The Physiological Response of Conifers to Fire

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Authorization to Submit Dissertation

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Abstract

One of the grand unknowns of ecosystem science is how fire kills trees. Answering this question is critical to parameterize climate-vegetation models given the observed changes in global fire regimes, the feedbacks between fire and forests in the global carbon cycle, and the potential role of forest management in moderating anthropogenic climate change. In this dissertation I conducted three studies using *Pinus ponderosa* saplings burned under controlled conditions to improve the understanding how fire effects on tree physiology. First, I assessed the impact of two fire intensities on sapling mortality under two water status pre-fire (well-watered and drought-stressed). The results showed that saplings under drought-stress pre-fire were more vulnerable to mortality when exposed to low fire intensities. However, 100% of mortality was observed regardless of the pre-fire water status when saplings were exposed to high fire intensity. Thus, the data also suggest that there is a fire intensity threshold where the pre-fire water stress can have a significant influence on sapling mortality. Second, we investigated the short (one-day post-fire) and long-term (21-months post-fire) effects of fire on sapling water transport. In the short-term, fire did not have impact on sapling xylem hydraulic conductivity or were more vulnerable to drought-induced embolism. However, in the long-term, saplings were more vulnerable to cavitation. But no damage in the xylem conduits cell walls were observed. Thus, it was hypothesized that the new traumatic xylem formed in the edges of the fire scar and the pre-fire xylem clogging with resin could be responsible for increasing vulnerability to cavitation in these plants. Lastly, I evaluated the impact of a lethal fire intensity on sapling hydraulic conductivity and non-structural carbohydrates periodically for 28-days post-fire. Hydraulic conductivity was not affected any day. This confirmed the results found in the second study. Fire caused a decline in total NSC in burned plants compared with unburned saplings, but it was significantly only 28-days post-fire. The results suggest that tree mortality from fire is likely not due to hydraulic failure but may be related to carbon imbalance.

Acknowledgements

Dedication

First, I would like to dedicate my work and thank my mother, Maria Ap. P. Feltrin, for always support my dreams, my goals and to teach me how to be a good person while she was here. I also would like to express my gratitude for all the support I had from my family, especially my father Ralph Ap. Feltrin, my brother Ralph P. Feltrin, and my aunts Maria L. Feltrin, Benedita Ap. Feltrin, the new friends I made during my Ph.D program, Mariana Dobre, Paola B. Stramandinoli, Rachel Midence, Brandon M. Souvenir, Brian F. Hanson, Jessie Godfrey, Monica R. Harmon, CDJ, and my old friends. I also would like to thank and dedicate my work to all the teachers, professors, and mentors I had from the first grade to my Ph.D program.

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Statement of Contribution

Appendix 1 represents a joint first author publication between Wade Steady and Raquel Partelli Feltrin. Steady and Feltrin co-led the development of the research through each conducting (designing, analyzing, and writing) a separate experiment that was then combined into a single publication. Their advisor Smith conducted the analysis of the logistic regressions in support of both students.

Chapter 1: Introduction

Droughts and wildfires are natural disturbances in many forest ecosystems. Global mean temperature rise associated with anthropogenic climate change are predicted to increase the magnitude of droughts and fire events (IPCC 2014). However, it is uncertain how forests will respond to more intense disturbances such as severe droughts (Brodribb et al. 2020) and more frequent and intense wildfires. Process-based models that incorporate physiological mechanism can help to better understand and help to take actions to mitigate the effects of these disturbances, particularly when they co-occur, in forest ecosystems. In the last decades, there was has been an increase in studies focused to understand the impact of droughts on tree physiology (McDowell et al. 2008, Adams et al. 2013). But only recently, scientists started focusing in understanding the fire effects on tree physiology (Kavanagh et al. 2010, Michaletz et al. 2012, Battipaglia et al. 2016, West et al. 2016, Bär et al. 2018). Thus, to improve the models and predictions more studies related physiological response of trees to fire are needed.

The goal of this dissertation is to provide understanding of some of the physiological response of post-fire tree mortality. In the first chapter, titled as "Drought increases in vulnerability of *Pinus ponderosa* saplings to fire-induced mortality", I assessed the impact of pre-fire drought stress on post-fire *Pinus ponderosa* saplings mortality exposed to two different fire intensities. I evaluated how the interaction of different fire intensity and pre-fire saplings water status impacted photosynthesis, recovery (flush of new buds), and mortality of saplings. In the second chapter, titled as "Short- and long-term effects of fire on stem hydraulics in *Pinus ponderosa* saplings", I evaluated the short and long-term effect of fire on *Pinus ponderosa* water transport. In the last decade, studies using fire proxies had provided evidence that fire can decrease hydraulic conductivity (Michaletz et al. 2012) and plants exposed to fire are more vulnerable to drought-induced embolism (West et al. 2016; Bär et al. 2018). I evaluated the effects of lethal and non-lethal fire intensity on *Pinus ponderosa* saplings' xylem water conductivity and xylem conduits cell wall. In the third chapter with the title "*Ponderosa pine* hydraulic conductivity and non-structural carbohydrate response to a lethal fire intensity", I assessed impact of a lethal fire intensity over time on non-structural

carbohydrates in saplings' leaf, stem and roots, and whole plant, xylem water conductivity, and photosynthesis. In addition, I included a previous joint first author study (Steady et al. 2019) that evaluated the impact of increasing levels of fire behavior on *Pinus ponderosa* saplings mortality and the impact on saplings' growth. This study also provided some baseline information for the studies here presented.

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Chapter 2:

Drought increases vulnerability of *Pinus ponderosa* saplings to fire-induced mortality

Abstract

The combination of drought and fire can cause drastic changes in forest vegetation cover. Given the predictions of more frequent and severe droughts and the resulting increase in fire activity particularly in western US, we assessed the impact of drought and different fire intensities on ponderosa pine saplings. In the laboratory, saplings were exposed to two different fire intensities. We monitored photosynthesis, recovery (bud development and survival) and mortality of saplings periodically during the first month post-fire, and 200- and 370-days post-fire. All the saplings burned at the high intensity (1.4 MJ m^{-2}) regardless of the pre-fire water status died. 70% of pre-fire well-watered saplings recovered after exposure to low intensity fire (0.7 MJ m^{-2}). However, all the pre-fire drought-stressed saplings died, even at the low fire intensity. Regardless of the fire intensity and water status, photosynthesis was significantly reduced in all saplings exposed to the fire. However, at the end of this experiment (370 days post-fire) burned saplings that recovered had similar photosynthesis rates as unburned plants. In addition, all plants that recovered or attempted to recover produce new foliage within 35 days post-fire treatments. Our results demonstrate that the pre-fire water status of saplings can have an impact on sapling recovery after fire.

Keywords: fire, drought, saplings, mortality, photosynthesis

Introduction

In the last decades we have witnessed widespread forest die-off caused by heat stress and droughts (Breshears et al., 2005; Allen et al., 2010). Although, high temperatures and severe droughts are of high concern in relation to tree mortality, wildfires can also be intensified as consequences of more severe droughts and higher temperatures caused by climate change (Running, 2006; Westerling et al., 2006). Additionally, the combination of prolonged drought

events and wildfires can cause drastic changes in forest cover (Davis et al., 2019). To project tree mortality caused by drought or fire, forest managers and scientists rely on tree mortality models. However, most post-fire tree mortality models are empirical models developed from a limited number of species (see Hood et al., 2018). Thus, they are limited in the accuracy of their predictions particularly under future climate scenarios. An alternative to improve post-fire tree mortality predictions is to use process-based models that include the physiological conditions of plant pre-fire, such as water stress and the physiological responses after the fire (Hood et al., 2018). However, there is not many studies available that assessed the pre-fire drought impact on trees mortality post-fire (Sparks et al. 2018) and about the physiological responses that can potentially drive post-fire tree mortality (Ryan 2000, Michaletz et al. 2012, Battipaglia et al. 2016, Bar et al. 2018).

Trees under drought stress can be more vulnerable to fire-induced mortality. During water shortage, trees close their stomata reducing CO_2 uptake and assimilation (Grieu et al., 1988; Rouhi et al., 2007). And after long periods of reduced photosynthesis plants can deplete their non-structural carbohydrate (NSC) reserves for metabolic maintenance (Li et al. 2018). When trees are exposed to fire a set of injuries in foliage, bole and root damage caused by the heat can occur (Michaletz and Johnson, 2007) and to recover from these injuries NSC is needed. Thus, if fire and drought co-occur, depletion of NSC reserves can occur and lead to tree mortality. In addition, fire can induce failure of water transport due to xylem embolism (Kavanagh *et al.,* 2010) and deformation of xylem conduits (Michaletz et al., 2012; West et al., 2016; Bär et al, 2018). Kavanagh et al. (2010) proposed that changes in vapor pressure deficit caused by convective heat during the fire can potentially increase the water column tension in the xylem and spread of air bubbles and potentially lead to tree mortality particularly in drought-stress trees. The heat-induced deformation of xylem conduit cell walls can decrease the water conductivity (Michaletz et al., 2012) and cause plants to be more vulnerable to drought-induced embolism (Bär et al. 2018).

Fire can also lead to tree mortality through damage to the meristematic tissues and tree crown. During the fire, heat can be transferred through the bark causing damage to the phloem and vascular cambium (Ryan and Frandsen, 1991; Dickinson and Johnson, 2004). If the phloem is damaged movement of sugars would be impaired and if severe enough, could

eventually result in tree mortality. Damage to the vascular cambium could result in either the production of traumatic xylem tissue which may be more vulnerable to embolism (Partelli-Feltrin et al. in prep., see Chapter 3) or the loss of ability to produce new xylem, if the entire cambium is killed. Severe crown damage can reduce photosynthesis significantly (Smith et al., 2017) forcing the plants to rely on their reserves to recover. Also, severe crown damage can cause death of buds preventing the plant from recovering its foliage.

In this study we assessed the influence of pre-fire water status on post-fire tree mortality and the effects of different fire intensity on sapling physiology. We exposed well-watered and drought-stressed *Pinus ponderosa* saplings to two different fire intensities and evaluated their recovery over more than one-year post-fire. Here we tested following hypotheses: (1) saplings under pre-fire drought stress are more likely to die than well-watered and (2) saplings under drought stress are less likely to develop new foliage than well-watered saplings post-fire.

Material and Methods

Plant material

In February of 2017, a total of 60 *Pinus ponderosa* saplings were purchased from Franklin H. Pitkin Forest Nursery – University of Idaho, Moscow, ID and potted in 3.7 L pots with 50% Sungro$^{@}$ Professional Growing mix media and 50% perlite. Saplings were grown in a climate-controlled greenhouse at University of Idaho for 6 months. The temperature average in the greenhouse ranged between 15 to 25 ℃ with a photoperiod of 16 hours. During this period, all saplings were watered every other day and fertilized once in a month with a 200 ppm solution of N,P,K (20:20:20) Technigro$^{@}$ fertilizer. At the time of the fire treatments root collar diameter and height mean ($\pm$ SE) were 1.11 ± 0.01 cm and 0.44 ± 0.06 m, respectively.

Experiment design and treatments

In fall 2017, saplings were randomly placed in two water status treatments: well-watered (n=30) and drought-stressed (n=30). Plants in the well-watered treatment were watered every other day to field capacity to avoid water stress. Water was withheld from all plants in the drought-stressed and predawn leaf water potential (Ψ_p) was monitored using a pressure

chamber (PMS Instruments Co., Albany, Oregon). For drought-stressed plants, we targeted the drought treatment so that plants would reach a Ψ_p equal or more negative than -1.6 MPa just prior to the fire experiment. This Ψ_p coincides with previous minimum water potentials measured on *Pinus ponderosa* saplings during an abnormally dry summer in Idaho (Baker et al. 2019). It should be noted that although this observation was during a very dry summer, no mortality was observed at that time from drought (KVB and DMJ, unpublished data).

Prior to exposure to the fire, saplings in each water status treatment were randomly divided into three fire treatments: unburned and burned with fire radiative energy (FRE) of 0.7 and 1.4 MJ m^{-2}. Each water status $\times$ fire treatment had 10 saplings. Saplings were subjected to a fire under controlled conditions in Idaho Fire Initiative for Research and Education (IFIRE) at University of Idaho, Moscow, ID. Saplings were individually placed in the middle of the circular area of 1 m^{-2} demarked in a concrete board with the soil leveled with the board. To generate FRE of 0.7 and 1.4 MJ m^{-2}, 152 and 304 g of *Pinus ponderosa* needles that had been oven dried at ~90 °C for 48 hours were used. Dried needles were evenly distributed in the circular area around the saplings and the fuel bed was ignited using ~ 2g of ethanol (see Steady et al. 2019). After the burns all plants were returned to the greenhouse and watered every other day until the end of the experiment.

Physiological measurements

On the day prior to sapling exposure to fire, Ψ_p and photosynthesis (A) were measured in 10 saplings in well-watered and 8 saplings in drought-stressed treatments. After the fire, A was measured 1, 14, and 27 days post-fire on 5 saplings in each water status/fire treatment. At day 370 post-fire, gas exchange was only measured in the remaining living plants. Photosynthesis was measured between 8:00 to 11:00 in the morning using LICOR 6400XT portable photosynthesis system (LI-COR, Lincoln, NE) with a photosynthetic photon flux of 1400 µmol m-2 s-1, CO2 concentration of 400 ppm, temperature of ~ 25 °C and relative humidity of 37%.

Buds development and sapling mortality

New foliage was evaluated at days 27, 35, 68, 125, 141, and 200 post-fire. At each day we counted the number of the buds that flushed. Here we define as bud flush as when new leaves start expanding into the fully developed new leaves. Number of buds per sapling (density)

and status (alive, dying, and dead) was recorded. Alive buds were defined as green, dying when some of the new needles started turning brown, and dead when all needles emerged from the bud were brown. Mortality was assessed at day 200 post-fire. Saplings were considered dead if all foliage died, saplings did not regenerate new shoots, and the vascular cambium and phloem were dead. To determine the death of vascular cambium and phloem all saplings with dead foliage and no development of new shoots were harvested and if the tissue between the bark and xylem, i.e. phloem and cambium cells, were not green then the cambium and phloem were considered dead.

Statistical analysis

Before plants were exposed to the fire treatments, we tested Ψ_p and A between well-watered and drought-stressed treatments using Student's t-test. To test the fire effects on Ψ_p and A in the following days post-fire, we used a mixed effect model with plants as random effects. Tukey's HSD *post-hoc* test was used to assess the differences in gas exchange and Ψ_p between treatments. We subsequently assessed the differences bud density between the treatments at increasing number of days post-fire using ANOVA and Tukey's HSD post-hoc test. R version 3.6.0 (R Core Team) and the packages lme4 (Mächler et al. 2015) and emmeans (Lenth 2019) were used to conduct the data analysis .

Results

Sapling physiology

The day before plants were exposed to the fire, Ψ_p and A differed significantly ($P < 0.0001$) between well-watered and drought-stressed plants (Fig. 1). In the following days after the fire treatments, comparisons between unburned plants showed that drought-stressed plants Ψ_p were significantly different (-0.84 ± 0.05 MPa) than well-watered plants (-0.45 ± 0.05 MPa) one-day post-fire with $P = 0.0004$ but similar 14, 27- and 370- days post fire. A similar result was observed in photosynthesis only differing at day 1 after the fire. Although, Ψ_p was measured in plants exposed to 0.7 MJ m^{-2}, we recognized the fire damage caused in the foliage may have contributed to produce inaccurate results. Thus, only A was compared between burned unburned and burned at 0.7 MJ m^{-2} plants. A was significantly reduced in

well-watered saplings burned at 0.7 MJ m^{-2} one-day post-fire compared to unburned well-watered plants and remained near zero at day 27 post-fire. A was similar 370 days post-fire between well-watered unburned and burned at 0.7 MJ m^{-2} plants (Fig. 1). Likewise, A was similar one-day post-fire in drought-stressed unburned and burned at 0.7 MJ m^{-2} saplings. At day 14 and 27 post fire, photosynthesis remained near zero in burned drought-stressed plants and significantly different than unburned drought-stressed saplings (Fig. 1). All plants exposed to 1.4 MJ m^{-2} had significant foliage loss and subsequently died, thus we could not measure post-fire Ψ_p and A.

Bud development and saplings mortality

Newly flushing buds were observed in burned plants between 27- and 35- days post-fire (Fig. 2). No saplings exposed to 1.4 MJ m^{-2} flushed any buds within 35 days. Of the 10 well-watered plants exposed to 0.7 MJ m^{-2}, nine of them had flushed buds (Fig.2a). However, in one of these plants we observed that all of the newly developed leaves that flushed were dead by day 125 post-fire. In another plant the new leaves started dying on day 125 and were dead by day 172 post-fire (Fig. 3). In drought-stressed saplings burned at 0.7 MJ m^{-2}, only 2 saplings had flushed buds within 35 days pos-fire. But we observed that the new leaves started dying at day 68 and 125 post-fire and by day 141 all new leaves developed in both saplings were dead (Fig. 2b). Although the percentage of green canopy remaining after the fire was not assessed in this study, we observed that plants that had remaining green canopy after the fire did not flush new buds, while the plants that had the majority of the canopy damaged flushed new buds (Fig. 3). Bud density in the well-watered treatment burned at 0.7 MJ m^{-2} was significantly greater than in drought-stressed burned at 0.7 MJ m^{-2} at 35, 68, and 125 days post-fire (Fig. 4). All unburned plants were alive at day 200 post-fire. Well-watered plants burned at 0.7 MJ m^{-2} had 30% mortality while plants burned at 1.4 MJ m^{-2} had 100% mortality. All burned plants in the drought-stressed treatment were dead at day 200 post-fire (Fig. 5).

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Discussion

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Here, we assessed the impact of pre-fire water status on sapling mortality and recovery after being exposed to two different fire intensities. We found that pre-fire drought stressed saplings were more susceptible to mortality than well-watered plants burned at the same fire

intensity. We also found that the pre-fire water status was not relevant to determine post-fire tree mortality when saplings were burned with the higher fire intensity. Regardless of the fire intensity and pre-fire water status, photosynthesis was reduced in all burned saplings for at least the next 27 days post-fire. In the end of the experiment (370 days post-fire), only 7 out of 10 of the well-watered saplings burned at low intensity recovered. In addition, we observed that all plants exposed to 0.7 MJ m^{-2} fire flushed new buds within 35 days post-fire treatments.

The increase in well-watered sapling mortality with increase in fire intensity found in this study supports previous studies conducted with conifer saplings (Sparks et al., 2016; Smith et al., 2017; Steady et al., 2019). To our knowledge, no studies exist that have evaluated sapling mortality under pre-fire water stress exposed to different fire intensities. However, a study conducted with *Larix occidentalis* saplings showed that plants under moderate drought stress were more susceptible to mortality than saplings not under water stress when subjected to the same fire intensities (Sparks et al., 2018). Our results suggest that the pre-fire water status can influence the recovery or mortality of ponderosa pine saplings after the fire. For instance, the water stressed plants exposed to the low fire intensity (0.7 MJ m^{-2}) had 100% of mortality while well-watered plants burned at the same intensity suffered only 30% of mortality. But our data also suggest that there is a fire intensity threshold, above which where the pre-fire water stress no longer influences on sapling mortality. As observed in this study, all plants exposed to 1.4 MJ m^{-2} regardless of the pre-fire water stressed died. Steady et al. (2019) found that in well-watered ponderosa pine saplings, there was 100% mortality when subjected to a fire intensity of 0.8MJ m^{-2}. Thus, it is likely that when ponderosa pine saplings are subjected to fire doses above 0.8 MJ m^{-2}, they will experience 100% mortality regardless the water status pre-fire.

We observed that regardless the pre-fire water status and fire intensity, all the saplings that were dead by the end of this experiment did not flush buds between 27- and 35- days post-fire. One of the potential fire injuries in these plants that could have led to death can be attributed to bud death. When 100% of the buds are either killed by the convection heat or contact with the flames during the fire, it can cause immediate mortality, particularly in non-resprouting species such as ponderosa pine (Hood et al., 2018). However, we also observed

that some saplings flushed new buds but then eventually died (Fig. 2). Thus, our data also suggests that cambium death and/or depletion of carbohydrates can be the cause of these saplings' mortality. The plants that flushed new buds and eventually died had most of their canopy damaged (Fig. 4a) and consequently a decrease in photosynthesis (Fig 1a). Such decline in photosynthesis can force the plant to depend on their NSC reserves to rebuild the foliage and repair the damaged caused in other organs such as stem and roots. Although our data suggests that cambium necrosis and/or depletion of NSC can be the potential causes of some sapling's mortality, more studies are needed to evaluate these mechanisms, particularly NSC depletion.

The pre-fire water status can contribute to make the saplings more vulnerable to mortality due to fire injuries. As we observed, all the water stressed plants burned at low intensity were dead in the end of this experiment while the well-watered plants only had 30% of mortality. It is possible that the low moisture content in the canopy due to the water stress contributed to more consumption of the canopy consequently increasing the damage of the buds. For instance, in high elevation conifers the canopies high moisture content just after the bud flush contributes with the low probability of crown fire in these forests (Agee et al., 2002). Also, the low moisture content in the bark might increase the magnitude of phloem and cambium heating and thus, tissue death in these plants. However more applied research is needed to identify these mechanisms in drought-stress plants.

Survival of saplings post-fire in the field could be different from the results of this study. For example, if a fire event occurs in the end of summer or fall, we expect new bud development to occur in the following spring, not within days after the fire as observed in this study. It is also possible that saplings in the field may have higher rates of survival if cambium and buds are not 100% damaged when exposed to the lower fire intensity (0.7 MJ m^{-2}). While our sapling roots were constrained in pots, in the field saplings can have deep roots that can be protected from heat damage. Also, saplings in the field can be hardened due to exposure to environment changes, i.e. water shortage, low and high temperatures, during the year. With predicted increases in drought events and the potential increase in fire activity in the coming decades (Flannigan et al., 2000 and 2013), these result gives us important insights into how saplings under drought stress might respond to fire in the future.

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Figure 2.1. Mean predawn leaf water potential (Ψ_p) and photosynthesis (*A*) in unburned (circle) and burned (triangle) *Pinus ponderosa* saplings. Ψ_p values are only reported for unburned plants. The burned plants data showed are related to plants burned at 0.7 MJ m^{-2}. Comparisons are made between unburned and burned saplings at the same day. Days are relative to the fire treatments. Bars are SE.
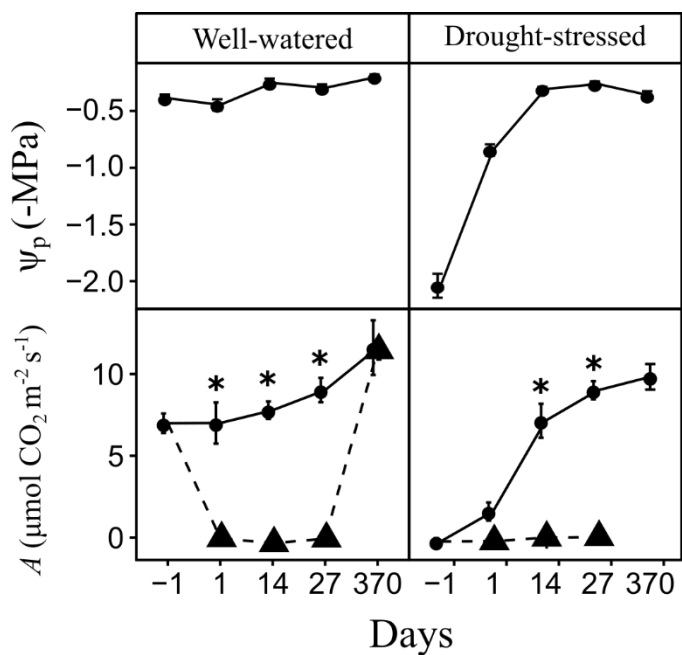
Figure 2.2. Bud emergence, development and mortality in each sapling post-fire treatment. (a) Well-watered plants burned at 0.7 MJ m^{-2}. (b) Drought-stressed plants burned at 0.7 MJ m^{-2}. It was considered new bud if at least one bud was flushed, bud started dying when new leaves started turning brown and bud dead when all the new leaves died. Approximate days were considered for each bud stages. Sapling 55 in panel b did not flushed any new buds but had some green canopy remaining.
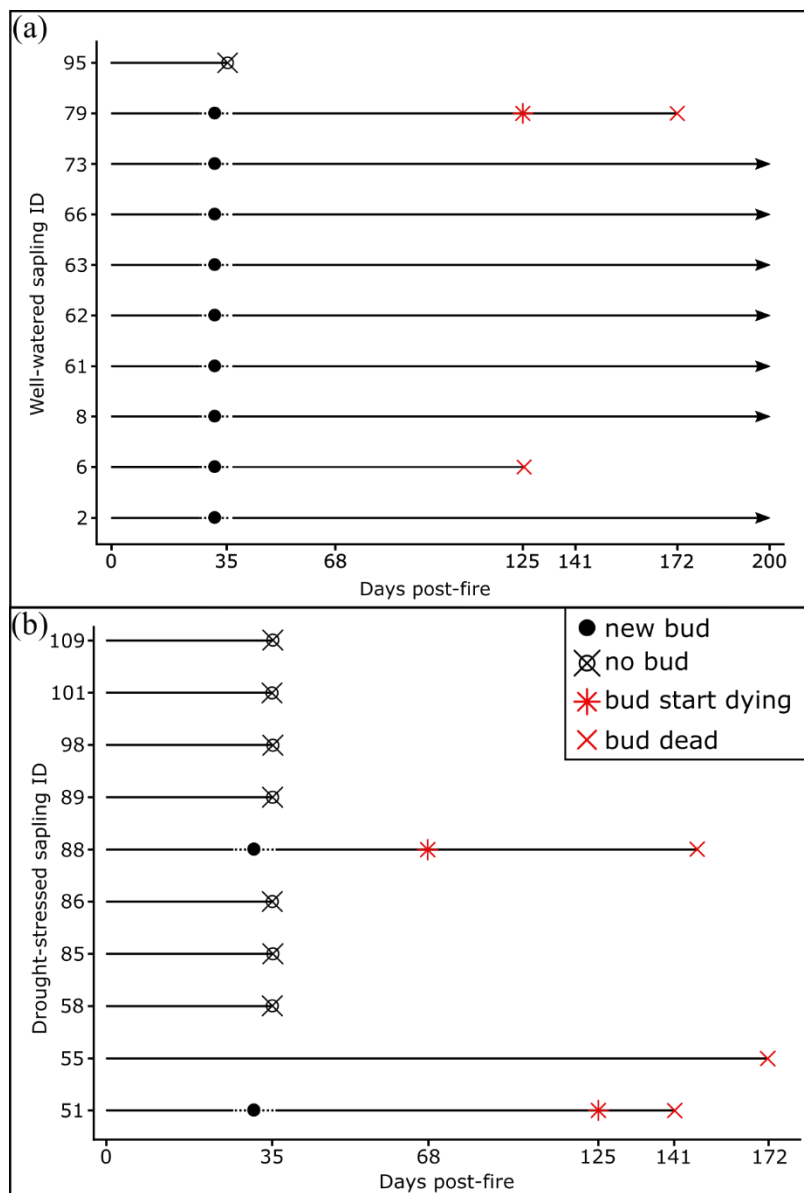
Figure 2.3. Bud and foliage mortality between 125- and 172-days post-fire in saplings burned at 0.7 MJ m^{-2}. Bud mortality observed in a well-watered sapling (sapling ID 79) and canopy mortality in a drought-stressed sapling (sapling ID 55). Numbers above each panel represent days post-fire.

Sapling 79 **125**   **141**   **172**
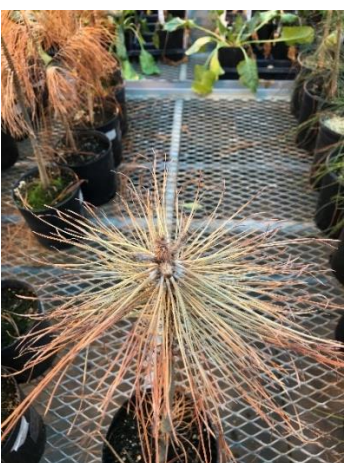
Sapling 55 **125**   **141**   **172**

Figure 2.4. Bud density in well-watered and drought-stressed *Pinus ponderosa* saplings burned at 0.7 MJ m^{-2} from day 35 to 200 days post-fire.
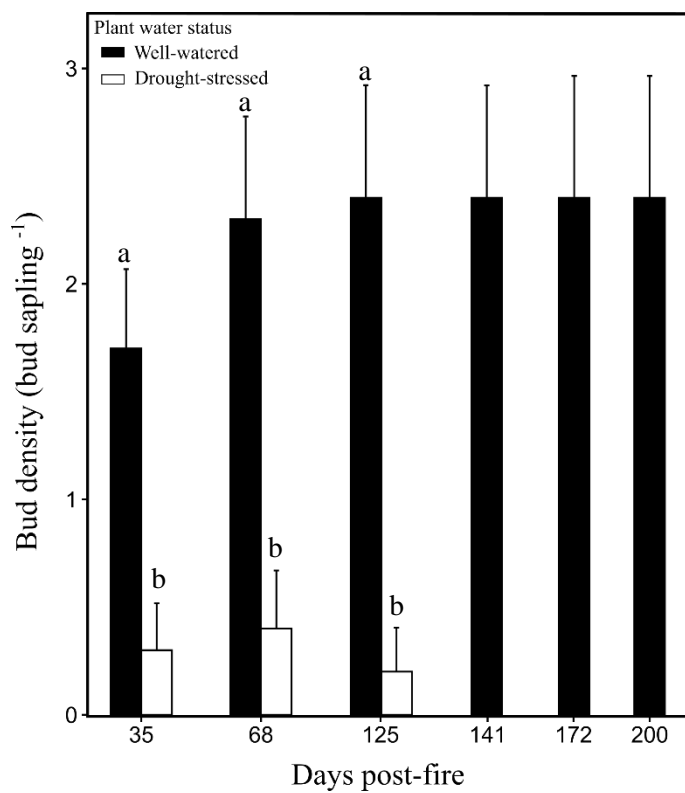
Figure 2.5. *Pinus ponderosa* sapling mortality after exposure to different fire intensity (n=10).
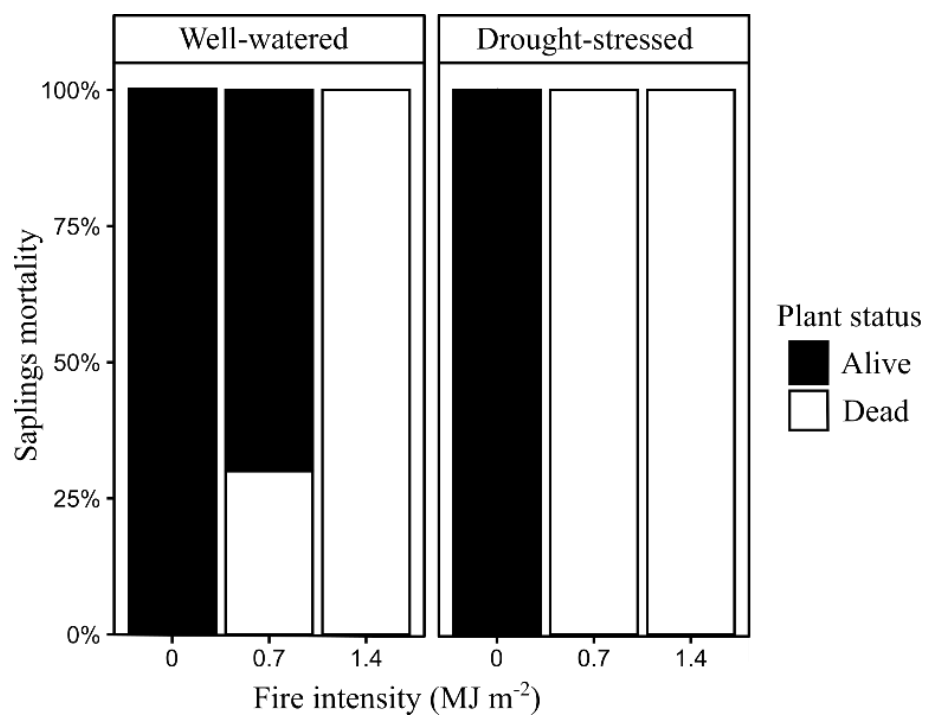
Chapter 3:

Short- and long-term effects of fire on stem hydraulics in *Pinus ponderosa* saplings

Abstract

Understanding how fire impacts tree physiological processes is critically and urgently needed to accurately model post-fire carbon processes and inform management decisions. Given trees can die immediately or at extended time periods after fire, we combined two experiments to assess the short- (one-day) and long-term (21-months) effects of fire on the xylem of *Pinus ponderosa* saplings. Under controlled conditions, saplings were burned at lethal and non-lethal fire intensities. Native percentage loss of conductivity, vulnerability to cavitation, and xylem conduit anatomy was assessed in unburned and burned saplings. Saplings evaluated one-day post-fire were not more vulnerable to xylem cavitation than unburned saplings. However, surviving saplings evaluated 21-months post-fire were more vulnerable to cavitation. Deformation of the xylem conduits in burned saplings was not observed in either experiment. Our results provided evidence that after 21-months, post-fire surviving saplings are more vulnerable to drought-induced embolism. Given conduit cell wall deformation was not observed, we hypothesize that the irregularity of the newly grown xylem cells in the vicinity of the fire wound and the potential crystallization of resin clogging the tracheids and/or pits in pre-fire xylem may be responsible for a decrease in both conductivity and resistance to embolism in burned plants.

Introduction

By the end of the 21st century, fire seasons worldwide are predicted to be longer with greater associated ecosystem and community impacts due to anthropogenic climate change (Flannigan et al., 2013, Bowman et al. 2017). Regional studies have already shown increases in fire activity over the past 30 years (Pausas, 2004, Abatzoglou & Williams, 2016). Increasing fire activity coupled with more extreme and frequent drought events (Sheffield & Wood, 2008) and fuel buildup associated with fire exclusion (Naficy et al., 2010) can

increase potential post-fire impacts, even for fire-adapted ecosystems. Accurate forecasts of post-fire impacts under changing and novel conditions will require that process-based models better incorporate how fires impact plant physiological changes. However, considerable uncertainties remain on how fires impact tree physiology and mortality (Hood et al., 2018, Bär et al., 2019). Improving this understanding is essential to accurately parameterize Earth system models to predict the immediate and extended impacts on terrestrial biogeochemical cycles and, ecosystem services, and to better inform post-fire land management decisions (Krieger, 2001, Smith et al., 2014, Smith et al., 2016, Stenzel et al., 2019).

Given trees can die many years post-fire (Ryan et al., 1988, McHugh & Kolb, 2003) and that there is the potential for droughts in the years after a fire, knowing the persistence of fire impacts on tree hydraulic systems is crucial to improve these predictions. Trees that are not killed immediately by fire are usually left with injuries in the crown and vascular cambium that can potentially lead to mortality (Ryan & Frandsen, 1991, Ryan, 2000, Dickinson & Johnson, 2004). Recently, it has been proposed that dysfunction of the plant hydraulic system is an important mechanism of post-fire tree mortality (Kavanagh et al., 2010, Michaletz et al., 2012, West et al., 2016, Bär et al., 2018). The impairment of the tree hydraulic system caused by fire has been proposed to occur through heat induced-embolism in the xylem water conduits and/or deformation of the conduits cell walls. Shifts in atmospheric conditions (high temperature and low relative humidity) caused by the convective heat (i.e. fire plume) may result in increased tension in the xylem water column (Kavanagh et al., 2010) and spread of air bubbles though air-seeding (Sperry & Tyree, 1988, Delzon et al., 2010). Tree death is highly likely if embolized xylem conduits reach critical thresholds where the embolism is not reversible (Urli et al., 2013, Brodribb & Cochard, 2009, Hammond et al., 2019). Furthermore, during fires it has been proposed that conductive heat transfer through the bark may cause permanent deformation of xylem conduits (Michaletz et al., 2012, West et al., 2016, Bär et al., 2018). This deformation is thought to occur because conduit cell walls are formed of polymers that, when exposed to specific temperatures, will undergo thermal softening (Irvine, 1985, Olsson & Salmén, 1997) and after cooling, the cells walls may stay permanently deformed. It has been further suggested that damaged xylem may result in decreased water conductivity and increased xylem vulnerability to embolism (Michaletz et al., 2012).

If decreased hydraulic conductivity caused by fire persists over extended time periods, other physiological processes may be affected and potentially contribute to post-fire tree mortality. Prior studies have found that stomatal conductance and photosynthesis declined with decreasing water conductivity (Hubbard et al., 2001). Furthermore, it has been reported that if photosynthesis is reduced for significantly long periods, non-structural carbohydrates (i.e. sugar and starch), can be depleted due to usage for metabolism maintenance in drought-stressed plants (Li et al. 2018). Such reductions in photosynthesis in burned plants have been shown to depend on the intensity of the fire (Smith et al., 2017). In this case, higher demand for of non-structural carbohydrates is expected in order to recover from the fire damage and to maintain the metabolism even if plants are not under water stress after the fire.

Limited studies have assessed the effects of actual fires (as opposed to the use of fire proxies such as radiant heaters, convection ovens, or water baths) on the living tree hydraulic system. Experiments conducted with species of *Pinus* exposed to fires in the field have found different results in vulnerability to embolism. Battipaglia et al. (2016) showed that *Pinus pinea* trees were not more vulnerable to drought-induced embolism one-month after exposure to a prescribed fire. While in another study, it was found that *Pinus Sylvestris* was more vulnerable to drought-induced embolism 12-months post-fire (Bär et al., 2018). As these experiments were conducted at different time scales, one month and 12-months post-fire respectively, it is possible that the effects of fire on the tree hydraulic system differ between immediate short-term 'shock' impacts and extended 'recovery' time periods (>6 months) after the trees have had time to regrow new xylem. Studies have suggested that heat-induced impairment of tree water transport can potentially lead to tree mortality (Michaletz et al., 2012, West et al., 2016, Bär et al., 2018). However, to our knowledge, no prior studies have evaluated the impacts of lethal intensities from actual fires on tree water transport.

In this study, we experimentally evaluated fire effects on the xylem function of well-watered *Pinus ponderosa* saplings. We combined two experiments that allowed us to assess both the short- and long-term impacts of fire. We assessed the short-term (1-day post-fire) impact of fire on hydraulic conductivity in plants exposed to a known lethal fire intensity (Steady et al.,2019). For the long-term evaluation (21-months post-fire), we only used saplings that were able to survive a lower intensity fire treatment. Although *Pinus ponderosa* is fire

resistant (Miller, 2000), small saplings are easily killed by surface fires (Gartner & Thompson, 1972, Battaglia et al., 2009). If the heat generated during fire can cause impairment of the tree hydraulic system, we would expect to observe a substantial impact in saplings exposed to lethal fire intensity, particularly in younger trees that have not developed fire-resistant traits. Thus, we hypothesized that hydraulic conductivity and vulnerability to embolism would be impacted negatively by fire in both the short-and long-term post-fire experiment. In addition, we performed a detailed anatomical analysis to assess the heat impact on xylem water conduits.

Methods and Materials

Plant material

In this study, we combined two experiments conducted with *Pinus ponderosa* saplings. For both experiments, 1-year-old saplings were obtained and grown at the University of Idaho's Center for Forest Nursery and Seedling Research facility in Moscow, ID, USA. A total of 54 and 12 seedlings were potted in 3.8-L pots with Sungro@ Professional Growing mix media beginning of spring 2017 and 2018, respectively, and periodically watered and fertilized. The 12 saplings obtained in 2018 were used in the experiment to evaluate the fire impact on xylem function 1-day post-fire (short-term experiment) and the 54 saplings obtained in 2017 were used to assess the 21-months post-fire treatment impact (long-term experiment).

Fire experiments

In fall 2017, the saplings obtained for the long-term experiment were randomly divided into an unburned control (n = 18) and two burned (n = 18 each) treatments. 18 plants per burned treatment were subjected to two fire intensities at fire radiative energy (FRE) dosage levels of 0.7 and 1.4 MJ m-2. At the time saplings were subjected to the fire, they had a mean root collar diameter (RCD) of 1.43 ± 0.30 cm and mean height of 0.58 ± 0.01 m. Following Smith et al. (2013), a linear regression between FRE and fuel consumption of P. ponderosa needles (kg m-2) was calculated using 10 burned pure fuel beds at ~0% moisture content, where FRE $= 5.74 \times$ fuel consumption. FRE data for this regression was obtained via dual-band thermometry using standard methods (Kremens et al. 2010, Smith et al. 2013). To then generate FRE dosage levels of 0.7 and 1.4 MJ m^{-2}, fuel loads of 0.152 and 0.304 kg m^{-2} of

oven dried P. ponderosa needles were used, respectively. Fuel was oven dried at ~95°-105° C for at least 48 hours to obtain moisture content ~0% (Steady et al., 2019).

Saplings were individually placed in a custom cut concrete board, such that the soil surface of the pot was level with the board, and fuel was evenly spread in a 1 m2 circular area surrounding the saplings. Fuel beds were ignited using ~ 2g of ethanol on the edge of the circular area (see Steady et al., 2019 for details). After each fire treatment, all plants were transported to the greenhouse at the forest nursery facility, kept inside for 17 months, and then moved to outside of the greenhouse in full sun for 4 months until August 2019. At 17 months post-fire, all the plants exposed to the 1.4 MJ m^{-2} dosage level were dead and thus the long-term experiment to assess xylem was only conducted with the unburned and 0.7 MJ m^{-2} FRE dosage level plants.

The 12 saplings obtained in 2018 for the short-term experiment were divided into two fire treatments: an unburned control (n = 6) and burned (n = 6) with a lethal fire intensity of 1.4 MJ m^{-2}. A known lethal fire intensity was chosen to evaluate whether the water conductivity had a major impact in driving post-fire tree mortality. Saplings were subjected to the fire treatment during fall 2018 and followed the same procedure previously described. After exposure to the fire, all saplings were harvested in the tree physiology laboratory at the University of Idaho to evaluate the short-term fire impact on saplings' xylem. RCD and height average of saplings in both experiments were similar (P < 0.05) at the time they were subjected to the fire treatment.

Stem hydraulics

In the laboratory, the unburned and burned saplings used to assess the short-term impact on xylem were watered and bagged with a black plastic bag overnight. Although predawn leaf water potential (Ψleaf; - MPa) was measured the next morning with a pressure chamber (PMS Instruments Co., Albany, Oregon), we recognize that fire-damaged needles may have produced spurious results. Saplings were then cut at the base of the stem and submerged in a perfusion solution containing 0.02M KCl and 0.012M HCl. Foliage was removed under the perfusion solution. The selection of what part of the stem to include to assess for hydraulic function was determined by where the highest stem temperatures were observed. Using an infrared thermometer (Etekcity Co, Anahein, CA) at the bark surface immediately after these

fire treatments occurred, it was determined that the maximum temperature occurred at approximately 9 cm above the soil surface of the pot. To cut the stem segments, we measured 9 cm from the base of the stem, marked that point, and from there the distal ends of the stems were re-cut under water until approximately 14 cm long. A fresh razor blade was used to cut the distal ends in order to remove any potential emboli caused during the stem segment sampling. The bark was not removed on the stem segments for the anatomical analysis in order to avoid physical damage of the first layers of the xylem structure. To perform the hydraulic measurements, about two centimeters of the bark was removed in each distal end of the stem segments.

From the 54 saplings in the long-term experiment, a total of 10 saplings (5 unburned and 5 burned at 0.7 MJ m^{-2}) were used to evaluate the long-term impacts on stem hydraulics. The night before this set of measurements, all saplings were watered at field capacity. The next morning, Ψ_{leaf} was measured, segments of ~30 cm were cut at the base of the stem, wrapped in a wet paper towel and placed inside an airtight bag and immediately transported to the laboratory. Stem segments were debarked and submerged in the perfusion solution. If the stem had a fire scar, it was included in the stem segment, otherwise we followed the same procedure previously described. Fire scars are wounds that are formed due to the heat-induced death of cambial cells at temperatures of 60 °C or higher (Dickinson & Johnson, 2004). In both experiments stem hydraulic measurements were performed in the main stem of the saplings.

After stem segments were re-cut under the perfusion solution, native xylem hydraulic conductivity (kn; kg s^{-1} m^{-1} MPa^{-1}) was measured using a degassed perfusion solution. We followed standard methods (Sperry et al., 1988) to measure stem water conductivity. After, stems were placed in a vacuum chamber with perfusion solution overnight at ~4°C to remove all xylem emboli. The next morning samples were placed in a container with the perfusion solution at room temperature and left for about 1 hour to thermally equilibrate. After equilibration, both stem ends were recut under the perfusion solution and maximum xylem hydraulic conductivity (kmax) was measured. All conductivity measurements were corrected

by the length of each stem segment and native percentage loss of xylem conductivity (nPLC) was calculated using the following equation:

$$nPLC = 100*[1- (k_n/ k_{max})]\qquad(1)$$

After k_{max} was measured, the stems were used to construct vulnerability curves using the centrifugation technique (Alder et al., 1997, Cochard et al., 2013). Stem segments were spun in a centrifuge to induce negative pressures of -0.5 and -1 to -4 (in increments of -1 MPa) in the xylem. After spun at each pressure, stems were submerged in the perfusion solution and the stem ends were recut. Xylem water conductivity (k') was measured at each applied pressure and corrected by the length of each stem. At each given applied pressure, we calculated percentage loss of stem conductivity (PLC) using the following equation:

$$PLC = 100*[1- (k'/ k_{max})]\qquad(2)$$

Xylem microscopy

From each stem segment used to measure hydraulic conductivity in both experiments, we cut small sections of ~5 cm long and placed in a solution of 1:1:1 of glycerol, ethanol, and deionized water until being processed. Stem cross-sections of 40 µm thick were cut with a microtome (America Optical Company, Buffalo, NY, USA), stained with 6% safranin solution for 2 min, and washed with distilled water and ethanol until excess of safranin was removed. Xylem water conduits, i.e. tracheids, were examined for physical cell wall deformation using an Olympus BX51confocal microscope equipped with an Olympus DP70 digital camera. Conduit cell wall deformation in the long-term experiment stem cross-sections were examined in the growth ring that was potentially exposed to the heat or where fire scars were observed. In the stems that had fire scars, only observed in long-term experiment, we also examined the xylem formed post-fire in the edge of the wound. In addition to staining with safranin, cross-sections with a fire scar were also stained with alcian blue to better observe which type of cells comprise the healing tissue in the edge of the fire scar.

Statistical analyses

All the statistical analyses for this study were performed using R version 3.6.0. We performed a t-test to determine whether unburned and burned nPLC and kmax were significantly different. A t-test was also conducted to confirm that saplings had similar RCD and height at the time they were subjected to the fire in the short- and long-term experiment. Means and ± SE were reported for Ψleaf, nPLC, kmax, RCD and height. In both experiments, fitplc package in R (Duursma & Choat, 2017) was used to fit the hydraulic vulnerability curves and estimate pressure needed to cause 12 (P_{12}), 25 (P_{25}), 50 (P_{50}), and 88 (P_{88}) PLC in each treatment. 95% confidence intervals were used to compare unburned and burned P_{12}, P_{25}, P_{50} and P_{88} to determine if saplings exposed to fire were more vulnerable to cavitation.

Results

Stem hydraulics

In the short-term experiment, mean Ψ_{leaf} was - 0.54 ± 0.03 MPa in unburned plants. k_{max} was not significantly different (*t*-test, $P = 0.06$) in burned (0.33 ± 0.03 kg s^{-1} m^{-1} MPa^{-1}) than unburned plants (0.246 ± 0.03 kg s^{-1} m^{-1} MPa^{-1}). Mean *n*PLC did not differ significantly (*t*-test, $P = 0.57$) in unburned (10.81 ± 5.38%) and burned (and 7.43± 3.03%) saplings (Fig. 3.1a). Hydraulic vulnerability curve analysis showed that one day after the fire, burned saplings one day after the fire were not more vulnerable to cavitation than unburned plants (Fig. 3.2a). The unburned and burned 95% confidence interval of each P_{12}, P_{25}, P_{50}, and P_{88} overlapped (Table 3.1).

In the long-term experiment, Ψl_{leaf} was similar (t-test, P = 0.57) with mean of -0.38 ± 0.07 MPa in unburned and -0.37 ± 0.04 MPa in burned treatments. Similar to the results found in the short-term experiment, k_{max} was not significantly different (*t*-test, $P = 0.52$) in unburned (0.50 ± 0.09 kg s^{-1} m^{-1} MPa^{-1}) and burned plants (0.431 ± 0.07 kg s^{-1} m^{-1} MPa^{-1}). Mean *n*PLC also did not differ (*t*-test, $P = 0.19$) between unburned (9.54 ± 9.04%) and burned (-4.62 ± 1.13%) treatments (Fig. 3.1b). However, vulnerability curve analysis showed that in the long-term, plants exposed to fire were more vulnerable to embolism than plants not subjected to the fire (Fig. 3.2b). Xylem pressures needed to cause 12, 25, and 50% loss of hydraulic

conductivity were less negative and significantly different than in unburned plants (Table 3.1).

Xylem microscopy

In both experiments, no visible damage was observed in the xylem of burned saplings (Fig. 3). Burned plants' xylem conduits did not show any cell wall damage and the appearance was similar to unburned plants in both experiments in this study. Three (of five) of the sapling stems evaluated 21-months post-fire had vascular cambium damage and formed a fire scar (Fig. 4a, Fig. S1). In the pre-fire xylem (i.e. the xylem present when saplings were subjected to the fire treatments) that had fire scars, a dark brownish coloring was observed (Fig. 3.6). In the microscopic cross-section of Fig. 3.4, we observed irregular xylem tissue formation in the vicinity of the fire scar (Fig. 3.4b). A more detailed analysis of the new xylem formed in the healing tissue showed a formation of traumatic xylem composed of irregular shaped tracheid and parenchymatous cells (Fig. 3.4c,d). In one stem, the xylem adjacent to the fire scar that formed after the fire showed regular tracheids but with deformed cell walls (Fig. 3.8). We also observed that in the pre-fire xylem, where the vascular cambium was damaged, the transverse resin canals were filled with resin. However, in the same cross-section, where the vascular cambium was not damaged, resin was not observed in the ducts (Fig. 3.5a,b).

Discussion and Conclusions

Native xylem embolism in well-watered burned P. ponderosa saplings was not affected by fire. nPLC analysis 1-day after the fire treatment showed no significant difference in hydraulic conductivity between unburned and burned plants subjected to lethal fire intensity (Fig. 3.1a). We know of no studies to date that have investigated the short-term response of water transport using actual fire at a lethal intensity. Our data suggest if an increase in nPLC during the fire through the mechanism previously described (Kavanagh et al., 2010) did occur in our first experiment, saplings were able to repair the hydraulic pathway overnight (Tyree et al.,1999, Nardini et al., 2011). A recent study found that saplings of *Pinus taeda* had 50% chance of mortality when the stems had lost 80% of hydraulic conductivity from drought and that most of the plants that died had more than 90 PLC (Hammond et al., 2019). Thus, we conclude that a catastrophic embolism generated during the fire would be needed to

cause conifer tree mortality through hydraulic failure, far greater than the ~11 PLC we observed one-day post-fire.

Hydraulic vulnerability analysis of the plants burned with a lethal fire intensity and harvested 1-day post-fire showed that stem vulnerability to embolism was not impacted (Fig. 3.2a, Table 3.1). For example, the value of water potential needed to induce 50% loss of xylem conductivity (P50) was not different in unburned and burned saplings (Table 3.1). A prior study observed similar results in live mature Pinus pinea stems 1-month after a prescribed fire (Battipaglia et al., 2016). Although we only assessed the short-term effect on vulnerability at a lethal fire intensity, we contend that any fire intensity lower than 1.4 MJ m^{-2} would be unlikely to cause greater hydraulic damage and should produce the same result we observed. However, vulnerability analyses of living plants 21-months post-fire revealed that saplings exposed to fire are less resistant to embolism. This agrees with a prior study that found similar results in branches of *Pinus sylvestris* one-year after a forest fire (Bär et al., 2018).

The decrease in resistance to embolism in trees exposed to fire has been attributed to heat-induced damage to the xylem conduits (Michaletz et al., 2014, West et al., 2016, Bär et al., 2018). Our analysis of xylem in both experiments failed to provide any evidence of conduit wall deformation (Fig.3.3). In contrast to our results, a recent study found that xylem wall conduits were deformed in branch segments of *Pinus sylvestris* subjected to water bath at 90 ºC for 1 hour (Bär et al., 2018). Other studies also reported deformation of xylem cell walls in branches of angiosperm species subjected to water bath treatments (Michaletz et al., 2014, West et al., 2016). One explanation is that using a water bath to simulate the effects of fire on trees, and/or the time of exposure used in these experiments, overestimates the quantity of heat on the xylem that would otherwise be observed in fires. Similar to our results, no post-fire conduit damage was observed in stems of *Pinus pinea* during a fire in the field (Battipaglia et al., 2016). We expected to observe conduit deformation in the vicinity of fire scars in the xylem that grew before the fire. The thermal softening of lignin and hemicellulose (polymers present in the cells walls) can occur at temperatures as low as 54 ℃ depending on their moisture content (Goring, 1965). Thus, it is likely that the xylem in the vicinity of the fire scars in our plants were exposed to temperatures of 60 ℃ or higher.

However, we did not find any deformed conduits in xylem that formed before the fire. Although our data support the hypothesis that at extended time periods after fire, the burned plants may be more vulnerable to cavitation, they do not support the hypothesis that this is caused by conduit deformation at the time of the fire.

A significant proportion of the xylem water conduits have to be damaged to cause levels of embolism that lead to mortality, particularly in well-watered plants. A prior drought study found that *Pinus taeda* which experienced more than 80 PLC ultimately died, and showed this response related to the amount of functional and non-functional xylem (Hammond et al., 2019). If a similar proportion of xylem has to be damaged in plants exposed to fire to cause mortality through hydraulic failure, it is likely that a long-term exposure to fire is needed to soften the conduit cell walls (Chatziefstratiou et al., 2013). Given we did not find conduit cell wall deformation in saplings exposed to lethal fire intensity dosages (Fig. 3 short-term/burned) other physiological mechanisms may be responsible for explaining the sapling mortality. Furthermore, if eventually a critical proportion threshold of xylem conduit deformation is reached, it is also likely that phloem and vascular cambium are already damaged because the heat would have to pass though both the phloem and cambium to reach the xylem. Thus, an alternative mortality mechanism could be damage to the phloem and cambium cells that causes dysfunction in carbohydrate transport and/or depletion of reserves (Sevanto et al., 2014). Prior studies have shown that similar aged saplings exposed to lethal fire intensities exhibited high foliage damage (Smith et al. 2017, Steady et al., 2019), which suggests that damage to meristematic tissues may also be responsible for sapling mortality.

An alternative explanation for the post-fire increase in vulnerability to cavitation observed in the long-term experiment is the traumatic xylem formed after the fire in the vicinity of the fire scar. Note that similar to our results, hydraulic conductivity was not affected in *Pinus pinea* one-month after a prescribed fire (Battipaglia et al., 2016) but an effect was observed in *Pinus sylvestris* one-year after a forest fire (Bär et al., 2018). According to other previous studies, cell wall deformation is expected in the xylem conduits present during the fire (Michaletz, 2012, West et al., 2016, Bär et al., 2018) not in the xylem formed post-fire. Our anatomical analysis on the fire wound xylem showed that the newly formed xylem at the wound edges was disorganized and composed of irregular tracheid shapes and

parenchymatous cells (Fig. 3.4c,d). Other studies with conifers also found irregular shaped tracheids in the traumatic xylem tissue formed in in the wound vicinity (Kuroda & Shimaji, 1984, Zajączkowska, 2014, Chano et al., 2015). Changes in the xylem formed after fires in the vicinity of wounds, such as smaller conduits, that potentially can cause decreases in plant hydraulic conductivity, which has been reported for different conifer trees (Arbellay et al., 2014). However, these changes are restricted to regions adjacent to the fire scar (Mundo et al., 2019), similar to what we also observed (Fig. 3.9). It is also important to note that in a study by Bär et al. (2018), the decrease in resistance to cavitation was observed in damaged compared with undamaged branches from the same individual tree and not from using an unburned control. It is likely that the 'undamaged' branches were still exposed to heat during the forest fire, but the amount of heat that reached those branches was not enough to cause cambial cell damage. Therefore, given the lack of definitive evidence of xylem conduit wall deformation in plants exposed to flames in real fires, we suggest that the malformed traumatic xylem tissue contributes to increased vulnerability to drought-induced embolism and that this is only possible if vascular cambium cells are damaged during the fire.

The possibility of pre-fire xylem contributions to decreased water conductivity and resistance to embolism remains unresolved. Our results also showed a slightly but not substantial k_{max} decrease in burned plants 21-months post-fire. This small difference in stem k_{max} may be also related to the nonconductive pre-fire xylem. The staining of an extra sapling with a fire scar from the long-term experiment showed that the pre-fire xylem around the fire scar was not conducting water (Fig. 3.10). The pre-fire xylem of the saplings that had the fire scars showed a dark coloration in the vicinity of the fire scars (Fig. 3.6). This dark coloration may be caused by deposition of resin in the pre-fire xylem. Because we did not observe any damage in the water conduits in the fire scar vicinity (Fig. 3.3 burned/long-term), we suggest that the clogging of xylem conduits and/or pits with resin can explain the dysfunction of the pre-fire xylem.

Clogging of xylem conduits and/or pits with resin can happen through normal resin canal rupture or pitted epithelial cells in the traumatic resin canals. The epithelial cells are responsible for synthesis and excretion of resin in the resin canals. The production of resin can be increased by wounding (Kolowski & Kramer, 2007) and more resin can be excreted in

the resin canal. We observed that in the region where cambial cells death occurs, resin canals were filled with resin (Fig. 3.5b). When resin canals are filled with resin, the epithelial cells exert a pressure in the resin making the resin flow (Büsgen & Münch, 1929, Cabrita, 2018). However, these canals can rupture and resin can flow out of the canals. Particularly in trees in the genus *Pinus*, resin canals can be part of the fusiform rays (Panshin & Zeeuw, 1980, Biermann, 1996) (see Fig. 3.11). Thus, it is likely when the resin canals rupture that the resin can be forced out into the ray parenchyma cells. Once inside the ray, resin can move into the tracheid though the pits that connect these two structures (Phillips, 1948) (Fig. 3.11). If eventually the tracheid that contains resin embolizes, resin is exposed to the air and can crystalize, clogging the tracheid pits and preventing the movement of water. Another pathway of resin flow into the ray is with formation of traumatic resin canals. Different than the normal resin canals, traumatic ducts have pitted epithelial cells (Panshin & Zeeuw, 1980) and this could facilitate the flow of resin inside the ray with no need of resin canal ruptures. However, to confirm this hypothesis, studies evaluating the pathway of resin into the water conduits and the potential crystallization of resin clogging the xylem conduits/pits are needed.

Our findings provide considerable improvements in the understanding of both the short- and long-term hydraulic responses in conifer saplings exposed to fire. The lack of substantial impacts on water conductivity (nPLC) after the fire, particularly in saplings subjected to a lethal fire intensity, suggests that hydraulic failure is not the main physiological driver of mortality in the near-term for conifer saplings exposed to fire. These results provide the first support to the remarks in a recent Tansley review that hypothesized that conifers may exhibit resistance to heat-induced cell wall deformation (Bär et al., 2019). We acknowledge that in non-conifer species, the results may be different, but we contend that research using lethal fire intensities from actual fires is needed to definitively confirm past studies using non-fire proxies of heat. We postulate that it is more likely that other physiological mechanisms, such as carbon depletion and/or destruction of meristematic tissues may be the primary mechanism for fire-induced tree mortality. Although fire did impact the hydraulics of the surviving saplings, it was not caused by thermal softening of conduit cell walls. Indeed, most studies that suggest vulnerability to cavitation is due to conduit cell wall deformation lack results from actual fires. On the other hand, we hypothesize that increases in vulnerability to

cavitation may occur because of the irregular xylem growth in the vicinity of fire scars. In addition, the pre-fire xylem dysfunction can also contribute to decrease in water conductivity and vulnerability to cavitation by clogging the conduit and pits with resin.

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Figure 3.1. Native percentage loss of hydraulic conductivity (*n*PLC) in unburned and burned *Pinus ponderosa* (a) one-day and (b) 21 months post-fire. Diamond point indicates mean values. Box plots show the median (horizontal line), first and third quartile (box edges), 1.5×interquartile (whiskers), and outliers (points).
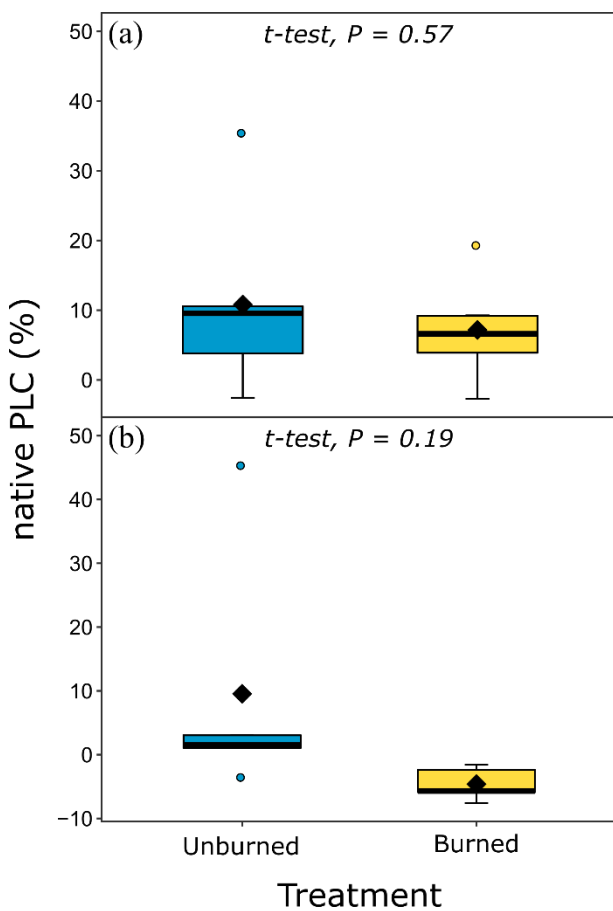
Figure 3.2. Percentage loss of stem hydraulic conductivity in *Pinus ponderosa* saplings (a) one-day and (b) 21-months post-fire. P50 was significantly ($P < 0.05$) different 21-months (b) post-fire but similar one-month (a) after plants were exposed to the fire. Filled circles indicate the value of hydraulic conductivity measured in each stem at each xylem pressure. Sample sizes are six in experiment 1 (a) and five in experiment 2 (b) for each treatment.
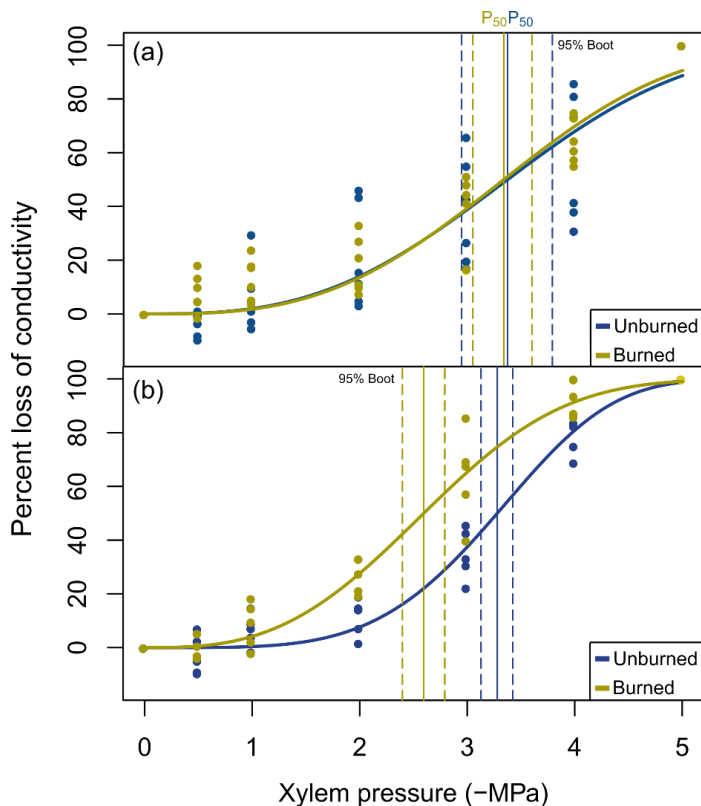
Figure 3.3. Stem xylem water conduits in unburned and burned *Pinus ponderosa* saplings one-day (short-term) and 21-months (long-term) post-fire. Asterisk indicates the phloem tissue and arrows the xylem conduits. The picture in the lower row in the burned treatment shows the xylem conduits where cambium cells were killed by heat. Bars, 20 µm.
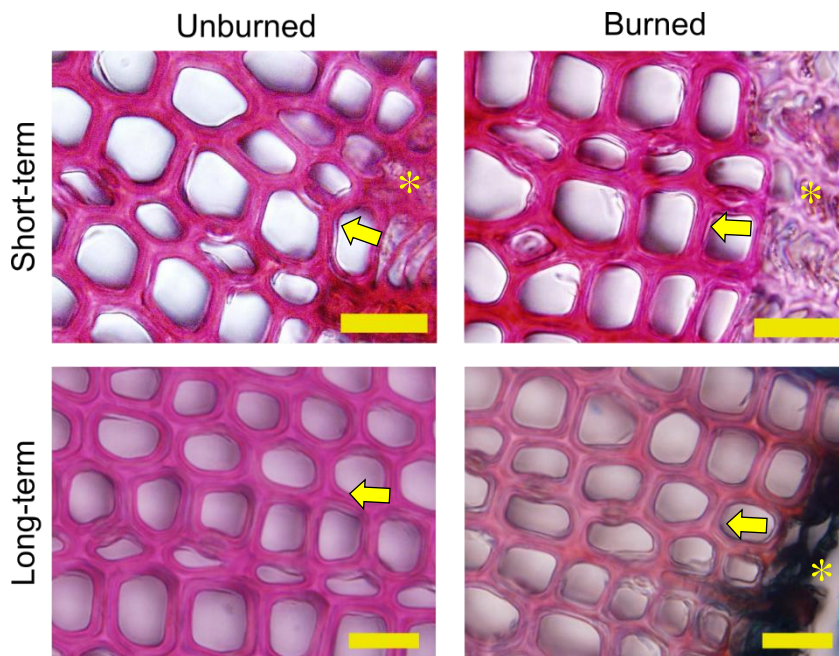
Figure 3.4. Stem cross-section of burned *Pinus ponderosa* saplings. (a) Stem cross-section show the fire scar (arrows) and dark brownish coloring that indicates the deposition of resin in the fire scar vicinity in the pre-fire xylem. (b) Anatomical cross-section of (a) generated with combined images of magnification x4 of the entire cross-section. Arrows indicate where vascular cambium was heat injured. (c) Stem cross-section stained with safranin and alcian blue shows the newly traumatic xylem tissue (asterisk) formed after the fire to close the wound. Fire scar is indicated by arrow. (d) Traumatic xylem tissue with irregular shaped tracheid (arrows) and parenchymatous cells (arrowhead). Bars, 200 µm.
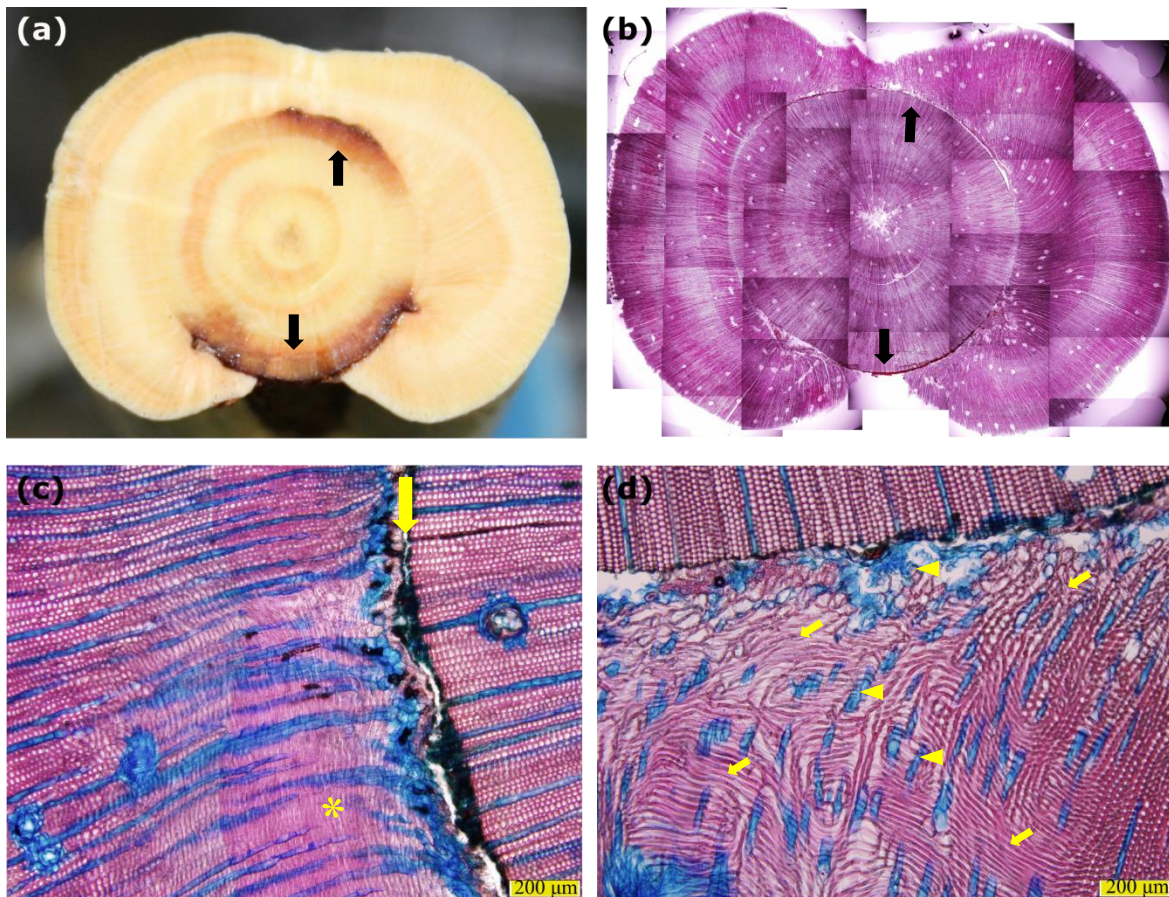
Figure 3.5. Traverse and longitudinal resin canals in burned *Pinus ponderosa* sapling. Resin canals in the xylem, near where vascular cambium cells death did not occur appear empty (a) while in areas near where cambial cells death occurred, the resin canals are filled with resin (b). Traverse and longitudinal resin canals are indicated by arrowhead. Dashed line indicates the position of cambial cells when plant was subjected to the fire (a). Xylem formed after the fire (a) is indicated by XF and fire scar (b) by arrow. Bars, 200 µm.

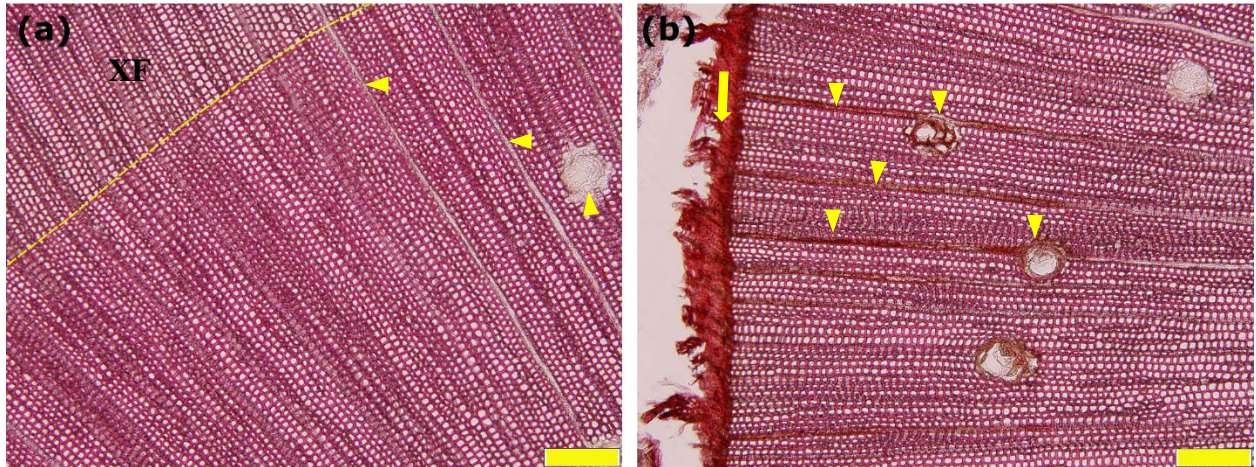**Figure 3.6.** Stem cross-sections of *Pinus ponderosa* saplings with fire scars 21-months post-fire. Fire scar and the dark brownish coloring potentially caused by deposition of resin in the pre-fire xylem are indicated by arrows.
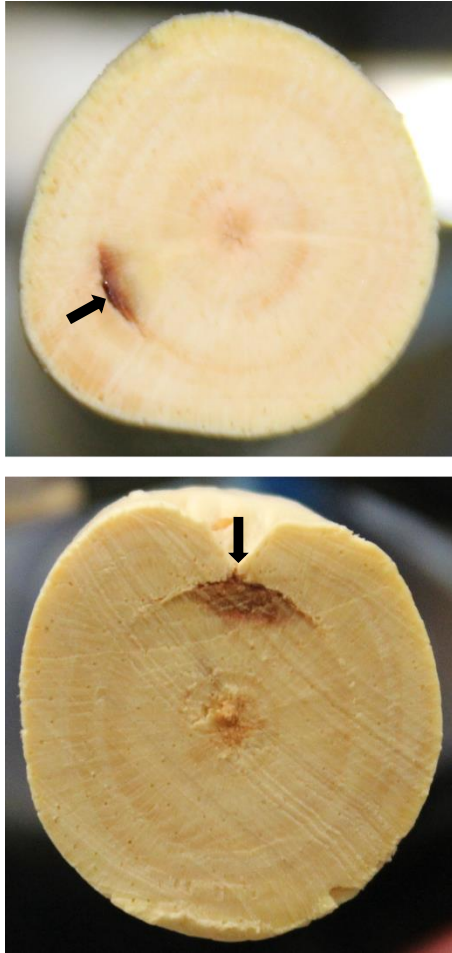
Figure 3.7. Healing vascular tissue in *Pinus ponderosa* saplings. Stem cross-section stained with safranin and alcian blue shows the pre-fire xylem (XP) with arranged and regular tracheids. After the thermal cambium cells death a fire scar formed (black arrow). The xylem post-fire (XF) is composed of parenchimatous cells (arrowhead) and irregular shaped tracheids (yellow arrows).
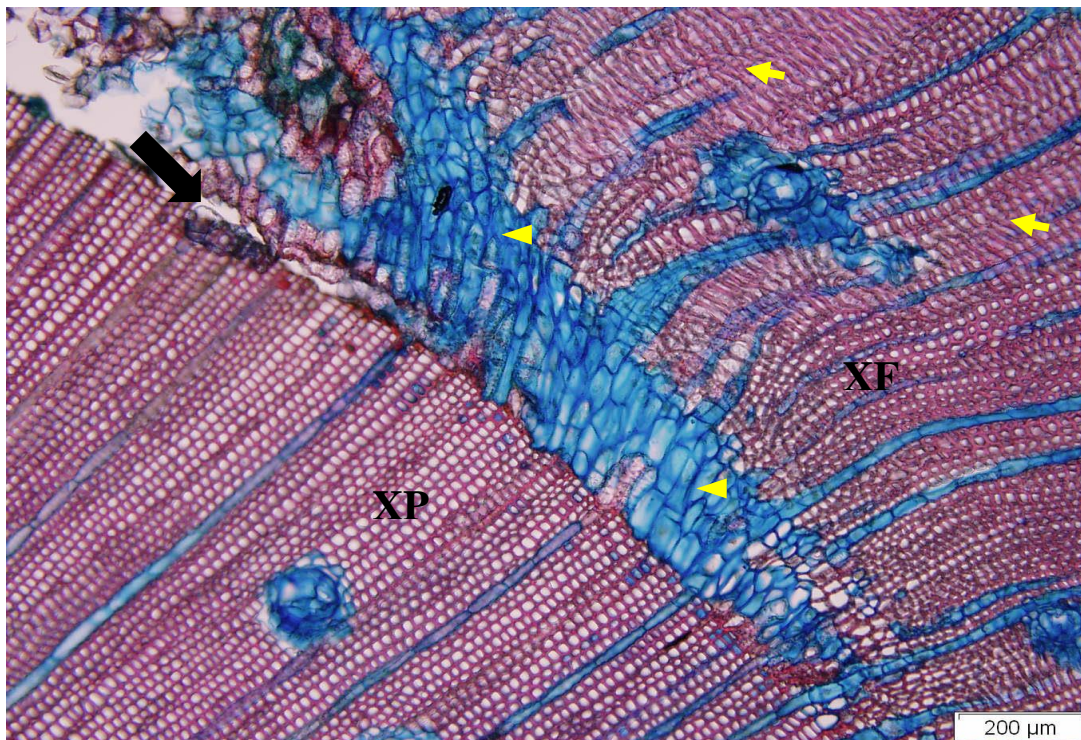
Figure 3.8. Traumatic xylem tissue adjacent to the fire scar in *Pinus ponderosa* saplings 21-months post-fire. (a) Xylem pre- (XP) and post-fire (XF) adjacent to the fire scar (FS). XP shows intact tracheids while XF tracheids have deformed cell walls. (b) x100 magnification of fig a showing the intact tracheid cell wall in XP (arrow) and deformed tracheid cell walls in XF (arrowhead).
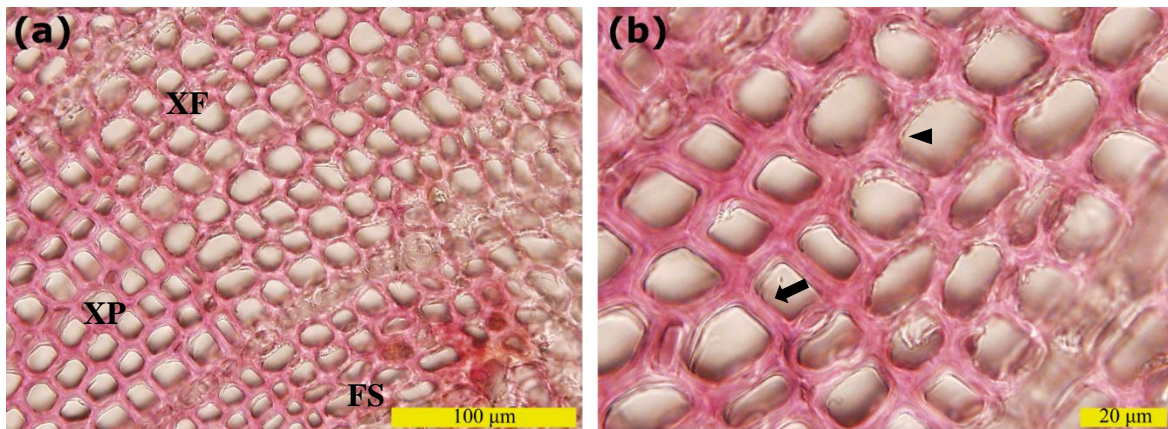
Figure 3.9. Traumatic xylem tissues restricted to region adjacent to fire wound. Dashed yellow line shows when regular tracheids started being formed. Traumatic xylem (TX) is confined around the fire scar (arrow). Arrows indicates when xylem tissue started forming regular tracheids.
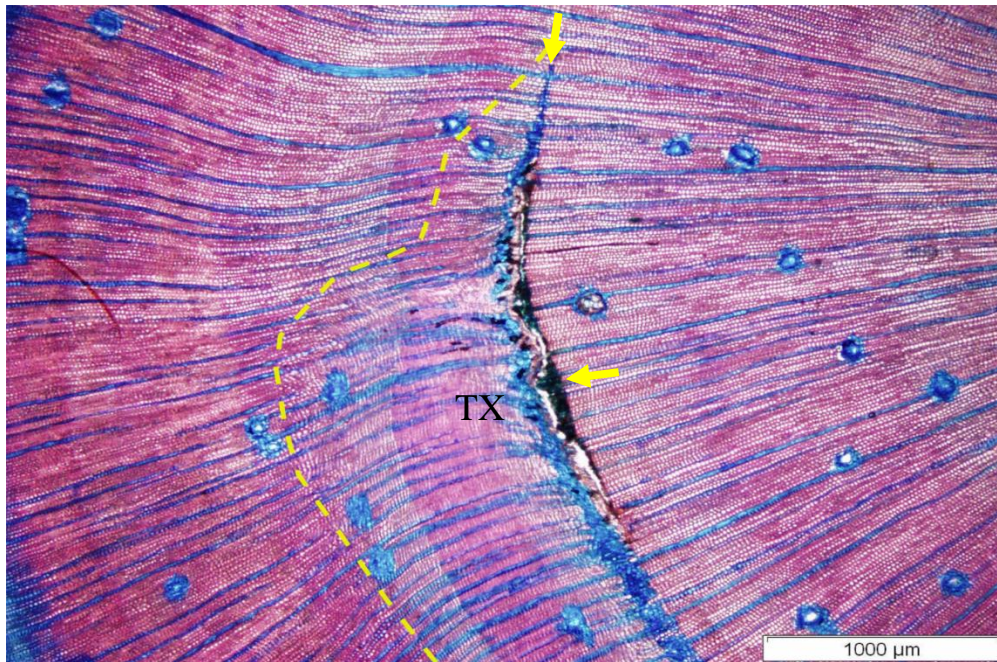
Figure 3.10. Active xylem in unburned and burned *Pinus ponderosa* saplings 21-months post-fire. (a-b) Active xylem is indicated by blue stained with 0.1% methalyne blue. (b) The pre-fire xylem in the vicinity of the fire wound was not stained. Fire scar is indicated by dashed line.
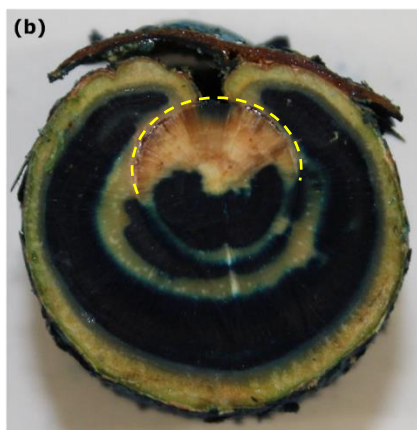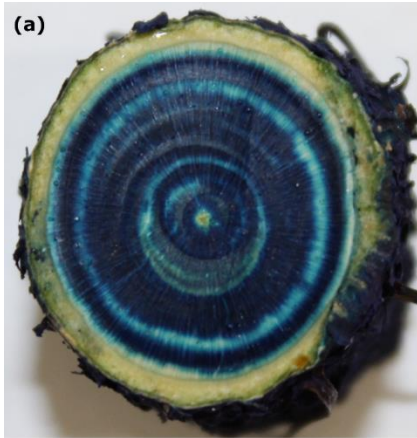
Figure 3.11. Fusiform ray and transverse resin canal (RC) in *Pinus ponderosa* sapling. Cross-section shows the pits (arrow) that connect ray parenchyma cells (RP) and tracheids (TR).
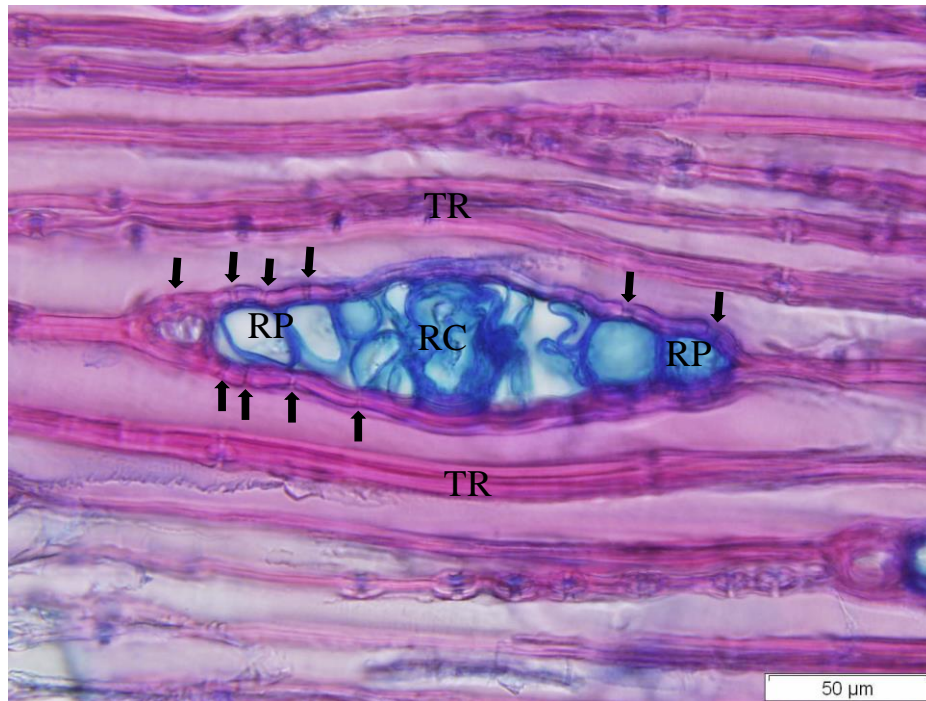
Table 3.1. Predawn water potential resulting in 12, 25, 50, 88% loss of stem conductivity (P_{12}, P_{25}, P_{50}, P_{88}; MPa) in *Pinus ponderosa* saplings one-day (short-term) and 21-months (long-term) post-fire. Reported values are means and units are MPa. 95% confidence intervals are giving between parentheses. Significant differences between unburned and burned means are indicated with asterisks. Upper Confidence intervals for P_{88} were not available when these values fell outside of the data range.

|		Treatments	
Experiment	Water potential	Unburned	Burned
	P_{12}	-1.88 (-1.33, -2.57)	-1.91 (-1.42, -2.39)
Short-term	P_{25}	-2.49 (-1.99, -3.09)	-2.50 (-2.05, -2.90)
	P_{50}	-3.37 (-2.97, -3.81)	-3.34 (-3.04, -3.58)
	P_{88}	-4.95 (-4.41, NA)	-4.82 (-4.55, NA)
Long-term	P_{12}	-2.23 (-1.95, -2.53)	-1.46 (-1.25, -1.69)*
	P_{25}	-2.68 (-2.46, -2.90)	-1.92 (-1.72, -2.16)*
	P_{50}	-3.28 (-3.11, -3.42)	-2.60 (-2.38, -2.81)*
	P_{88}	-4.23 (-3.93, -4.51)	-3.80 (-3.36, -4.12)

Chapter 4: Ponderosa pine hydraulic conductivity and non-structural carbohydrates response to a lethal fire intensity

Abstract

The vascular system of trees can be damaged during forest fires and lead to tree mortality through disruption of water transport and carbohydrate depletion. Although it is suggested that these physiological mechanisms can drive post-fire tree mortality very little is known about their contributions to tree death. Using *Pinus ponderosa* saplings exposed to a lethal fire intensity, we evaluated the impact of fire on non-structural carbohydrates (NSC) and water transport over time. Hydraulic conductivity, and soluble sugars and starch concentrations in leaves, stem and roots were monitored for 28 days post-fire. Soluble sugars and starch were significantly lower post-fire in stems and roots of burned plants as compared with control plants. Photosynthesis was significantly reduced all the days when compared to control plants. Hydraulic conductivity in burned plants after fire was not different from control and plants measured at the same time. Fire caused a significant decline in whole-plant NSC in burned plants compared with control. Our results suggest that tree mortality from fire is likely not primarily driven hydraulic failure in the xylem but appears strongly related to carbon assimilation and storage. Although we demonstrated that fire caused depletion of NSC, more studies are needed to elucidate the role of carbohydrates in post-fire tree mortality.

Introduction

After fires, trees are left with many injuries that can potentially lead to mortality. Non-structural carbohydrate (NSC) storage can play an important role in post-fire tree survival. During a fire, tree tissues directly related to carbohydrate production (leaves) and transport (phloem) can be damaged leading trees to rely partially or completely on their carbon reserves to recover following a fire event. Phloem is the vascular tissue responsible for the transport of photosynthates produced in the leaves to the roots. When phloem is damaged around the entire bole, the transport of photosynthates from the leaves to the roots is disrupted (Rademacher et al. 2019). However, what dictates the permanent disruption of

carbohydrate transport is the damaged caused in the vascular cambium cells that are responsible to produce new phloem. Thus, if during the fire the vascular cambium is not completely damaged, new phloem could be regenerated and the transport of carbohydrates restored. Disruption of photosynthate transport caused by phloem and cambium cell death during fire contribute to tree mortality (Ryan & Frandsen, 1991). Some studies have shown the influence of partial and complete vascular cambium damage in conifer tree mortality (Ducrey et al., 1996; Ryan, 2000). However, trees can still survive for a period after complete cambium and phloem damage (Noel, 1970; Ryan, 2000) using carbohydrates stored in their roots (Mei et al. 2015). However, after depletion, the roots die and water and nutrient transport to the rest of the plant ceases leading to tree mortality.

Reduction in carbohydrates reserves could also occur following and lead to post-fire tree mortality when the source of photosynthate (leaves) is damaged. NSC reserves are reduced when photosynthesis is halted for a long period (Li et al. 2018, Weber et al. 2018). During fire, crown consumption and scorch damages leaves and reduces or halts carbohydrate production. Studies have shown that the probability of tree mortality is higher with increase in volume of canopy damaged by fire (Peterson & Ryan, 1986; Harrington, 1993). Smith et al. (2017) found that conifer saplings exposed to a high fire "dose" had significant decline in photosynthesis with 100% mortality. Similar results were also found when increase in canopy scorch reduced photosynthesis in conifer saplings (Sparks et al. 2016). Although these studies evaluated the tree physiological responses that are related to carbohydrate production, i.e. photosynthesis, there is no direct evidence of fire effects on carbohydrate storage. However, it is known that depletion of NSC can occur in defoliated plants (Gregory and Wargo 1985, Wiley 2013).

It has been proposed that water transport can be compromised in trees exposed to the fire. Fire-induced embolism may occur during the fire due to the increase in vapor pressure deficit (VPD) around the foliage caused by the heat plume (Kavanagh et al. 2010). It is assumed that because of the high VPD during fire, the water column tension in the xylem increases causing embolism of the xylem conduits through air seeding (Sperry and Tyree 1990, Delzon et al. 2010). This is potentially more harmful for plants under water stress (Feltrin et al. in prep, see Chapter 2). It has also been suggested that xylem water conductivity is negatively

affected through the heat-induced deformation of xylem cells wall (Michaletz et al. 2012). Other studies have shown that trees exposed to heat simulating fire can be more vulnerable to drought due to the heat damage caused in the xylem conduits (West et al. 2016, Bär et al. 2018). Decreases in water conductivity can decrease photosynthesis (Hubbard et al. 2001) that consequently it can induce depletion of carbohydrates reserves (McDowell 2011).

With the predictions of more severe drought that favors the occurrence of wildfires because of climate change (IPCC 2014), understanding tree physiological responses to fire such as altered water transport and carbohydrate production and utilization is crucial for better predictions of post-fire tree mortality under future climate change scenarios. Our goal was to evaluate the impact of a lethal fire intensity on NSC and water transport over time. We hypothesized that 1) *Pinus ponderosa* sapling hydraulic conductivity decreases after exposure to lethal fire; 2) saplings exposed to the fire would have lower carbohydrate concentration over time compared to plants not exposed to the fire. In addition, we tested the hypothesis that saplings exposed to the fire would have lower carbohydrate concentration compared to defoliated plants. We expected that burned saplings would have lower NSC than defoliated plants due to the fire damage caused in multiple plant tissues while defoliated plants had only canopy loss.

Material and Methods

Plant material

In April 2018, 100 two-year-old *Pinus ponderosa* saplings potted in 3.8 L pots with Sungro[@] forestry mix number 3 and grown outside for one year were purchased. Then saplings were kept for five months in a climate-controlled greenhouse at University of Idaho with maximum and minimum temperatures of 25 and 15 °C, respectively. Saplings were watered every other day and fertilized once every month with 20:20:20 Technigro[@] fertilizer. In summer 2018 when saplings were subjected to the treatments, they had root collar diameter mean ($\pm$ SE) of 1.28 ± 0.06 cm and height of 0.55 ± 0.03 m. To assess the effects of fire on xylem hydraulic conductivity, we used a 2x3 factorial design. 30 saplings were randomly divided in to two treatment groups – control and burned, and 6, 13, and 27 days post-fire. A 3x3 factor design was used to evaluate the effects of fire on NSC. 50 saplings were randomly divided in three treatments – control, burned, and defoliated – and 7, 14, and 28 days post-

fire. In addition 5 saplings were measured for hydraulic conductivity and harvested for NSC one day before fire treatment to assess pre-fire physiological status.

Treatments

Fire treatments were conducted under controlled laboratory conditions at the University of Idaho Fire Initiative for Research and Education laboratory, Moscow, ID. Well-watered saplings were subjected to surface fires with a fire radiative energy (FRE) of 1.4 MJ m^{-2}. A previous study found that FRE of 1.4 MJ m^{-2} is lethal for *Pinus ponderosa* saplings (Steady et al. 2019). FRE was calculated using linear regression between FRE and consumption of *P. ponderosa* needles (kg m^{-2}) acquired from 10 burned fuelbeds, where FRE = 5.74 × fuel consumption (Smith et al. 2013, Steady et al. 2019). Consequently, a fuel load of 0.304 kg m^{-2} of *P. ponderosa* needles at ~0% moisture content was used. Needles were oven dried at ~100°C for at least 48 hours to obtain a moisture content ~0% (Smith et al. 2013, Steady et al. 2019, Matthews 2010). Saplings were individually placed in a custom cut concrete board, such that the soil surface of the sapling pot was level with the board, and dry needles were evenly spread in a 1 m^2 circular area surrounding the sapling. After the burn, all saplings were returned to the greenhouse and watered every other day. On the same day plants were exposed to the fire, 100% of the needles in saplings placed in the defoliation treatment were removed. Removal of needles did not cause any damage to the apical bud. Saplings placed in the control treatment were not burned.

Hydraulic conductivity

Xylem hydraulic conductivity (*k*) was only performed in control and burned saplings. *k* was measured in the saplings' main stem. In the morning prior to *k* measurements, predawn leaf water potential (Ψ_p; - MPa) was measured using a pressure chamber (PMS Instruments Co., Albany, Oregon). Due to heat-induced damage to the burned sapling needles, there is a potential risk of measurement error and thus only values for the control plants are reported. Stem segments ~20 cm in length were excised from each sapling and immersed in deionized water at ambient temperature (22° C). Xylem hydraulic conductivity (*k,* kg m^{-2} MPa^{-1} s^{-1}) was measured using a Sperry apparatus and using degassed deionized water (Sperry et al. 1988). Prior to the measurements, the pressure head reservoir of water was connected to a

vacuum pump for 48 hours for de-gassing. Native conductivity (k_n) was measured just after stem segments were excised. After k_n was measured, stems were placed in a vacuum chamber with deionized water overnight to clear any air emboli. The following morning, stems were placed in another container with deionized water at ambient temperature, both ends were shaved using a fresh razor blade, and maximum hydraulic conductivity (k_{max}) was measured. All hydraulic conductivity was corrected by the length of each stem segment and native xylem conductivity (nPLC) was calculated using the following equation:

$$nPLC= 100[1 - \left(\frac{kn}{kmax}\right)] \tag{1}$$

Non-structural carbohydrates analysis and photosynthesis

Predawn and midday leaf water potential (Ψ) and photosynthesis (A) were measured one-day before saplings were harvested for NSC sampling. Photosynthesis measurements were performed with a LICOR 6400XT (LI-COR, Lincoln, NE) with constant photosynthetic flux of 1400 µmol m^{-2} s^{-1} and CO_2 of 400 ppm.

Non-structural carbohydrates (soluble sugars and starch) were analyzed in the needles, stem (including the bark) and roots. Each organ was harvested and microwaved to stop the enzymatic process, placed in paper bags and oven dried at 90°C for 2 days. After, samples were stored inside of an oven at 30 °C until being ground in a fine powder. NSC analysis was conducted in the Environmental Ecology Laboratory at Oklahoma State University. To determine the sugar and starch concentration 5 g of plant material of each organ was used. NSC analysis was conducted with alcohol extraction following the sulfuric acid method described in Landhäusser et al. (2018). Sugar and starch concentration were calculated by multiplying the concentration (%) by the dry mass of each organ. The whole-plant NSC concentration was the sum of sugar and starch concentration in all organs, divided by total plant mass.

Statistical analysis and calculations

The statistical significance between treatments at each day and in the same treatment overtime of leaf predawn and midday water potential, nPLC, sugar and starch concentration in each plant organ, and whole-plant NSC were performed using a pairwise comparison. Statistical analysis was conducted using the software R version 3.6.0 and the package emmeans. Statistical analysis was conducted to compare total NSC between the treatments at each day including the leaves and without the leaves NSC concentration. Some values of the dry mass were missing so a linear regression was used to estimate the values of the organs that it was missing.

<div align="center">

Results

</div>

A was significantly reduced in burned treatment compare to control at all days post-fire (Fig 4.1). Although photosynthesis was greatly reduced as compared to controls, some burned saplings still had net positive photosynthesis. Native hydraulic conductivity (nPLC) was not significant different between control and burned treatments at day 6- and 27- after the fire treatment. However, at day 13 post-fire control treatment had significantly higher ($P = 0.02$) PLC than burned saplings (Fig. 4.1). Predawn and midday Ψ in control saplings were similar ($P > 0.05$) over time (Fig. 4.1).

Sugar and starch concentration of leaves in the burned treatment was similar to control at each day post-fire (Fig 4.2). Starch concentration of stem decreased significantly in burned and defoliated treatments compared to control treatment at each day with exception of day 14. Sugar concentration of stems were similar between unburned and defoliated treatment but differed significantly from burned treatment at day 7 and 14 post-fire. At day 28, the defoliated treatment had significantly higher sugar concentration of stems than the burned treatment (Fig. 4.2). Starch concentration of roots was similar among all treatments at day 7 post-fire. The defoliated treatment had significantly lower root starch concentration on day 14 compared to control but did not differ from the burned treatment. Control and burned root starch concentration were similar at day 14. Starch and sugar concentration of roots in burned and defoliated treatments were similar and lower than unburned plants at day 28 post-fire (Fig. 4.2). Burned plants had significantly lower root sugar concentration on day 7 compared

to the defoliated treatment but were similar to controls. At day 14, root sugar concentration was only statistically significant in burned treatment compared to control and defoliated.

Analysis of whole-plant NSC without including leaf total NSC showed that burned treatment had significant lower NSC all the days post-fire compared with control. At day 7 and 14 post-fire total NSC was similar in burned and defoliated treatments but significantly different at day 28. Total NSC in control and defoliated treatments were similar all the days post defoliation treatments (Fig 4.3). Whole-plant NSC including lead showed that burned and defoliated plants were only differed from control treatment at day 28 post-fire and defoliation treatments. Burned and defoliated treatments were similar all days (Fig 4.3). The leaf was the sapling organ that contributed the most for the total NSC in control and burned treatments ranging from 56.6 to 74.5% between day 7 and 28 post-fire (Fig 4.4). Stem NSC in control plants ranged from 10.1 to 18% and roots from between 14.4 to 15.5% of the total NSC. Stem in burned treatments contributed between 16.3 and 20.5% while roots contributed with 27.2 % at day 7 and 16.67% at day 28 post-fire. At day 7- and 28-days post-fire, roots contributed to the most to total NSC in defoliated treatments while at day 14 root and stem had almost equal contributions.

Discussion

In the current study, we found that native hydraulic conductivity was not reduced in burned plants. A previous study conducted with conifers also showed that fire had no significant effect on branch conductivity of conifer trees one-year post-fire (Bar et al. 2018). Another recent study conducted with ponderosa pine saplings found that native hydraulic conductivity was not affected even in saplings exposed to a lethal fire intensity (Feltrin, in prep., see Chapter 3). Although several studies have shown declines in hydraulic conductivity after exposing plants to different forms of heat (e.g. water bath; Michaletz et al 2012, West et al. 2016, Bar et al 2018), there is no evidence of declines in conductivity from exposure to flame, even at lethal fire energy doses.

We found that burned plants had lower soluble sugars and starch concentration than control and defoliated saplings except in leaves. Fire did not affect soluble sugars and starch concentration in the leaves at any day compared to the control treatment. Alexou and Dimitrakoupoulos (2014) also found that fire did not have a significant effect in soluble

sugars in the leaves of *Pinus brutia* within a week after the fire treatment. Overall, starch and sugars were lower in stems of burned saplings than either controls or defoliated plants (with a few non-significant but marginal differences. Such differences could be explained by usage of carbohydrates to recover from the damaged cause in the stem. The fact that defoliated saplings had greater amounts of sugar in stems supports this idea. Defoliated

Phloem death due to fire impacts the transport of carbohydrates. Girdling can cause starch accumulation in the organs above (leaves) and depletion below the girdling (root) (Rademacher et al. 2019). Although there was an apparent damage in the phloem in our saplings (Fig. 4.5), it was not observed an accumulation of starch in the leaves (Fig 4.2). Contrary, our results showed that leaf starch slightly decreased overtime (Fig 4.2) even with lower rates of photosynthesis (Fig 4.1). This suggests that it is possible that cambium and phloem was not complete damage or the new photosynthate produced could be used to repair the fire-damage in the canopy but probably was not enough to supply the demand for a complete recovery.

Defoliation and fire caused a decrease in total NSC in *Pinus ponderosa* saplings (Fig 4.3). Considering whole-plant NSC including total NSC in leaves, our defoliated results are consistent with previous study where 100% defoliated plants had lower whole-plant NSC compared with plants not defoliated (Jacquet et al. 2014). We found that fire also reduced the total NSC in *Pinus ponderosa* saplings but less than defoliation (Fig 4.3b). We expected that whole-plant NSC would be less affected by defoliation than fire because of the greater damage that fire can cause in all the plants organ. However, our analysis of whole-plant NSC not including the total NSC in leaves showed total NSC was more affected by fire than defoliation. Also considering that leaves contributed for the most percentage of NSC in burned saplings (Fig 4.4), it is clear that the damaged caused in the stem and roots are significant particularly when compared with defoliated saplings that only had 100% of defoliation with no damage in the root and stem.

In summary, fire had no negative effects on hydraulic conductivity in *Pinus ponderosa* saplings, consistent with prior work suggesting that hydraulic failure is not one of the main mechanisms driving tree mortality after fire. However, our data showed that fire had a significant effect on NSC implying that depletion of carbohydrates can contribute to post-fire

tree mortality. However, more studies are needed to understand what doses of fire cause phloem and vascular cambium damage. Also, experiments are needed where girdled (or carbon-depleted) plants exposed to non-lethal fire intensity are compared to plants burned with lethal fire intensity are needed to elucidate the role of NSC in post-fire tree mortality. We also acknowledge that caution must be made to extrapolate the results found in this study to mature trees, considering the different carbohydrates dynamics in saplings and mature trees (Hartmann et al. 2018).

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Figure 4.1 Mean predawn and midday leaf water potential (Ψ), mean photosynthesis (*A*) and native xylem percentage loss of hydraulic conductivity (*n*PLC) in *Pinus ponderosa* saplings from -1 to 28 days relative to the fire day. The panel a shows the mean predawn and midday Ψ only in control saplings. Figure b and c shows *A* and *n*PLC for control and burned plants. Red dot lines represent the day plants were exposed to fire treatments. Measurements were taken in 5 saplings at day -1 and 5 saplings in each treatment at day 6 – 27 post-fire. Asterisks show the significant difference ($P < 0.05$) between treatments at each day. Bars are SE.
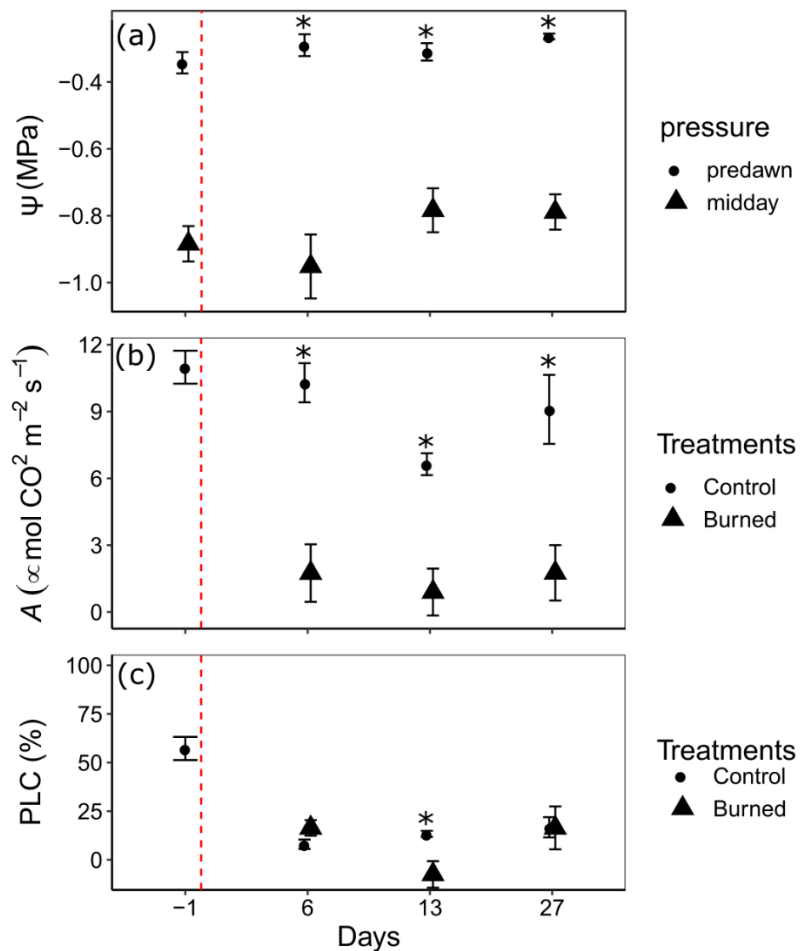
Figure 4.2 Mean leaf, stem and root sugar and starch concentration of dry mass in *Pinus ponderosa* saplings. Measurements were taken in 5 saplings at day -1 and in 5 control and 5 burned plants at day 7 – 28, except at day 7 for defoliated stem and day 28 for control root that was measured in 4 plants. Significant difference between the treatments at each day for sugar and starch for each organ are shown with letters ($P < 0.05$). Bars are SE.
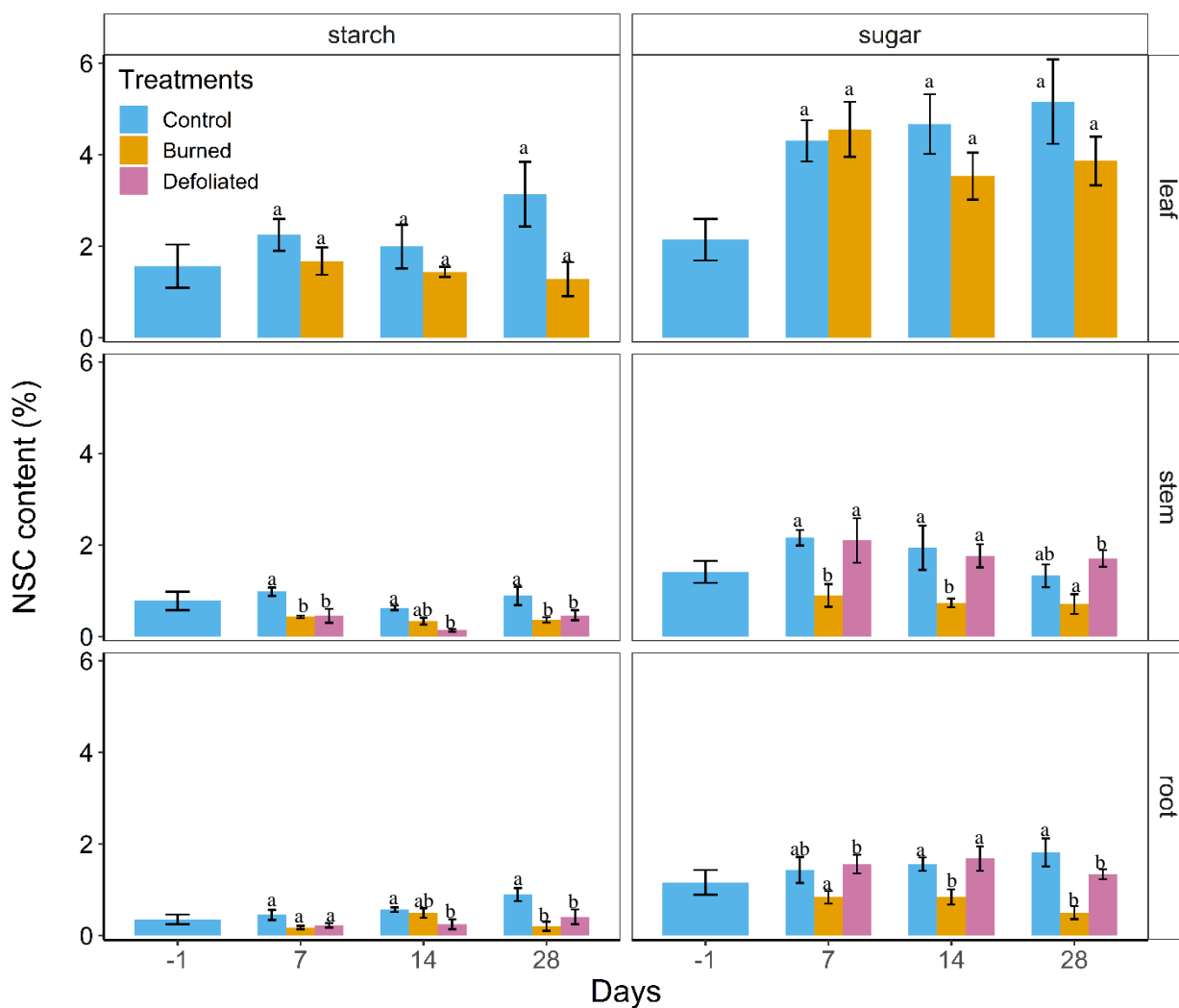
Figure 4.3 Mean total NSC content (%) in whole plants without including total NSC in the leaves (a) and including the leaves (b). Measurements were taken in 5 saplings at day -1, 5 unburned and 5 burned plants at day 7 – 28. Day 7 defoliated and day 28 burned treatment are only 4 saplings. Significant difference between the treatments at each day for total NSC is shown with letters ($P < 0.05$). Bars are SE.
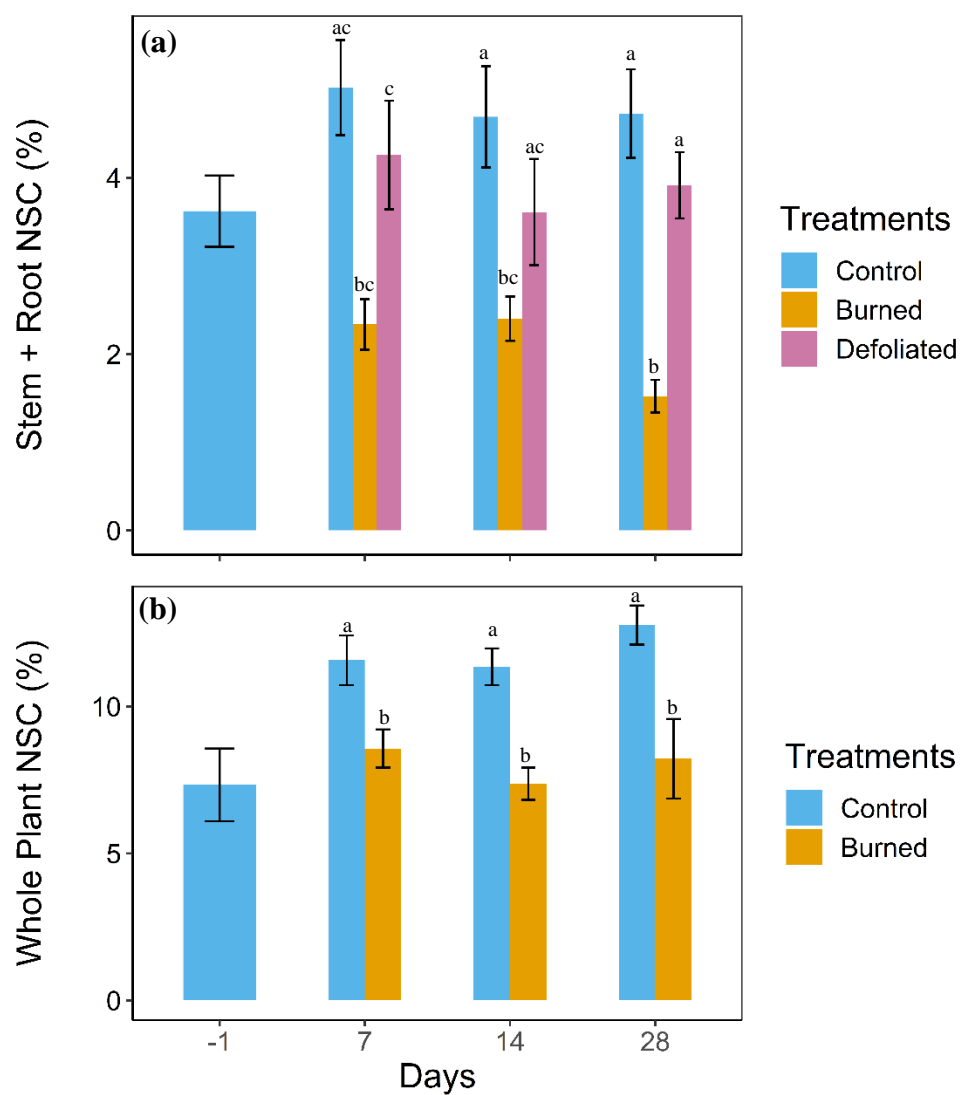
Figure 4.4 Percentage of total non-structure carbohydrate (NSC) in each *Pinus ponderosa* sapling organ related to the total NSC content at day 7, 14, and 28 days post-fire in each treatment. Numbers inside the pie char represents the total NSC in each organ in in percentage.
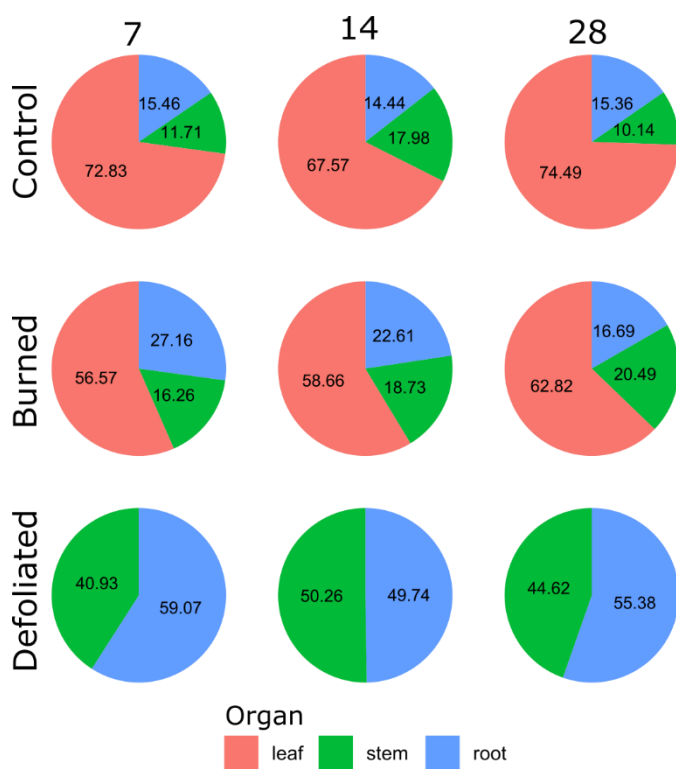
Figure 4.5 Stem cross-sections of control (a) and burned (b) *Ponderosa pine* saplings. Arrows indicate a healthy phloem in the control that shows a greenish coloration (a) and a damaged phloem in the burned plant that has a brownish coloration (b).

a)

b)

Chapter 5: Conclusions

This dissertation aimed to provide better understanding in the impact of fire on tree physiology. The three studies here discussed provided a considerable understanding and a baseline for future studies. In summary, we observed that pre-fire water status did have a significant effect on *Pinus ponderosa* saplings mortality. However, this effect is only observed in a fire intensity threshold. Our results together with Steady et al. (2019) suggests that 18-month old saplings exposed to fire intensities above 0.8 MJ m^{-2} will experience 100% mortality regardless of the pre-fire water-status. Some of the potential fire injuries that could have contributed to sapling mortality in pre-drought stress was related to low foliage and bark moisture that could increase the chances of meristematic tissues necrosis, i.e. buds and vascular cambium. We also found that in a short-term lethal fire intensity did not cause a significant effect on water conductivity. However, in the long-term, saplings that survived after a fire exposure were more vulnerable to drought-induced embolism. Contrary to previous studies (Michaletz et al. 2012, West et al. 2016, Bär et al. 2018), our study did not provide evidence that vulnerability in saplings exposed to fire were caused by xylem conduits deformation. But we hypothesize that the vulnerability to cavitation in *Pinus ponderosa* saplings was related to the new xylem (i.e. traumatic xylem) formed in the edges of the fire scar and pre-fire xylem tracheids clogging with resin in the vicinity of the fire scars. And lastly, our results confirmed that over time (within 27 days post-fire) water conductivity in well-watered *Pinus ponderosa* saplings exposed to a lethal fire intensity was not affected. However, we observed a significant depletion of whole plant non-structural carbohydrates. Particularly in the root reserved in burned saplings. Thus, this implies that depletion of carbohydrates may contribute to post-fire tree mortality.

Our findings highlight that fire can be more harmful for plants under drought-stress even under low fire intensity. These results are particularly important considering the predictions of increase in temperature and more severe droughts (IPCC). With such predictions, our results not only provide valuable understanding how fire would affect plants under drought-stress during the fire, but also how vulnerable to mortality the plants are if drought occurs in

the years following the fire. Although, our results provided valuable understanding how fire affects the physiological responses of *Pinus ponderosa* more studies with different tree species and focused on some responses here suggested, for instance vulnerability caused by the new traumatic tissue, are urgently needed.

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