit (Figure 9). When the measured stem vulnerability curve parameters for *K. africana* were used, while there was good fit with expected PLC at 65°C there was a common underestimation of the expected PLC at 80°C (Figure 10). However, the effects of surface tension, deformation and VPD induced cavitation (*D*) showed the strongest fit with the expected data, even at 80°C. The largest discrepancy between expected PLC and model simulation was with the underestimation of the effect of VPD induced cavitation only.
When the estimated parameters for leaves and petiole were used in model simulations there was a much better fit to the expected PLC at 65 and 80°C (Figure 10). While the effect of VPD only showed the largest underestimation of expected PLC, the combination of VPD and deformation as well as VPD and surface tension all showed strong agreement with the expected PLC at both 65 and 80°C (Figure 10).

E. cladocalyx:

Measured vulnerability curve and fitted model curves were similar and showed a good fit (Figure 9). When the measured stem vulnerability curve parameters for *E. cladocalyx* were used, there was a significant underestimation of expected PLC in all effect simulations except the combination of VPD and surface tension only, which showed strong agreement at 80°C only. When the estimated parameters for leaves and petiole were used in model simulations there was a much better

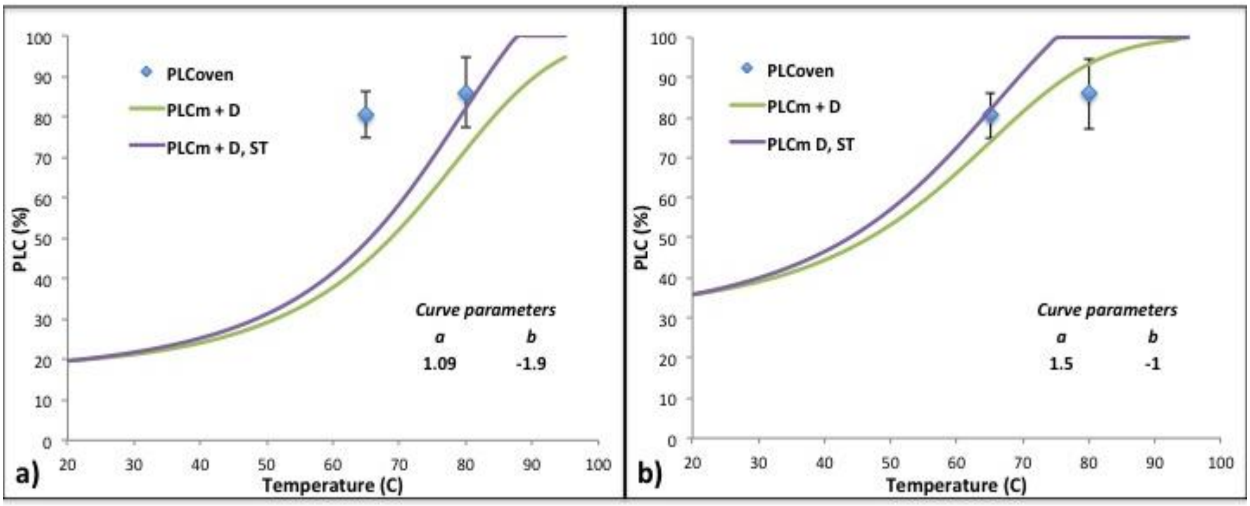

Figure 11: Multiple effects model simulations of temperature vs. PLC for *E. cladocalyx* a) stem and b) leaf/petiole curve parameters. Legend: "PLCoven" represents actual measured PLC ±SE and "PLCm" represents model predicted PLC as a function of temperature. "D" is VPD effect, "ST" is surface tension effect and "DF" is xylem deformation effect.

fit to the expected PLC at 65 and 80°C (Figure 11). Both the simulations of VPD only as well as VPD and surface tension in combination showed very similar expected PLC and were significantly similar to the expected data at 65 and 80°C

Discussion

The hydraulic death hypothesis argues that post-fire tree mortality is due to the failure of the hydraulic systems integrity, resulting in a reduction in xylem conductance. It implies that fire-induced drought-stress is the ultimate cause of mortality rather than cambium necrosis. In this investigation, we demonstrate both experimentally and through model exploration that there are at least two mechanisms in which xylem conductance can be reduced following a fire. The first is plume-induced cavitation in which a fire-plume passing through a canopy indirectly causes xylem cavitation due to a strong water gradient between the foliage and plume. The second mechanism is xylem deformation in which conduit walls and diameters are directly altered during heating, making them permanently less efficient at water transport.

Indirect reduction in hydraulic conductance: Plume-induced cavitation.

Plume-induced cavitation was shown to occur in both *E. cladocalyx* and *K. africana* stems when exposed to 70°C and 100°C in the oven (Figure 4 and 5). This is evident because when these stems were flushed after the treatment, their conductance fully recovered to rates significantly similar to that of the controls. The fact that the reduction in conductance was recovered, via flushing and removal of air emboli, implies that the physical structure of the xylem remained unaltered and that cavitation was the cause of the initial reduction.

Although both species displayed the effect, what is interesting to note is that *E. cladocalyx* showed significantly more cavitation at 65°C than *K. africana* (80.5 and 48.8% respectively). It can be argued that while *K. africana* was able to limit cavitation at lower temperatures, in comparison *E. cladocalyx* seems to have significantly more vulnerable xylem. This is perhaps surprising at first because the stem vulnerability curves for *E. cladocalyx* and *K. africana* are significantly similar in curve characteristics (Table 3) and one would expect relatively similar responses. While it is possible that a higher leaf area would allow a greater rate of water loss and thus cavitation, both *E. cladocalyx* and *K. africana* had similar average leaf areas for stems treated in the oven (363.67cm^2 and 385.64cm^2 respectively). Perhaps what this rather implies is that *E. cladocalyx* has greater vulnerability segmentation between the leaves and the stem compared to *K. africana.*

Do leaves and petioles act as safety valves during a fire event?

The idea of vulnerability segmentation was first proposed by Tyree & Sperry (1989) and denotes the fact that leaves and petioles tend to be more vulnerable to hydraulic loss than stems (Chen *et al.*, 2009; Bucci *et al.*, 2012). The concept relates to the idea that leaves function as safety valves in order to protect the stem from hydraulic loss and many studies have demonstrated support for the hypothesis (Chen *et al.*, 2009; 2010; McCulloh *et al.*, 2011; Johnson *et al.*, 2012). For example, in a study by Bucci *et al.* (2012), they showed that leaves of a number of *Nothofagus* species were more vulnerable to embolism than the stems to which they were attached. The explanation for this was that, under prolonged drought, the reliability of the more expensive woody portion of the water transport pathway can be maintained at the expense of the more replaceable portions (i.e. distal stems and leaves). Leaves can be shed if leaf water potential reaches a threshold value and this would restrict water-loss (i.e. transpiration decreases), thereby maintaining the integrity of the stem.

Taking this into consideration, it was noted that the vulnerability curves produced for each species were created using large stem segments rather than entire intact distal shoots (which include petioles and leaves). According to the vulnerability segmentation hypothesis, this should lead to a discrepancy between the measured and actual vulnerability that leaves and petioles would demonstrate. Because of this, the hydraulic death model curve parameters (*a* and *b*) were adjusted to fit the measured data. A range of probable parameter values was determined from literature (Table 2) and when leaf and petiole limits were used, there was much stronger agreement between the model and the measured data (Figure 10 and 11). This means that the model agrees with the proposal that there is strong vulnerability segmentation between the leaves and stems in both species. However, the model also shows that *E. cladocalyx* has greater vulnerability segmentation compared to *K. Africana* and when we examine the model simulations, we can see that the difference between the stem and leaf/petiole results is more pronounced in *E. cladocalyx* (Figure 11). Due to the recent "open vessel" artifact associated with the air-injection method for determining xylem vulnerability (Choat *et al.*, 2010), it is possible that the measured stem vulnerability is an overestimation of the actual vulnerability. If this were the case, it would imply an even greater degree of vulnerability segmentation between the stem and leaves, especially in *E. cladocalyx*.

Direct reduction in hydraulic conductance: Xylem deformation.

Deformation of xylem conduits was only shown to occur in *K. africana* stems exposed to 100°C in the water bath treatments while no *E. cladocalyx* stems from any treatment showed signs of deformation (Figure 7 and 8). *K. africana* stems placed in a 100°C water baths showed a significant reduction in xylem conductance following the treatments, however, when treated stems were subsequently flushed, the conductance remained significantly similar to the treated stems and did not recover to levels similar to controls (Figure 5). This is not surprising as the water bath treatment was designed to exclude any water loss from the stem and therefore cavitation was not possible. The permanent nature of the reduction implies that the xylem vessels were physically damaged as a consequence of the heat applied during the treatment duration, and subsequent microscopy reveals that conduits were indeed deformed. Intriguingly, while stems of *K. africana* that were exposed to 70°C showed no significant difference in conductance compared to control (Figure 5), there was a slight increase in PLC (Figure 6) and examination of their xylem revealed that there were indeed signs of deformation in their xylem walls. However, it was to a lesser extent than stems heated to 100°C and SEM images confirm that the vessel walls remain relatively smooth and intact in comparison (Figure 7). Pit-pore shape also appeared to be unchanged in heating treatments which is expected and it is also shown in Michaletz *et al.* (2012) and pictured in figure 7 and 8 (SEM). An important consideration to be taken, however, is that the deformation pictured in figure 7 is of the "worst-case" scenario, in which the maximum degree of deformation that was observed is shown. Moreover, images are of petiole cross sections and it is likely that they would have been more vulnerable to deformation than wooded stems as would heat up faster.

What is interesting to note about *K. africana* is that there is no sign of deformation when stems are treated in the oven, even at 100°C, while stems placed in a 100°C water bath show significant signs of deformation (Figure 5). Why then do we not find any deformation in the oven treatment but we do in the water bath? While the water bath treatment is expected to raise the internal temperature of the stems faster than the oven would, stems placed in the oven reach temperatures that are well within the range of thermal softening of lignin. On top of this, whole intact branch segments that included leaves and petioles were used in the treatments, and they would have heated up considerably faster. We can see evidence for this in the temperature curves for the oven treatments where stems of both species that had a diameter of 0.4cm heated up considerably faster than stems twice the size (Figure 3). This means that it is unlikely that maximum temperature and duration are the cause of the difference.

A second consideration to be taken is that while there was no negative tension created in the xylem vessels during the water bath treatment, it is likely that stems would have contained a certain amount of native embolism. When bubbles of gas are heated, they expand in order to keep the internal pressure constant, just like a balloon (i.e. PV = nRT). This means that when air emboli are heated within xylem stems, they will also expand accordingly (Arefmanesh & Advani, 1991). Provided the vessel is intact and able to be pressurised, it is likely that the expansion of emboli could spread to adjacent vessels. With functioning pit-pores, this could possibly lead to the formation of positive pressure within the air filled vessels due to resistance.

The final consideration is that transpiration and cavitation were able to reduce the temperature that the oven treated stem’s reached compared to stems treated in the water bath. This is because water moving through the xylem as a consequence of rapid transpiration would have carried heat with it as it moved up the stem and out the leaves. Water has a very high thermal capacity and latent heat of vaporization, which makes it a very good transporter of thermal energy (Weast *et al.*, 1988). For example, Wesolowski *et al.* (2014) demonstrated a “cooling effect” in which the periderm slows down the heat transfer into the cambium during a fire simulation. The cooling effect was related to high bark moisture allowing greater evaporative cooling and was shown to vary between *Eucalyptus* species. However, these properties also have a contradictory effect as the same high bark moisture content can increase conduction and storage of heat in. As a result, high moisture content of bark may effect in reduced fire resistance as stem to stay hotter for longer (Lawes *et al.*, 2011). This makes it difficult to understand the role of bark moisture as well as capacitance and whether it is likely to increase or decrease the heating effect. It is also possible that cavitation acts to reduce heat transfer as air is considerably less efficient at storing and transferring heat (Weast *et al.*, 1988). This means that embolised vessels would in fact heat up slower.

Mechanism of deformation prevention/reduced heat transfer:

When considering the best explanations for the observed differences in deformation, both between treatments and species, the evidence suggest that a chain of events act as a mechanism for preventing the onset of deformation. During a fire event, heat is rapidly lost along with water through the leaves, and before very high tension in the water column is reached, cavitation occurs (Hacke & Sperry, 2000; Sperry *et al.*, 2003; Jacobsen, 2005). If tension is relieved before the xylem walls reach high enough temperatures to become plastic, it is possible that the extent of deformation would be reduced compared to individual who maintain tension in the column for longer. On top of this, once a vessel becomes embolised via cavitation, the embolism expands and fills other conduits, inevitably displacing sap with air. Air-filled vessel would not only heat up slower and less effectively, but also retain heat for shorted periods than sap filled vessels would. There is also the possibility that a slight pressurization of intact vessels could ameliorate the onset of deformation. It is clear that both species were able to maintain positive pressure within vessels as stems took some time to relax after air-injection treatments (outgassing from cut ends).

Taking both VPD-induced cavitation and deformation into consideration, it would seem that cavitation can play a protective role for plant hydraulic systems exposed to high temperatures. Is this perhaps indication for a role of vulnerability segmentation in post-fire persistence? Further evidence for the protective role of cavitation emerges when we consider the model simulations.

When looking at the model predictions in which leaf/petiole vulnerability are used (Figure 10b and 11b), we find that both species have different outcomes for the added effects. While *E. cladocalyx* shows the strongest agreement with predicted data when including VPD effect only (Figure 11b, PLCm +D), *K. africana* shows the strongest agreement with the effect combination of VPD and heat induced deformation (Figure 10b, PLCm + D, DF). In other words, the model predicts that *K. africana* experienced reduced hydraulic conductance as a result of VPD induced cavitation as well as heat induced deformation, whereas, *E. cladocalyx* lost conductance mostly as a result of VPD induced cavitation. Although deformation was not found in *K. africana* stems placed in the oven, the model suggests that deformation is relevant and it is interesting that between the two species, it is the one that has the lower degree of vulnerability segmentation which demonstrates it. Although the lack of evidence from the oven treatments, by maintaining a higher degree of functioning vessels *K. africana* is likely to retain tension in vessels for longer resulting in prolonged stress on walls. The high degree of segmentation in *E. cladocalyx* caused vessels to cavitate rapidly in the oven, thereby relaxing tension. When considering these robust agreement, the model provides strong support for the protective role that cavitation has been proposed to play in this investigation. Perhaps longer treatment durations in the oven might show signs of deformation in *K. africana*, however, this would probably need to be done *in vivo* with trees that have their entire hydraulic system intact, as tension could be maintained for longer.

The effect of surface tension coupled with VPD induced cavitation also shows relatively strong agreement with measured data, however, *E. cladocalyx* predictions were considerably over estimated at higher temperatures (Figure 10b and 11b, PLCm + D, ST). When both surface tension and deformation were coupled to VPD, *K. africana* also shows an overestimated prediction. While the effect of changing surface tension with increasing temperature has been shown to increase the vulnerability of xylem to cavitation (Michaletz *et al.*, 2012), it needs to be questioned whether the method used (ethanol-water perfusion) was perhaps slightly derived, and it appears that the effect is not as important as previously proposed.

Together, evidence from the treatments and model suggest that it is both the spatial and temporal nature of cavitation that could determine the extent of deformation experienced. But this does not explain why *E. cladocalyx* shows no signs of deformation in the water bath treatment. Because the treatment insured cavitation was prevented as well as the fact that stems of both species reached similar temperatures for similar durations, it is unlikely that bark properties (such as thickness and moisture content) can explain the lack of deformation. Considering that both *E. cladocalyx* and *K. africana* experienced the same conditions, it is most likely that the lack of deformation in *E. cladocalyx* is due to its vessel characteristics. When examining the wood anatomy of the two species. *K. africana* has relatively hard wood with a density of about 845 kg/m^3 (Munalula & Meincken, 2009) while *E. cladocalyx* has less dense wood of about 744kg/m^3 (Martorell *et al.*, 2013). Wood density has been shown to influence water storage capacity, hydraulic conductivity, resistance to drought-induced embolism as well as growth rate. For example, (McCulloh *et al.* 2012) demonstrated that the higher wood density of *Miconia argentea* was associated with smaller diameter vessels and fibers, more water stress-resistant leaves and stems, and it had roughly half the capacitance of the a lower wood density species, *Anacardium excelsum*. When we examine the xylem vessels of both species, we find that *E. cladocalyx* has significantly larger vessel diameters and much thicker vessel walls compared to *K. africana* (Figure 7 and 8).

What this implies is that larger diameter vessels with thick strong walls are more suited to preventing deformation. Perhaps this is because more sap and thus heat would be moved away from the xylem via rapid transpiration, vessels would cavitate sooner relieving tension, and more air would fill vessels. Further, thicker vessel walls are considerably stronger and would not only resist heat damage for longer but are also less likely to collapse (Hacke *et al.*, 2006). This may provide an explanation for the remarkable resilience to heat induced deformation that *E. cladocalyx* displayed. An important thing to note is that these characteristics can slow the rate of heat transfer into the stem and would not only be beneficial for preventing deformation, but also for preventing cambium and phloem necrosis.

The hydraulic death hypothesis:

While it is clear that fire does indeed cause significant cavitation and possibly even deformation in some species, why are some plants able to survive while others succumb to these effects? The answer may be revealed when considering the unique anatomy and life histories of the two species, as well as the consequences of having high degree of vulnerability segmentation.

While both species have similar vessel lengths (Table 3), *E. cladocalyx* has significantly larger vessel diameters (Figure 7 and 8). According to the safety versus efficiency trade-off hypothesis (Tyree *et al.*, 1994; Tyree and Sperry, 1989), larger vessels are generally more susceptible to cavitation. This is because they have a larger surface area which has been shown to correlate with an increasing pit membrane area per vessel, and thus increased vulnerability to cavitation (Hacke *et al.*, 2006; Wheeler *et al.*, 2013). As a consequence of its highly vulnerable xylem, *E. cladocalyx* will often experience large amounts of embolism in distal shoots during times of drought (Martorell *et al.*, 2013). While this may temporarily reduce conductance, it is common for plants to go through cycles of embolism formation and recovery throughout the day (Johnson *et al.*, 2012). The occurrence of active vessel refilling has been observed in the bole and distal parts of many species (Hao *et al.*, 2013). It has been shown that eucalypts are particularly good at refilling vessels and in a study by Martorell *et al.* (2013), they demonstrated that the stem hydraulic conductance of *Eucalyptus pauciflora* seedlings exposed to severe drought managed to recovered to control levels within 6 hours after re-watering, suggesting an active mechanism of embolism repair. This might explain why *E. cladocalyx* is able to survive well during extended periods of drought, despite the apparent vulnerability of the distal shoots, by using a combination of strong hydraulic vulnerability segmentation as well as an extremely effective mechanism for refilling cavitated vessels. While refilling has not been shown conclusively to occur post-fire, it is very likely that it is possible and there is evidence that *Adenostoma fasciculatum* seedlings are able to reverse some fire-induced embolism if rainfall occurs soon after the fire (Williams *et al.*, 1997).

Perhaps it is the ability to refill cavitated vessels that determines whether hydraulic death occurs in trees exposed to fire? It has recently been demonstrated that declining carbohydrate reserves during drought could cause hydraulic failure through impaired vessel refilling (McDowell, 2011; Sevanto *et al.*, 2013). This is because carbohydrates may play an important role in the refilling of embolised conduits (Secchi & Zwieniecki, 2011), and if they cannot be transported to sites where they are needed, refilling could fail. Perhaps more importantly is that refilling requires water. Hydraulic isolation limits xylem water reserves and refilling one conduit could just lead to cavitation of an adjacent one. This could exhaust carbohydrate reserves, but would not lead to additional loss of conductivity unless water leaks outside the initial apoplast-symplast volume. Thus, mortality of living cells associated with carbon limitations could temporarily lead to additional water available for refilling elsewhere in the plant. However, if cell mortality promotes leakage outside the plant (such as through heat-damaged leaves, bark or roots), it could lead to rapid hydraulic failure (Sevanto *et al.*, 2013).

In accordance with this, it has been shown that pre-fire climatic water deficit is related to an increase in post-fire mortality (van Mantgem *et al.*, 2013). Over the past 20 years, there has been an increasing frequency of climate-related continental-scale forest mortality events (Allen *et al*. 2010). These events are associated with drought and have been observed in tropical rainforests (Phillips *et al*. 2009), temperate mountainous and Mediterranean forests (van Mantgem *et al*. 2009; Carnicer *et al*. 2011) as well as boreal forests (Peng *et al*. 2011).
The explanation for this was that hydraulic failure was probably related to an increased susceptibility to cavitation coupled with the inability to repair damaged vessels as a result of water-stress. This implies that the pre-fire climatic conditions would need to be favorable to refilling. The importance of this is further illustrated by the fact that post-fire climatic conditions are significantly more water-stressed as a result of the fire. For example, Kremens *et al.* (2003) recorded temperatures of 100°C and 0% relative humidity in a wildfire and these conditions persisted for 2 hours after the fire event.

Interestingly, in a study by Higgins *et al.* (2012) on the traits that determine shifts in abundance of fire-prone savanna tree species, the contrary was found and there was no evidence for a seasonal effect of fire on the probability of surviving. A possible explanation for this contradiction in results could be due to the species that were used in each respective investigation. While van Mantgem *et al.* (2013) used coniferous species, Higgins *et al.* (2012) used resprouting angiosperms. This is important because although hydraulic safety margins tend to be much greater in conifers compared to angiosperms, conifers tend to experience more embolisms in leaves and roots on a daily basis, probably due to weak stomatal responses to changing air humidity. On top of this, compared to angiosperms, conifers tend to have little parenchyma or nonstructural carbohydrates in their wood. The ability to rapidly repair embolisms may rely on having nearby parenchyma cells, which could explain the need for greater safety margins in conifer wood as compared to angiosperms (Johnson *et al.*, 2012). But it is not only the high embolism and weak refilling capacity of conifers that can explain this difference. This is because water is still needed in order to refill vessels, yet resprouting angiosperms do not have higher mortality rates when soil moisture is low. This is because lignotubers and other storage organs are found in resprouters and thus a source of water as well as carbohydrates, that is much less dependent on soil moisture, is available to them (Clarke *et al.*, 2012).

While refilling provides a potential mechanism through which trees can recover hydraulic function post-fire, this process would be absolutely dependent on the survival of tissues that allow refilling (i.e. cambium and phloem). It has often been assumed that vascular cambium tissues are killed at a threshold temperature of about 60°C. Does this mean that if this temperature is reached, cambium and phloem would be dead and therefore unable to refill vessels? The problem with this temperature threshold concept is that longer exposures to temperatures below the threshold or shorter exposures to temperatures above may also result in cambium death (Dickinson & Johnson, 2004).

Although the concept seems to have leeway, there is no doubt that trees protect their cambium and phloem when frequently exposed to fires (Midgley *et al.,* 2010). This can be illustrated by examining the life histories of the two species. While *E. cladocalyx* is a savanna tree that is often exposed to fire, *K. africana* is an established forest species that has not evolved to tolerate fires but rather low summer rainfall (Campbell & Moll, 1977). This translates into very different kinds of responses to fire. While it is known that *K. africana* is particularly poor at surviving fires, *E. cladocalyx* is an epicormic resprouter that is able to regenerate new growth from aerial buds buried deep in the bark (Waters *et al.*, 2010). In order to recover foliage after a fire, resprouters need to have buds that are not only alive but also supplied with enough water to allow bud burst. This means that not only the buds but also the hydraulic pathway leading to buds needs to be robust and able to resist heat induced failure. Increasing bark thickness solves this and it has been shown that one of the best predictors of fire survival is absolute bark thickness (bark thickness growth rate), where individuals with thicker absolute bark display the lowest mortality rates (Midgley *et al.*, 2010). The implication of this is that trees need to invest a great deal of resources into bark growth, in order to protect the underlying hydraulics and buds.

The cost of this protection is that it places a constraint on the rate at which height can be acquired (Archibald and Bond, 2003). As a result, *E. cladocalyx* would be expected to have much thicker absolute bark compared to *K. africana*, resulting in a higher resistance to heat induced deformation. However, while this prediction would imply a contradiction to our results (i.e. *K. africana* and *E. cladocalyx* average bark thickness is 0.048cm and 0.045cm respectively for oven treated stems), it is important to note that eucalypts are some of the tallest trees on earth even though they frequently deal with fire (Barnard & Ryan, 2003). This denotes that they are able to maintaining sufficient protection at a reduced cost. Eucalypts have accomplished this through the unique evolution of bud strands in which epicormic buds are found deep within the vascular cambium, where they are protected by maximum bark thickness (Burrows, 2002). This means that they are able to effectively protect buds using relatively thin bark, and it has been shown that eucalypts have remarkably thin absolute bark thickness compared to non-eucalypts in the same habitat. While buds may be protected by thicker bark, bark thickness is positively correlated with age and diameter (Wesolowski *et al.*, 2014). This means that distal shoots and branches would have much thinner bark and therefore would not only be more susceptible to heat induced bud necrosis but the xylem would also be more vulnerable to deformation (Figure 11). When considering the unusually thin bark of eucalypts, it becomes clear that its ability to survive a low-intensity fire is not due to bark thickness but rather the survival of the underlying tissues (i.e. bud strands and associated hydraulics).

This is interesting when considered in light of refilling: perhaps cambium and phloem necrosis does play a role in fire-survival but has simply been misinterpreted in rapid mortality scenarios. Here we propose that the loss of living cells adjacent to xylem vessels would inhibit refilling, and therefore, rapid mortality following a low-intensity fire is as a consequence of this. It would seem then that cambial and phloem necrosis is actually linked to hydraulic death. It is necrosis of the adjacent cells to xylem vessels that inhibits refilling of vessels leading to progressive desiccation and eventually death. This suggests that the hydraulic death hypothesis and carbon starvation hypothesis are not as a result of different processes but rather reflect a temporal constraint on the same mechanisms. In other words, hydraulic death can be seen as a result of rapid cambium and phloem necrosis leading to the failure of refilling, whereas carbon starvation reflects the same processes, however, over a much longer period of time.

Conclusion:

While it appears that VPD induced cavitation caused by a hot plume rising above a fire is the main mechanism by which xylem conductance is reduced following low intensity fires, deformation cannot be disregarded and both have been shown to affect the hydraulic conductance of trees exposed to fires.

Here we propose that there are three main mechanisms by which trees survive fires: 1) cavitation plays a protective role for plant hydraulics as vulnerability segmentation implies that leaves act as "safety valves". Strong vulnerability segmentation is therefore essential for trees exposed to fires as cavitation needs to be prevented from spreading into the bole. 2) Taken together, bark properties and cavitation appear to slow the rate of stem heating which in turn protects the hydraulics, however, xylem traits such as wall thickness seem to be the main preventative adaptation of deformation. 3) This mechanism for slowing heat transfer also points to a protective role over cambium and phloem as they lie adjacent to xylem vessels. This is very important because we propose that it is the inability to refill cavitated vessels after a fire that is the ultimate cause of mortality and this is dependent on the survival of these cells. This means that both the hydraulic death hypothesis and the carbon starvation hypothesis are reflections of the same processes, however, while carbon starvation would imply a partial ability to recover post-fire, hydraulic death does not and this is why we find both rapid and prolonged rates of mortality.

With refilling being constrained by drought, it would seem that the survival of trees exposed to fire also strongly depends on available water and carbohydrate reserves. As resprouters, such as *E. cladocalyx*, contain water and carbohydrate reserves within boles and lignotubers, they are able to resist the effects of drought on refilling which greatly increases their chance of surviving hydraulic failure. On the other hand, non-resprouters would be highly dependent on pre-fire climatic conditions. With global change driving increased drought frequency in forests around the world, it is likely that non-resprouters face bigger consequences with regards to fire-induced mortality.

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