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Pyrohydraulic traits: The role of hydraulic segmentation as a hydraulic fuse in resisting fires

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BLYSHO004

This dissertation is presented for the degree of Master of Science
in the department of Biological Sciences,
University of Cape Town
March 2021

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Vincent van Gogh said: "There is peace even in the storm."

But do you think there is peace in a fire?

PLAGIARISM DECLARATION

I know the meaning of plagiarism and declare that all the work in the dissertation, save for the properly acknowledged, is my own.

Shonese Thora Bloy

Signed by candidate

ACKNOWLEDMENTS

My acknowledgements at first were at the end of the dissertation, my brain believed that the thanks came at the end. First off, if you are reading this, you are probably my examiner. Thank you to you my examiners who are about to embark on my journey. I hope you enjoy the read.

I am deeply grateful for the funders towards my MSc degree, without them I would have been unable to complete this degree. Thanks to my first funder, our Vice Chancellor Professor Mamokgethi Phakeng. Her determination to see people of colour in STEM, has provided me with a platform to be such a person. I am blessed to have received support and financial assistance in the form of the M-Phakeng Wrap Around Scholarship and VC Scholarship. I would like to acknowledge the VC in all her hard work, and how she pursues to see the country progress. She has seen the gap and takes up her position to see it filled. I would like to honour and thank Ma VC for being a role model and setting the standard. Secondly, thank you to the UCT Financial aid for assisting me in my first year of my master's degree. Thirdly, to Associate Professor Adam West, my supervisor, for providing financial support in my final stretch.

A hearty thanks to all my supervisors that assisted in methodological set-up and design, and for assistance in writing. My primary supervisor, Associate Professor Adam West, thank you for your support, your encouragement, for your golden nuggets of wisdom and financial support. Thank you to Professor Emeritus Jeremy Midgley for the casual check-ins and involvement in discussions tailored to my project, as well as providing writing guidance. Thank you to Doctor Rob Skelton for assistance with my second data chapter, commenting on my draft work and giving me writing advice. And even though Michelle Louw was not appointed as a supervisor for my master's degree, I would like to acknowledge her for proofreading my drafts, as well as giving guidance during my dissertation, helping me in the laboratory, with sampling and discussing my data. Her close assistance did not go unnoticed.

I am deeply grateful for my family, for their love and their support. I know moving to Cape Town and attending UCT may have added some financial strain, but I thank them for their patience, love, and unconditional support. I only wish to give back to them what they invested in me and more. A special thanks to my parents who would have probably helped me climb a tree if they were based in Cape Town. Their encouragement kept me working during the 2020 level 5 Lockdown. My friends who are like family, Deanna Lasania and Larnelle Garnie, who encouraged me and were there for me when times were tough. I appreciate Deanna who would

bring me snacks. She would sit with me, providing moral support, while I was running conductance measurements, hoping together everything would run smoothly for that day. And Larnelle for helping me escape and just listening to me vent. And lastly to Dennis, thank you for being a supportive boyfriend, he tried his best to help me where he could. His encouragement for me to be level-headed and calm when I over-analysed situations, did not go unnoticed.

I am appreciative for everyone who physically helped me during my degree, by assisting me in carry two-metre-long branches or with lab work (Dennis Matogo, Deanna Lasania, Jess Lund, Mandisa Mteshana, Dean Erasmus, Michael Ross, Caton Schutte, and Chrysanthemum Munatsi). Thank you to my 'West Lab' colleagues (Kayla Atkins, Tshepiso Radebe, Michelle Louw, Simcelile Chenge, Justin van Blerk, Agustin Cavallaro, Sally Mashele and Joshua Mirhin). They not only assisted me in the field but also provided me with encouragement, laughter, and advice. A special and specific thanks to Chrysanthemum, Sally and Michelle. These three lovely people invested a large amount of time in assisting me in the lab during measurements. An individual thanks to Joshua who climbed high up some trees for me. Thank you for not having vertigo as I have, or else I would not have gotten those branches. My last acknowledgment in relation to my experimental phase of my dissertation goes to Boitumelo Marope and Bongani Tom, the department's research technicians, for ensuring the smooth running of my experiments.

Lastly, I would like to acknowledge God. For granting me peace when I needed it the most and for guiding me during my degree.

FUNDING

2019: The VC scholarship and UCT Financial Aid

2020: Mamokgethi Phakeng wrap around Scholarship and The VC scholarship

ABSTRACT

Fire is known to kill trees, but the mechanisms underlying plant death and survival remain unclear. The central claim of the hydraulic death hypothesis (HDH) is that xylem embolism may kill plants by preventing tissues that survive fires from remaining hydrated and has been suggested to explain rapid post-fire mortality. A corollary of the HDH is that it would be beneficial for plants to invest in a suite of traits, 'pyrohydraulic' traits, that may prevent embolism because this could aid post-fire survival. It was hypothesized that 1) the most distal parts along the branch of a fire-tolerant tree species, *Eucalyptus cladocalyx*, will act as a hydraulic fuse post-fire, in contrast to a fire sensitive species, *Kiggelaria africana*, where the hydraulic fuse post-fire would not be exhibited, and 2) the mechanism for this hydraulic fuse was vulnerability segmentation. A xylem conductance experiment post heat-plume (Chapter 2) and a vulnerability experiment (Chapter 3) were conducted on branches from both species. I predicted that 1) embolism would be localized to the distal parts of a branch for *E. cladocalyx* but not *K. africana* 2) non-suberized tissue of the distal tissue parts of *E. cladocalyx* branches will be more vulnerable to embolism than the suberized tissue, but *K. africana* would not exhibit this differentiation. Results indicate that embolism was localized to the most distal parts of a branch in *E. cladocalyx*, as well as that the non-suberized tissue was more vulnerable to embolism. *E. cladocalyx* suffered a 19% loss of conductance, while *K. africana* suffered a much greater 68% loss of conductance after heating. The P_{50} values of the non-suberized and suberized tissue sections were statistically similar in both species, but the P_{12} values differed between the sections for *E. cladocalyx*. *E. cladocalyx* was shown to be more vulnerable to embolism than *K. africana*. These results indicate that developing tissue of *E. cladocalyx* is more vulnerable to heat-plume induced embolism than developed stem tissue, consistent with the hypothesis that vulnerability segmentation is a pyrohydraulic trait. Therefore, the most distal parts of a *E. cladocalyx* branch acts as a hydraulic fuse and contributes to post-fire recovery. The phenomenon may be used by other fire-tolerant species as a strategy to survive post-fire, thereby enabling them to endure a fire and recover through protecting their hydraulic continuum.

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CHAPTER 1: Post-fire Tree Mortality

Fire is an important disturbance that occurs within certain ecosystems and claims a major role in the ecology of these ecosystems (Bond, 2019). It may maintain or alter structure and function in fire-prone ecosystems, influencing biome distribution, biodiversity and productivity (Bond & Keeley, 2005; Bowman et al., 2017). The vegetation within these ecosystems should exhibit traits that aid in post-fire survival or post-fire regeneration. Such traits include serotiny, post-fire resprouting, thick-bark and fire stimulated germination or flowering (Lawes, Adie, et al., 2011; Lawes, Richards, et al., 2011). It has been debated whether these specific plant traits (i.e., resprouting, serotiny) are indeed plant traits adapted for fire. For example, fire adapted traits can have complex origins and other environmental factors may have facilitated the evolution of them (Bradshaw et al., 2011), however this has been criticised as plants could be adapted to fire regimes and not just fire in general (Keeley et al., 2011). Emphasising that plant species in ecosystems that are associated with specific fire regimes have fire-adaptive traits, stressing the importance of fire intensity and fire frequency (Keeley et al., 2011). Conversely, plant species that have evolved in the absence of fire, might not be able to survive or re-establish after a fire. Thus, some plants die, and some survive, post-fire; this is dependent on the sensitivity of plants in an ecosystem as well as their life strategy traits.

The frequency of fires has increased due to climate change (Shlisky et al., 2007; Bowman et al., 2017; Ruffault et al., 2018). An increase in fire occurrence can be detrimental to some ecosystems (Bond & Van Wilgen, 1996; Shlisky et al., 2007). Although evidence shows that the Mediterranean basin is resilient to repeated fire, some evidence shows that a change in a fire regime within in some Mediterranean communities possibly generate harmful impacts (Pausas et al., 2008). A major concern is that communities adapted for a specific fire regime, such as a low intensity fire, could suffer in response to change in intensity caused by climate change. A shift in fire frequencies can possibly also cause a shift in species that can survive in those areas (Fensham et al., 2003). An alteration in a fire regime can also allow for the spread of alien invasive species (Keeley & Brennan, 2012). Fire intensity is thus an important factor to consider. Low to moderate fire intensities may not result in a lethal outcome, but could cause injuries to trees which impact the functioning of the trees (Bär, Michaletz & Mayr, 2019). Thus, an understanding of global change impacts to fire regimes especially fire intensity is important.

What kills trees during fire?

Trees, as an entity, are important in ecosystems as they have vital roles in an environment, such as playing a major role in the carbon sequestration and the carbon cycling of an environment (Dixon et al., 1994), and trees within forest ecosystems protect the landscape, preventing rapid run-off, soil erosion and loss of resources (Belmont et al., 2005). Tree presence within ecosystems is sensitive to fire regimes. Increased intervals between fires can result in habitat changes from open to closed ecosystems, leading to a less flammable state (Bond, 2019). Conversely, frequent wildfires may maintain an open habitat by controlling tree populations (Bond, 2019). Therefore, the presence and absence of trees are important as they may regulate habitats from switching to an alternate state. For example, the savanna ecosystem can transition from a grass dominant state to a tree dominant state (Bond, 2019). Wildfires have been around for centuries, it is a natural force influencing plant traits over many years (Bond, 2019).

Understanding fire behaviour as well as post-fire tree mortality and the underlying mechanisms thereof are crucial. Michaletz *et al* (2007) reviews the manners in which trees may die post-fire, by exploring several biophysical processes that link fire behaviour and tree mortality. During fires, heat is produced by combustion and transferred to the tree, potentially severely injuring the roots, bole or the canopy of the tree which can result in death (Michaletz & Johnson, 2007; Hood et al., 2018). Fires are not uniform and are inclusive of many processes such as smouldering and flaming combustion, conduction, convection, and radiation. Heat transfer to the crown from a fire occurs by radiation and convection, this results in crown scorching or combustion. The heat transfer by conduction through the soil to the roots will result in root necrosis, although little is known about the fire effects on roots (Michaletz & Johnson, 2007; Bär, Michaletz & Mayr, 2019). And finally, heat transfer from a fire to the bole of a tree occurs by radiation and convection this may result in tree death.

This death is hypothesised to be caused by cambium necrosis (Michaletz & Johnson, 2007; Bär, Michaletz & Mayr, 2019). Even low intensity fires can result in rapid post-fire tree mortality (Michaletz & Johnson, 2007; Michaletz, Johnson & Tyree, 2012; West et al., 2016). It has been long believed that the cambium necrosis hypothesis is the mechanism for post-fire tree mortality. Cambium necrosis post a low intensity fire, should lead to plant death over a long period of time due to an absence of differentiating new conducting tissue and thus does not explain rapid post-fire death over a few days (Dickinson & Johnson, 2004; Michaletz & Johnson, 2007; Midgley, Kruger & Skelton, 2011; Michaletz, Johnson & Tyree, 2012). Cambium necrosis is the death of living cambium and phloem cells due to heat conduction around the circumference of the stem, ultimately leading to root starvation due to lack of differentiation of new xylem and phloem. Cambium cell death consequently impairs the translocation of photosynthates, forcing the plant to rely on carbon reserves. Eventually, reserve depletion occurs, leading to plant death (Pinard & Huffman, 1997; Dickinson & Johnson, 2004; Dickinson, Jolliff & Bova, 2004; Sevanto et al., 2014). This death is a slow process and does not explain rapid death. Recently hydraulic death has been suggested as a mechanism of post-fire rapid death.

Hydraulic Death Hypothesis

As forest fires can be classified into three classes: ground fires, surface fires and crown fire, tree injury may occur in any one of these ways (Bär, Michaletz & Mayr, 2019), but the main interest in my study is injury due to the exposure to dry air. As a wildfire passes a heat-plume is created in the canopy by combustion in the fire front. Heat-plumes are plumes, comprising hot dry air rising in the canopy, these plumes cause direct and indirect injury to the trees. This dry hot air creates a rapid atmospheric drought environment. This leads to stress within the canopy as the vapour pressure deficit (VPD) increases. The increase of temperature has a negative effect on the sap surface tension and this impacts vulnerability to embolism (Michaletz, Johnson & Tyree, 2012; West et al., 2016; Lodge, Dickinson & Kavanagh, 2018; Bär, Michaletz & Mayr, 2019). The increased VPD in the foliage, results in water being rapidly lost through the leaves and petioles, creating a sudden transpiration burst (Michaletz & Johnson, 2007; Kavanagh, Dickinson & Bova, 2010; Midgley, Kruger & Skelton, 2011). This theoretically results in the water potential abruptly decreasing and causing plume-induced embolism or conduit collapse (Kavanagh, Dickinson & Bova, 2010; Michaletz, Johnson & Tyree, 2012; West et al., 2016). For the hydraulics of a plant to be fully functional, the water column of the plant must remain intact (i.e., xylem must be completely water-filled), thereby, ensuring optimal water transport to the photosynthetic parts of the plant. A hydraulic failure can therefore result in death, from desiccation, as the embolism could not be repaired or circumvented through regrowth (Balfour & Midgley, 2006; Kavanagh, Dickinson & Bova, 2010; Midgley, Kruger & Skelton, 2011; Michaletz, Johnson & Tyree, 2012; West et al., 2016). Thus, recent evidence shows that a hydraulic mechanism may better explain rapid post-fire mortality (Midgley, Kruger & Skelton, 2011; West et al., 2016). A hydraulic failure can occur by xylem deformation or embolism, linking to rapid death (Balfour & Midgley, 2006;

Kavanagh, Dickinson & Bova, 2010; Midgley, Kruger & Skelton, 2011; Michaletz, Johnson & Tyree, 2012; West et al., 2016; Bär, Michaletz & Mayr, 2019).

Recent evidence shows that wildfires result in conduit wall deformation causing permanent reduction in xylem conductivity (Michaletz, Johnson & Tyree, 2012; West et al., 2016). Xylem deformation happens when heat from a fire warms xylem to the point where thermal softening of lignin, hemicellulose, and cellulose polymers in conduit walls occur (Hillis & Rozsa, 1985; Lewin & Goldstein, 1991). The conduits will deform, and possibly collapse due to the tension in the water column (Hacke et al., 2006). The conduit will then cool, and solidify in this state, inhibiting water transport (Michaletz, Johnson & Tyree, 2012; Bär, Michaletz & Mayr, 2019). This reduction in hydraulic conductivity increases xylem water tension and limits water transport resulting in downstream tissue dehydration and mortality. Therefore, these mechanisms of death are possibly not mutually exclusive, and both hydraulic failure and carbon starvation, may act together and result in some species death (Sevanto et al., 2014).

Hydraulic failure can also occur when air bubbles block water flow through the xylem conduits (Sperry, Stiller & Hacke, 2003). The blockage prevents flow, limiting water transport and directly reducing xylem conductance (Sperry, Donnelly & Tyree, 1988; Cochard, 1992; Kavanagh & Zaerr, 1997; Sperry, Stiller & Hacke, 2003; West et al., 2016). Embolism occurs when air is pulled into the xylem through the pit membranes, or air coming out of solution in the xylem, and expanding to fill the xylem conduit thus embolising by air seeding the water-filled xylem conduit (Cochard, Cruiziat & Tyree, 1992; Sperry & Saliendra, 1994; Tyree & Zimmermann, 2002). Plants generally prevent this embolism in the water column by closing their stomata to halt water loss through transpiration in times when water is limited. However, if a plant experiences extreme water limitation for a prolonged period, such as a drought, the tension in the water column increases resulting in embolism, which may lead to tree death (Cochard, 1992; Kavanagh, Dickinson & Bova, 2010). However, it is not known whether stomata close fast enough to prevent embolism, after they have been exposed to a fire caused heat-plume.

The occurrence of embolism in plants is dependent on the outside air pressure (P_{air}) and sap water pressure (P_{sap}). If the pressure difference between air and sap water is greater than the pressure for embolism (P_{cav}) then embolism will occur (see Eqn 1; M.T. Tyree and Zimmermann, 2002; Michaletz, Johnson and Tyree, 2012).

$$P_{air} - P_{sap} > P_{cav} \quad \text{Eqn 1}$$

Embolism can be modelled indirectly using xylem water potential, (see Eqn 2).

$$\Psi_X = \Psi_S - (\mathrm{g}_S \mathrm{cVPD})/\mathrm{K}_{leaf} \quad \text{Eqn 2}$$

Where, xylem water potential Ψ_X is calculated by using soil water potential (Ψ_S), canopy stomatal conductance (g_S – rate at which carbon dioxide or water vapour enter or leave, respectively, through stomata of the leaf), constant (c – $1/P_{air}$), Vapour Pressure Deficit (VPD – difference of moisture in the air and the amount of moisture air can hold when saturated), and leaf specific canopy conductance (K_{leaf} – xylem conductance of plant standardized to the leaf area of the plant). Therefore, if Ψ_X becomes more negative, due to a high VPD, it can exceed P_{cav} and result in embolism (see Eqn 2).

Pyrohydraulic traits

Traits that aid in the survival of tree stems post-fire include bark thickness, bud protection and post-fire seed germination, these are fire adaptive traits (Lawes, Adie, et al., 2011; Lawes, Richards, et al., 2011). However, hydraulic traits are also important in surviving fire as argued above in hydraulic death hypothesis. West *et al* (2016) argued that certain hydraulic traits may confer benefits in surviving fires, hence they coined the term “pyrohydraulic traits.” Such traits found in West *et al* (2016) were vulnerability to embolism and thermal tolerance.

In the West *et al* (2016) study, experimental evidence shows that simulated heat-plumes result in reduced conductance due to embolism (West et al., 2016). This was completed by exposing stems from two different tree species to two simulated heat-plumes (70 and 100°C) for six minutes. Thereafter the xylem conductance was measured, and the branches were flushed. This determined the extent to which the loss of conductance was recoverable by flushing. As expected, the stems suffered a reduced conductance when subjected to a simulated heat-plume, and the loss of conductance was recovered with flushing. Both tree species in 100°C heat-plume experiment experienced an approximate 80% loss of conductance and xylem conductance was recovered by flushing. When exposed to the 70°C heat-plumes, they differed.

K. africana had a 49% loss of conductance while *E. cladocalyx* had an 80% loss of conductance, therefore indicating *E. cladocalyx* had a highly vulnerable response to the heat-plume. Apart from the simulated heat-plume, they also ran a water bath treatment experiment. Both species were subject to two water bath treatments (70 and 100^{o}C) for six minutes. The xylem conductance was once again measured, and the branch was flushed and re-measured. Again, to determine the extent conductance could be recovered and informing if xylem deformation took place. Only *K. africana* experienced a significant reduction in conductance, indicating that *K. africana* was more susceptible to xylem deformation, as conductance could not be recovered after a 100^{o}C water bath treatment. The results obtained in West *et al* (2016) indicated that the cause of reduction was embolism, and it was suggested that vulnerability of some species to embolism was a pyrohydraulic trait, a kind of a fuse. Also, thermal tolerance against xylem deformation protects branches from excessive heating this could also be a pyrohydraulic trait.

West *et al* (2016) simulated the effects of a heat-plume on the percentage loss of leaf specific canopy conductance using a hydraulic model. The model was best able to explain the experimental data by suggesting that the xylem of *E. cladocalyx* was more vulnerable. This was not the case for *K. africana*. The simulated hydraulic fuse for *E. cladocalyx,* was found by reducing the P_{50} value (xylem water potential at 50% loss of conductance), localizing the hydraulic failure to the distal parts of the stem. The hydraulic failure in the distal parts would be due to embolism, this would block water flow to the distal ends, but preserve the main stem. Therefore, hydraulic segmentation was the suggested mechanism. They found that simulating hydraulic segmentation as a phenomenon for *E. cladocalyx* better explained the percentage loss of conductance (PLC) for the species, as the model simulations underestimated PLC at 70^{o}C heat-plume. PLC at 100^{o}C heat-plume appeared to be overestimated.

This led to idea that hydraulic segmentation might act as a pyrohydraulic trait and protect *E. cladocalyx* by localizing embolism to the distal tips. The lack of hydraulic segmentation might be one way *K. africana* is unable to survive fires. This dissertation aims to test whether the distal ends could act as a hydraulic fuse preventing embolism from propagating throughout the tree.

Hydraulic Segmentation

In this dissertation, the term “hydraulic segmentation” is used to describe the phenomenon that is the loss of conductivity localized in the distal portions of the hydraulic continuum, thereby protecting the remainder of the hydraulic continuum from hydraulic failure. This usage is consistent with West *et al* (2016) and is analogous to the concept of the “hydraulic fuse” (Zimmermann, 1983; Tyree & Zimmermann, 2002). Segmentation enables localization of the embolism to distal part of the pathway, possibly to distal expendable organ parts, protecting larger organs that are a major carbon investment and took many years to grow (Tyree & Ewers, 1991; Tyree & Zimmermann, 2002). Leaves have been shown to act as a hydraulic fuse in some species (Mcculloh et al., 2012; Wolfe, Sperry & Kursar, 2016), and there is evidence in certain species that leaves tend to be more vulnerable to embolism than stems of a branch (Woodruff et al., 2007; Chen et al., 2009). However, there is also evidence that indicated some species exhibit a lack of vulnerability segmentation (Skelton, Brodribb & Choat, 2017). Although these studies indicate that leaves act as a “safety valve” they have been conducted in the context of drought and plant response to dehydration. This dissertation explores if the distal sections of a branch may act as hydraulic fuses during fires, thus resulting in hydraulic segmentation.

The phenomenon of hydraulic segmentation could come about through two distinct, but non-exclusive, mechanisms. The first is resistance segmentation, which occurs when there is an increase in hydraulic resistance to flow in the distal portions of the hydraulic continuum. This increase in resistance results in an increased water potential gradient across the tissue in question, for a given flow, this has an influence in potentially causing embolism in these tissues (Zimmermann, 1978, 1983). The second mechanism is vulnerability segmentation. Vulnerability segmentation occurs when there is an increase in the vulnerability of the distal tissue, presumably due to anatomical changes to the xylem (Tyree & Ewers, 1991; Tyree & Zimmermann, 2002). This will result in preferential embolism in these tissues (even if there is no flow and all tissues in the plant are in equilibrium with each other). Although resistance and vulnerability segmentation can be directly related to each other, it is plausible that they may also operate independently (Levionnois et al., 2020). However, both result in the phenomenon of hydraulic segmentation, as defined in this dissertation, where loss of conductivity is localized in the distal tissues of the plant, thereby protecting most of the hydraulic continuum. It is hypothesized that fire-adapted trees protect themselves, by limiting embolism to distal tissues. Since embolism is limited to the distal part of the tree it can facilitate resprouting from epicormic buds embedded in larger branches or the main stem or even from the roots. Evidence for hydraulic segmentation under drought conditions has been found, showing that, where it occurs, embolism is more likely to occur in distal shoots, petioles or leaves (Tyree & Sperry, 1989; Johnson et al., 2009; Bucci et al., 2012, 2013; Pivovaroff, Sack & Santiago, 2014; Levionnois et al., 2020). However, hydraulic segmentation as a 'pyrohydraulic trait', as a phenomenon, for post-fire survival has not yet been explored. This implies that post heat-plume - generated in a fire-front - a fire-tolerant tree experiences plume-induced embolism. However, the embolism will be localized to the distal parts of the tree causing these parts to die but protecting the main bole from further embolism. Hydraulic segmentation as a 'pyrohydraulic trait' may aid in the survival of a tree post-fire.

The goal of this investigation is therefore to determine if hydraulic segmentation differs between a fire tolerant species (*E. cladocalyx*) and a fire sensitive species (*K. africana*). It is predicted that the fire tolerant species will exhibit hydraulic segmentation. This trait will be beneficial for this species, because the embolized distal part of the tree can die off and the tree can resprout using the intact water column. Whereas the fire sensitive species is predicted to exhibit a lack of hydraulic segmentation and thus the tree will experience an extreme loss of conductance throughout the tree.

Synopsis of Data Chapters

In the two data chapters, through experimentation it was tested whether embolism would be localized to the distal sections of a branch post heat-plume. This was further compared between two tree species with different abilities to survive fire. The presence of this segmentation would suggest that this might function in fire-survival and thus be a pyrohydraulic trait. In Chapter 2, an induced heat-plume experiment was used. Xylem conductance (Kolb, Sperry & Lamont, 1996) of branch segments were subsequently measured, and the post heat-plume xylem conductance was compared to the flushed xylem conductance of the same branch.

In Chapter 3, the vulnerability segmentation hypothesis was tested as a mechanism and as a pyrohydraulic trait. The optical technique (Brodribb et al., 2016, 2017; Brodribb, Bienaimé & Marmottant, 2016) was conducted, imaging two sections of a branch. The two parts of the stem tissue was imaged, a non-suberized tissue section of the branch and a suberized tissue section of the branch. The branch was left to dry out, cumulative embolism was recorded, and water potential was logged. I predicted that the non-suberized tissue section of the fire-tolerant branch would be more vulnerable to embolism compared to the suberized tissue, and the fire sensitive tree species would not show this differentiation between tissue sections.

CHAPTER 2: Testing for Hydraulic Segmentation as a Hydraulic Fuse

INTRODUCTION

Pyrohydraulic traits may be important in the *in-situ* survival of plants from fire-prone environments, post-fire. When a fire event occurs, the heat-plume generated in the fire front can result in xylem embolism in the canopy of nearby trees. This sudden pocket of hot dry air causes the tree to experience a sudden burst of transpiration resulting in embolism (Kavanagh, Dickinson & Bova, 2010; Midgley, Kruger & Skelton, 2011; West et al., 2016). Embolism events can prevent a tree from transporting water to its photosynthetic organs to replace that lost through transpiration (Sperry, Stiller & Hacke, 2003). If this embolism could not be repaired, or circumvented through regrowth, this would result in rapid death due to hydraulic failure (Balfour & Midgley, 2006; Kavanagh, Dickinson & Bova, 2010; Midgley, Kruger & Skelton, 2011; Michaletz, Johnson & Tyree, 2012; West et al., 2016). As a result, it should be beneficial for a tree experiencing fires to invest in traits that would minimize this risk.

One such trait may be segmentation (Zimmermann, 1983). Hydraulic segmentation is when embolism is localized to the distal parts of the plant's hydraulic pathway, acting as a "hydraulic fuse", thereby protecting the hydraulic pathway in the main part of the stem (Zimmermann, 1983; Tyree & Ewers, 1991; Tyree et al., 1993; Tyree & Zimmermann, 2002). By regrowing and replacing these damaged distal portions of the xylem, the tree could theoretically regain full function post-fire. In contrast, a tree that was not able to hydraulically segment might experience embolism in the more central parts of the stem, areas that are not as easy to repair or regrow. This might result in impaired function post-fire, which can result in either rapid (Midgley, Kruger & Skelton, 2011), or delayed (Bär, Nardini & Mayr, 2018) mortality.

Hydraulic segmentation was proposed as a hydraulic fuse phenomenon explaining the embolism responses of the fire intolerant *Kiggelaria africana* and fire-tolerant *Eucalyptus cladocalyx* in an experimental heat-plume (West et al., 2016). In this experiment, the measured loss of conductivity in the two species, when exposed to a heat-plume, was best simulated by suggesting more vulnerable xylem in the distal shoots of *E. cladocalyx*, but not *K. africana*. Thus, it is proposed that *E. cladocalyx* showed a greater degree of hydraulic segmentation than *K. africana*, and that this might account for the ability of former species to persist and resprout post-fire.

The aim of this study was to test the hypothesis proposed by West *et al* (2016) *that E. cladocalyx* is more hydraulically segmented than *K. africana*. I tested this by examining where embolism was localized in shoots of *E. cladocalyx* and *K. africana* exposed to a simulated heat-plume. I considered that a high percent loss of conductivity (PLC) in the distal parts of the shoot together with a low PLC in the central parts would be evidence of hydraulic segmentation. Conversely, little difference in PLC between the distal and central parts would be indicative of little hydraulic segmentation. I hypothesised that *E. cladocalyx* would show a greater degree of hydraulic segmentation than *K. africana*, consistent with the hypothesis that hydraulic segmentation is an important pyrohydraulic trait enabling fire survival in this species.

METHOD

Study Species

The study species used for my research were tree species from the extremes of the fire strategies, a fire-tolerant species, and a fire sensitive species (see West *et al* 2016). The tree species sampled were all located on the University of Cape Town grounds. The first species was *Eucalyptus cladocalyx* F. Muell, an alien invasive species in South Africa. This species is a fire-tolerant species, capable of epicormic resprouting, recovering and maintaining the population structure post-fire (Burrows, 2000; Ruthrof, Loneragan and Yates, 2003; Fig. 1). The second species was *Kiggelaria africana* L., an Afromontane Forest species and it is fire intolerant. This species is drought tolerant and suffers from mortality post-fire (van Wilgen *et al.*, 1992: Fig. 1).

Figure 1: The two study species located on the University of Cape grounds are tree species A) *Eucalyptus cladocalyx,* a fire-tolerant species and B) *Kiggelaria africana*, a fire sensitive species.

Field Collection

Branches from the two species were collected during late winter and early spring of 2019. The branch samples cut from the trees were approximately two metres in length. After sampling, the branches were placed in a bucket of water and transported to the lab where they were re-cut under water to prevent accidental embolism. Stem water potential of the collected branches were measured using a Scholander Pressure Chamber (PMS Instrument Company Model 1505D) to determine if the branches were water-stressed at the time of sampling. The branches were left submerged in water under a hood light in the lab at ambient temperature (± 27°C) for four hours. Leaving the branches for this duration ensured that their stomata were open, and transpiration was taking place, thereby, ensuring acclimatization to the same initial conditions for all branches prior to experimentation.

Experimental setup

A sequence of experiments was conducted. Each experiment included control and treatment branches and followed the methodology of West *et al* (2016). Post acclimatization, the branches for the control and treatment were trimmed down under water to approximately 60cm, this prevented accidental embolism. Before the treatment, the cut end of the treatment branches was wrapped in Parafilm ™ (Bemis), and the branches were inserted in a glass beaker filled with polystyrene pieces. The polystyrene ensured that the branches did not touch the glass, thereby, preventing conductive heating. The treatment branches in the glass beaker were exposed to a simulated heat-plume for six minutes, this was a surrogate for a heat-plume during a fire. The simulated heat-plume was generated using a convection oven set at 100°C. The control branches were treated in the same manner without the exposure to the simulated heat-plume. The control branches allowed us to determine whether the branches were initially embolised. The heat-treated branches allowed us to determine whether embolism was present post heat-plume. The heat-treated branch samples and the control branches were cut and defoliated under water in preparation for the xylem conductance measurements (following the methods described in Kolb *et al*, 1996).

Xylem Conductance

The control and heat-treated branch samples were attached to an airtight sealed tube using a rubber connector and this tube was connected to a beaker containing 0.01M KCl solution. The solution was made by dissolving 0.745g KCl in one litre reverse osmosis water and filtering it through a 0.22µm Whatman membrane filter. The beaker containing the KCl solution was placed on an electronic balance linked to a computer and the mass values were logged at 10 second intervals. The branch sample was placed in a cylindrical vacuum chamber. A partial vacuum was created, pulling KCl solution from the reservoir beaker on the balance through the xylem at 20kPa, 40kPa and 60kPa vacuum pressures, respectively. The flow rate at these pressures were recorded by dividing the change in the reservoir mass by the associated 10 second time interval. From this flow rate a linear trend across the different pressures was created. The gradient of the trend line is equal to the xylem conductance - pressure-flow gradient (Kolb, Sperry & Lamont, 1996). Xylem conductance was standardized to leaf specific xylem conductance K_{leaf} (mmol m^{-2} s^{-1} Mpa^{-1}) by dividing it by leaf area (m^2). This was the initial conductance measurement for the branch and will be referred to as initial. Thereafter, the branch samples were stored overnight in a 4^oC room in distilled water. The following day a thin slice of the cut ends of the branch samples was sliced off using a blade to ensure the branch was free from damage and debris from initial measurements. The branch samples were then flushed with 0.01M KCl solution at 175kPa for one hour, allowing for removal of possible embolism and thus a maximum conductance could be assumed.

Leaf area

Heat-treated branches were defoliated post heat-plume, before xylem conductance measurements, and control branches were defoliated before xylem conductance measurements. This was to standardize each branch to its leaf area, resulting in obtaining a leaf specific xylem conductance (K_{leaf}). Defoliation of the branches was done by removing the lamina at its junction with the petiole, incorporating distal portions of the xylem vessel in the measurement. The leaf area of each branch was measured using a leaf area meter (LI-COR LI3100C).

Percentage Loss of Conductance

The Percentage Loss of conductance (PLC) was calculated to compare treatment effects between the experiments. The Percentage Loss of Conductance (PLC) was calculated for each experiment using the following equation:

$$\mathrm{PLC} = \frac{\mathrm{K_{max}} - \mathrm{K_T}}{\mathrm{K_{max}}} \times 100 \qquad \text{Eqn 3}$$

where $\mathrm{K_{max}}$ is the average conductance for the flushed controls and $\mathrm{K_T}$ is the average conductance for the treatment.

Error of propagation for PLC was calculated as follows:

$$\mathcal{E} = \sqrt{\sigma_{\mathrm{K_{max}}}{}^2 + \sigma_{\mathrm{K_T}}{}^2 + \mathrm{PLC}\ \mathrm{x} \sqrt{\left({}^{\sigma_{\mathrm{K_{max}}}}/_{\mathrm{K_{max}}}\right)^2 + \left({}^{\sigma_{\mathrm{K_T}}}/_{\mathrm{K_T}}\right)^2}} \qquad \text{Eqn 4}$$

where σ is the standard deviation of the variables.

Experiments

Four sequential experiments were conducted to determine whether the branch sample exhibited hydraulic segmentation, wherein embolism would be localized in the distal parts of the branch. To test this, the distal parts of the branches were progressively trimmed back in certain experiments to determine changes in the PLC.

Experiment 1:

The first experiment was designed to determine if branches experienced embolism post a simulated heat-plume, this also aimed to replicate the results found in West *et al* (2016). The whole shoot branch, with no trimming off distal ends, was examined (Fig. 2). An initial and flushed xylem conductance for each control and heat-treated branch were measured. Therefore, acquiring two measurements for each whole branch. It was hypothesised that both species would have a reduced conductance (the flushed conductance and the not-flushed conductance would be different) and an extremely high PLC.

Experiment 2:

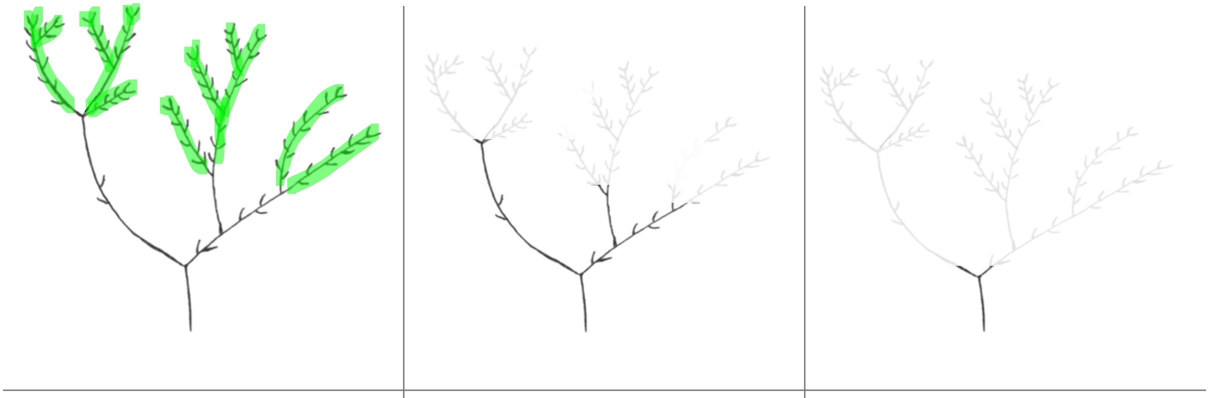
In the second experiment the aim was to determine if branches localize embolism in the youngest shoots of the branch. Similar methods were followed as in the first experiment; however, these branches were trimmed. The youngest most distal parts of the branch were trimmed off under water post heat-plume - before the xylem conductance measurements. This is the non-suberized segments of the branch, the less thickened (woody) parts of the branch, as there has not been a deposition of suberin (Fig 2: Experiment 2). All trimming and defoliation, of control and heat-treated branches, was done under water, to prevent accidental embolism. It was hypothesised that both species would have a reduced conductance, however the PLC of *E. cladocalyx* would be lower than the PLC of *K. africana*.

Experiment 3:

In the third experiment, the aim was to determine whether branches localize embolism in the distal parts of the branch. If embolism obtained from the heat-plume was localized to the distal part of the branch, then the embolism would be trimmed off by this experiment. As these were sequential experiments, similar methods as the second experiment were followed, except that, these branches were also trimmed back by a further terminal node (Fig. 2: Experiment 3). These branches were trimmed a node under water post simulated heat-plume and then xylem conductance measurements were recorded. Control branches were trimmed under water and xylem conductance measurements were conducted. It was hypothesised that *E. cladocalyx* would have no to a very low reduced conductance (the flushed conductance and the not-flushed conductance will be similar) and a low PLC, inferring hydraulic segmentation. *K. africana* would have a reduced conductance and a high PLC, inferring some hydraulic segmentation but at a very low degree.

Experiment 4:

Finally, a fourth experiment was conducted to investigate whether results were compromised by experimentally caused open vessels. Xylem vessels are elongated, taper at the ends and have pits that allow water flow. An open vessel is a xylem vessel that has been cut on both ends, i.e., vessel free of vessel taper and pits, resembling a straw-like structure. In the third experiment, the trim resulted in the length of the branch being much less than the species actual maximum xylem vessel length. Maximum xylem vessel length for *E. cladocalyx* and *K. africana* are 61 and 56 cm, respectively (West et al., 2016). Experiment 3 may have resulted in inaccurate measurements due to the artificially open vessels. I aimed to 1) evaluate and measure whether trimming the branches resulted in open vessels, and 2) investigate whether the measurement pressures were sufficient to refill any open vessels, resulting in an artificially high conductance for trimmed shoots. Open vessels will resemble a straw and are no longer compartmentalised by the presence of the pits. Therefore, liquid under far lower pressure than that required to refill a conduit through a pit membrane, might freely refill the vessel. Thus, an inaccurate measurement would occur due to the vessel filling with liquid during the conductance measurement. The presence of open vessels was determined by trimming branches as done in Experiment 3; however, these branches were not heat-plumed. The trimmed off distal ends were submerged into water. The proximal end of the branch was connected to a tube supplying N_2 gas at 20kPa – a low pressure so not to damage the conduits and any present pits. The N_2 gas could flow through, allowing us to see the N_2 gas bubbles at the other end. The presence of the bubbles would indicate open vessels. Once the presence of open vessels was confirmed, I investigated whether it compromised the conductance measurements and showed no PLC. Thus, I tested for this by measuring the xylem conductance and the flushed conductance of these branches. It was expected that if open vessels were a problem, then there would be no difference between conductance of branches that were flushed and branches that were not flushed, as the flushed vessels would simply refill under the pressures experienced during measurement. A difference between flushed conductance and not flushed would indicate open vessels did not compromise Experiment 3.



Experiment 1 Experiment 2 Experiment 3

Figure 2: Schematic drawing of a generic branch for each experiment, the branch was sequentially trimmed back. In Experiment 1, the branch was only defoliated. In Experiment 2, the branch was trimmed back by removing the non-suberized section (shown in green) of the branch. In Experiment 3, the branch was trimmed even further back by trimming a node back. The faded-out sections indicate the points at which trimming was done for Experiment 2 and 3.

Statistical Analysis

The flushed conductance measurement of each branch was compared to the average of the flushed controls of the experiment. If the flushed conductance measurement of a branch was five times more than the average of the flushed controls, the entire sample was discarded from the analysis. The removal of these branches ensured that artificially high conductance due to leaking from xylem, or leakage from the hydraulic line during a measurement were not part of the analysis. An ANOVA test followed by a post-hoc Tukey test were conducted to determine the difference between treatments within experiments. The ANOVA and post-hoc Tukey tests showed the direction of change and compared treated branches to control branches. However, due to variance within experiments paired t-tests were conducted to detect the difference between paired flushed and unflushed branches within each experiment. The paired t-test has more power to detect consistent relative changes between branches with considerable variance in conductance than ANOVA. PLC for each heat-treatment experiment was conducted. An ANOVA test followed by a post-hoc Tukey test were conducted to compare the difference in PLC between treatments within experiments All statistical analyses were conducted in R statistical software (R Core Team, 2018).

RESULTS

Experiment 1: Whole shoot branch conductance

The leaf specific conductance (K_{leaf}) between the control, control flushed, and treatment flushed were not significantly different for *E. cladocalyx* or *K. africana* (Fig. 3). This indicated that the branches treated with a heat-plume were able to recover conductance post flushing of the branches for both species. The reduction and recovery of K_{leaf} indicates that the loss of conductance was due to embolism, rather than to heat-deformation. No significant difference between the control and control flushed indicated that the branches had no significant embolism present before experimentation, embolism was not induced by sampling and flushing the branches did not create artefactual reduced conductance. The flushing protocol did not result in reductions of K_{leaf}. Therefore, the significantly lower conductance of *K. africana* treatment branches when compared to the controls and the flushed treatment was due to the heat-plume treatment (ANOVA: F = 20.13, $p < 0.001$). Contrastingly, the conductance of *E. cladocalyx* treatment branches were not significantly different from the flushed treatment branches but did however also differ significantly from the controls (ANOVA: *E. cladocalyx*: F = 6.76, $p < 0.01$). *E. cladocalyx* had a higher conductance than *K. africana*.

Paired t-tests were conducted to test for differences between the control or treated conductance of the branches to their flushed conductance. The K_{leaf} between the flushed and initial of the control branches for both species, *E. cladocalyx* and *K. africana* were not significantly different (Paired t-test: *E. cladocalyx*: t = 1.98, df = 5, $p > 0.5$; *K. africana*: t = -0.34, df = 5, $p < 0.5$; Fig. 3). The heat-treated branches resulted in a significant loss of conductance and conductance was recovered by flushing the branches (Paired t-test: *E. cladocalyx*: t = -4.78, df = 5, $p < 0.001$; *K. africana*: t = -6.05, df = 5, $p < 0.001$; Fig. 3). Experiment 1 reproduced the results of West *et al* (2016) and showed that embolism occurred with a simulated heat-plume, inferring that a fire-induced heat-plume results in embolism. Post-fire, both species suffered xylem conductance loss, however at this point the location of the embolism events were unknown.

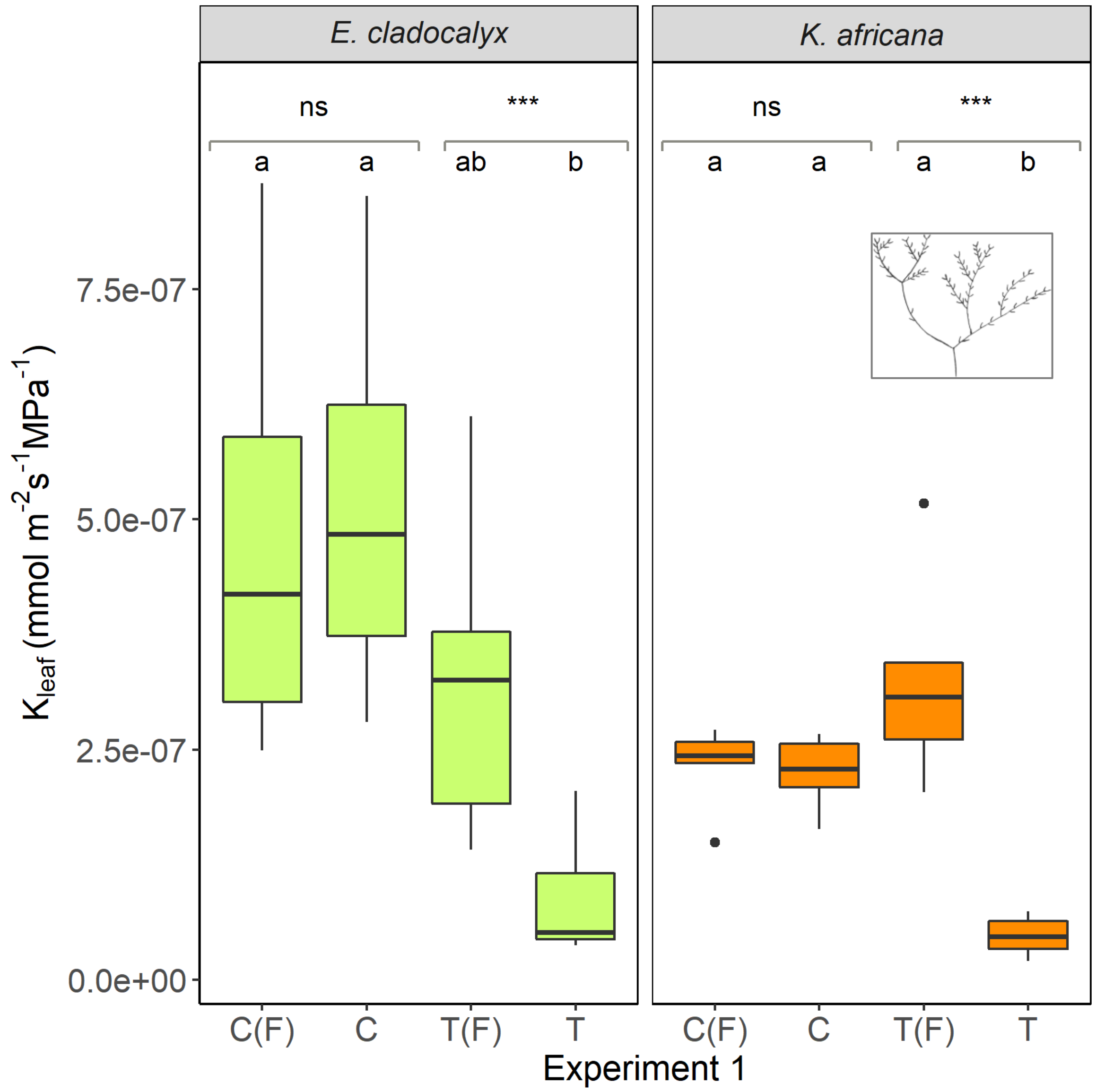


Figure 3: Experiment 1: Leaf specific canopy conductance (K_{leaf}) post 100°C heat-plume for whole shoot branches (schematic of a branch inserted showing the whole shoot branch) of *Eucalyptus cladocalyx* and *Kiggelaria africana*. C(F) = "Control flushed", C = "Control", T(F) = "Treatment flushed", T = "Treatment". Plots show the median values (bold horizonal line), interquartile range (box), range (whiskers) and outliers (closed circles). Paired t-tests were conducted comparing the treated and flushed conductance of the branches, the significant differences are indicated by *, where *** is $p<0.001$, ** is $p<0.01$, * is $p<0.05$ and ns is not significant. ANOVA test and a post-hoc Tukey test were conducted, differing letters indicate that there is a significant difference between the treatment levels. Refer to Appendix Table A and B for raw data at each experiment level for both species.

Experiment 2: Branch conductance without non-suberized tissue

Trimming off the non-suberized portion of the branches in Experiment 2 had no impact on the relative patterns of K_{leaf} in both species and returned similar results to Experiment 1 (Fig. 4). The non-suberized part of this branch has been trimmed off post heat simulation. There were no significant differences in K_{leaf} between the control, control flushed, and treatment flushed for either *E. cladocalyx* or *K. africana*. This indicated that the heat-treated branches were able to recover conductance post flushing of the branches. The treatment branches were statistically different, for both species, to the flushed of the heat-treated branches as well as the controls (ANOVA: *E. cladocalyx*: $F = 5.52$, $p < 0.01$; *K. africana:* $F = 7.12$ $p < 0.01$).

The K_{leaf} between the flushed and initial of the control trimmed branches for *E. cladocalyx* and *K. africana* were not significantly different (Paired t-test: *E. cladocalyx*: $t = 1.84$, $df = 6$, $p > 0.5$; *K. africana*: $t = 0.60$, $df = 5$, $p > 0.5$; Fig. 4). Both species suffered a significant reduction in conductance (Paired t-test: *E. cladocalyx*: $t = -6.51$, $df = 6$, $p < 0.001$; *K. africana:* $t = -18.80$, $df = 5$, $p < 0.001$; Fig. 4). This indicated that the trimmed branches still had a significant amount of embolism present even with trimming the non-suberized, youngest, distal parts of the branch. The conductance was recovered by flushing the branches, this indicating that embolism was the cause for reduced conductance.

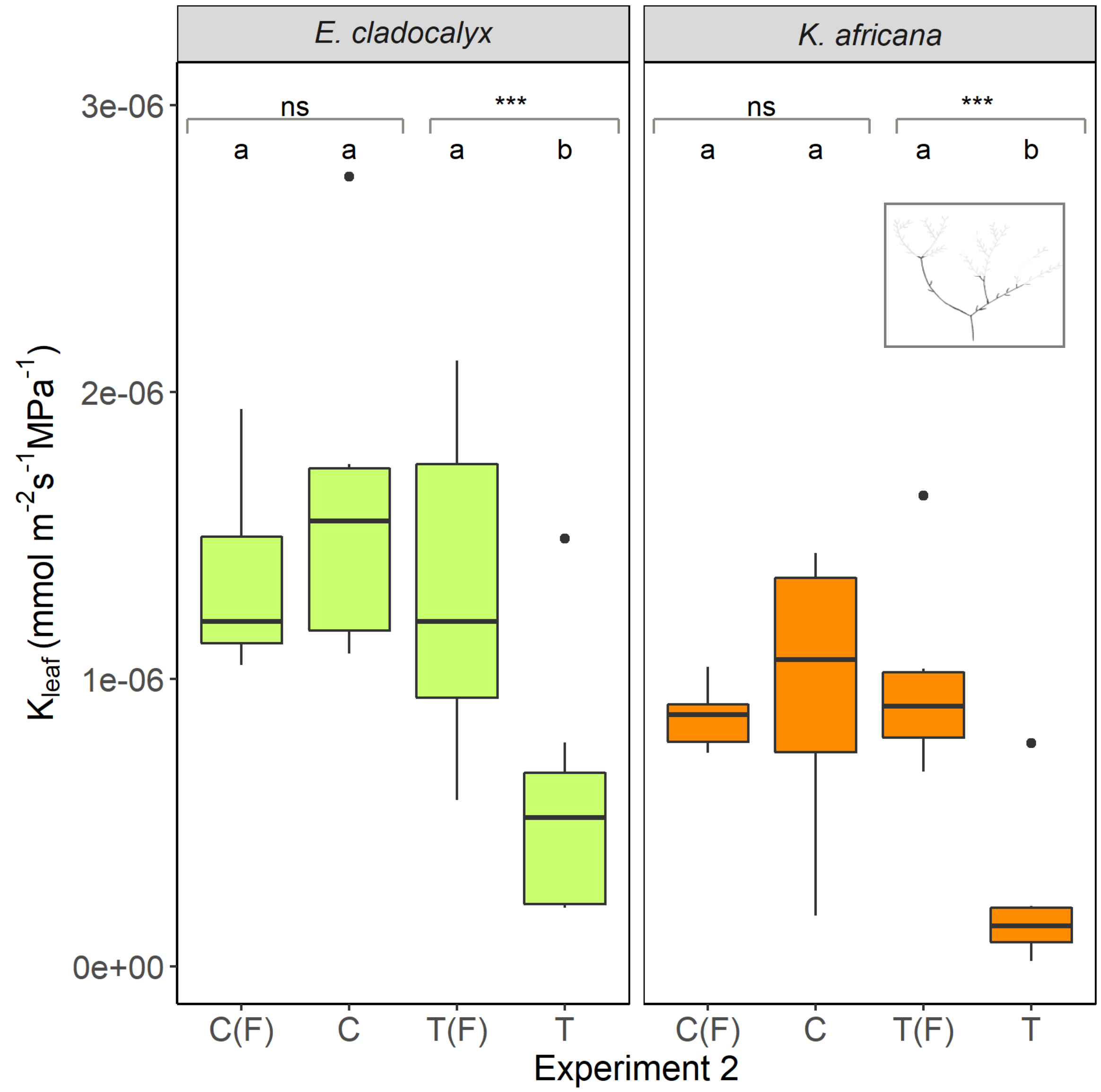


Figure 4: Experiment 2: Leaf specific canopy conductance (K_{leaf}) post 100℃ heat-plume for non-suberized trimmed off branches (schematic of a branch inserted showing the trimmed area) of *Eucalyptus cladocalyx* and *Kiggelaria africana*. C(F) = “Control flushed”, C = “Control”, T(F) = “Treatment flushed”, T = “Treatment”. Plots show the median values (bold horizonal line), interquartile range (box), range (whiskers) and outliers (closed circles). Paired t-tests were conducted comparing the treated and flushed conductance of the branches, the significant differences are indicated by *, where *** is $p<0.001$, ** is $p<0.01$, * is $p<0.05$ and ns is not significant. ANOVA test and a post-hoc Tukey test were conducted, differing letters indicate that there is a significant difference between the treatment levels. Refer to Appendix Table A and B for raw data at each experiment level for both species.

Experiment 3: Branch conductance with full distal node removed

Trimming off a full distal node resulted in far less percentage loss of conductance in heat-treated branches for *E. cladocalyx*, but not for *K. africana* (Fig. 5). In contrast to Experiment 1 and Experiment 2, the K_{leaf} between controls and heat-treated branches for *E. cladocalyx* in Experiment 3 were not significantly different (ANOVA: F = 1.19, $p < 0.5$), indicating that any loss of conductance in the heat-treated branches was removed by trimming off the distal node. This provided strong evidence that the loss of conductance seen in Experiment 1 and Experiment 2 in *E. cladocalyx* branches was localized in this distal node. In contrast, the difference between the heat-treated, treatment flushed, and controls branches of *K. africana* seen in Experiment 1 and 2 remained in Experiment 3 (ANOVA: $F = 9.41$ $p < 0.001$). Thus, the trimming of the distal node did not affect the loss of conductance in the heat-treated branches, suggesting that the loss of conductance in *K. africana* branches was not localised to the distal node.

There was considerable variance between individual branches when trimming off the distal node. Thus, we also compared the effect of flushing within branches using the paired t-test as an indication of whether there was a loss of conductance in the heat-treated branch that could be removed by flushing. K_{leaf} between the control and flushed control branches was not significantly different for both *E. cladocalyx* and *K. africana* (Paired t-test: *E. cladocalyx*: t = 3.08, df = 7, $p > 0.5$; *Kiggelaria africana*: t = 2.97, df = 5, $p > 0.5$). In contrast to the results from the ANOVA, *E. cladocalyx* experienced a slight significant increase in conductance in the flushed heat-treated branches when compared to the initial conductance (t = -2.93, df = 7, $p < 0.05$; Fig. 5). The paired t-test results for *K. africana* supported that of the ANOVA in showing a significant increase in conductance in flushed heat-treated branches (t = -4.88, df = 5, $p < 0.001$; Fig. 5). These results suggest that trimming the distal node of *E. cladocalyx* had a far greater impact on removing loss of conductance than it did for *K. africana*.

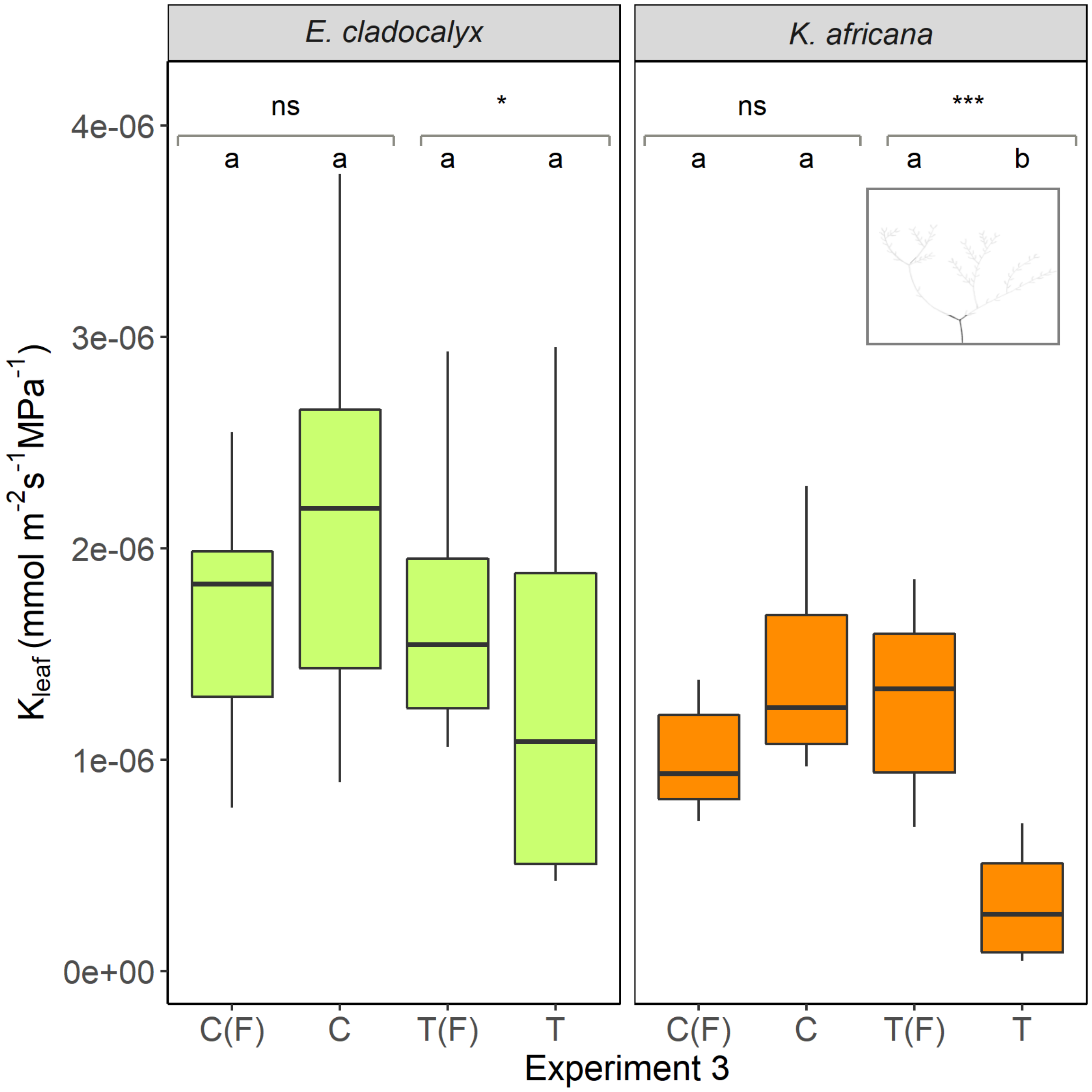


Figure 5: Experiment 3: Leaf specific canopy conductance (K_{leaf}) post 100°C heat-plume for trimmed back by a node branch (schematic of a branch inserted showing the trimmed area) of *Eucalyptus cladocalyx* and *Kiggelaria africana*. C(F) = “Control flushed”, C = “Control”, T(F) = “Treatment flushed”, and T = “Treatment”. Plots show the median values (bold horizonal line), interquartile range (box), and range (whiskers). Paired t-tests were conducted comparing the treated and flushed conductance of the branches, the significant differences are indicated by *, where *** is $p<0.001$, ** is $p<0.01$, * is $p<0.05$ and ns is not significant. ANOVA test and a post-hoc Tukey test were conducted, differing letters indicate that there is a significant difference between the treatment levels. Refer to Appendix Table A and B for raw data at each experiment level for both species.

Experiment 4: Testing the impact of open vessels on branch conductance

As mentioned, Experiment 3 was a concern for open vessels. The open vessels may have led to an artificially low PLC in the treated branches, but this was not true. For both species, blowing low pressure N_2 gas into the treatment stems (i.e., air-filling open vessels) resulted in a measurable loss of conductance, that was only regained upon flushing (Fig. 6). This was similar to the results seen in Experiment 1 and 2 and differed from the results of Experiment 3. The K_{leaf} between the control, control flushed, and treatment flushed were not significantly different for *E. cladocalyx* or *K. africana*. This indicated that the heat-treated branches were able to recover conductance post flushing. For *K. africana*, heat-treated branches were statistically different to their flushed as well as the controls of the experiment (ANOVA: *K. africana: F* = 15.25 $p < 0.001$). However, for *E. cladocalyx* heat-treated branches were not significantly different from the treatment flushed, but statistically different from the controls (ANOVA: *E. cladocalyx*: F = 7.39, $p < 0.01$). This experiment was performed on trimmed branches for both species, which resulted in a significant loss of conductance and the conductance was recovered by flushing (Paired t-test: *E. cladocalyx*: t = -7.39, df = 6, $p < 0.001$; *K. africana*: t = -17.05, df = 5, $p < 0.001$; Fig. 6). This indicated that we were not refilling open vessels during experimentation, but successfully measuring embolism in the branches.

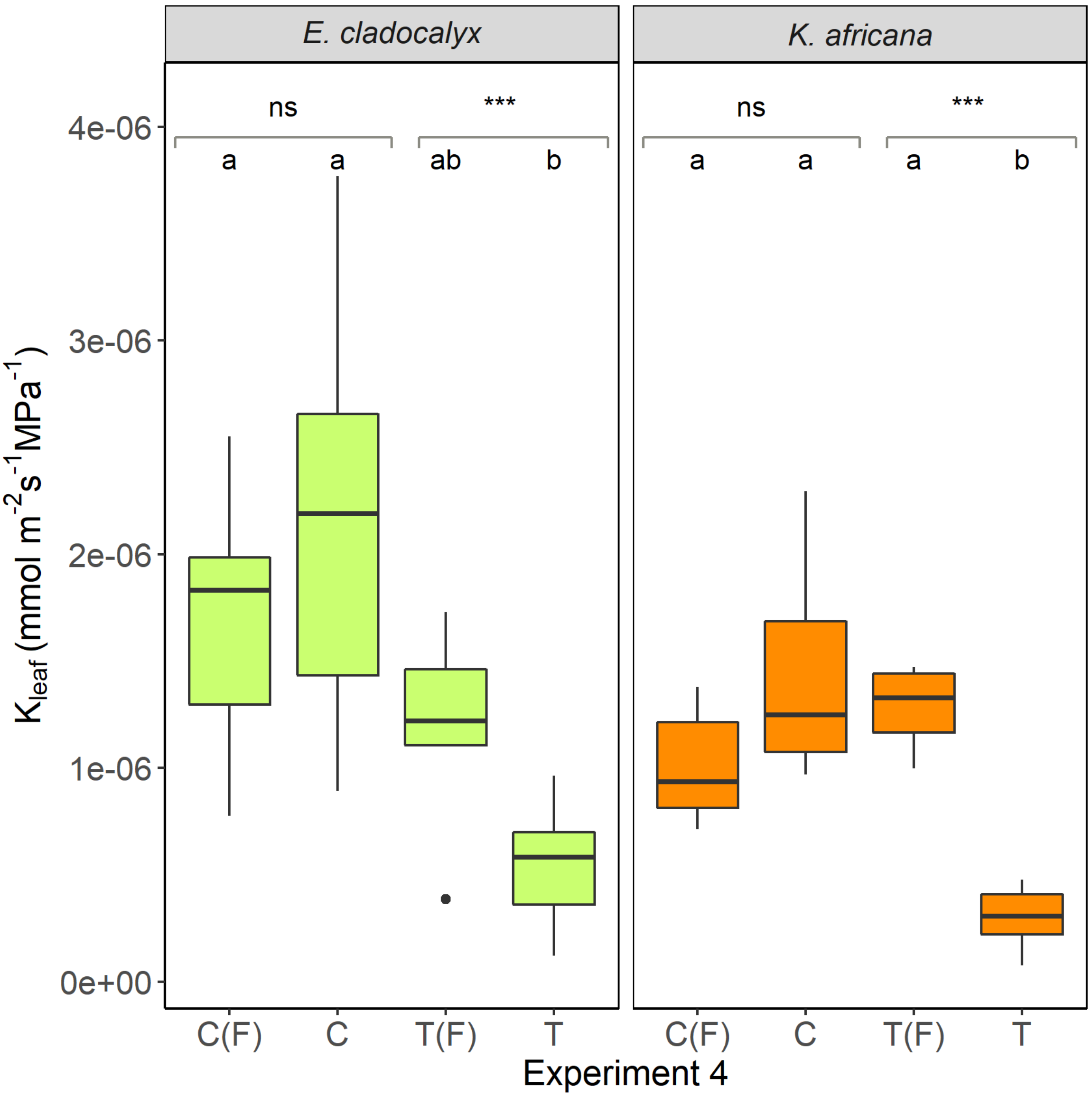


Figure 6: Experiment 4: Leaf specific canopy conductance (K_{leaf}) post blowing low pressure air into branches of *Eucalyptus cladocalyx* and *Kiggelaria africana*. C(F) = "Control flushed", C = "Control", T(F) = "Treatment flushed", and T = "Treatment". Plots show the median values (bold horizonal line), interquartile range (box), range (whiskers) and outliers (closed circles). Paired t-tests were conducted comparing the treated and flushed conductance of the branches, the significant differences are indicated by *, where *** is p<0.001, ** is p<0.01, * is p<0.05 and ns is not significant. ANOVA test and a post-hoc Tukey test were conducted, differing letters indicate that there is a significant difference between the treatment levels. Refer to Appendix Table A and B for raw data at each experiment level for both species.

Percentage Loss of Conductance in the different Experiments

E. cladocalyx had approximately 82% conductance loss in Experiment 1, 58% loss in Experiment 2 and a low 19% loss in Experiment 3 (Fig. 7), indicating that the reduced conductance post heat-plume differed between the experiments. An ANOVA test showed that there is a difference between the three experiments and a post-hoc TUKEY showed that Experiment 3 significantly differs from the other experiments (ANOVA: *E. cladocalyx*: F = 13.72, $p < 0.001$; Fig. 7). This implies that most embolism occurred in the distal part of the branch and that embolism can be removed with trimming. Contrastingly, *K. africana* had an approximate 80% conductance loss in Experiment 1, 74% loss in Experiment 2 and 68% loss in Experiment 3. An ANOVA test was conducted to test for difference between the means of the experiments and no statistical significance difference was shown (ANOVA: *K. africana: F* = 0.618, $p > 0.5$; Fig 7). This implies that embolism occurs throughout the branch and is not localized, and therefore could not be trimmed off.

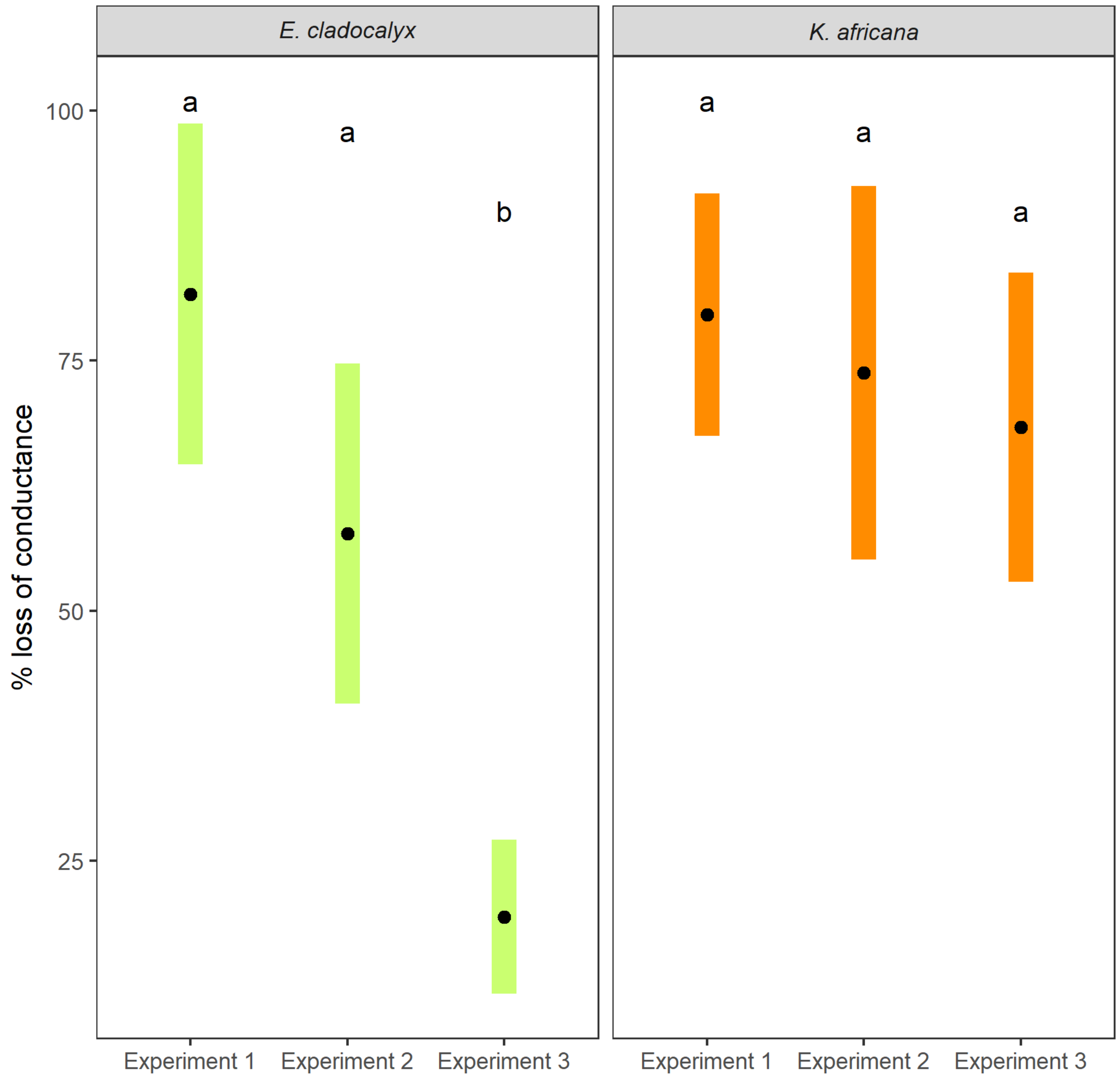


Figure 7: Percentage loss of conductance (PLC), calculated as the leaf specific canopy conductance (K_{leaf}) of the heat-treated branches relative to K_{leaf} of the flushed control branches, in *Eucalyptus cladocalyx* and *K. africana* across all three heat-plumed experiments. Points indicate the PLC value for each experiment and the shaded area indicate 95% CI around each point. An ANOVA and post-hoc Tukey test were conducted to test significance difference between experiments, letters were used to indicate difference in significant levels.

DISCUSSION

The purposes of this study were to determine whether embolism is localized to the distal parts of a branch post a heat-plume, and whether this differed between the two study species. This localization of embolism to distal ends was successfully shown as well as key findings in West *et al* (2016) being replicated. This further supports the hypothesis that increased VPD in a fire-plume leads to a transpiration burst, which ultimately results in extensive embolism and links hydraulic failure to post-fire mortality.

Both, *E. cladocalyx* and *K. africana*, experienced a severe loss of conductance post simulated 100°C heat-plume. In accordance with the *West et al* (2016) study, I found a c. 80% loss of conductance for *E. cladocalyx* and *K. africana*. This magnitude of embolism could be fatal (Urli et al., 2013). The large reduction in xylem conductance was recovered with flushing, which affirmed that the reduction was a result of embolism and not deformation.

The distal ends of branches were trimmed post simulated heat-plume and the conductance of the remaining segment of branch was measured. It was found that embolism was localized to these ends in *E. cladocalyx* as the PLC for the remaining branch was only 19%. Contrastingly, *K. africana* showed a 68% loss of conductance. The results for both species supported the proposed hydraulic segmentation hypothesis from West *et al* (2016), where through model simulations hydraulic segmentation was inferred for *E. cladocalyx* but not for *K. africana*. It was considered that a high PLC in the distal parts of the shoot together with a low PLC in the central parts would be evidence of hydraulic segmentation. Conversely, little difference in PLC between the distal and central parts would be indicative of little hydraulic segmentation. Hydraulic segmentation is exhibited in *E. cladocalyx.* A low degree of hydraulic segmentation was exhibited in *K. africana.* Fire resistant trees may be more capable of withstanding a fire and recovering as they can segment their hydraulic pathway, protecting a hydraulic continuum.

Artefacts are a concern in plant hydraulics. There are two possible artefacts that could occur, these are excision and open vessel artefacts (Wheeler et al., 2013; Hacke et al., 2015). Embolism may result from cutting while xylem vessels are under tension (Wheeler et al., 2013). The sampling protocol did not result in embolism. In Experiment 3, it was a concern that open vessels were a possible artefact, as these branches were trimmed a node back. This reduced the length of the branch to being much less than the maximum xylem vessel length of the branch. Open vessels are when vessels are not compartmentalised by the presence of the pits. Water may freely flow through the vessel at a very low pressure (Tyree & Zimmermann, 2002). We mitigated for both these artefacts in this study, and with confidence I can assume that the branches were not affected by these artefacts.

Do the distal portions of a fire-tolerant tree species act as a hydraulic fuse?

Hydraulic segmentation, in my dissertation, is used to describe the phenomenon that is analogous to the concept of the hydraulic fuse. It is the loss of conductivity in the distal portions of the hydraulic continuum. The distal sections of a branch have been shown to act as hydraulic fuses, thus resulting in hydraulic segmentation. Hydraulic segmentation may be key to the survival of trees post-fire and act as safety valve. From Ruthrof (2003), *E. cladocalyx* is shown to be fire-resilient with fast canopy recovery post-fire. High rates of canopy recovery may possibly provide good indication that the plant invests in hydraulic segmentation as a pyrohydraulic trait. Evidence through this study shows that *E. cladocalyx* does invest in hydraulic segmentation as a trait. The recovery can be assumed to have occurred by the resprouting of distal shoots from a position of where the water column is still intact. *E. cladocalyx* is a fire-tolerant species and has been shown to do well with high frequency fire. They are more resilient than other Eucalyptus species and the fire process seems to favour the driving of the population dynamic of *E. cladocalyx* (Ruthrof, Loneragan & Yates, 2003). This species proves to be a successful alien invader post-fire, and caution should be practised when planting them in fire-prone environments. *K. africana*, a fire intolerant species, shows a lack of this trait and this may count towards its inability to survive a fire event (van Wilgen et al., 1992).

Hydraulic segmentation could be important and necessary for the survival of fire-prone species. Hydraulic segmentation will help a tree species post-fire as it will preserve the most vital part of the plant hydraulic pathway. The plant then stands a chance to recover post-fire. As the embolism is localized to the distal parts of the canopy, further exploration in canopy dieback should be considered. Canopy dieback could be connected to hydraulic segmentation and regrowth from the intact water column. As mentioned, live buds can be located where the water column is still intact. Plant physiology plays a vital role in fire adaptation.

It is vital to recognise that fire is complex and not uniform (O’Brien et al., 2018). Hydraulic segmentation could only be one trait among many pyrohydraulic traits that aid in survival. We need to better understand hydraulic linked death as well as fire behaviour. This will improve modelling of tree death mortality. Hydraulic segmentation is a hydraulic fuse which localizes embolism, such fuses and the mechanisms related to it should be further explored. Hydraulic segmentation facilitates in the protection of plant hydraulics by localizing embolism. This improves the survival of the tree post-fire and allows for the tree to recover. As hydraulic segmentation is not seen in both species, there must be a cost-benefit to investing in hydraulic segmentation. Consequently, there may be potential risks to having sensitive distal tissue as the tree may lose function when it is unnecessary such as in a mild drought or heat wave. Therefore, the cost of hydraulic segmentation should be explored as some trees lack hydraulic segmentation. This trait is beneficial as it assists in rapid recovery. Finally, this study further supports the hydraulic death hypothesis.

CHAPTER 3: Is Vulnerability Segmentation a Mechanism that promotes Post-fire Survival?

INTRODUCTION

Embolism reduces xylem hydraulic conductance by causing air blockages that hinder the movement of water through the plant and can ultimately lead to death of tissues downstream of the blockage. In Chapter 2, I investigated whether embolism was localized to the distal parts of the plant's hydraulic pathway when exposed to a heat-plume. It was proposed that this form of hydraulic segmentation acts as a hydraulic fuse (Tyree & Ewers, 1991; Tyree et al., 1993; Tyree & Zimmermann, 2002) that would allow the tree to recover post-fire. As embolism would be localised to distal parts, these embolized ends could then be shed off and new growth could occur from the previously dormant buds hidden in the stem, and these buds would be supplied by the remaining intact water column.

In Chapter 2, it was found that *E. cladocalyx* exhibits hydraulic segmentation, because loss of function was localized to the distal parts of the branch. *K. africana*, on the other hand, showed a lack of hydraulic segmentation. As described in Chapter 1, two distinct, but not mutually exclusive mechanisms may explain this hydraulic segmentation phenomenon: resistance segmentation and vulnerability segmentation. In this chapter, vulnerability segmentation was examined as a potential mechanism that may explain the exhibited hydraulic segmentation.

In order to explore vulnerability segmentation, I concurrently generated vulnerability curves (VCs) at different points along the stem to determine whether embolism was localised or continuous along the branch. From these vulnerability curves, three parameters were extracted the P_{12}, P_{50} and P_{88}. These are the pressures at 12, 50 and 88% loss of conductance (P_{12}, P_{50} and P_{88}). P_{12} is the proxy of pressure at which air entry occurs producing embolism. P_{50} is the standardized midpoint measure of vulnerability of VCs. P_{88} is the measure at which embolism is irreversibly lethal in many angiosperm species and represents a point of major hydraulic failure in the plant (Urli et al., 2013). I tested this by examining, through the minimally invasive optical technique, by means of comparing the generated VCs from this method and extracting the parameters. This allowed me to look at distinct portions of a long stem simultaneously. This method is based on the principle that a distinct colour change in visible conduits will occur when there is a transition from a water-filled conduit to an air-filled conduit (Brodribb et al., 2017). The optical technique has been used to record spatial and temporal patterns of embolism formation within leaves as well as stems, but rarely simultaneously and on the same individual plant. (Brodribb et al., 2016, 2017; Brodribb, Bienaimé & Marmottant, 2016; Skelton, Brodribb & Choat, 2017).

The aim of Chapter 3 was to explore whether the patterns of hydraulic segmentation seen in Chapter 2 were due to the mechanism vulnerability segmentation. I investigated if the different sections were more or less vulnerable to embolism than each other or if embolism occurred same water potential. I tested this by examining whether different parts of the equilibrated (i.e., non-transpiring) branch's stem embolised at the same or at different water potentials. Recall in Chapter 2, hydraulic conductance measurements were conducted on whole shoot and trimmed branches, thus in Chapter 3 I aimed to focus on the crucial sections of the branch that related to the trimmed sections results from Chapter 2. Specifically, I looked at two tissue sections of the branch, non-suberized and suberized tissue. Non-suberized tissue section of the branch is the most distal part of a branch, it is the part of the branch that has not yet been modified by the deposition of suberin, they are less woody and considered to be part of the youngest shoot. Whereas the suberized tissue section of the branch is modified by the deposition of suberin and contains fully mature bark. In Chapter 2, three simulated heat-plume experiments were conducted that, tested the xylem conductance of a 1) whole shoot branch, 2) a branch with the youngest shoots trimmed off, and lastly 3) a branch that was trimmed a node back (see Fig. 8).

Analysing these two sections were done to determine whether the most distal part of the branch was more vulnerable to embolism. It is expected that the non-suberized section, which is the most distal part, of the branch would be more vulnerable to embolism, as these younger, more distal tissues are more readily replaced. In Experiment 1 of the xylem conductance measurements both species experienced a severe loss of conductance, and in Experiment 2 *E. cladocalyx* experienced a 58% loss in conductance and *K. africana* a 74% loss. I hypothesised that *E. cladocalyx* non-suberized tissue would be more vulnerable to early embolism compared to its suberized tissue. For *K. africana* I hypothesised that the non-suberized tissue would have a similar vulnerability to embolism as its suberized tissue. This would be consistent with the hypothesis that *E. cladocalyx* exhibits hydraulic segmentation caused by vulnerability segmentation. To test this, it was considered that a higher P_{12}, P_{50} and P_{88} for the non-suberized tissue than the suberized tissue would be evidence of vulnerability segmentation. These values of each tissue section of each branch were determined by constructing vulnerability curves. It is expected that the mean values for P_{12}, P_{50} and P_{88} of the non-suberized section of *E.*

cladocalyx would be higher than the suberized section of *E. cladocalyx*. Conversely for *K. africana*, it is expected that the mean values for P_{12}, P_{50} and P_{88} of the non-suberized section and suberized section would be similar. This would then support the hypothesis that *E. cladocalyx* is more vulnerable to embolism at the distal ends than *K. africana*.

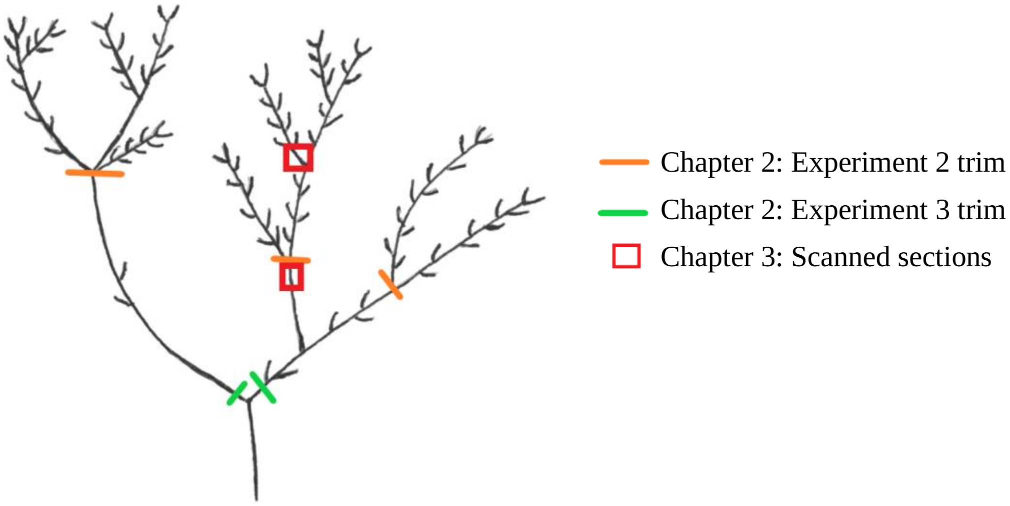


Figure 8: Schematic drawing of the generic branches showing what sections of the branches were tested separately in Chapter 2 and in Chapter 3. In Chapter 2, the branch was sequentially trimmed back. In Experiment 1, the branch was defoliated, thus the conductance of the whole shoot was measured. In Experiment 2, the branch was trimmed back by removing the non-suberized section of the branch, indicted with orange lines. In Experiment 3, the branch was trimmed even further back by trimming a node back, indicated with green lines. In Chapter 3, the branches were placed down on the flatbed scanner. The red blocks indicate the sections at which the xylem was exposed, and images of the embolism events were captured.

METHOD

Two-meter-long branches of *Eucalyptus cladocalyx* and *Kiggelaria africana* were collected from healthy, large trees growing on the University of Cape Town campus, following the field collection methodologies in Chapter 2. These branches were collected during the summer of 2020 and then later during late winter of 2020, the time interval between sampling sets was a result of the COVID-19 pandemic lockdown. These branches were collected before sunrise at periods when the trees were most hydrated. Upon collection the branches were placed in a bucket of water and transported to the lab, where they were re-cut under water to prevent accidental embolism. Branches were kept between 1.5 and 2m for the experiment, ensuring that the branch was above the maximum vessel length. Stem water potential of shoots from the collected branches were measured using a Scholander Pressure Chamber (PMS Instrument Company Model 1505D) to determine if the branches were water-stressed at the time of sampling. The stem water potential was then measured every 10 minutes until the completion of setup. The branch was kept in a dark room in a bucket of water, while the scanners and psychrometer were set up, this ensured the stomata were closed and the branch was fully hydrated.

Experimental setup

While the branch was submerged in water, two small sections where the xylem would be exposed for scanning, were chosen. A non-suberized and suberized part of the stem were chosen (Fig. 9: label 1 and 2, respectively). The bark length of approximately 6mm sections were delicately removed, by peeling the layer of bark away without touching the xylem. This revealed two exposed portions of xylem which were ca. 10cm apart from each other. The location of the non-suberized section could be found along the intact branch in Experiment 1 of the xylem conductance measurements, as these branches were not trimmed (see Fig. 8). The location of the suberized section could be found along the branch segment that was still intact and not trimmed off in Experiment 2 of the xylem conductance measurements (see Fig. 8). These pieces of exposed xylem were continuously kept wet with wet paper towel, until the branch was secured on the scanners.

Capturing images using a desktop scanner

For this experiment, a flatbed scanner (Epson Perfection V800) was used to capture images of the exposed xylem of both sections of each plant. By collecting repeat images every few minutes and subtracting subsequent images from each other, the scanner would detect the distinct colour change when conduits transition from water-filled to air-filled. Full details, including an overview of the technique, image processing, as well as scripts to for step-by-step image capture and analysis, are available at http://www.opensourceov.org. Briefly, the branch was arranged over the scanner, the branch was taped and secured down to ensure no movement while scanning. The exposed portions of xylem were placed face down on the scanner in a well of Tensieve® conductive adhesive gel (Parker Laboratories, Inc.) surrounded by a containing well of Prestik (Bostik). The sections were pushed as close to the scanner face as possible; this ensured the window of exposed xylem was in focus. The cut end of the branch was sealed with Parafilm^{TM} (Bemis) and Dow Corning® high vacuum grease, to minimize drying-out from the proximal end. For the image collection, AutoIt automation software (AutoIt Consulting Ltd), VueScan (Hamrick Software) was used to interact with the scanners to set and run the image capturing process. The AutoIt script was edited to capture an image every five minutes while the branches were drying out. The branch was left to dry down for several days, ensuring all embolism events were captured during this time. Once image capture was completed, the image sequences were processed. The images were downloaded and loaded onto Fiji ImageJ (National Institutes of Health) as an Image sequence. OpenSourceOV (OSOV) toolbox from opensourceov.org was installed in Image J and used to process the images. Each image was compared with each subsequent image to generate a pixel difference between successive images, revealing when the reflectance of light changed from xylem that was water filled to xylem that was air filled. The pixels of embolism were selected through this process and the remaining pixels (e.g., noise from minor movement of the dehydrating branch) were cleared. The pixels of embolism were summed up in this image stack. The saved image stacks were later used to create montages using sequence colouring.

Stem Psychrometer Installation

Further down the bole, a larger section, approximately five centimetres in diameter, of bark was removed. This was removed by using a blade to delicately cut through the bark and followed by peeling away the bark to reveal the xylem tissue. Damage to the xylem was carefully avoided. This section was more than 60cm away from the cut end of the branch which was submerged in water. A Stem Psychrometer (ICT PSY1) was fitted on to this exposed region of xylem on the stem (Fig. 9: label 3). This was then kept damp, while high vacuum grease was lightly applied to the rim of the psychrometer and connected to the data logger and personal computer. ICT was installed on the computer and the program was used to set up the logger. It was set to log stem water potential every 10 min, with a 5 second for Peltier cooling and 6 second of waiting time. The psychrometer was attached to this exposed xylem region and high vacuum grease was used around the edges of the psychrometer to ensure a complete vacuum seal. This also sealed up the surrounding exposed xylem, preventing the xylem from drying out due to air exposure. The psychrometer was then wrapped, to ensure environmental changes did not impact the readings.

While the psychrometer was equilibrating over the space of an hour, water potential of shoots was measured using a Scholander pressure chamber (PMS instruments). This was completed by trimming off a shoot with a cluster of leaves and placing it into the pressure chamber. This was done to 1) ensure leaf and stem water potential were similar, which was expected after the stomata had closed, and 2) ensure the psychrometer was correctly attached.

In cases where psychrometer did not attach correctly and the results from the psychrometer did not match with the initial pressure bomb measurements, the water potential of the plant was sequentially measured using a pressure bomb. Pressure bomb measurements were initially conducted every 10 minutes until the measurements were similar and then the time interval was increased. These were the intervals: the water potential was measured every 15 minutes, 20 minutes, 30 minutes, 40 minutes, and finally at hour intervals.

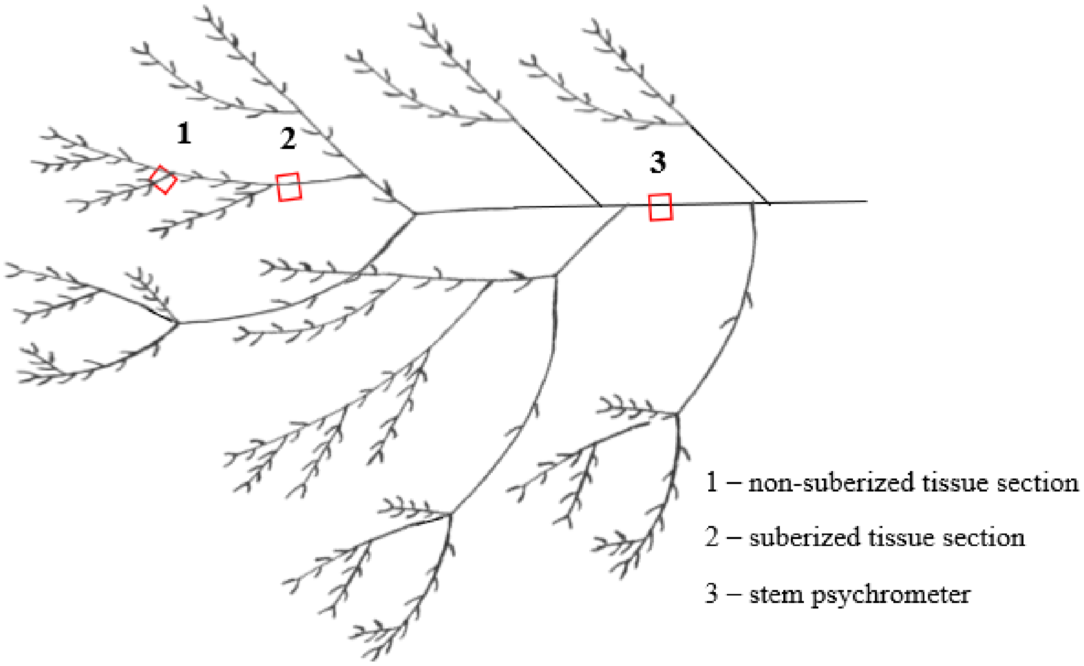


Figure 9: A schematic drawing of a generic distal branch that was placed down on a flatbed scanner. The red blocks indicate the sections at which the xylem was exposed, 1) non-suberized tissue section, 2) the suberized tissue section and 3) the area on which the stem psychrometer was placed.

Vulnerability curve analysis

The water potential (MPa) measured from the pressure bomb and stem psychrometer was plotted against time (minutes). This had three clear linear phases. These three linear phases were fitted and used to create water potential, Ψ, vs time relationship that could be used to predict Ψ for every image. The slope and the intercept were used to calculate the Ψ for each image using the images timestamp and the following equation:

$$\Psi = \text{Timestamp} * \text{Slope} + \text{Intercept} \qquad \text{Eqn 5}$$

The percentage of embolism was calculated by summing up pixels that changed colour, this would provide results per image and the maximum amount of embolism in pixels recorded. A water potential and percentage of embolism could be extracted for every captured image (embolism event). Vulnerability curves (VCs) were fitted using a sigmoid function (Pammenter & Willigen, 1998, Eqn 6) using the nonlinear least square (nls) function in R (R Core Team, 2018). This modelled the relationship between the percentage of embolized area and water potential for each section.

$$y = \frac{100}{1+e^{(a*(x-b))}} \qquad \text{Eqn 6}$$

Where a relates to the steepness of the curve and b is the water potential (MPa) at 50% loss of conductance. This shows the percentage loss of conductance as a function of xylem pressure (Delzon et al., 2010). The pressure at 12% loss of conductance (P_{12}), 50% loss of conductance (P_{50}) and 88% loss of conductance (P_{88}) were extracted from these VCs. The P_{50} was extracted from the result parameters, which was done by fitting a sigmoid function using the nls function. The P_{12} and P_{88} were extracted by finding the tangent to the response curve at 50% of the maximum embolism (Domec & Gartner, 2001). The slope of the tangent is given by the derivative of equation 6 as described in Domec & Gartner (2001):

$$\frac{d}{dx} = \frac{100*a*e^{-a(x-b)}}{(e^{-a(x-b)}+1)^2} \qquad \text{Eqn 7}$$

Water potential where the embolized area is 50% both $e^{-a(x-b)}$ terms will equal to one, thus the slope of the tangent was:

$$\text{slope} = a*100/4 \qquad \text{Eqn 8}$$

using this slope and the point at P_{50}, as the line passes through this point (b, P_{50}), the P_{12} and P_{88} could be extracted from the tangent line.

Statistical Analysis

The average P_{12}, P_{50} and P_{88} each tissue section was calculated, to measure the vulnerability of the species. Paired t-tests were conducted, to detect the difference between paired sections of the branch, comparing the non-suberized to the suberized tissue section of the branch. All statistical analyses were conducted in R statistical software (R Core Team, 2018).

RESULTS

There was close agreement between the pressure bomb data and the stem psychrometer data. The three linear phases fit well and provide a good prediction of water potential per image (Fig. 10). The raw water potential had phases, a first phase that was an extreme steep decrease, this was followed by a second phase which was slightly less steep and finally a third which was a shallow to levelling-off decrease. This was used in the vulnerability analysis. *E. cladocalyx* dried down to approximately −6MPa, and *K. africana* dried down to approximately between −6.5 and −7MPa. *K. africana* took four to five days to dry down to its most negative water potential and *E. cladocalyx* took five to seven days. *K. africana* experienced a bigger drop in water potential over a shorter period compared to *E. cladocalyx* (Fig. 10).

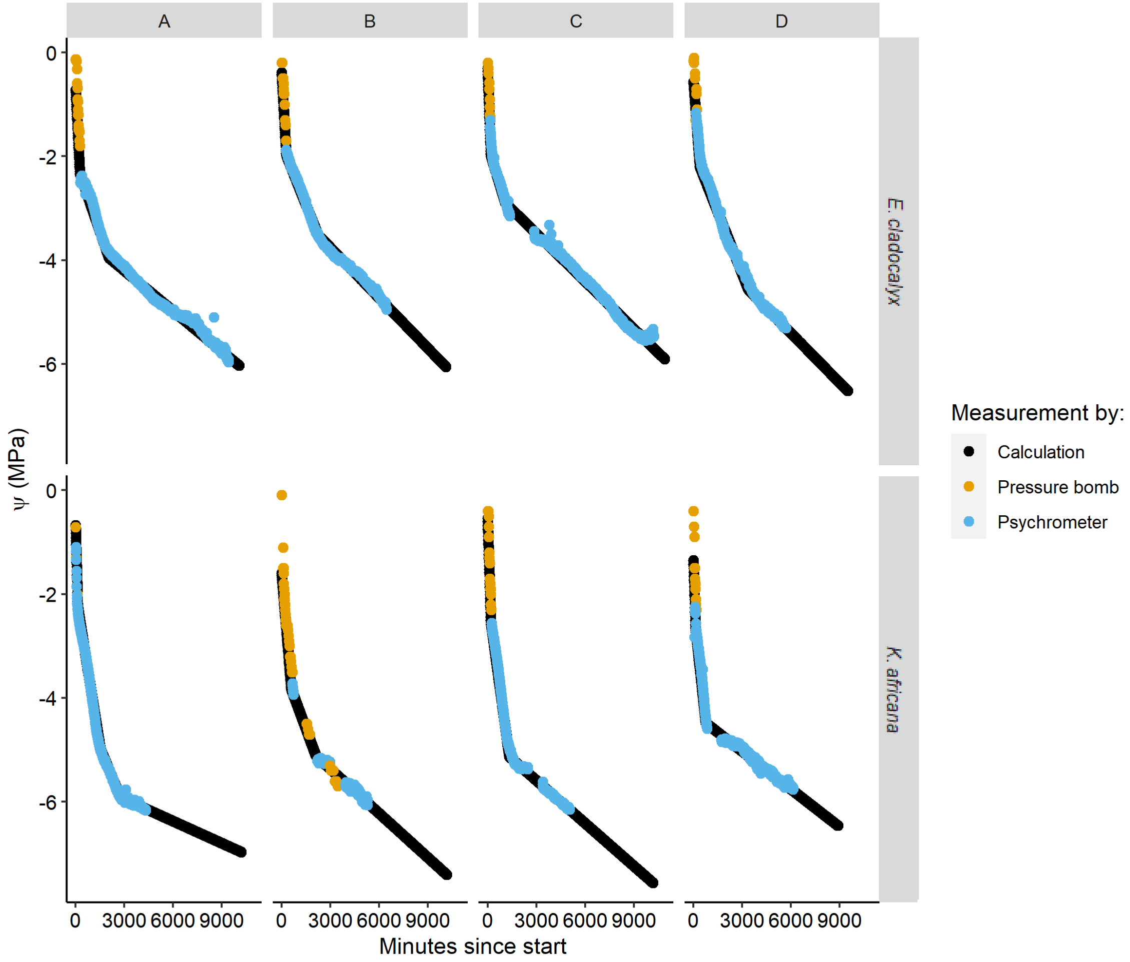


Figure 10: The water potential plotted against time since the start of the experiment for each branch sample (A -D) of each species, *E. cladocalyx* (top) and *K. africana* (bottom). The water potential was measured using a pressure bomb (orange) and a stem psychrometer (blue), these measurements were then used to calculate water potential (black) for the timestamp of the image.

Embolism events of captured conduits were seen in both species, all sections of all samples reached maximum amount of embolism as the cumulative area of embolised conduits plateaued (Fig. 11), this implies that the methodology used was correct. Both species reached the maximum embolism recorded over a short period of time and then plateaued (Fig. 11). Within each species the suberized sections had more embolized conduits than non-suberized sections. This could have been expected as suberized sections had a larger surface area, than the non-suberized sections. Sample B of *E. cladocalyx* is an evident outlier, as the curve does not follow the same pattern of curve shape as the other samples which is expected (Fig. 11).

Embolism events for both species were captured before the branch reached a water potential of −1MPa, thus early embolism events were caught. If the branch suffered embolism events before images were taken, these embolism events could not be captured or analysed. This supports that the field sampling did not result in major damage to the plant before the experiment was performed and that the plants were hydrated and unstressed at the time of collection. The area of embolism highlighted in coloured pixels covers the exposed window of the sections (Fig. 12).

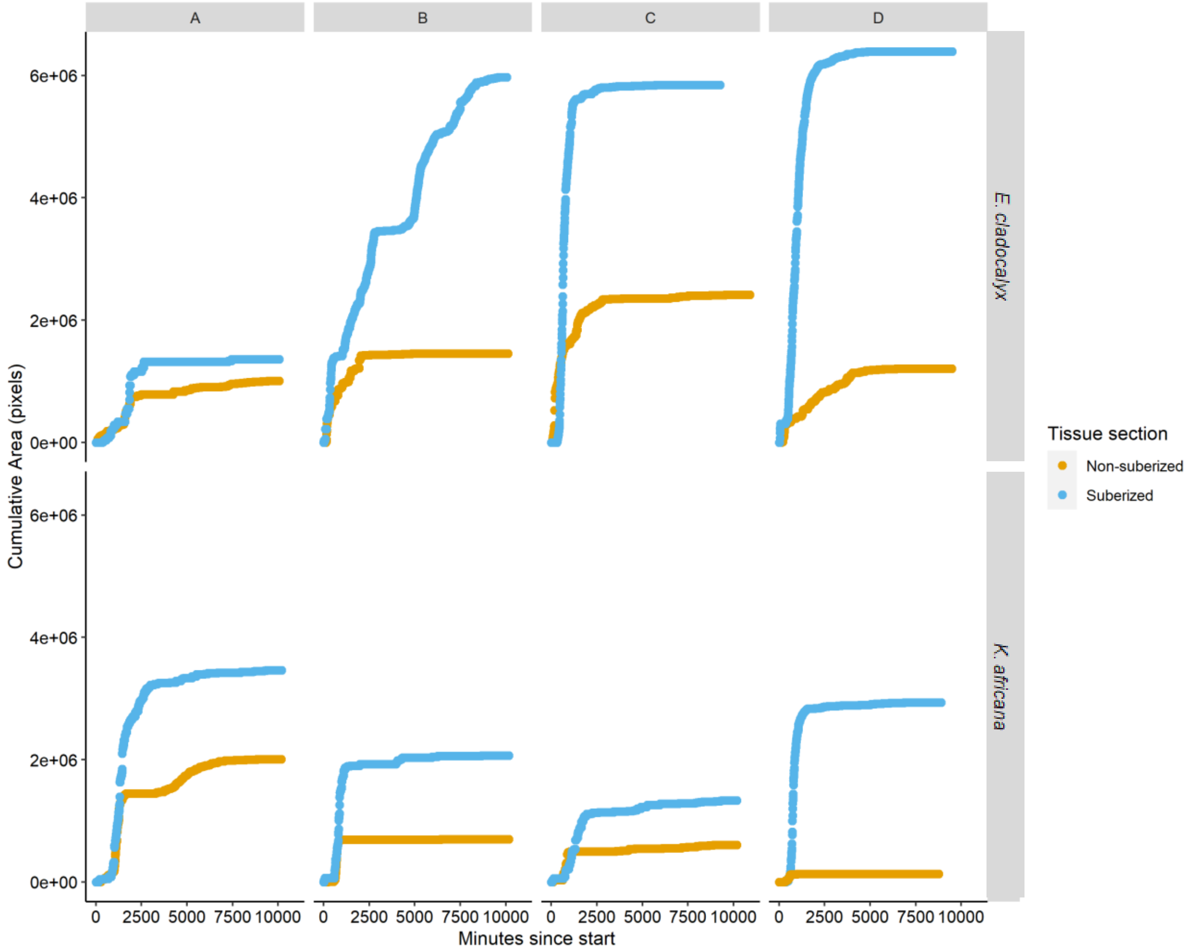


Figure 11: The area of exposed xylem captured summed as the cumulative area of embolized conduits over time since the start of the experiment for each branch sample (A -D) of each species, *E. cladocalyx* (top) and *K. africana* (bottom). Showing the non-suberized (orange) and suberized (blue) tissue sections for each sample.

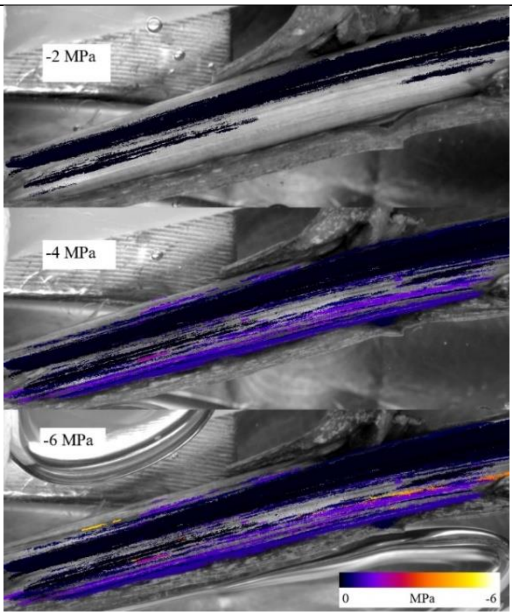
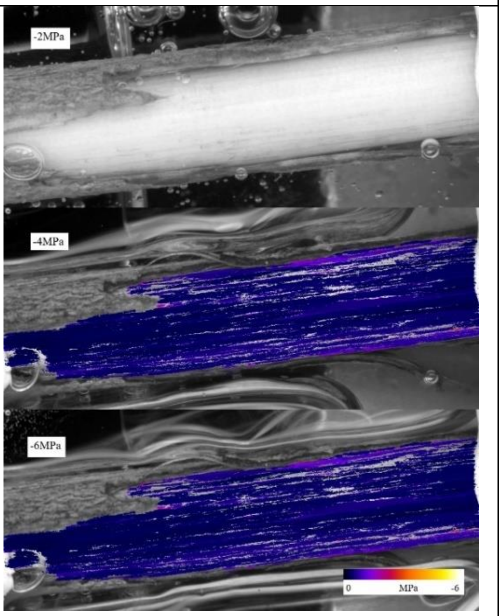
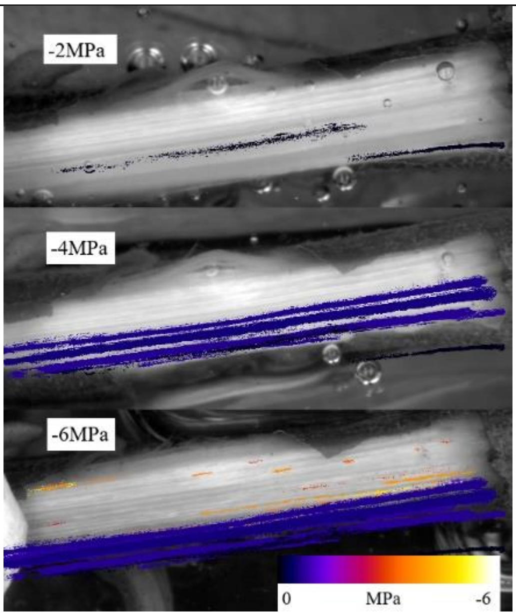
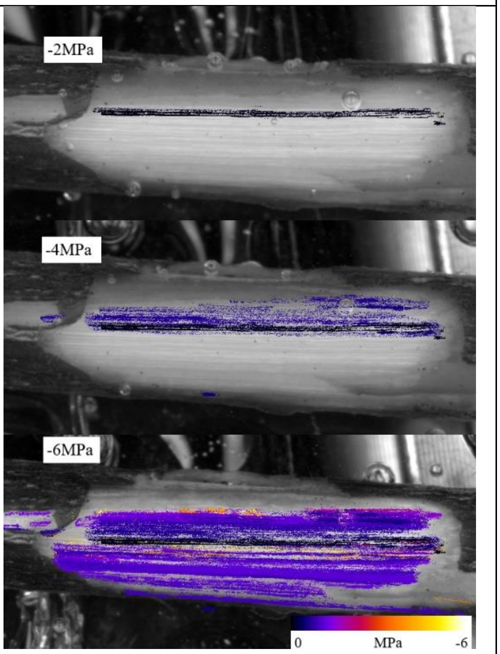
Species	**non-suberized tissue**	**suberized tissue**
E. cladocalyx		
K. africana		

Figure 12: Embolism events of sample C of each species, *E. cladocalyx* and *K. africana*, for each tissue section, non-suberized and suberized, as observed using the optical technique. This shows the embolism accumulated at different water potentials, WP, namely −2, −4 and −6MPa. The scale bar indicates the embolized pixels coloured in relation to the water potential at which the embolism occurred (Refer to Appendix Figure A and B for all samples).

E. cladocalyx visually seemed to experience more embolism in the non-suberized tissue at less negative water potentials than in the suberized tissue (Fig. 13). On a species level, *K. africana* displayed embolism events at a more negative water potential then *E. cladocalyx* (Fig. 13 & Table 1). However, *E. cladocalyx* has shallower curves than *K. africana*, indicating that *E. cladocalyx* is slightly more resistant to embolism overall. *E. cladocalyx* experienced embolism events over a longer period than *K. africana* (Fig. 12 & 13).

Paired t-tests were conducted to test for mean differences between the non-suberized and suberized tissue section of the branches. The P_{50} mean value between the non-suberized and suberized tissue section branches for both species, *E. cladocalyx* and *K. africana* were not significantly different (Paired t-test: *E. cladocalyx*: $t = -0.62$, $df = 3$, $p < 0.5$; *K. africana*: $t = 1.94$, $df = 3$, $p < 0.1$; Fig. 14, Table 2). The P_{12} mean between the non-suberized and suberized tissue section branches for *K. africana* was not significantly different, however, was significant difference for E. *cladocalyx* (Paired t-test: E. *cladocalyx*: $t = 10.54$, $df = 3$, $p < 0.001$; *K. africana*: $t = 2.25$, $df = 3$, $p < 0.1$; Fig. 14, Table 2). The P_{88} mean between the non-suberized and suberized tissue section branches for both species were not significantly different (Paired t-test: *E. cladocalyx*: $t = -0.21$, $df = 3$, $p > 0.5$; *K. africana*: $t = 0.21$, $df = 3$, $p < 0.5$; Fig. 14, Table 2).

Embolism events between the exposed sections of both species were similar, apart from the P_{12} value of *E. cladocalyx*. Embolism events first occurred in the non-suberized tissue section of *E. cladocalyx* (Fig. 14).

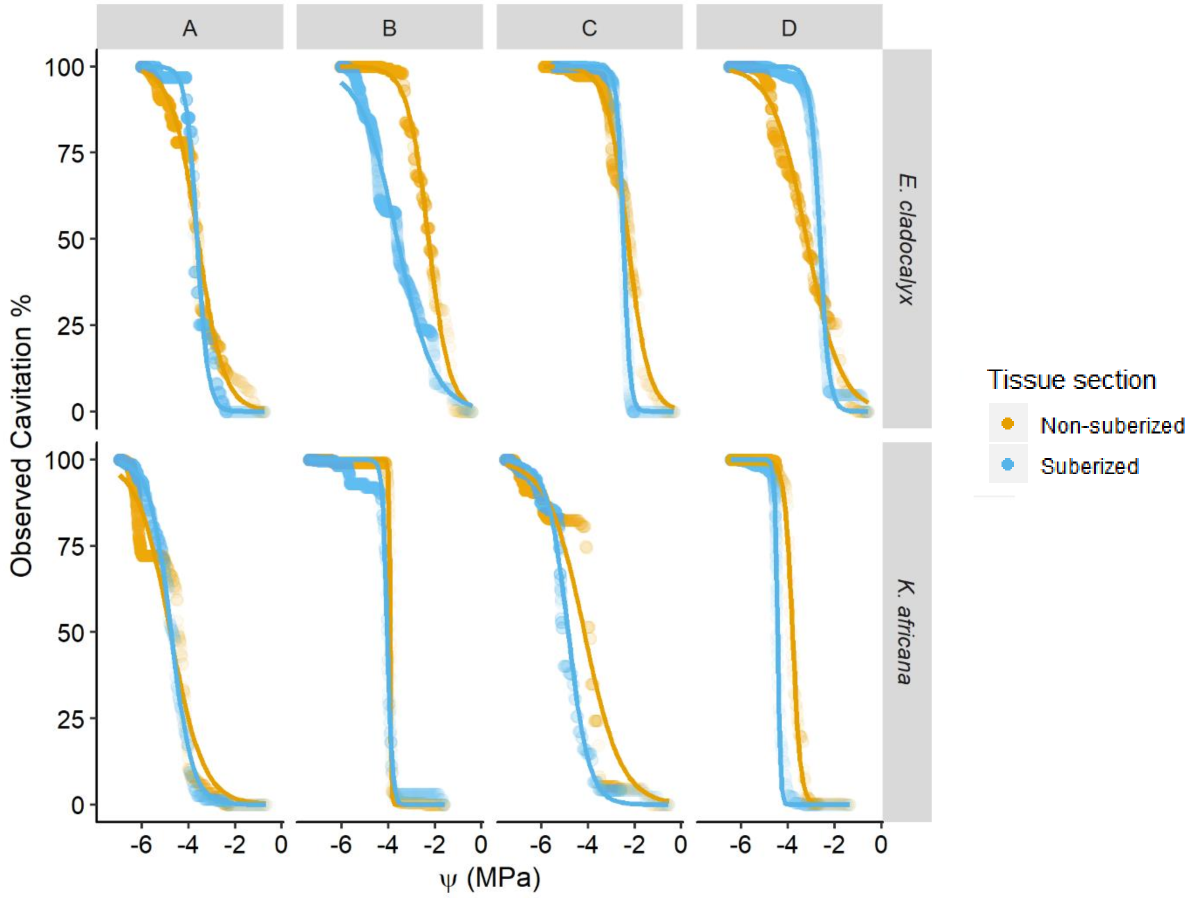


Figure 13: The percentage of cumulated area plotted against water potential (MPa) for each branch sample (A -D) of each species, *E. cladocalyx* (top) and *K. africana* (bottom). Showing the non-suberized (orange) and suberized (blue) tissue sections for each sample. Sigmoid curves were fitted for each section.

Table 1: The extracted parameters, steepness of the curve (a) and pressure (MPa) at 50% loss of conductance P_{50} (b), from sigmoid function as well as the P_{12} and P_{88} for each sample of each tissue section, non-suberized and suberized, of each species *E. cladocalyx* and *K. africana*.

		non-suberized				suberized			
Species	Sample	a	b (MPa)	P_{12} (MPa)	P_{88} (MPa)	a	b (MPa)	P_{12} (MPa)	P_{88} (MPa)
E. cladocalyx	A	1.80	−3.61	−2.50	−4.72	4.21	−3.64	−3.16	−4.11
	B	2.21	−2.28	−1.38	−3.18	1.26	−3.62	−2.03	−5.21
	C	2.23	−2.31	−1.41	−3.21	7.10	−2.49	−2.21	−2.77
	D	1.37	−3.20	−1.74	−4.66	4.93	−2.64	−2.24	−3.05
K. africana	A	1.42	−4.78	−3.37	−6.19	2.20	−4.75	−3.84	−5.66
	B	33.28	−3.93	−3.87	−3.99	9.54	−4.05	−3.84	−4.27
	C	1.27	−4.17	−2.59	−5.74	2.21	−4.86	−3.96	−5.76
	D	5.69	−3.81	−3.46	−4.17	15.54	−4.46	−4.33	−4.58

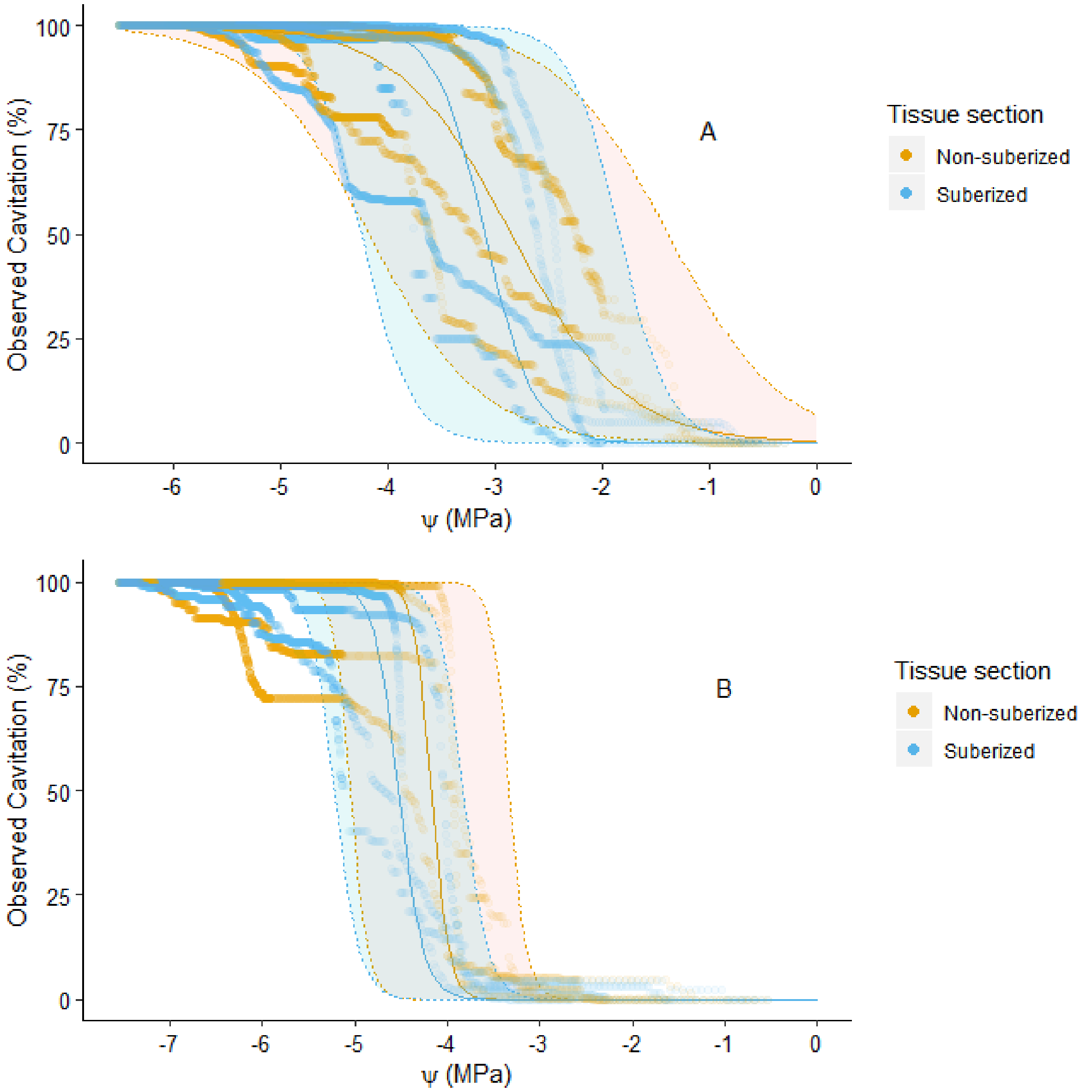


Figure 14: Relationship between the percentage of embolized area and the xylem water potential (ψ) for the four samples of each species, (A) *E. cladocalyx* and (B) *K. africana* of the two different tissue sections, non-suberized (orange) and suberized (blue) that were imaged. Sigmoid curves using the mean data for each section, non-suberized (orange) and suberized (blue) and the colour shaded areas with the dotted-line border indicates the 95% confidence intervals, non-suberized (orange) and suberized (blue).

Table 2: Showing the mean, standard error, and significance level of the pressure at 50% loss of conductance (P_{50}), pressure at 12% loss of conductance (P_{12}) and the pressure at 88% loss of conductance (P_{88}) for each tissue section (non-suberized and suberized) of each species, *E. cladocalyx* and *K. africana*. Paired t-tests were conducted comparing the non-suberized and suberized sections of the branch, the significant differences are indicated by *, where *** is p<0.001, ** is p<0.01, * is p<0.05 and ns is not significant.

		P_{50} (MPa)		**P_{12} (MPa)**		**P_{88} (MPa)**	
Species	Tissue Section	Mean	*p*	Mean	*p*	Mean	*p*
E. cladocalyx	non-suberized	−2.85 ± 0.33	ns	−1.76 ± 0.26	***	−3.94 ± 0.43	ns
	suberized	−3.10 ± 0.31		−2.41 ± 0.25		−3.79 ± 0.55	
K. africana	non-suberized	−4.17 ± 0.22	ns	−3.32 ± 0.27	ns	−5.02 ± 0.55	ns
	suberized	−4.53 ± 0.18		−3.99 ± 0.12		-5.07 ± 0.38	

DISCUSSION

The purpose of this study was to determine the mechanism underlying hydraulic segmentation observed in Chapter 2. The underlying mechanism that was tested for was vulnerability segmentation. Both, *E. cladocalyx* and *K. africana,* showed statistically similar P_{50} and P_{88} results between the non-suberized and suberized tissue sections. However, *E. cladocalyx* showed a significant difference for the P_{12} between non-suberized and suberized tissue sections and *K. africana* did not. This is consistent with the vulnerability segmentation hypothesis in *E. cladocalyx.* As P_{12} signifies the air entry point, the non-suberized, distal, section first experiences embolism, the embolism can then propagate in the distal tips. Therefore, suggesting that the distal ends are more vulnerable and may act as a hydraulic fuse. In *E. cladocalyx*, embolism initially starts early in the non-suberized section of the branch, and then the suberized section that was analysed closely follows.

It is expected that this might follow through to the P_{50} and $P_{88,}$ but this lack of significant difference could be due to the selection of sections. Either the chosen sections were too close to one another, or there was too much variation in the curves to pick up small differences in vulnerability (i.e., sample size limitations). There is a difference of the embolism pattern between the mature and developing tissue. The non-suberized section of *E. cladocalyx* may be more vulnerable to embolism and likely to point to vulnerability segmentation.

The P_{50} measured in West *et al* (2016) did not match the P_{50} values extracted from this study but were on the same rank. West *et al* (2016) measured thick stems far upstream and my study measured the most distal tips of the same species. Therefore, the difference in P_{50} makes sense, as the optical technique provided limitations. The depth of view of the scanned images is short, restricting observations to small, distal tissues (< 5 mm in diameter). Thicker diameter stems could not be captured, as the scanner would not be able to pick up all layers of xylem vessels, and this would not reflect accurate results. Therefore, in Chapter 3, due to the limitation of not being able to examine further down the bole, only the distal parts of the branches were captured and analysed. Thus, the non-suberized section of the branch coincided with branches from Experiment 1 in Chapter 2 and suberized sections coincided with Experiment 2 in Chapter 2 (see Fig. 8), and these sections were close to one another due to being unable to examine further down the bole of the stem. West *et al* (2016) measurements were done on larger shoots. *E. cladocalyx* exhibited vulnerability segmentation in the distal region and this supports the hydraulic segmentation hypothesis, whereas *K. africana* showed a lower degree of hydraulic segmentation and no vulnerability segmentation.

Alternate evidence supports vulnerability segmentation between the leaf and stem of several Eucalyptus species under drought conditions (Blackman et al., 2019). This may further support that fire tolerant species may exhibit hydraulic segmentation post-fire as the leaves are more vulnerable than the stem. The distal ends may also be more vulnerable to embolism post heat-plume which may facilitate in post-fire survival.

This chapter suggests that *E. cladocalyx* does invest in vulnerability segmentation as a trait, as the non-suberized tissue showed embolism events earlier than the suberized (P_{12} was higher in non-suberized, distal tissue for this species). This localization of embolism protects the bole of the tree by maintaining an intact water column. This is beneficial because the preservation of this intact water column may have live buds located along the pathway. These live buds can then resprout post-fire. Vulnerability segmentation is only seen in one species, so there must be a cost to investing in vulnerability segmentation. This may be related to having sensitive tissue located in the distal ends and unnecessarily fusing these ends when a drought or heat wave passes.

CHAPTER 4: Synthesis

Experimental evidence of traits assisting in survival post-fire

In this dissertation, the term “hydraulic segmentation” is used to describe the phenomenon consistent with West *et al* (2016) and is analogous to the concept of the “hydraulic fuse” (Zimmermann, 1983; Tyree & Zimmermann, 2002). I set out to determine if 1) hydraulic segmentation, where the loss of conductivity is localized in the distal portions of the hydraulic continuum, differed between two tree species with differing abilities to survive fire, and 2) if vulnerability segmentation was the mechanism underlying the observed hydraulic segmentation. I demonstrated that there was a greater degree of hydraulic segmentation in the fire-tolerant *Eucalyptus cladocalyx* than the fire-sensitive *Kiggelaria africana* (Chapter 2), and that there was some evidence for vulnerability segmentation being the mechanism for hydraulic segmentation in *E. cladocalyx* (Chapter 3).

What could make distal ends more vulnerable to embolism?

The question remains, what would make these distal parts of a tree more vulnerable to embolism that would lead to exhibiting hydraulic segmentation. Angiosperms and gymnosperms differ in response to stresses, evidence has been found that angiosperms experience a reduced conductivity when compared to gymnosperms. Angiosperm vulnerability to embolism is determined by the porosity of pit membranes (Bär, Nardini & Mayr, 2018). Vulnerability to embolism in relation to drought has been linked pit architecture, pit membrane thickness, and distribution and pore sizes (Wheeler et al., 2005; Hacke et al., 2006; Li et al., 2016). Future studies on the effects of heat in relation to fire on pit structure is required. Studies have found ways in which xylem can become more vulnerable to embolism if vessel-associated parenchyma cells are killed by heat. This could negatively impact the surfactants that coat and stabilize nanobubbles. This breakdown will increase vulnerability to embolism within the xylem vessels (Schenk et al., 2017; Bär, Michaletz & Mayr, 2019). Wood density may also be an important morphological characteristic to consider post-fire, wood density aids in the prevention of heat-induced embolism (Hacke et al., 2001; Brando et al., 2012). The higher the density the more resistant to embolism (Zwieniecki & Secchi, 2015). These different factors could play a role in the difference between the non-suberized tissue and suberized tissue of a fire-resistant trees. The non-suberized tissue of *E. cladocalyx* was notably green, the youngest part of the branch and had thinner bark surrounding the xylem, compared to the suberized section which had notably thicker bark. Extreme differences between the sections may have not been evident due to the limitations in optical technique.

Future work

Drought conditions and fire frequency are increasing due to climate change (Bowman et al., 2017; Ruffault et al., 2018). Understanding the combined effect of drought stressed plants followed by a fire is crucial. Plants that are drought-stressed prior to a fire will suffer more than a plant that is fully functional. A recent study found that conifers were less likely to survive a fire when they experienced a drought before the fire event (van Mantgem et al., 2018). Species are more vulnerable to drought induced embolism after experimental heating (Bär, Nardini & Mayr, 2018). Therefore, understanding the hydraulics of a plant post-drought and post-fire is crucial, as they may interact on the hydraulics of a plant. The study of hydraulics post-fire is still novel, and more understanding is needed. This will allow us to be better informed about the interaction of drought and fire and model predications of tree death better.

Vulnerability segmentation has been heavily studied in terms of drought situations, the lack and the presence thereof (Hao et al., 2008; Chen et al., 2009; Bucci et al., 2012; Johnson et al., 2016; Skelton, Brodribb & Choat, 2017; Skelton et al., 2018). For both species, the branches were subject to slowly drying down in Chapter 3, the branches were subject to drought-like conditions over fire conditions. An improved study will include drying the branch down in a hot and low humidity-controlled room, to reflect fire-like conditions. The location of embolism may differ at different humidity levels (Vincent et al., 2014). The result from this study supports that *K. africana* is drought resistant, *K. africana* exhibited embolism events at higher negative water potential. Vulnerability segmentation as a pyrohydraulic trait needs to be further investigated and tested in fire-tolerant species that are subject to fire events.

There has been support for hydraulic segmentation, especially emphasising leaves as a “hydraulic fuse” (Tyree et al., 1993; Chen et al., 2009, 2010; Bucci et al., 2012, 2013; Mcculloh et al., 2012). Recent studies have shown a lack of segmentation under drought conditions (Skelton, Brodribb & Choat, 2017; Skelton et al., 2018; Li et al., 2020). The lack of segmentation is also important to understand. A lack of segmentation has been seen between leaf and stem (Skelton, Brodribb & Choat, 2017; Smith-Martin et al., 2020), and between petiole and stem (Li et al., 2020) of some plant species. Different species can display a variation of hydraulic segmentation. *Kiggelaria africana* shows support to a lack of hydraulic segmentation and vulnerability segmentation in this study, as it exhibits a low degree of hydraulic segmentation post-fire. The lack of hydraulic segmentation is curious, as this trait is believed to be beneficial for a tree to possess. The lack of hydraulic segmentation could be beneficial under mild events of drought, as this reduces the PLC. Therefore, there may be a cost associated with the investment of hydraulic segmentation. The trade-offs in investing in hydraulic segmentation should be questioned. The xylem vessel anatomy at different 'hydraulic segmented' sections possibly differ. Or, perhaps the fuse is associated with only certain species due to some plants evolving by natural selection to have hydraulic segmentation as a trait. The lack of vulnerability segmentation could be a result of even investment in tissues (Skelton, Brodribb & Choat, 2017). *K. africana* could exhibit this similar even investment in tissues, thus not displaying vulnerability segmentation. An even investment in tissue will allow for a quicker return on leaves or indicate slow growth. The butterfly species, *Acraea horta*, are attracted to *K. africana* and their caterpillars often eat the tree bare of leaves (Van Wyk & Van Wyk, 1997). Thus, *K. africana* is constantly subject to this defoliation and would therefore need a greater return on leaves, this implies that there is an even investment in tissue and that lack of vulnerability segmentation might be favoured. Hydraulic segmentation may not be present in every tree, more work should be done to explore the presence of hydraulic segmentation in fire-tolerant species over fire intolerant species.

The main aim of this study investigates the effects of a passing fire, in an event of a fire the leaves are most likely to be scorched. There is scarce information regarding the spatial and temporal propagation of embolism within a whole plant, yet this might be a way to avoid losing function of essential tissues. Evidence has been found that grapevine had differing hydraulic traits along a single growing season (Sorek et al., 2021). A difference of vulnerability to embolism along the hydraulic continuum is seen in conifer tree species, the mature trunk is more vulnerable than the branches of the tree, but the shoots also have a high vulnerability (Mcculloh et al., 2014). I found a slight difference of embolism patterns within individual plants between developing and mature tissue. This can play a role in promoting post-fire survival of core tissues. However, this highlights the importance of acknowledging that there might be differences along the architecture of a plant, and this will need to be accounted for in future sampling. Therefore, it could possibly be important to take note of tissue age or stage of development when sampling.

Further work is needed to understand the association of fire and other ecosystems. Another such ecosystem is the savanna ecosystem. Savanna ecosystems are flammable ecosystems that experience recurring fire events. In these ecosystems, fire maintains a balance of the vegetation and prevents the ecosystem from switching to an alternate state (West, Midgley & Bond, 2012; Skowno et al., 2016). In the absence of fire, there is an establishment of woodlands as the savanna trees grow and escape the fire trap. This results in high tree density in this once mixed grassland-woodland ecosystem (Bond, Midgley & Woodward, 2003; Bond, Woodward & Midgley, 2005). The hydraulic response to fire in these ecosystems has not been explored. Pyrohydraulic traits are possibly beneficial in the survival of these plants.

Conclusion

Plant mortality as a result of fire has been widely investigated, informing on the mechanisms of death (Michaletz & Johnson, 2007; Midgley, Kruger & Skelton, 2011; Bär, Nardini & Mayr, 2018; Hood et al., 2018; Karavani et al., 2018). Future studies of tree hydraulics will inform on the mechanism of post-fire survival. This will enhance the knowledge in how flammable ecosystems function and maintain a balance. *E. cladocalyx* is capable of epicormic resprouting post-fire, and the live buds could be located along the water column that was preserved due to hydraulic segmentation. The hydraulic death hypothesis is supported by this study, as well as the hypothesis of pyrohydraulic traits that aid in the survival of trees post-fire. One of these being hydraulic segmentation as a hydraulic fuse and vulnerability segmentation as its mechanism, these were both supported in this dissertation, however further investigation is required. Understanding pyrohydraulic traits will provide basis for future model studies. This can be applied when investigating the effects of fire on ecosystems.

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APPENDIX

Table A: The mean, standard deviation, variance, and sample size of the raw data of *E. cladocalyx* for the control not flushed, control flushed, treatment not flushed, and treatment flushed at every Experiment level (1-4).

Species: *E. cladocalyx*

Experiment 1

Treatment level	Mean	Standard dev	Variance	n
Control	5.18e-07	2.116e-07	4.4713e-14	6
Control Flushed	4.77e-07	2.35e-07	5.5402e-14	6
Treatment	8.74e-08	6.82e-08	4.6536e-15	6
Treatment Flushed	3.24e-07	1.73e-07	2.9963e-14	6

Experiment 2

Treatment level	Mean	Standard dev	Variance	n
Control	1.60e-06	5.77e-07	3.3317e-13	7
Control Flushed	1.35e-06	3.20e-07	1.0246e-13	7
Treatment	5.70e-07	4.61e-07	2.1280e-13	7
Treatment Flushed	1.32e-06	5.95e-07	3.5427e-13	7

Experiment 3

Treatment level	Mean	Standard dev	Variance	n
Control	2.12e-06	9.61e-07	9.2358e-13	8
Control Flushed	1.67e-06	5.78e-07	3.3367e-13	8
Treatment	1.35e-06	9.70e-07	9.4135e-13	8
Treatment Flushed	1.74e-06	7.21e-07	5.2027e-13	8

Experiment 4

Treatment level	Mean	Standard dev	Variance	n
Control	2.12e-06	9.61e-07	9.2358e-13	8
Control Flushed	1.67e-06	5.78e-07	3.3367e-13	8
Treatment	5.41e-07	2.88e-07	8.3009e-14	7
Treatment Flushed	1.21e-06	4.29e-07	1.8396e-13	7

Table B: The mean, standard deviation, variance. and sample size of the raw data of *K. africana* for the control not flushed, control flushed, treatment not flushed, and treatment flushed at every Experiment level (1-4).

Species: *K. africana*

Experiment 1

Treatment level	Mean	Standard dev	Variance	n
Control	2.26e-07	3.88e-08	1.5069e-15	6
Control Flushed	2.34e-07	4.39e-08	1.9256e-15	6
Treatment	4.77e-08	2.11e-08	4.4614e-16	6
Treatment Flushed	3.23e-07	1.09e-07	1.1961e-14	6

Experiment 2

Treatment level	Mean	Standard dev	Variance	n
Control	9.73e-07	4.85e-07	2.3549e-13	6
Control Flushed	8.69e-07	1.11e-07	1.2229e-14	6
Treatment	2.28e-07	2.78e-07	7.7253e-14	6
Treatment Flushed	9.92e-07	3.43e-07	1.1794e-13	6

Experiment 3

Treatment level	Mean	Standard dev	Variance	n
Control	1.43e-06	5.15e-07	2.6567e-13	6
Control Flushed	1.01e-06	2.71e-07	7.3221e-14	6
Treatment	3.18e-07	2.77e-07	7.6740e-14	6
Treatment Flushed	1.28e-06	4.56e-07	2.0823e-13	6

Experiment 4

Treatment level	Mean	Standard dev	Variance	n
Control	1.43e-06	5.15e-07	2.6567e-13	6
Control Flushed	1.01e-06	2.71e-07	7.3221e-14	6
Treatment	3.01e-07	1.50e-07	2.2354e-14	6
Treatment Flushed	1.29e-06	1.93e-07	3.7141e-14	6

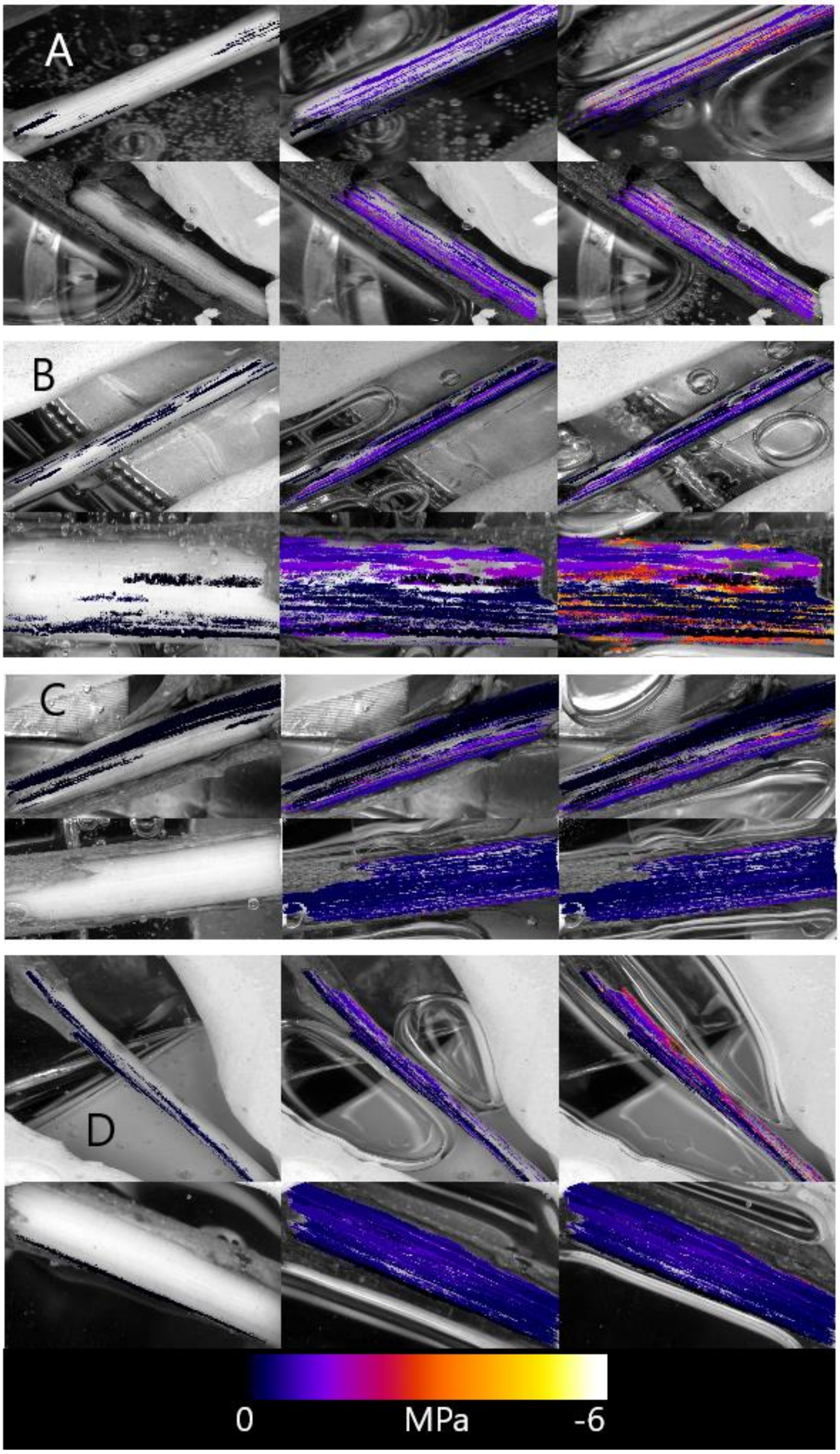

Figure A: Showing the embolism events in highlighted pixels for non-suberized followed by the suberized tissue section for each sample (A, B, C, and D) for *E. cladocalyx* at three different water potentials −2, −4, and −6MPa. This shows the embolism accumulated at the different water potentials. The scale bar indicates the embolized pixels coloured in relation to the water potential at which the embolism occurred.

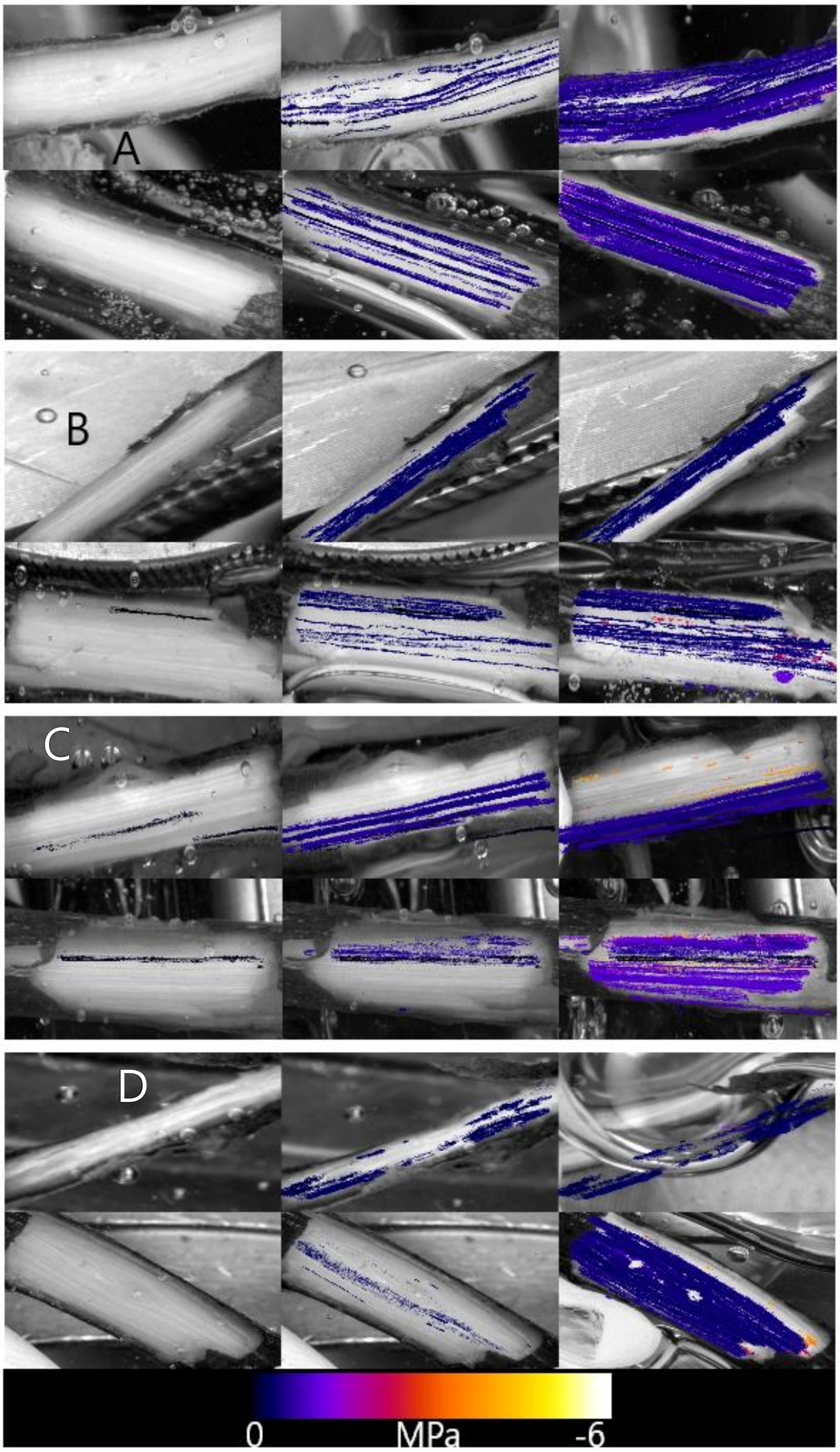

Figure B: Showing the embolism events in highlighted pixels for non-suberized followed by the suberized tissue section for each sample (A, B, C, and D) for *K. africana* at three different water potentials −2, −4, and −6MPa. This shows the embolism accumulated at the different water potentials. The scale bar indicates the embolized pixels coloured in relation to the water potential at which the embolism occurred.