UNIVERSIDADE FEDERAL DE LAVRAS

AMANDA CRISTIANE RODRIGUES

ANALYSES OF FIRE-INDUCED TREE MORTALITY ASSOCIATED WITH HIDRAULIC FAILURE OF XYLEM FUNCTION

LAVRAS – MG
2014

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Thesis presented at Universidade Federal de Lavras, as part of the requirements of the Graduate Program in Agronomia/Fisiologia Vegetal, to obtain the Doctor degree.

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LAVRAS
MINAS GERAIS - BRAZIL
2014

Ficha Catalográfica Preparada pela Divisão de Processos Técnicos da Biblioteca Central da UFLA

Rodrigues, Amanda Cristiane.
Analysis of fire-induced tree mortality associated with hydraulic failure of xylem function / Amanda Cristiane Rodrigues. – Lavras : UFLA, 2014.
59 p. : il.

Tese (doutorado) – Universidade Federal de Lavras, 2014.
Orientador: Amauri Alves de Alvarenga.
Bibliografia.

1. Fire. 2. Tree mortality. 3. Xylem failure. 4. Embolism. I. Universidade Federal de Lavras. II. Título.

CDD – 581.1

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APROVADA em 24 de Fevereiro de 2014

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LAVRAS
MINAS GERAIS - BRASIL

“Bem aventurados os mansos, porque eles herdarão a terra” Mateus 5:5

Aos meus pais, José e Ernestina.
Aos meus irmãos Josiane, Fernanda e Alexandre.
Aos meus sobrinhos, Matheus e Ana Clara.
To Andriy.
OFEREÇO

Aos amigos Kamila e Jean.
DEDICO

ACKNOWLEDGMENT

Agradeço primeiramente a Deus pela oportunidade da vida que é tão simples e traz tantas oportunidades.

Agradeço aos meus queridos pais, José e Ernestina, pessoas simples e boas de coração que sempre me insentivaram a construir um novo amanhã. Aos meus irmãos, Josiane, Fernanda, Alexandre e Maurício, pela amizade e apoio constantes. Aos meus sobrinhos Matheus e Ana Clara, meu ouro e minha princesa, que sempre tem algo espetacular a dizer, e que nunca falham na missão de tornar os meus dias incríveis. Os pequenos são os que eu mais agradeço pela sua simplicidade e grandiosidade. I would like to thanks Andriy, my sweetheart for all help, fellowship and love.

Às indispensáveis amigas Kamila, Michele, Carla, Fabiane, Angélica e Tetê, pela convivência sempre suave e carinhosa. A saudade de vocês é quase sempre suprida com um sorriso no meu rosto causada pela lembrança dos momentos vividos com vocês.

Ao Prof. Dr. Amauri Alves de Alvarenga pela orientação, confiança e amizade nesses 5 anos e meio. To Dr William Hoffmann, I have no words to describe how important you are. I would like to thank you for the opportunity, to all knowledge, patience and attention given. I am going to be taking you as a mirror for a long time. Ao Prof. Dr. Marcelo Polo, pela amizade de sempre e por estar sempre por perto mesmo que de longe.

Aos queridos ‘crocodiles’ do laboratório, especialmente à Sara, Jean, Mariana, Túlio, Fernanda, Lara, Fabrício, e todos os outros que passaram pelo Laboratório de Crescimento e Desenvolvimento de Plantas e Pessoal de Auto Nível. Agradeço também aos alunos do BIC Júnior pelos ensinamentos.

A todos os professores do setor de Fisiologia Vegetal pelos conhecimentos transmitidos, principalmente aos professores João Paulo, Donizete e Luis Edson, pela relação extra-classe. Aos funcionários Manu, Dani, Joel, Odorêncio, Lena, Barrinha, Evaristo e Dartagnan pela ajuda constante. Aos colegas do setor de Fisiologia Vegetal pelo convívio.

I would like to thanks the Hoffmann’s lab students, Alice, Brad, Michael, Renne, Sam, Steph and Jenny, for the patient, help, knowledge and friendship. I also would like to thanks all the great people I met in North Caroline through the year I spent there, special to Andriy, Lilian, Andrea, Maude, Jorge, Tuane, Antoine, Chris, Jucilene, Bill, Rita, Amanda, Hanna, Zach, Marília and Klécia.

Aos membros da banca examinadora pela disponibilidade. À Universidade Federal de Lavras, ao setor de Fisiologia Vegetal e a North Carolina State University pela oportunidade.

À FAPEMIG e à CAPES pela concessão de bolsa de estudos.

RESUMO

O fogo atuando como um modificador de ecossistemas ainda é pouco entendido em relação ao mecanismo que resulta em mortalidade de espécies vegetais arbóreas, uma vez que não é possível correlacionar a mortalidade pelo fogo à simples combustão de material vivo. O anelamento tem sido então atribuído como a principal causa de mortalidade e trata-se do mecanismo mais utilizado por modelos. No entanto, árvores aneladas podem sobreviver meses ou até mesmo anos. Após a passagem do fogo são observados sinais que indicam estresse hídrico, como por exemplo murcha de folhas, o que aponta falha hidráulica do xilema como um dos possíveis causadores de mortalidade de espécies arbóreas. O presente trabalho apresenta resultados, em condições de campo e laboratório, apontando parâmetros fisiológicos que levam a uma melhor compreensão do processo de falha hidráulica causando mortalidade. Foi observado que a primeira resposta, após o sistema caulinar ser submetido à altas temperaturas (80ºC), é o fechamento estomático. Ao longo do tempo (semanas) ocorreu uma diminuição da condutividade hidráulica do xilema, concomitante com diminuição do potencial hídrico das folhas resultando em 100% de mortalidade dos indivíduos estudados. A mortalidade observada foi atribuída a embolismos, uma vez que caules refluxados retomaram a condutividade e não foram observadas perdas estruturais dos vasos xilemáticos. Porém, não foi observada mortalidade quando da retirada de 1cm da casca. Ao contrário do que foi observado nas curvas de vulnerabilidade, onde a combinação de calor e um gradiente decrescente de pressão causou mais danos que o calor com subsequente gradiente decrescente de pressão, a ausência das folhas, excluindo esse gradiente, não resultou em menores taxas de mortalidade. Quando as folhas foram incineradas pelo fogo, foi observado um aumento no fluxo hídrico do xilema, o que pode causar maior vulnerabilidade do xilema aos processos de cavitação, influenciando nas razões de mortalidade observadas após incêndios.

Palavras-chave: Fogo. Mortalidade de Árvores. Falha do Xilema. Embolismo.

ABSTRACT

Although the importance of fire transforming landscape, and predicted to affect more areas due to climate changes, fire tree mortality process is not well understood. Correlate fire mortality with tissue combustion might explain well herbaceous mortality, but fails by explaining tree mortality very often observed after fire. Girdle leading to mortality by root starvation has been considered the most important mechanism to explain tree mortality and to predict tree mortality by models, although, more recent studies discuss hydraulic failure as a more likely hypothesis. With a breakthrough in the understanding of the mortality process, our work brings results in field conditions that reinforce some physiology parameters that drive tree mortality process. We observed a decrease in stomatal conductance right after heat was applied to the stem. We also observed a constant decrease in the specific conductivity, concomitant with a decrease in the water potential. No mortality was observed in the girdle treatments, although 100% mortality was observed in stem heat treatments. Unexpected no differences were observed between trees with presence and absence of leaves. This result is unlike what we found in the vulnerability curves that were more harmful when heat was combined with a decreasing pressure gradient when compared with heat followed by a decreasing pressure gradient. Beyond stem damage, we also found an increase in the xylem flow while incinerating the leaves, what might increase xylem vulnerability to cavitation processes. In conclusion, we can affirm that tree mortality by heat of the studied species is caused by cavitation processes, leading to a complete hydraulic failure.

Key-words: Fire. Tree Mortality. Xylem Failure. Embolism.

SUMARY

Chapter 1: Background

1 Introduction

Fire operates as the main boundary for plant species success and mortality as a consequence of fire determine, in part, the structure and composition of fire-prone vegetation (HOFFMANN et al., 2012; PIVELLO, 2011; STAVER; ARCHIBALD; LEVIN, 2011). When vegetation is affected by fire, shrubs are consumed, while the effects at trees differ in accord with height, diameter, and capacity of bark insulation protecting the live tissues from the high temperatures. Trees unable to protect the live tissues lose the above ground, process known as 'topkill' resprouting latter or dying if there is incapability to resprout. Tree mortality changes the structure, as well as carbon, water and mineral fluxes of the ecosystem. Thus, understanding and improving the predictive capacity of fire causing mortality is essential to understand and predict the effects of fire in carbon fluxes, vegetation dynamics and comprehension of tree adaptations in fire-prone ecosystems.

An accurate mortality prediction involves a mechanist analysis of the mortality process. If tissue incineration does not result in mortality, the loss of trees is attributed to girdle of the cambium, the mechanism most used in fire tree mortality models (BOVA; DICKINSON, 2005; COSTA et al., 1991; DICKINSON; JOHNSON, 2004; DICKINSON; JOLLIFF; BOVA, 2004; JONES et al., 2004, 2006; MANTGEM; SCHWARTZ, 2003; MICHALETZ; JOHNSON, 2007, 2008; REGO; RIGOLOT, 1990; RYAN; FRANDSEN, 1991). However, girdled trees survive for months or even years, while mortality after fire can be observed as a fast process (days or weeks).

Thus, the loss of live tissues, as phloem and meristems, is insufficient to explain mortality. Accordingly, hydraulic failure appears to be the most acceptable hypothesis to explain the mechanism of mortality after fire, when is common observe leaf desiccation in similar patterns with water stress as indication of hydraulic failure (DUCREY et al., 1996; MIDGLEY; KRUGER; SKELTON, 2011). If water flow through xylem is the main issue for tree survival after fire, beyond bark thickness, physiologic parameters should be considered while predicting mortality after fire.

Michaletz, Johnson and Tyree (2012) working with vulnerability curves in heated stems attributed the loss of conductivity observed to mechanical damages in the xylem structure and changes in sap surface tension.

Michaletz, Johnson and Tyree (2012) found a conductivity lack of 20%. Besides damages in the stems, Midgley, Kruger and Skelton (2011) point that xylem damages combined with excessive water lost by transpiration or evaporation in high temperatures could increase cavitation vulnerability. Also, leaf petioles are more vulnerable to cavitation than stems (HAO et al., 2008; SACK; HOLBROOK, 2006), what could increase the probability of a hydraulic failure after fire when leaves are affected.

The presence of embolisms in the xylem is common to occur during high transpiration conditions and a low availability of water. Embolisms appear mostly due to a ‘timelag’ existent between transpiration and water absorption (SWAEF et al., 2013). During a fire, the flow at the xylem might be accelerated, favoring the break of the water column. Cavitated vessels expel the bubbles avoiding embolism process in 3 different ways: vapour water condensation, air dissolution or expelling the air through bark or stomata (SHEN et al., 2008). However, all these 3 processes require a positive pressure (TYREE; ZIMMERMANN, 2002). A positive pressure could be created by phloem unloading (NARDINI; GULLO; SALLEO, 2011), but not insulated phloem tissue die after fire, compromising the process of xylem conductivity recovery.

Besides the process of hydraulic failure after fire has not been demonstrated yet in field conditions, the comprehension of tree mortality mechanism is extremely important in a climate changes scenario, when fires are expected to happen more frequently affecting non-fire-prone areas.

1.1 The relationship fire and vegetation

Fire affects vast areas of the globe every year, modifying the ecosystems by killing trees and other plants. Even when just ‘topkill’ is observed, the loss of the above ground removes a significant amount of biomass and carbon invested. Some forest and savanna ecosystems coexist with fire as a natural process part of its history, thus trees growing in fire-prone areas have specific traits to co-occur with fire as serotine, thick bark, resprout capacity and meristems protection by thick bark or by soil (underground meristems).

Fires occur naturally, usually after the dry season and beginning of rain season (PIVELLO, 2011) when lightning are more frequently. Ramos-Neto and Pivello (2000) registered at the Parque Nacional das Emas an occurrence of 91% of natural burns in the total of fires occurred, while Medeiros e Fiedler (2004) found similar results in the Parque Nacional da Serra da Canastra. However, most of the ignitions have human origin. Beyond natural and human origin, fire is also used as a periodically prescription, presenting low intensity, once the burn frequency maintain a low biomass quantity offering less risks to trees and other plants (RENNINGER et al., 2013).

After a fire there is a reduction on trees growth, outcome recovering of damaged leaves, stems and fine roots, modifying the carbon partitioning and assimilation (CLINTON et al., 2011). Depending on tree height, interval since last burn and fire intensity, substantial crown and root damage may occur (CLINTON et al., 2011; HOOD; SMITH; CLUCK, 2010). However, independent of the fire intensity, the stem is certainly subject to direct flame damage.

Humans and climate exert a direct influence at the fire patterns, and as a feedback the fire influences the climate by releasing carbon, accelerating processes involving the primary production and respiration process (BOND; KEELEY, 2005; BOWMAN et al., 2010). However, we have a small knowledge of how fire modifies tree physiology, and how the leaf, stem and root damage interact at tree mortality after high temperatures.

Frequent burns were observed to decrease the number of tree species at the Brazilian Cerrado (MIRANDA; BUSTAMANTE; MIRANDA, 2002; MOREIRA, 2000; SAN JOSE; FARIÑAS, 1991; SILVA; BATALHA, 2010). Yet, the high similarity among the phytophysiognomies in this biome suggests high vegetation resilience in different fire frequencies. The Cerrado resilience can be explained by its evolution with frequent burns (since 8 million years ago) (BEERLING; OSBORNE, 2006; SIMON et al., 2009), existing species selection to individuals with tolerance to fire condition (SILVA; BATALHA, 2010). Simon and Pennington (2012) present evidences that thick bark and resprout capacity are features derived from fire adaptation. Thus, fire suppression may lead to emergence of forests habited by fire susceptible individuals (HOFFMANN et al., 2012).

1.2 Fire tree mortality or “topkill”

Post-fire mortality is a prediction component of models. However is also a limiting point due to species diversity and susceptibility to future insect attacks, diseases, climate changes and intraspecific competition (VARNER et al., 2009).

Bark thickness has large influence in tree mortality after fire. In the Cerrado for example, trees with >6.2mm of bark thickness has 50% probability to survive after prescribed fire (HOFFMANN et al., 2009). Beyond bark thickness, high trees have leaves in an advantageous position avoiding damages and also having more efficient heatsinks mechanisms, as biomass increase (COSTA et al., 1991; MARTIN, 1963). Fire resistance is an important feature that governs savannas and forest structure (BOND, 2008; HOFFMANN et al., 2012; STAVER; ARCHIBALD; LEVIN, 2011).

Mortality is a result of combined damages in roots, stems and leaves (KAVANAGH; DICKINSON; BOVA, 2010) being considered as death indicators the loss of a 100% crown, stem girdle, and damages at the 30cm depth roots (VARNER et al., 2009). The loss of leaves, roots, stem girdle and damages in the live tissues explain partially tree mortality. Hydraulic failure and its mechanisms thus are pointed as the main elements to be analyzed and as a more efficient mechanism to explain tree mortality (BALFOR; MIDGLEY, 2006; DUCREY et al., 1996; MICHALETZ; JOHNSON; TYREE, 2012; MIDGLEY; KRUGER; SKELTON, 2011).

Other than some species that tolerate 45-50ºC (*Pinus ponderosa*) (DICKINSON; JOLLIFF; BOVA, 2004), live cells tolerate 35-40ºC, when higher temperatures lead to cell mortality. In temperatures higher than 60ºC the polymers constituents of the cell wall are susceptible to have it structure modified, leading changes in the capacity of xylem flow resulted from vessels structure modifications (MICHALETZ; JOHNSON; TYREE, 2012). So, live tissues, as phloem and parenchyma, and the lignified tissues, as xylem, are sensitive to fire, once fire can generate temperatures much higher than those listed above.

Even when fires do not cause mortality, it reduces the growth (carbon lost) and modifies water and carbon flow (VARNER et al., 2009). Studies involving variations in carbohydrates concentrations show that sugar pool is directed to injuries repair, being late a possibility of secondary damages than those caused directly by fire (VARNER et al., 2009).

A fire regime used without knowledge about its effects cause soil degradation, increase of invasive species and most of all the loss of biodiversity (PIVELLO, 2011). In addition, fire acts as the main evolution agent of ecosystems that evolved with constant burns (SIMON; PENNINGTON, 2012). Thus, tree mortality processes has to be analyzed aiming understand the effects of fire along it range as a structure modifier.

1.3 Hydraulic conductivity in tree species

The water flow process has been fascinating physiologists and physics. In the water flow, cavitation is the transition from a metastable state to a gas more stable state influencing the water column integrity. The process of cavitation is responsible for mortality and large losses in the forest production (COCHARD et al., 2013; HOFFMANN et al., 2011).

A common behavior in trees is stomatal closure before the tracheid tension reach values when cavitation occur (BOND; KAVANAGH, 1999; TYREE; SPERRY 1988). However, the difference between the tension that stomata close and the tension that hydraulic failures ensue is relatively small (SPERRY; HACKE, 2004), thus with a small tension addition in the water column it might occur cavitation. Although the stomatal close limits the photosynthesis, it permits the hydraulic system of trees to be less susceptible to cavitation, maintaining the water flow and the survival after limitations are overcome (KAVANAGH; DICKINSON; BOVA, 2010).

In highly negative pressures, air bubbles can be sucked to the xylem interrupting the continuous flow. In accordance with cohesion and tension theory, once the water column is broke, it rejoins only if xylem pressure reaches values above the water vapour pressure. The differences of pressure necessary to rejoin the column are created at the transpiration flux. The process of how bubbles are sucked to the xylem is unstable and the water superficial tension acts collapsing the bubbles spontaneously (TYREE, 1997). However, high temperature modify the surface tension force (MICHALETZ; JOHNSON;

TYREE, 2012), probably preventing the recovery of possible embolisms formed after fire and leading to hydraulic failure. The lack of a coordination between stomata and hydraulic system leads to a higher susceptibility to cavitation processes. Johnson et al. (2012) affirm that once altered water physics conditions, the stomata closes decreasing the risks of cavitation and hydraulic failure.

High temperatures also act in the xylem vulnerability, once it might alter lignin structure, with loss in vessels conformation (MICHALETZ; JOHNSON; TYREE, 2012). In situ, vessel refilling recovering cavitated vessels is a process known to happen, but that is still not well understood, and consists in one of the main questions unresolved in the plant hydraulic field (BRODESSEN et al., 2010; MEINZER et al., 2013; NARDINI; GULLO; SALLEO, 2011; SECHI; ZWIENIECKI, 2011; ZWIENIECKI; HOLBROOK, 2009).

2 Action strategies

This thesis aimed to study physiologic parameters governing tree death after fire. We evaluated, in field and laboratory conditions, the hydraulic failure in *Oxydendrum arboreum* and *Liquidambar styraciflua* species. Our working hypotheses were thirsty death caused by embolism, and a directly involvement of leaves influencing the percentage mortality and the hydraulic processes.

To test our hypotheses, we evaluated four experiments described in two papers. First paper consisted in evaluate the stomatal conductance (g_s), water potential (ψ), hydraulic conductivity (k_s), % mortality, and vulnerability curves, in heat treated young trees of *O. arboreum* and *L. styraciflua*, with and without leaves. The second paper consisted in analyze the behavior of sap flow while burning leaves and after heat was applied to the stem.

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Chapter 2: How fire kill trees. An examination of fire-induced hydraulic failure

Abstract: Fire-prone areas structure is transformed by fire mainly through losses of tree species. Tree mortality is usually attributed by death of live tissues, as phloem and meristems, resulting in root starvation. However, processes of root starvation killing a tree might take months or even years. Thus, water stress seems to explain mortality right after fire. To evaluate hydraulic failure inducing mortality after high temperatures, we tested two hypotheses in *O. arboreum* and *L. styraciflua*: 80ºC in the xylem tissue leads to xylem dysfunction as a consequence of loss of xylem conductivity, and the presence of a pressure gradient upcoming from leaf transpiration affects the vulnerability to cavitation increasing %M (mortality). Our first hypothesis was proved right once we observed a decrease in stomatal conductance, specific conductivity and water potential after heat was applied, resulting in 100% mortality. The second hypothesis failed with no differences observed between trees with presence and absence of leaves. Although, vulnerability curves showed more damage when heat was applied simultaneous with pressure gradient. After observed refilling of cavitated vessels and with no differences observed in the xylem anatomy, we conclude that tree death is due to cavitation processes leading to hydraulic failure.

1 Introduction

Vegetation fires affect vast areas of the globe each year, transforming landscapes by killing trees and other plants (BRANDO et al., 2012; HARDESTY; MYERS; FULKS, 2005; HOFFMANN et al., 2012). Even when trees are not fully killed by fire, death of aerial stems represents a massive removal of carbon from the biomass pool, while individual plants undergo a loss of substantial invested resources. Consequently, understanding and predicting stem death after fire is important for modeling ecosystem carbon fluxes, vegetation dynamics, and optimal plant strategies in fire-prone ecosystems.

It has been widely accepted that fire causes tree death due to girdling of the vascular cambium of the trunk (BOVA; DICKINSON, 2005; DICKINSON; JOHNSON, 2004; DICKINSON; JOLLIFF; BOVA, 2004; JONES et al., 2004, 2006; MICHALETZ; JOHNSON, 2007, 2008), but this has recently been called into question (BALFOUR; MIDGLEY, 2006; MIDGLEY; KRUGER; SKELTON, 2011; MICHALETZ; JOHNSON; TYREE, 2012). Specifically, this explanation is incomplete because mechanical girdling alone does not result in the instantaneous stem death that is often apparent immediately after fire. Instead, mechanical girdling of the cambium generally requires several months or more to kill a tree (CHEN et al., 2010; DOMEC; PRUYN, 2008).

Hydraulic failure of stem xylem provides an alternative explanation of fire-induced stem death. Michaletz, Johnson and Tyree (2012) found that heating stems to 60ºC cause structural deformation of xylem, making it considerably more susceptible to embolism by air seeding across pit membranes. Although that study found large effects of stem heating, it may have underestimated the susceptibility for fire-induced hydraulic failure because vulnerability to air seeding was tested after heating concluding wall deformation and stems were no longer exposed to elevated temperatures. It is likely that structural deformation of xylem would be greatest when warming coincides with pressure. A high pressure gradient across a pit membrane at a time when the secondary cell wall is softened by heating should result in greater flexing of the cell wall, making cavitation more likely (HILLIS; ROZSA, 1985; LEWIN; GOLDSTEIN, 1991; WOLCOTT; KAMKE; DILLARD, 1990).

Beyond stem damage, fire might coincide with foliage injury by scorching or by heat plumes impacts, implying in changes at the fluxes of water and carbon, with straight consequences to hydraulic system (CHEN; ZHANG; HE, 2013; LI et al., 1981). It is possible that during a fire event the xylem flow find to be enhanced by the atmospheric temperature and relative humidity that during a wildfire was recorded to be 100ºC and 0%, respectively (KREMENS et al., 2003), resulting in a vapour pressure deficit of 270 kPa (KAVANAGH; DICKINSON; BOVA, 2010). Also, the hot plume generated by a wildfire may place a significant strain on a tree drought stress mechanisms (KAVANAGH; DICKINSON; BOVA, 2010). Thus, trees without leaves should have a higher survival prospect.

In this study, we performed experiments to test the mechanisms leading to stem death in two situations: a high temperature applied to stem and cambium girdle, both in combination with presence and absence of leaves. We considered hydraulic parameters that might be related to hydraulic failure such as stomatal conductance, leaf water potential, xylem specific conductivity, and vulnerability curves. Our working hypothesis presumed that a xylem temperature of 80ºC is lethal, and mortality is caused by hydraulic failure, consequence of cavitation process, while the loss of the live tissue causes a late mortality. We were assuming that the presence of the leaves attenuate the hydraulic failure, outcome from combination of pressure gradient by transpiration and heat damages at the xylem polymers.

2 Methods

The study species, *Oxydendrum arboreum* (Ericaceae) and *Liquidambar styraciflua* (Altingiaceae) are deciduous trees common in forests of eastern USA. As saplings both species are readily topkilled by fire but regenerate vigorously from basal sprouts.

In the experiments below, we used 80^{o}C as our target temperature for simulating fire effects. This temperature is sufficiently higher than the lethal temperature for plant tissues, and is sufficient to reduce the structural integrity of xylem, once lignin undergo to a glass for a rubbery state in > 60^{o}C (KELLEY; RIALS; GLASSER, 1987).

Experiment 1. Stem responses to girdling and stem heating

The purpose of this experiment was to test whether stem responses to burning could be explained simply by death of the cambium. The experiment was performed on *O. arboreum* at Fort Bragg, Fayetteville – NC, in summer, 2012. We chose 15 multi-stemmed individuals of *O. arboreum* that were resprouting following a fire that occurred 2 years previously. Each individual had at least six stems with stem diameter varying from 9.8 to 18.1mm. Each of the six largest stems was assigned one treatment in a 3x2 factorial design. For the first factor, each stem was girdled, heated, or left undisturbed. For the second factor, each stem was either defoliated or not. The individual, which comprised six stems, thus represented an experimental block. For the girdling treatment, a 1-cm band of bark was carefully removed at a height of 35cm using a razor blade. The heat treatment was applied at the same height using a heating element comprising 160-ohm resistors, connected in parallel in two wires 2cm length with a spacing of 5mm. To monitor the temperature inside the stem at the site of heating, small hole was drilled, and a thermocouple was inserted into the center of the stem, while another was positioned on the outer surface of the stem. The heating element was then wrapped around the stem, which was then covered with foam insulation. To heat the stem, a direct current of 12V was supplied by a battery. Current was supplied until the temperature at the stem center reached 75ºC. Based on initial trials, this allowed residual heat to bring the maximum temperature to approximately 80°C. The average time to reach 80ºC and return the initial temperature was 3 min. To control for a possible effect of drilling the hole for thermocouple placement in heated stems, we drilled a similar hole in each girdled and control stem. For the defoliation treatment, all terminal branches were removed using a pruning shears. After treatments were applied we measured stomatal conductance (g_s), water potential (ψ), hydraulic conductivity (k_s) and % mortality, as described below.

The g_s was measured with an AP4 Leaf Porometer (Delta-T Devices). Measurements were evaluated on three leaves per stem, at different heights (top, middle and bottom of the canopy). The measurements were evaluated immediately after applying the treatments and 7-day intervals for 21 days when all the heated stems had died. Initial treatments and all measurements were performed between 10:00 and 15:00. ψ was measured in the field immediately following the measurement of g_s and on one leaf per stem, using a pressure chamber (PMS Instruments, model 1000). The intervals for ψ followed the same for g_s.

Stem survival after 21 days was assessed by visual inspection of leaves and branches. Any plant lacking live leaves and possessing only dry, brittle branches was considered dead.

The k_s was measured on the stems at the end of the experiment. The stems were cut under water, packed in black plastic bags with moist paper and transported to the laboratory. In the laboratory the stems were recut under water and the conductivity was measured. The conductivity was measured using a 0.1-µm-filtered KCl (20 mM) solution in ultrapure water under a pressure of 3.5 kPa. The flow rate was determined by timing the rate of efflux into a graduated pipette. Calculations followed Tyree and Ewers (1991). After measurement of initial conductivity the stems were refilled for 24 hours in a vacuum chamber with pressure of 2 kPa then measured again. We calculated xylem cross-sectional area from stem diameter (after bark removal) and pith diameter measured with digital calipers.

We also performed experiment 1 with *L. styraciflua* that was conducted in old field conditions at a site near Raleigh, NC. Due to a lower number of suitable stems, we did not apply the girdling treatment, and instead assigned plants to only heat treatment without defoliation, heat treatment with defoliation, and control. Furthermore, this species responded more quickly to the heating treatments, so subsequent measurements were performed over a shorter period. Therefore, the ψ and g_s were measured 1, 3, 7 and 10 days after the treatments, while %M and k_s were measured after 10 days.

As expected, there was a nearly complete loss of conductivity in the stems at the end of the experiments for both species, so we performed another experiment to examine the temporal patterns of conductivity loss. As before, we assigned plants in Raleigh-NC to heat treatment with and without defoliation, as well as controls. We evaluated the k_s after 1, 7 and 21 days after the treatments. The methodology to obtain the k_s was the same described above, except that refilling of embolized vessels was performed by flushing stems with degassed KCl solution under a pressure of 100 kPa for 20 min prior to remeasuring conductivity. No refilling was performed in *L. styraciflua* once after refilling the stems had a lower conductivity then before refilling (data not show).

Experiment 2. Vulnerability Curves

We used the air-injection method to test whether stem heating results in increased vulnerability to cavitation. Furthermore, we tested whether the effect of stem heating is larger when it occurs simultaneously with pressure gradients than when it occurs prior to decrease pressure gradients. For both species, vulnerability was evaluated using stems collected in Raleigh-NC. We had three sets of stems, heated during pressurization, heated prior to pressurization, and pressurized without heat. Heated during pressurization simulated a fire burning trees at different water potential and heated prior to pressurization simulated when tree goes by a condition of low water potential after heat.

For both species, we collected 15 stems, 5 to each treatment. To collect we followed the methodology for k_s stems. For the pressure apply we used the air-injection method (SPERRY et al., 1996). The stems attached to the chambers and connected at a pressure bomb were immersed in a water bath adjusted for 80ºC, applying 0, -0.5, -1.0, -2.0, -3.0 and -4.0MPa for 10 min each. All stems used had superior length to the larger vessel (*L. styraciflua* 20.7 to 29.4cm; *O. arboreum* 31 to 55cm, measured applying air with a syringe – N=10). For *L. styraciflua* we collected new stems for each pressure applied, justified by stem conductivity in this specie decreases just by sitting on the water (after 2 days 27% conductivity and within 3 days 85%). The data were expressed in percent loss conductivity (PLC, %).

Statistics Analyses

The effect of the treatments in the variables g_s, ψ, k_s and % mortality were analyzed using a one-way analyze of variance (ANOVA). To compare the means of each treatment we used a t-Test. The k_s data before and after refilling were compared using a t-Test paired two samples by means. All the statistics analyses were performed in SISVAR software (FERREIRA, 2011).

3 Results

All stems subjected to heat treatments were dead by the end of the experiments (21 days for *O. arboreum* and 10 days for *L. styraciflua*), as inferred from desiccated stems and foliage. In contrast, the mechanical girdling treatment, which was applied only to *O. arboreum*, did not result in any stem death during the study period (Table 1) and terminal branches were well hydrated and apparently alive, some even resprouting new leaves.

Table 1 Stomatal conductance (g_s), water potential (Ψ), specific conductivity (k_s) and the % mortality of *O. arboreum* after 21 days applied treatments of heated, girdle and control, with presence of leaves (L_P) and absence of leaves (L_A). The underline (__) indicates the parameter was not measured.

	Control		Heated		Girdle	
	L_P	L_A	L_P	L_A	L_P	L_A
% Mortality	0	0	100	100	0	0
g (µmol $m^{-2}s^{-1}$)	356.2	__	9.7**	__	89.2**	__
Ψ (MPa)	-1.6	__	-6.3*	__	-0.9	__
k_s (Kg m^{-2} MPa^{-1})	1.16	1.05	0*	0.03*	1.92*	1.61

*significantly different at the $P<0.05$ level
**significantly different at the $P<0.0001$ level

Although no mortality was observed in girdle treatments, g_s decreased, while for ψ no significant differences were observed. The k_s increased significantly at girdled trees with leaves, while no differences were observed in the treatment without leaves (Table 1).

Heat induced a reduction over time in g_s, ψ and k_s, but g_s was the primary response with a significant decrease readily from the first measurement

(Figures 1, 2 and 3). Although both species died in dissimilar average time, no significant differences were observed between them (*P*=0.732).

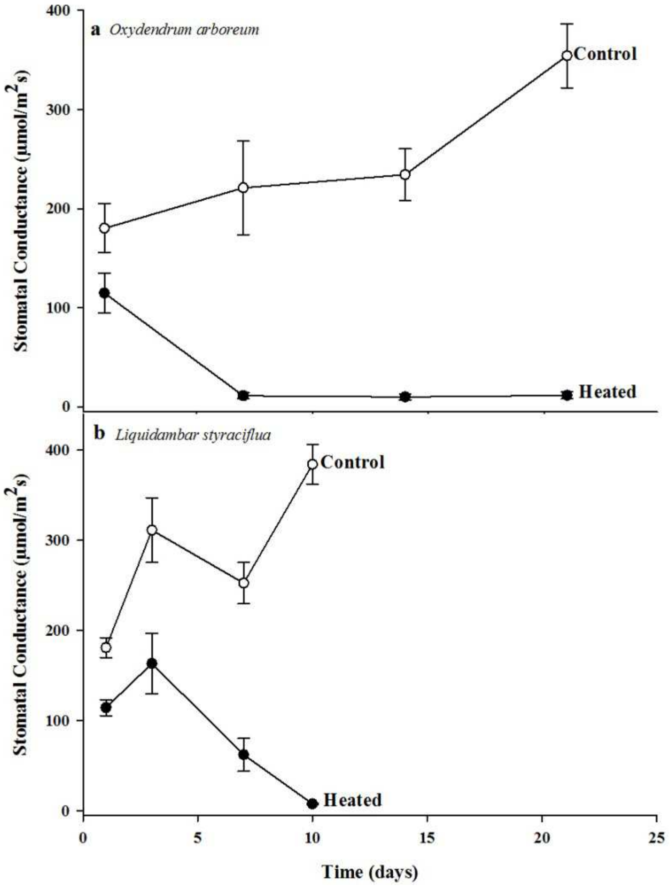

Figure 1 Stomatal conductance (µmol $m^{-2}s^{-1}$) measured during 21 days for *O. arboreum* (a) and 10 days for *L. styraciflua* (b) after heat (●) and control (○). Each point represents the mean of the samples and the bars represent standard error.

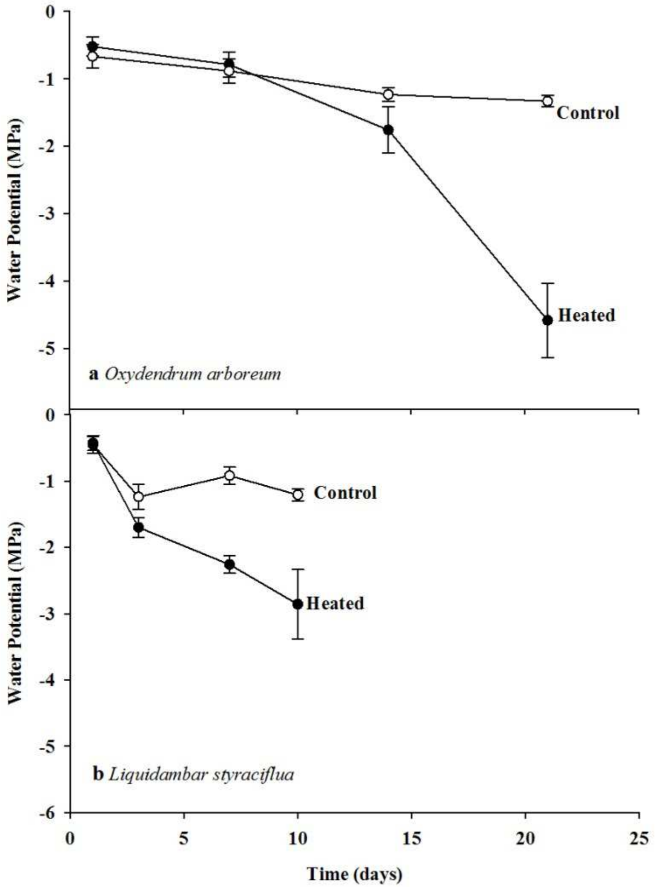

Figure 2 (a) *O. arboreum* and (b) *L. styraciflua* water potential (MPa) along days after heated treatment (●) and control (○). Each point represents the mean of treatments and the bars indicate the standard error.

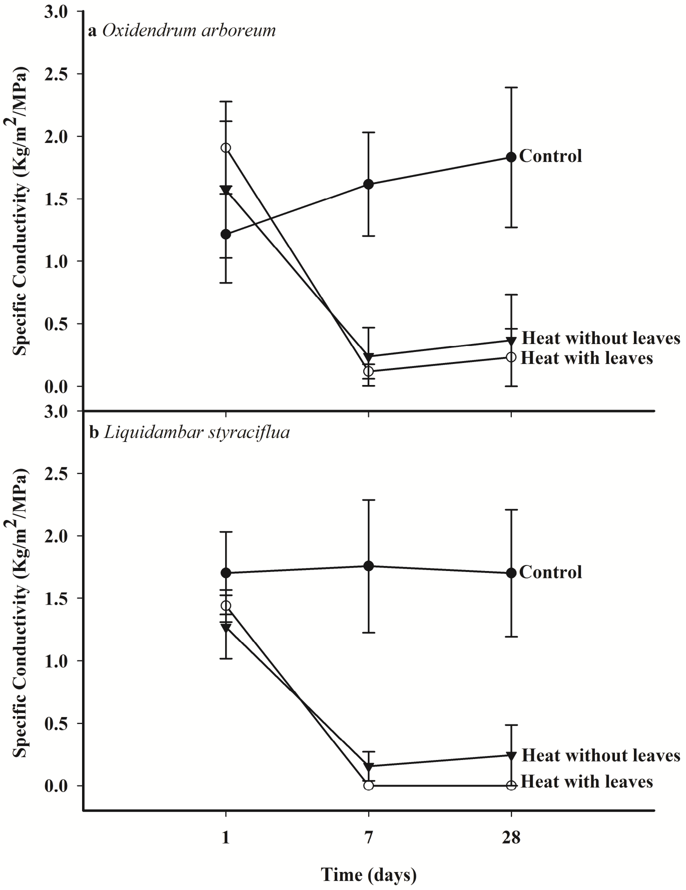

Figure 3 (a) *O. arboreum* and (b) *L. styraciflua* specific conductivity (Kg m^{-2}MPa) 1, 7 and 28 days after heat with leaves (○), without leaves (▼), and control (●). Each point represents the mean of the samples and bars represent the standard error.

All heated samples exhibited a k_s nearly to 0 by the end of the experiment. We were expecting to see differences between the treatments with and without leaves, but our hypothesis failed (Figure 3) and only a slightly difference (not significant) was observed. A week after heat applied the conductivity dropped about 7 times, and was already close to 0. Subsequently refiling the stems after 7 days heat was applied, we observed a conductivity recovery, equivalent with control stems (Figure 4). After one month heat was applied no recovery was observed after refiling. No vessel cavitation was detected in the heated stems after one day, inferred by no changes observed in k_s (data not show).

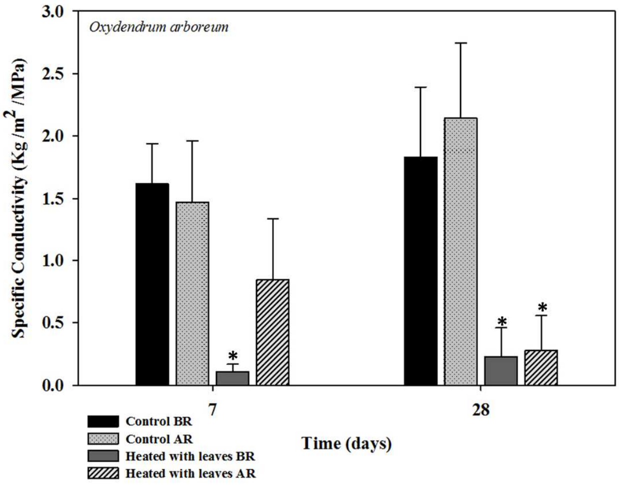

Figure 4 *O. arboreum* hydraulic conductance (Kg/m^2/MPa^1) after 7 and 28 days in control and heated stems, both before refilling (BR) and after refilling (AR). Each point represents the mean of the samples and the bars represent the standard error.

We evaluated xylem anatomy to verify deformations in the conduit walls decreasing the conductivity, but no sign of integrity loss was observed (data not show). However, a bottleneck was observed on the heated stems where heating element was wrapped, caused by loss of the live tissue by necrosis observed in the anatomy cuts. The loss of conductivity could also be attributed to damages at pit membranes, but if so will be expected a conductivity decline right after heat apply, reply no observed in our results (Figure 3).

The combination of heat and pressure caused more vulnerability to cavitation (Figure 5). It is clear in our results that the most likely hydraulic failure is whenever the interaction of the xylem under pressure and high temperature occurs, but heat by itself had effect in the xylem vulnerability curves.

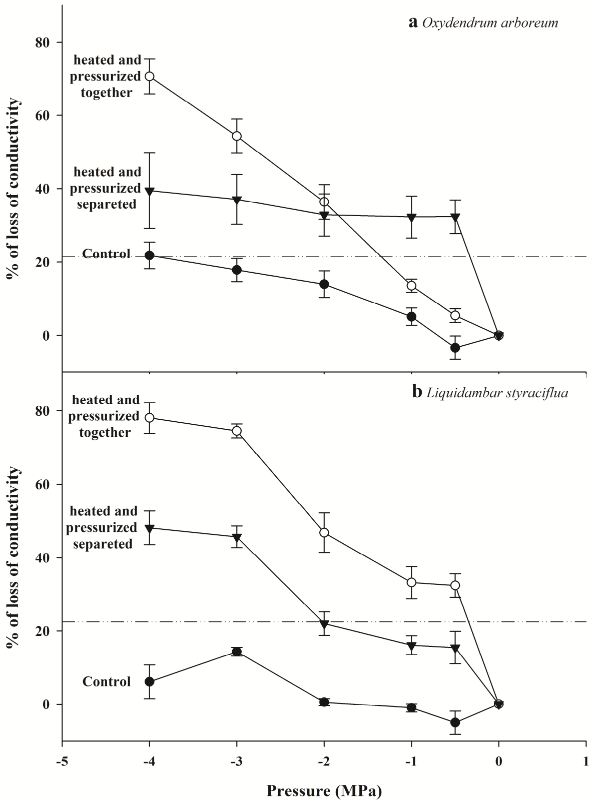

Figure 5: (a) *O. arboreum* and (b) *L. styraciflua* percentage of loss of conductivity along a pressure gradient (MPa) with heated (80ºC) and pressure in the same time (○), heated followed by a pressure gradient (▼) and the control, with stems submitted to a pressure gradient (●). The 0 of the heated treatments correspond to the conductivity before heated. Each point is the mean of the treatments and the bars are standard error bars. Dashed line indicates the mean of all treatments.

4 Discussion

The behavior observed in both species indicates that stem heating induced severe water deficits that led to stem death. The water stress indicates that main damage was in the hydraulic system. Whereas was not observed differences in the xylem anatomy, the water stress can be attributed to embolisms, leading the system to fail.

Embolisms happen to appear in a regular basis during warm days and are usually eliminated when transpiration rates decrease (TYREE, 1997). Despite no changes were found at k_s in the first measurement (Figure 3), there is certainly a hydraulic issue after high temperatures, once g_s decreased immediately after heat was applied (Figure 1). High temperatures can induce changes in the surface tension modifying the forces acting at the sap flow (MICHALETZ; JOHNSON; TYREE, 2012), increasing the chances of a water column break and reducing the transpiration rates.

Decrease in the stomatal conductance is thought to be the earliest response in drought conditions (SU et al., 2013), operating as a pressure regulator avoiding ψ decrease (SPERRY et al., 2002). Trees able to maintain ψ elevate are likely to be less susceptible to embolisms (HOFFMANN et al., 2011). Despite increase transpiration induces vulnerability to cavitation (JOHNSON et al., 2012) a decrease in stomatal conductance and maintenance of ψ were insufficient to avoid mortality (table 1).

After one week heat was applied, while refilling heated stems, we observed a conductivity recovery to similar levels to the control (Figure 4), supporting our hypothesis that embolisms, not structural changes, lead to mortality. Our main question is: if no loss of conductivity was observed right after heat was applied, and the cavitation was observed over time, why the heated trees have no *in situ* vessel refilling as usually is registered in trees? The way of how the embolized vessels are refilled is not known yet, but there is a hypothesis that phloem unloading creating a pressure gradient might be involved in the process (MEINZER et al., 2013; NARDINI; GULLO; SALLEO, 2011; ZWIENIECKI; HOLBROOK, 2009).

Although the girdle as a hypothesis to topkill had been refuted before (BALFOUR; MIDGLEY, 2006; MIDGLEY; KRUGER; SKELTON, 2011), the loss of the phloem and/or other living cells, either by mechanical girdling or by necrosis, prevent the phloem unloading. As we observed in our results, girdle stems with leaves showed an increase in k_s (table 1). The photosynthesis products in girdle trees are blocked at above girdle tissues, while reserves at the roots are depolymerize to guaranty the survival until tree death by root starvation (CHEN; ZHANG; HE, 2013). This situation creates a pool of soluble carbohydrates at the leaves and at the roots, and might create a pressure gradient sufficient to refill native embolized vessels, as a pressure gradient pull up water to the roots and stems. So, if our hypothesis is valid, mortality will be a result from the loss of living tissues and impossibility to refill embolized vessels.

The presence or absence of leaves did not affect the k_s or %M in heat treatment. Nevertheless, trees with a 100% crown scorch had been correlated with mortality over time (STEPHENS; FINNEY, 2002). The lack on transpiration allows the tree to maintain the water status, but also influences the carbon uptake by photosynthesis, and consequently the amount of nonstructural carbohydrates available (URLI et al., 2013). Thus, we have two factors that could lead tree mortality: the lack at carbohydrate production and the loss of water by transpiration. Still we have a question why no differences were observed between stems with and without leaves as we observed in the girdle treatments. But, considering the lack in g_s both treatments had a decrease in the pool of carbohydrates committing the embolism refill.

Differences in %M after fire considering the seasons have been found (HARRINGTON, 1993; SHEPERD; MILLER; THETFORD, 2012; TOLSMA; TOLHURST; READ, 2010), presenting the growing season as the most harmful. A dormant state is favorable once no water is lost by evaporation, and trees already have a pool of carbohydrates stored during the senescence of leaves before winter, creating a favorable condition to maintenance of the hydraulic system. Tyree and Zimmermann (2002) discussed about the low probability of carbohydrates generate a sufficient pressure gradient to influence absorption of water and recovery of embolisms. They justify that, due to the modulus of elasticity, the pressure created is limited and lost to adjacent cells, thus carbohydrates pressure gradient is not sufficient to refill embolisms. Nevertheless an important role for sugars released from starch stored in xylem parenchyma has been discussed in water conductivity recovery process (MEINZER; MCCULLOH, 2013; NARDINI; GULLO; SALLEO, 2011; SALLEO et al., 2009; SECCHI; ZWIENIECKI, 2012).

A classic cavitation case discussed in the literature is the freeze cavitation happening as a result of air expelled from the water during freezing processes (TYREE; ZIMMERMANN, 2002). In the same way, while heating water until 80 ºC in a Becker in the laboratory we observed bubbles coming out from the solution. The bubbles formed during the heating might not be harmful at a first time, but expanding over time would prompt a completely loss of conductivity, as we observed here. Bubbles are usually expelled from the xylem by two ways: (1) air diffuses from the surface bubble driven by air-concentration gradient and then released at the atmosphere; (2) air is transported by mass flow to evaporative surfaces (TYREE; ZIMMERMANN, 2002). Once the sap surface tension is modified by temperature (MICHALETZ; JOHNSON; TYREE, 2012) and the stomata is closed (Figure 1), the two options are impaired and obviously failed in our samples.

Surprisingly, no difference was observed in k_s between trees with and without leaves, despite the combination of pressure gradient and heat was the most damage to the PLC (Figure 5). The stomata closed after heat, and this means there was no pressure gradient from transpiration, concluding that just heat causes sufficient damage to lead a hydraulic failure. The situation is probably different if we consider high diameter trees, once the abundance of leaves transpiring, even in low rates, might create a drawback in k_s, once plants transpiring exhibit a loss of 24-40% in hydraulic conductivity during the minimum daily ψ (MEINZE et al., 2008). After heat, the impossibility to recover from embolisms leads trees to death.

It is clear a hydraulic failure in trees leading tree mortality after heat, but the mechanisms involving hydraulic resilience of species to refill embolized vessels are dependent of multiple physiological and structure traits not well understood (MEINZER; MCCULLOH, 2013). Seems to us that stomatal control have an important function in fire affected trees and is possible that trees with different stomatal behavior will respond differently to high temperatures. Depending in how long is the mortality delay it could imply in enough time, for example, to spread seeds and maintain seed storage on the soil. The mortality of young trees reflect at ecosystem carbon pool and also at forest structure, and in a scenario of global climate changes predicting more areas affected by fire, more efforts have to be invested in understanding the mechanisms of cavitation process in tree mortality in order to avoid unknown damages. Based in our results, we encourage an investigation considering groups of species, directing fire-prone and not fire-prone areas, to understand what features make some species more vulnerably then others to fire mortality.

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Chapter 3: Fire and Water: how water flows in the xylem when leaves are on flames.

Abstract: The mechanism of tree mortality after fire, despite its importance in the structure and evolution of ecosystems, is further more complex than what had been receiving attention. Also mortality had been studied as a consequence of isolated facts, mainly directed to stem causing mortality. Besides stem damage, this work aimed to evaluate crown scorch influencing on tree mortality. As experimental samples, two species were assigned, *O. arboreum* and *L. styraciflua*, both submitted to crown scorch and stem heat damage. In both experiments we evaluated the sap flow in the xylem using Dynamax Dinagage Sap Flow Gages, and for crown scorch experiment we also evaluated the water flow using a balance coupled to a datalogger. Besides no mortality in crown scorch experiment, an increase in the water flow was observed in all situations leaves were burned. Stem heated at 80ºC resulted in 100% mortality as a consequence of hydraulic failure, drove by embolisms leading to cavitation. Cavitation as a cause for mortality, might integrate the enhancement of water flow through the xylem, once an increase in water loss leads to increase in xylem vulnerability to embolisms. More work is necessary to evaluate crown scorch in a higher amount of leaves and its influence, when combined with stem damage, in the time between fire and mortality, as well as differences in groups of species with different % mortality after fire.

1 Introduction

Fire, as part of the history of savannas and forests, modify the ecosystems structure mostly through tree mortality, and tree mortality after fire is attributed as a consequence of stem damage by cambium girdling (BOVA; DICKINSON, 2005; DICKINSON; JOLLIFF; BOVA, 2004; DICKINSON; JOHNSON, 2004; JONES et al., 2004, 2006; MICHALETZ; JOHNSON, 2007, 2008) or by hydraulic failure preventing xylem flow (DUCREY et al., 1996; MICHALETZ; JOHNSON; TYREE, 2012; MIDGLEY; KRUGER; SKELTON, 2011). Cambium girdling causing root starvation is widely accepted, while hydraulic failure causing water stress is a hypothesis still being established. However, both theories limit fire plant mortality as a stem damage consequence. Therefore, root starvation and water stress do not explain the entirely process of mortality once they exclude leaf and root damage. Thus, as an interaction of factors, tree mortality mechanism is not clear yet.

Usually roots undergo to superficial damage, restricted to the fine roots located in the first 30cm soil layer. Roots are more affected by secondary fire effects, as fuel consumption, soil mineral composition, soil fauna biodiversity and soil hydrophobicity (CERTINI, 2005; SOLERA et al., 2011). While roots are not highly affected, leaves are usually consumed or at least exposed to damages by hot plume.

In fire-prone ecosystems, tree species have a thick bark protecting the stem live tissues from lethal temperatures (HOFFMANN et al., 2009). Even in trees that a thick bark is absent, the stem tissue is still less vulnerable to fire than the leaf tissue. Although stem girdling and hydraulic failure have been considered the reason for tree mortality, loss of leaves, in cases of a 100% crown scorch, was also correlated to tree mortality (STEPHENS; FINNEY, 2002). Tree mortality in that condition was attributed to impossibility of leaves recovery due to a lack at the carbohydrate production. Mortality by carbon starvation, especially in fire-prone ecosystems, seems to overestimate the plasticity of trees, once fire-prone ecosystems are mostly populated by species with a high amount of reserves stored at root system, due to resprout ability (CLARKE et al., 2013; HOFFMANN et al., 2009).

Scarce information is available about leaves contributing to tree mortality (ORDÓÑEZ; RETANA; ESPELTA, 2005; STEPHENS; FINNEY, 2002). Despite leaves driving the water flow by stomatal transpiration and increasing xylem cavitation vulnerability, its importance inducing hydraulic failure observed after fire is yet unverified. It is well established that leaves transpiring create a force (pressure gradient) to pull up water from the soil to the roots and stems. Pressure gradient has a direct effect in the formation and exclusion of embolisms in the xylem, thus influencing the water flow and xylem hydraulic. Further point is that leaf petioles are usually more susceptible to embolisms than stems (HAO et al., 2008; SACK et al., 2003), and if the leaves blade burning lead to formation of embolisms in the remained petioles, these embolisms could expand inducing hydraulic failure.

If not consumed, leaves might be affected by hot plumes, and exhibit an enhancement at the time-lag between water loss and absorption as suggested, but not experimented, by Midgley, Kruger and Skelton (2011). A high transpiration rate increases xylem vulnerability to embolisms and so contributes to hydraulic failure.

Mortality prediction helps understanding fire acting at the structure and evolutionary traits of fire-prone ecosystems. However few data are available to understand vulnerability of tree species to neither fire nor the physiological and morphological characteristics that determine this vulnerability (BRANDO et al., 2012).

Due to the scarcity of data about the mechanism of water stress after fire, we settled this work studding hydraulic failure leading to tree mortality. We tested two hypotheses involved in tree mortality: leaves burning increasing the water flow and consequently cavitation vulnerability of xylem, and stem heating causing embolisms leading to cavitation. If supported, these hypotheses would implicate in consider leaves consumption in the models, as well as cavitation as the main cause for fire mortality in tree species. Furthermore, these hypotheses might reinforce the role of tree height in mortality scape by protecting leaves from damage, consequently reducing the vulnerability of the hydraulic system. To test our hypotheses, we studied the effect of fire at leaves and high temperature at stems inducing mortality of two species, *Oxydendrum arboreum* and *Liquidambar styraciflua*. We also tested high temperatures influencing xylem water flow, evaluating xylem flow in heated stems and leaves burned.

2 Methods

The study species, *O. arboreum* (Ericaceae) and *L. styraciflua* (Altingiaceae) are deciduous trees common in forests of eastern USA. As saplings both species have all the leaves consumed during a fire and are topkilled.

Due to differences in the methodology on the followed experiments, we used F_L referring to flow measured using a balance and S_F to flow measured using a Dynamax Dynagage Sap Flow Gages.

Experiment 1: F_L measured by water absorbed

To test F while burning leaves, a balance was used. Branches were collected from a site near Raleigh (NC) a day before the experiment was evaluated, and maintained in a bucket with water. The balance (0.001g accuracy) was placed in a chamber flow, and connected to a datalogger (Campbell Scientific) set to uptake data each two seconds. An Erlenmeyer filled with water pre-measured was used as water source. Branch was suspended by a ring stand with a clamp holding the stem positioned to avoid contact with Erlenmeyer walls. Balance plate was then covered with paperboard to avoid aches and wind interference. To validate the method and warranty the decrease in the weight is due to leaf transpiration and not surface evaporation, a branch without leaves was monitored during one hour, and an Erlenmeyer lacking sample was monitored for 4 hours. No differences were observed.

The leaves at the branch sample were evaluated for instance to obtain stomatal conductance (g_s) and water potential (ψ) before fire. g_s was obtained using an AP4 Leaf Porometer from Delta-T Devices measuring 5 leaves per branch, describing the results in µmol $m^{-2}s^{-1}$. ψ was obtained using a pressure bomb model 1000 measuring 4 leaves per stem, and describing the results in

MPa. We also obtained the number of leaves (L_N) of each branch and leaf area of each species. Leaf area (L_A) was attained from 100 leaves for each species, measured in triplicates, using a CI-202 leaf area meter (CID, Camas, WA, USA). At the flow chamber, we measured light, humidity and temperature, using a portable Li-1400 datalogger, with a LI-190SA Quantum Sensor and a 1400-104 Relative Humidity and Air Temperature Sensor. Using the data obtained from the chamber flow surrounding we calculated the VPD (vapour pressure deficit).

Branches were burned using a torch with a mixed of orange and blue propanol flame. Blade leaves were incinerated, remaining only the petioles. After burn the blade, petioles were cut off using a pruning shears. The total time expend to uptake flow measures from intact leaves (F_L), during fire (F_{DF}), after fire (F_{AF}), and after cutting remained petioles (F_{WP}), was about 1 hour. After data collected, a slope of each 12 data was calculated.

Experiment 2: Sap flow under crown scorch and stem heating

Field experiments were conducted in old field conditions at a site near Raleigh, NC in summer 2012. Samples about 2 meters height and 1-3cm diameter were assigned to the experiments. Sap flow (S_F) was measured using a Dynamax Dynagage Sap Flow Gages, and gages used were SGEX, varying 9-16mm. The gages were installed three days before leaf burning treatments were applied. Gages installation followed instructions of the equipment manual, taking place 50cm above the soil, and the data were expressed S_F in g/h.

To evaluate S_F while burning leaves, an experiment was designed with leaves burned and leaves untouched. Thirty two trees were assigned to this experiment, 16 of *O. arboreum* and 16 of *L. styraciflua*, half control and half treated. Fire was then applied using the same torch described in experiment 1.

The Dynagage Datalogger was set to save data each minute, but during the fire data were also collected each second to visualize a better resolution of S_F during the fire. As in the experiment 1, the leaves had entirely blade incinerated. After blades were burned S_F was measured for subsequent 3 days, but in this case the petioles remained at the trees.

Heat inducing changes in the S_F was also assessed. To verify a possibility of hydraulic failure after high temperature, stems were heated until 80ºC, temperature higher than the lethal for plant tissues. The heat treatment was applied at a height above gage placement using a heating element comprising 160-ohm resistors, connected in parallel in two wires 10cm length with a spacing of 5mm. To monitor the temperature inside the stem a small hole was drilled, and a thermocouple was inserted into the center of the stem, while another was positioned on the outer surface of the stem. The heating element was then wrapped around the stem, which was then covered with foam insulation. To heat the stem, a direct current of 12V was supplied by a battery. Current was supplied until the temperature at the stem center reached 75ºC. Based on initial trials, this allowed residual heat to bring the maximum temperature to approximately 80^{o}C. The average time to reach 80ºC and return the initial temperature was 3 min. To control for a possible effect of drilling the hole for thermocouple placement in heated stems, we drilled a similar hole in the control stems. The gages were then attached and the S_F was measured until the stems were visual dead, with dried leaves and dried stems. The temperature chosen is between the temperature of lignin softening (60ºC) and evaporation water temperature (100 ºC) tested by Michaletz, Johnson and Tyree (2012).

3 Results

No trees subjected to leaf burn died and the samples were even resprouting new leaves weeks later. Yet, all trees subjected to stem heat died in about 21 days for *O. arboreum*, and 13 days for *L. styraciflua*. Both species studied presented similar S_F and F, thus only one representative figure is displayed over the results. Despite the similarity at leaf area (L_A) and number of leaves (L_N) between the species, the stomatal conductance was higher in *L. styraciflua* following also a higher value for the mean flux during the fire (Table 1).

Table 1 Stomatal conductance (g_s), water potential (ψ), number of leaves (L_N) and leaf area (L_A) measures before burning the leaves and F_L (leaf flow), F_{DF} (flow during fire), F_{AF} (flow after fire) and F_{WP} (flow without petioles) collected while burning the leaves. Light, humidity, temperature and VPD (vapour pressure deficit) collected from the surrounding conditions of the flow chamber where the experiments were evaluated. Flow data were obtained using the balance method. Data are represented by means from all samples burned. Bold numbers indicate the standard error.

Branch Characteristics		
	O. arboreum	***L. styraciflua***
g_s (µmol $m^{-2}s^{-1}$)	21.4 (±**1.89**)	41.8 (±**3.67**)
ψ (MPa)	-0.59 (±**0.037**)	-0.40 (±**0.023**)
L_N	41 (±**5.3**)	40.7 (±**4.34**)
L_A (mm^2)	45.88(±**1.92**)	40.84(±**4.72**)
F_L (g/h)	0.0018 (±**0.00064**)	0.0036 (±**0.00001**)
F_{DF} (g/h)	0.0037 (±**0.00072**)	0.0087 (±**0.00012**)
F_{AF} (g/h)	0.0015 (±**0.00047**)	0.0015 (±**0.00002**)
F_{WP} (g/h)	0.0003 (±**0.00010**)	0.0002 (±**0.00001**)
Flow Chamber		
Light (µmol $m^{-2}s^{-1}$)	44.50 (±**15.43**)	
Temperature (ºC)	27 (±**2.79**)	
Humidity (%)	25.1 (±**3.08**)	

Although leaf burn causes no mortality, the water flow increased when burning leaves, in both cases, laboratory and field experiments (Figure 1, 2 and 3). The peak observed may perhaps be resulting from an alteration at VPD, but no differences were observed in this parameter inside the chamber flow (Table 1). Flow had a maximum of 4 times and a mean of 2 times increase in the water consumption during fire (Figure 1 and 2; Table 1). Surprisingly, we observed a continuity of the F_{AF} in amounts similar with F_L (Figure 1 and Table 1). The flow stopped only when the remained petioles, F_{WP}, were removed from the branches (Figure 1 and Table 1).

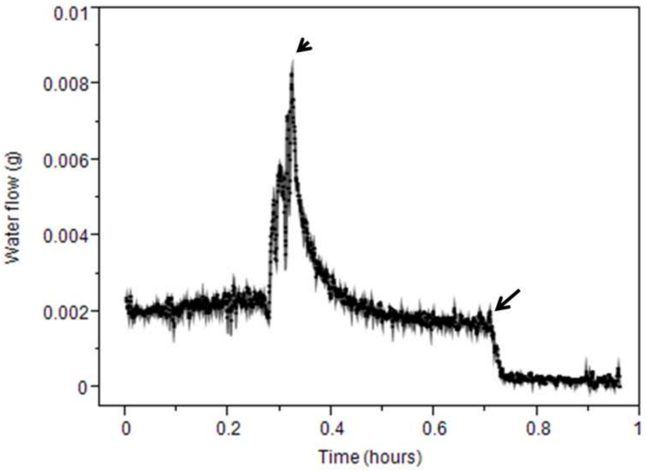

Figure 1 *O. arboreum* water flow in g measured at a balance. First straight line indicates flow by transpiration and the peak represents while leaf blade burning. Second straight line is the flow after leaf blade burned. Last straight line represents flow after cut petioles. Data is represented by the slope. Arrow cap indicates the maximum flow during the fire and arrow indicates the point when petioles were cut.

Differently from the laboratory experiment, leaves burning in trees at the field do not show flow continuity dependent of the petiole after burning the leaves (Figure 3). The flow stops without requiring petioles remove, as we observed in the 3 days following flow after leaves burn (data not show). The peak observed at the flow in the field was similar with peak observed in the laboratory, despite differences in the water source and experimental samples (whole tree and branch). Upon our knowledge, water flow while leaves are in flames had never been described before.

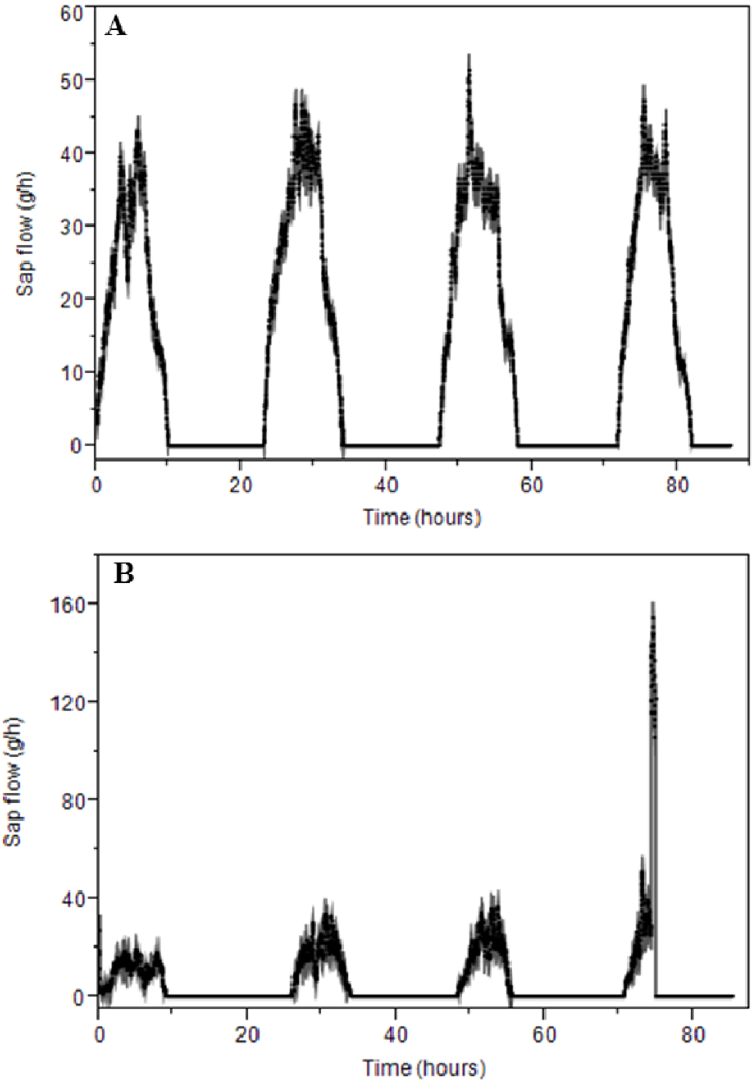

Figure 2 Sap flow (grams/hour) at (**A**) *L. styraciflua* untouched tree during 4 days and (**B**) peak at the fourth day represents the time when the fire was applied. The straight line, after the peak indicates the sap flow equal zero, as the straight line between daytime also representing no flow at night. Each peak represents one day.

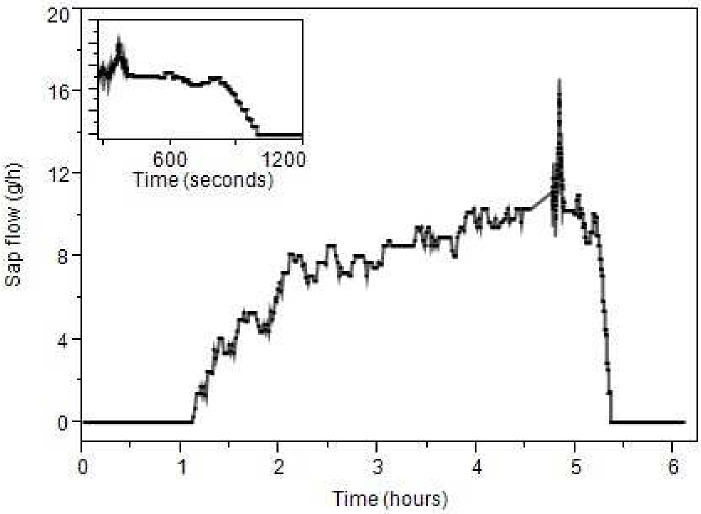

Figure 3 Sap flow of *L. styraciflua* in 6 hours in a scale of seconds. The peak indicates the time when the fire was applied, and is also demonstrated at the miniature figure. The continuity of the flow at the small picture indicates the flow continued after the fire for about 1000 seconds (15 minutes).

Stem heating showed a continuous decrease in the xylem conductivity. After heat was applied, S_F maintained regular ratios in the first two days (Figure 4). After that period, flow decreased continuously (Figure 4B) exhibiting water stress signs, observed as turgor leaf loss and stem desiccation. Once no decrease was observed after heat was applied, we neglect the probability of structure damages causing hydraulic failure and reinforce the cavitation hypothesis leading plant mortality after fire.

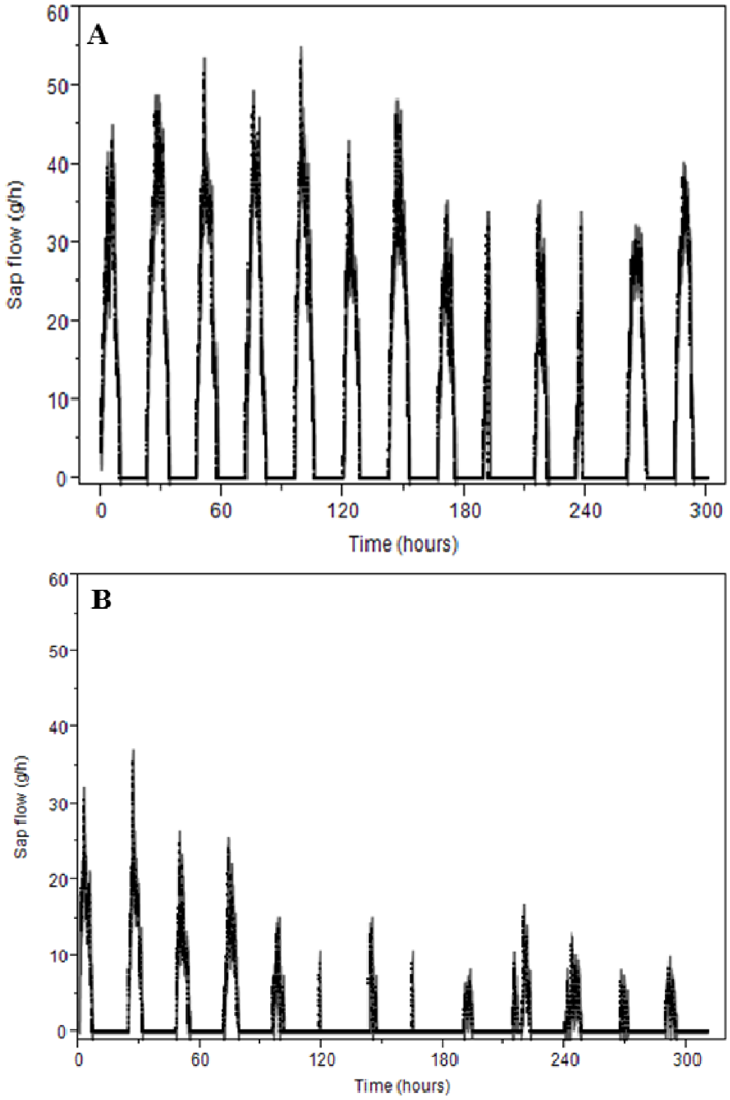

Figure 4 Sap flow of *L. styraciflua* trees at (**A**) untouched tree and (**B**) after stem damage by high temperatures (80ºC) showing a continuous decrease at sap flow.

4 Discussion

Our hypothesis that leaf burn cause tree mortality failed once mortality was not observed. Moreover, stem heat induced 100% of tree mortality in the species studied, and according to our findings the death was a result of a hydraulic failure, as hypothesized. Once no changes were observed in the S_F on the first two days, and due to a follow steady decrease in S_F, we assume that hydraulic failure is a consequence of embolisms leading to cavitation. Yet leaves burning did not cause mortality, the increase in S_F might interfere on tree survival.

Extensive and massive crown fires result in homogeneous burned landscapes with a low survival of trees inside the area affected (ROMME et al., 1998). It is expected that a crown fire attend vast damage to tree stem, but the implications of increasing flow to tree mortality are still not clear. We assume that an increase in the S_F would enhance the vulnerability to cavitation, as suggested by Midgley, Kruger and Skelton (2011). Further, leaves are a big source of loss of water during the fire, what might also affect the soil water reservoir.

The increase observed in S_F is a consequence derived from two main reasons, a temperature gradient and/or a pressure gradient enhancing the flow. Temperature gradients in water filled vessels do not induce the S_F, yet temperature increase does increase the rate of S_F by pressure gradients because the viscosity of water decreases about 2.4% for every degree Celsius in temperature. Also, as the temperature rises above 4 degrees Celsius, water or entrapped air bubbles in vessels do expand causing transitory water displacement (TYREE; ZIMERMMAN, 2002). Water displacement creates a favorable condition for embolisms, increasing the vulnerability to cavitation.

Beyond the water evaporation observed in the leaf blade, the petioles also have a function in the hydraulic system observed in the ongoing F_{AF} (Table

1). Petioles are more vulnerable to cavitation compared with stems, thus the continuity in F_{AF} and the interruption on F_{WP} could be attributed to vessel refilling of embolisms formed while burning the leaves. It is suggested in the literature that the resistance to water transport in leaves is ‘‘disproportionately’’ high compared to other plant organs (HAO et al., 2008; SACK et al., 2003), once the leaf blade is incinerated, water flow might be favored.

The water tension created at the transpiring leaf is ultimately transferred to the roots where it lowers the water potential of the roots to below the water potential of the soil. This cause water uptake from the soil to the roots to the leaves to replace water evaporated at the surface of the leaves (TYREE; ZIMMERMAN, 2002). Thus, the water loss during the fire is replaced in the insulated stems (high diameter trees) without greater damages. However, due to increase in water evaporation, fire might create transient water depletion in the soil. Trees remained have an increase in the water efficiency (GHARUN; TURNBULL; ADAMS, 2013), and after the first rain the water soil reservoir probably returns to previous levels. Nevertheless, frequent burned soil show an increase in the rain water runoff due to the presence of ash and burned material creating a crust (ONDA; DIETRICH; BOOKER, 2008). It is possibly that subsequent crown scorch fires increasing water depletion contribute to soil hydrophobicity usually observed in fire-prone areas (SOLERA et al., 2011).

Although the effects of fire on water quality have been well studied, surprisingly little is known about how fire will affect water quantity (MITCHELL et al., 2014). Only two studies (BRACHO et al., 2008; CLINTON et al., 2011) on components of watershed hydrology associated with wildland fire are registered (MITCHELL et al., 2014). Clinton et al. (2011) examined the effects of crown scorching in longleaf pine forests, and found that with a reduction in 77% of leaf area, sap flow was similar between scorch and control trees due to changes in transpiration by the remaining foliage. With vegetation and water balance so closely coupled, understanding the direct and indirect feedbacks between vegetation and water quantity associated with fire becomes increasingly important, particularly under predicted altered fire regimes (MITCHELL et al., 2014).

There is no doubt that stem damage is more important driving the process of tree mortality subjected to high temperature. Also, the evidences that mortality is caused by hydraulic failure is greatly strengthened by our results. From the observations made in this study, the question arises under what are the contributions of S_F increase combined with damages in the stem? Despite no differences were observed in the VPD, post-fire forest VPD was found to be 0%, taking about 2 hours to recovery for pre-fire levels (KREMENS et al., 2003). Depletion on VPD can increase the flow of remained leaves enhancing the vulnerability to cavitation (KAVANAGH; DICKINSON; BOVA, 2010). Cavitated vessels, in a soil with water availability, are refilled avoiding hydraulic failure. However, as we observed in our results, damage in the stem seems to cause irretrievable cavitation, once even though there was water availability in the studied sites, hydraulic failure happened and induced tree mortality. Combined, leaves and stem damage, might enhance the mortality percentage in fire-prone areas.

Sack and Holbrook (2006) discussed about ways to recovery from embolisms, like nocturnal root pressure, ion pumping or transient pressures, associated with increasing starch degradation. However, stem damage seems to incapable, for some unknown cause, any refilling mechanism.

Besides, different rates of transpiration could also implicate in species-specific mortality rates. We observed no difference in the mortality mean time for both species, whereas *L. styraciflua* died in a shorter period of time and presented a higher rate of transpiration. More studies are necessary to investigate differences in mortality rates implicated by differences species-specific concerning physiological and structural traits. The studies have to attain to difference in species plasticity to understand and manage fire without unexpected results.

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Chapter 4: Conclusions and Perspectives

Although no differences were observed in xylem anatomy, momentary changes in the cell wall structure are likely to happen. However, if so these changes cause no fluctuations in sap conductivity. Anyhow, cavitation causing system hydraulic failure is evident.

It is still not clear the influence of xylem flow increasing hydraulic failure after fire, once we did not measure the water potential or the hydraulic conductivity after crown scorch. As we observed in stem damage experiments, for the species studied, tree mortality does not depend on presence or absence of leaves. Although, in a fire situation both, stem and leaves, are subjected to fire damage, and in this case based in our results the presence of leaves affects the hydraulic system. In conclusion, all the physiologic modifications fire causes instantly are directly initiating tree mortality. These modifications include the increase in water flow, formation of embolisms, changes in the water forces combined with possible changes in the xylem structure, and lack in carbohydrates production caused by stomatal closure.

The decrease in the stomatal conductance as primary response may be a consequence of temporary changes in the water surface and xylem structure. A question that is still not answered is why stomata close when they do. What would be the thermal sign for stomatal closure? This should be a question for future work. Also, there is a paradox at stomatal closure between saving water and reducing photosynthesis. The implications of this paradox to tree survival still need to be investigated.

Further work should be directed to comparisons among different groups of species. Finding parameters that characterize tree mortality after fire will help models to increase the reliability of prediction. Although, new models should be propose considering hydraulic failure as the main issue for tree survival after fire.

Besides, future research should also focus in correlating the incapability of vessel refilling with carbohydrates production, as well as try to understand why in a soil with water availability no refilling is observed. Special features in trees that survive more often than others should also be investigated. Tree in a burned stand likely to experience a decrease competition from the surrounding shrubs increasing the water availability and consequently the probability of avoiding a hydraulic failure by refiling embolisms formed during the fire. It is essential to understand the parameters involved in fire resistance and fire susceptibility separated, as well as its interaction to understand fire impacts in fire-prone and not fire-prone ecosystems.