Using heat plumes to simulate post-fire effects on cambial viability and hydraulic performance in *Sequoia sempervirens* stems

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Running Head

Simulated fire effects on *Sequoia sempervirens*

Keywords

cambial viability, cambium necrosis, fire, neutral red stain, xylem cavitation

Abstract

Injury to the xylem and vascular cambium is proposed to explain mortality following low severity fires. These tissues have been assessed independently, but the relative significance of the xylem and cambium is still uncertain. The goal of this study is to evaluate the xylem dysfunction hypothesis and cambium necrosis hypothesis simultaneously. The hot dry conditions of a low severity fire were simulated in a drying oven, exposing *Sequoia sempervirens* shoots to 70°C and 100°C for 6 – 60 minutes. Cambial viability was measured with Neutral Red stain and water transport capacity was assessed by calculating the loss of hydraulic conductivity. Vulnerability curves were also constructed to determine susceptibility to drought-induced embolism following heat exposure. The vascular cambium died completely at 100°C after only 6 minutes of heat exposure, while cells remained viable at 70°C temperatures for up to 15 minutes. Sixty minutes of exposure to 70°C reduced stem hydraulic conductivity by 40%, while 45 minutes at 100°C caused complete loss of conductivity. The heat treatments dropped hydraulic conductivity irrecoverably but did not significantly impact post-fire vulnerability to embolism. Overall, the damaging effects of high temperature occurred more rapidly in the vascular cambium than xylem following heat exposure. Importantly, the xylem remained functional until the most extreme treatments, far after the vascular cambium has died. Our results suggest that the viability of the vascular cambium may be more critical to post-fire survival than xylem function in *S. sempervirens*. Given the complexity of fire, we recommend ground-truthing the cambial and xylem post-fire response on a diverse range of species.

Keywords

cambial viability, cambium necrosis, fire, neutral red stain, xylem cavitation

Introduction

Global wildfire has recently increased in size and frequency and is expected to escalate into the future (Westerling et al. 2006). Consequently, forest health and resilience are major concerns, whether in response to low and mixed severity fires or to controlled burns after which dominant trees survive. In these circumstances, surviving trees can suffer injuries to various tissues (Hood et al. 2018), leading to death, months or years after fire. Some of the impacts include fine root death (Swezy and Agee 1991), canopy loss (Clarke et al. 2013), and necrosis of the vascular cambium (Dickinson and Johnson 2004). Consequently, these damages can lead to carbon starvation (McDowell 2011) and xylem dysfunction (West et al. 2016, Bär et al. 2018). In particular, the xylem dysfunction hypothesis and cambium necrosis hypothesis have gained considerable interest among physiologists in recent years. Impacts to xylem and cambium have been studied independently and primarily on branches in the lab, rather than in real fires. To better characterize the cause of fire-induced tree mortality, it is important to understand how each of these vital functions suffer in relation to each other due to high temperatures.

Xylem function during fire has received little attention until recently, yet it is essential to our fundamental understanding of how fire affects trees (Kavanagh et al. 2010, Midgley et al. 2011, Michaletz et al. 2012). The post-fire xylem dysfunction hypothesis suggests that trees exposed to non-lethal fire experience xylem damage in two ways. First, fires create high temperature and low humidity conditions, which cause the xylem to lose water (Michaletz and Johnson 2007, Kavanagh et al. 2010, Midgley et al. 2011). Thus, the xylem tension increases, leading to the formation of air vapor emboli (Tyree and Zimmermann 2002, Cochard et al. 2015, Chacon et al. 2020). When air vapor emboli block water flow, the tree canopy experiences water stress and stomatal closure, thus reducing photosynthesis (Tyree and Zimmermann 2002). Embolized xylem will affect tree hydraulic function until the xylem is repaired or new xylem is grown. Second, laboratory studies have shown that heat causes lignin and hemicellulose in the cell walls to soften (Hillis and Rozsa 1985, Irvine 1985, Olsson and Salmén 2004), resulting in permanent warping and damage to the xylem conduits (Michaletz et al. 2012). This is thought to have lasting effects on water transport and subsequent resilience of the xylem to drought (West et al. 2016, Bär et al. 2018).

While much interest lies in post-fire xylem dysfunction, a second hypothesis – the cambium necrosis hypothesis – has long been an accepted mechanism for tree death (Ryan et al. 1988, Dickinson and Johnson 2004, Michaletz and Johnson 2007). Vascular cambium is the tissue responsible for growth in trees by producing xylem towards the interior and phloem towards the bark (Spicer and Groover 2010). The cambium necrosis hypothesis states that heat from fire kills the cambium cells, and the tree is no longer able to grow, either above ground or below ground (Michaletz and Johnson 2007, McDowell et al. 2008). When roots are unable to grow, the capacity for water uptake is reduced (Smirnova et al. 2008), affecting tree water relations and reducing the likelihood of tree recovery and subsequent growth. However, this is a complex process in which cambium necrosis may only partially encompass the circumference of a tree and still result in the tree continuing to survive (Ryan 2000). Importantly, heat damage to the vascular cambium likely also results in phloem injury because phloem is located in closer proximity to the heat than the vascular cambium.

Numerous empirical and modeling studies have identified either xylem damage or cambium death as critical lesions following a fire (Dickinson and Johnson 2004, Battipaglia et al. 2016, West et al. 2016, Bär et al. 2018, Partelli-Feltrin et al. 2021). Fire effects on xylem damage have been done via heat treatments in the lab and have shown heat to cause vulnerable and irreversibly damaged xylem (West et al. 2016, Bär et al. 2018). The original laboratory heat treatment studies simulated fire-induced thermal effects with hot water baths to facilitate the conductive transfer of heat (Michaletz et al. 2012, Bär et al. 2018, Lodge et al. 2018). More recently, other approaches have been used, including using a drying oven (West et al. 2016) and igniting saplings or individual branches (Partelli-Feltrin et al. 2021, Hoffmann et al. 2021). In contrast to these empirical experiments, post-fire cambium viability studies are often done through modeling approaches (Dickinson and Johnson 2004, Dickinson et al. 2004), or using excised cambium tissue (Achchige et al. 2021).

Previous work has examined either cambium necrosis or xylem dysfunction, but critically, the concurrent impact of these mechanisms has not been studied simultaneously. While each hypothesis has been shown to be valid in independent studies, it is important to understand the relative impact of each heat damage mechanism in relation to the other in order to better characterize the impact of heat on living trees. By determining critical thresholds for temperature and duration of heat exposure causing both cambium necrosis and xylem dysfunction, we can better understand the risks of wildfire and prescribed burns across a variety of species.

This study examined the effect of a controlled heat treatment on coast redwood (*Sequoia sempervirens*). We chose *S. sempervirens* because fires have recently impacted redwood forests (Mahdizadeh and Russell 2021), and redwoods often resprout from the main bole after a fire. Furthermore, climate change threatens to increase fire frequency in coastal California (Bedia et al. 2014, Turco et al. 2014), making it important to understand how this species responds to fire. Moreover, redwoods grow in the Mediterranean climate, which is characterized by mild wet winters and warm dry summers. Dry summers in combination with severe drought in California (Swain 2021) could make redwoods susceptible to drought-induced embolism after being weakened by fire, similar to the species reported by Bär et al. (2018).

We used a drying oven to heat foliated branches at 70°C and 100°C, with treatments lasting from 6 minutes to 60 minutes. Segments of each branch were used to simultaneously measure hydraulic conductivity (K), vulnerability to embolism, and vascular cambium viability in both control (unheated) and heated portions of the stem. We chose to use a drying oven as our method of heating because it provides hot and dry conditions, similar to those experienced by trees during fire (West et al. 2016). This laboratory approach does not capture every aspect of a real fire. For example, the flaming combustion of fire transfers heat quicker than hot air in an oven (Michaletz and Johnson 2007). Fires also provide uneven heating around the circumference of the tree and branches burn differently than the trunk (Gutsell and Johnson 1996). While the heterogenous nature of fire cannot be readily simulated in a laboratory experiment, the benefit of a controlled study is that some of the variation that occurs can be removed to more precisely identify the physiological responses to fire. Our goal was not to simulate fire, but rather to identify the post-fire relative response of cambium and xylem.

Our work addresses three questions: (1) Is the cambium or the xylem more prone to becoming dysfunctional with heat treatment? (2) What are the threshold temperatures and duration of heat exposure required to cause vascular cambium necrosis and hydraulic impairments? (3) Does heat exposure influence future drought resistance? We predicted that higher temperatures and duration of heat would lead to both increased vascular and cambial damage.

Materials and Methods

Experiment overview

The goal of our experiment was to simulate the effect of heat on xylem and cambial function in a manner that most realistically represents conditions during fire. To that end, we chose to follow the method of West et al. (2016), in which foliated stems were placed in a drying oven of varying temperatures and durations. Our approach differed in that we used different segments on the same stem material to measure hydraulic function before and after the heat treatment (Fig. 1). Additional experiments used the centrifuge to examine the heat-treated stems' vulnerability to embolism; this was motivated by the field observations of Bär et al. (2018). Simultaneous to the hydraulic measures, we developed a quantitative method to determine vascular cambium viability using Neutral Red stain.

Sample collection

Sequoia sempervirens branches were collected from the University of California, Santa Cruz campus. Branches were selected from the lower canopy, less than 6 meters high. They were at least 1 meter long and 6-8 mm in diameter. Branches were chosen to have minimal tapering, allowing for diameter across the length of the branch to be as uniform as possible (Fig. 1). Bark thickness was 0.1 mm on all branches. The branches were immediately bagged upon cutting and transported to the lab, where the ends of the branches were recut by 5 cm under water to remove any embolism caused by cutting. A sample size of 8 was used for each treatment.

Sample preparation

The branch ends were placed in water and bagged overnight to ensure fully hydrated stems before any heat treatments were applied. Xylem water potential was subsequently measured on small twigs with a pressure chamber (Soil Moisture Equipment Corp, www.soilmoisture.com) to verify that the branches were hydrated with a minimum balance pressure of 0.75 MPa. Across all samples, the initial xylem water potential was -0.27 ± 0.14 MPa. Applying heat treatments (as described below) required that we use different stem segments to compare control and heat-treated stems. As such, we divided each branch into multiple sections, each for a specified measure (Fig. 1). Towards the basal end of the branch, a 15 cm segment was reserved for control (unheated) conductivity measurements (K_{native}) and a 2 cm segment was reserved for control (unheated) vascular cambium viability measurements. The remainder of the branch was placed into the drying oven at its specified heat treatment with leaves intact. The cut end was wrapped in parafilm to prevent water loss, following the methods of West et al. (2016). To verify the effectiveness of parafilm preventing water loss from the cut end, we ran a pilot experiment to compare the parafilm method with one in which we kept the cut end submerged in water during the heat treatment. The percent loss of conductivity (PLC) due to embolism did not differ between the two treatments, so we continued to follow the cited methods (See Figure S1 available as Supplementary Data at *Tree Physiology* Online).

To perform heat treatments (see below), branches were placed in the oven in a beaker such that they were upright, avoiding contact with the hot oven walls. Furthermore, the beaker was lined with Styrofoam to prevent conductive heating of the stem (West et al. 2016). After the heat treatment, the branch was removed from the oven. The basal 15 cm of the stem was discarded because we observed the Styrofoam-lined beaker insulated the base of the stem from the imposed heat treatment. The remaining stem material yielded a 15 cm segment for hydraulic measures (K_{heat}) and a 2 cm segment for assessment of vascular cambium viability. After hydraulic conductivity was measured (see below), each 15 cm segment was degassed in deionized water under vacuum for one hour to refill embolism. The degassed control and heated stems were measured once more to record K_{max} and $K_{heatmax}$, respectively. Finally, the degassed stems were cut back to 14.2 cm in length and used to create vulnerability curves.

Heat Treatments

Heat treatments were performed in a drying oven following methods from West et al. (2016). Branches were placed in an oven with leaves intact to create a lab condition most representative of embolism occurring in the field. Heat treatments were applied as follows: 70°C for 6, 15, 30, 45, and 60 minutes, and 100°C for 6, 15, 30, 45, and 60 minutes. Temperatures were chosen to align with those in West et al. (2016), and also encompass the range of temperatures used in Michaletz et al. (2012) and Bär et al. (2018). The duration of heat exposure was chosen to align with 6 minutes in West et al. (2016) and 60 minutes in Bär et al. (2018), as well as additional intermediate durations to more accurately identify any threshold conditions in which xylem or cambial function fails. In separate stems of similar dimensions, a thermocouple was inserted under the bark to measure the cambium/xylem temperature under each of the heat treatments (Fig. 2). The thermocouple was placed in the outermost region of the xylem because the cambium was too close to the surface of the bark to make a deep enough incision to record a reliable temperature reading. Temperatures of the outer xylem likely approximate the cambial temperatures or slightly underestimate them. Notably, the 70°C treatments only heated the cambium to 58°C, just short of 60°C when proteins denature and cells die (Rosenberg et al. 1971). However, cells can also die at lower temperatures if they are exposed to heat for longer durations (Hare 1961, Dickinson and Johnson 2004, Achchige et al. 2021). Furthermore, lignin softens between 72°C and 100°C(Salmén 1984), which could lead to xylem deformation (West et al. 2016, Bär et al. 2018). The 100°C treatments heated the cambium up to 84°C, well past the limits of cell viability and the lignin softening. Importantly, these are maximum temperatures that the stems were exposed to during the heat treatments. Temperatures were lower than 58°C and 84°C, respectively, in the 70°C and 100°C treatments after 6 minutes, and approached maximum temperatures after about 20 minutes. This increase in temperature over time is representative of how living tissues and xylem heat during fire, with the caveat that the heat transfer caused by actual burning of the stem might heat tissues faster than the convective heat of an oven (Michaletz and Johnson 2007). For simplicity, we will refer to these treatments as 70°C and 100°C, despite the dynamic nature of the increasing temperature.

Although some treatments last up to an hour, we recognize that the shorter durations are likely the most ecologically relevant, as residence times of fire at the temperatures we are using are most often in the 6-15 minute range (Battipaglia et al. 2016). Furthermore, the residence time of flaming combustion (300°C) only lasts approximately 3 minutes (Fahnestock and Hare 1964, Wotton et al. 2012, Battipaglia et al. 2016).

Hydraulics

The flow rate through the segment was measured gravimetrically using 20mM potassium chloride under a pressure head of approximately 5 kPa. Flow rate was measured using a flow meter (SLI-1000, sensirion.com) and converted to hydraulic conductivity (K) by accounting for the pressure head and stem length (Sperry et al. 1988), and correcting for temperature. To standardize PLC comparisons, leaf specific conductivity (K_{leaf}) was calculated by dividing K by leaf area. K_{leaf} was confirmed to be similar between basal and distal portions of an unheated control stem, ensuring leaf area as a reliable standardizing metric (See Figure S2 available as Supplementary Data at *Tree Physiology* Online). Stains such as saffranin, acid fuchsin or crystal violet are often used to identify the functional xylem area before and after heat exposure (Sano et al. 2005, Jacobsen et al. 2018)

However, xylem staining in our samples was inconsistent, so xylem-specific conductivity was not determined. The impact of the heat treatment was assessed by calculating the PLC in the heat-treated stems compared with the control stems. A sample size of 8 stems was used for each treatment. Vulnerability curves were produced using the standard centrifuge method (Alder et al. 1997). Stems were trimmed under water with a razor blade to 14.2 cm long, and spun in a centrifuge for 4 minutes with a custom rotor, at progressively higher speeds to simulate reductions in water potential. After each spin, K was measured. Vulnerability curves were constructed using a Weibull fit in the fitplc R package (Duursma and Choat 2017). The P_{50}, which indicates the water potential at which a stem loses 50 percent of its conductivity, was used to compare treatments. Vulnerability curves were only constructed for the 6 and 15 minute heat treatments, which are more likely to represent residence times of these conditions in nature (Battipaglia et al. 2016). Four (n = 4) stems were used for each vulnerability curve, with the exception of the 100°C, 15 minute treatment which included some outlying data in 2 stems. For that treatment, the sample size was increased to 6 stems. The 2 outlying stems remain in the figures to show variability, but statistical analyses were performed on the four stems behaving normally.

Vascular Cambium Viability

Vascular cambium viability was determined using Neutral Red stain. We used 0.01% Neutral Red in a solution of 20mM phosphate buffer pH 7.5, 600mM KNO3, and 1mM CaCl2 (Basham and Bateman 1975). The saline components serve as a plasmolyzing solution to allow metabolically active cells to actively incorporate the stain across the tonoplast and into their vacuoles, thus staining living cells' vacuoles red (Tribe 1955). We included phloem cells in the analysis because phloem likely experiences the same conditions as vascular cambium during heat events, as they are adjacent to each other with no insulation between them. Longitudinal sections were prepared using a microtome (Lab-Microtome, Swiss Federal Research Institute, www.wsl.ch). Sections were taken close to the bark to ensure a large surface area of cambium and phloem. Sections were 80 um thick – large enough to ensure cells and their vacuoles stayed intact to display successful staining. Sections were submerged in the Neutral Red solution for 20 minutes. They were then rinsed with 20mM phosphate buffer pH 7.5, 600mM KNO3, and 1mM CaCl2. Stained sections were mounted on slides and imaged on a compound light microscope (Leica, www.leica.com). Sections were stained immediately after sectioning and were photographed within 30 minutes of the completion of staining. Vacuole area was measured manually (Fig. 3) on images photographed at 200x magnification using ImageJ (National Institutes of Health, imageJ.nih.gov) to determine the percentage of cambium/phloem area that stained red. Eight stems were stained per treatment. Each stem was photographed five times in four different locations on the stem for a total of 20 images for each stem. Five of those images were randomly selected for analysis. To analyze the images, a 0.35 mm^2 rectangular boundary surrounding cambium and phloem area was demarcated. The red vacuoles were manually traced to determine the total stained area in each image (Fig. 3). The average fraction of cambium/phloem area that stained red was reported. Some viable cells may not have stained if their vacuoles were cut during the sectioning process.

Results

Hydraulics

The heat treatments exposed the stems to both high temperature and dry air, conditions that might be experienced by some stems during a fire. T-tests showed that K_{leaf} is significantly lower in heated stems than control stems for all treatments (Fig. 4a,b). However, the degree to which this variation is functionally meaningful is debatable since the absolute differences in conductivities are relatively small. Embolism remained relatively low (23-36 percent loss in conductivity) in all of the 70°C treatments and the 6 and 15 minute 100°C treatments (Fig. 4c,d). The longer 100°C treatments induced a greater response in PLC, with a PLC of 44.2 ± 18.6 after 30 minutes, and a PLC of 93.6 ± 6.1 after 45 minutes. Therefore, we conclude that the treatments typical of heat exposure during a fire in nature, that is 6 to 15 minutes of exposure to 70°C – 100°C temperatures, produce a minimal effect on PLC. Indeed, it took 45 minutes at 100 degrees to almost completely embolize the samples.

The vulnerability curves revealed that *S. sempervirens* stems that were heated to 70°C for 6 and 15 minutes were no more vulnerable to cavitation than unheated controls. Indeed, the P_{50} is the same in the control and the heated stems in the 6 minute treatment (-6.54 ± 0.09 MPa and -6.66 ± 1.13 MPa, respectively) and the 15 minute treatment (-5.28 ± 0.65 MPa and -5.31 ± 1.21 MPa, respectively; Fig. 5a,b). This suggests that short-duration exposure to mild temperatures has little to no impact on air entry into the xylem.

In contrast, stems heated to 100°C were slightly but insignificantly more vulnerable P_{50}'s than the unheated control stems after both the 6 and 15 minute treatments. The stems heated to 100°C for 6 minutes (P_{50} = -4.95 ± 0.56 MPa) were slightly more vulnerable to cavitation than control stems (P_{50} = -5.73 ± 0.31 MPa; P = 0.12 ; Fig. 5c) and the stems heated to 100°C for 15 minutes (P_{50} = -4.68 ± 0.57 MPa) were also slightly more vulnerable to cavitation than control stems (P_{50} = -5.37 ± 2.04 MPa; P = 0.07; Fig. 5d). However, we note that degassing K_{heat} stems did not fully restore conductivity in the stems to K_{max} (Fig. 6). Hence, the $K_{heatmax}$ values, used as the initial points in the heat vulnerability curves, are reflective of post-heat maximum conductivity rather than a fully refilled stem. Although we observed similar P_{50}'s in the control and heat stems, the absolute conductivity at which those P_{50}'s occurred was reduced in the heated stems, typically by 20-40 percent. This suggests that drought events after fire may predispose trees to greater water stress because heat-induced embolism reduces the xylem's capacity to deliver water to the canopy. We suspect that either refilling or flow was impeded by aspirated pit membranes, which sealed, at least in part, the pit aperture (Delzon et al. 2010). Lastly, we viewed heated xylem under a scanning electron microscope and did not observe any obvious lesions in the tracheid walls or pit membranes (See Figure S3 available as Supplementary Data at *Tree Physiology* Online), but we suspect refilling could be impacted by pit membrane dysfunction.

Vascular Cambium Viability

The cambial area remained viable only at the mildest heat treatments, as indicated by Neutral Red staining (Fig. 7,8). The percentage of stained area was similar between the controls and 70°C treatment for 6 and 15 minutes, but beyond 15 minutes, nearly all vascular cambium died. An ANOVA confirmed the dramatic difference between treatment groups ($P < 0.001$). In each of the 30 and 45 minute exposures, only one of the eight stems displayed evidence of surviving vascular cambium. Furthermore, no vascular cambium survived following the 100°C treatment, aside from just a small number of cells in the 6 minute treatment. Cambial viability sharply declined after 70°C treatments for 6 and 15 minutes, indicating it is highly susceptible to fires of high intensity or duration.

Xylem dysfunction and cambium necrosis appear to differ substantially with respect to the threshold heat conditions needed to cause damage to each tissue (Fig. 9). Specifically, the xylem does not experience a substantial loss of hydraulic conductivity until after 30 minutes of exposure to 100°C treatment; this condition reflects the extreme spectrum of the experimental treatment and is not experienced by trees in situ. However, cambium necrosis occurs under relatively mild heat conditions with nearly all cambium dying at 100°C temperatures, even after short durations. Taken together, the data indicate that in *S. sempervirens* stems, the vascular cambium is more readily susceptible to heat damage at a given temperature than the xylem tissue, which suffers only mild embolism at the equivalent temperature.

Discussion

Previous studies have investigated the impacts of heat on xylem function (Battipaglia et al. 2016, West et al. 2016, Bär et al. 2018, Lodge et al. 2018, Partelli-Feltrin et al. 2021, Hoffmann et al. 2021) and vascular cambium viability (Dickinson et al. 2004, Achchige et al. 2021). Here, we have built upon these established findings and examined the effects of cambium and xylem concurrently. Assessing the viability of the vascular cambium provides us with another tool to assess tree health after a fire. We found that while heat does impact both xylem function and vascular cambium viability, both tissues respond to far different heat conditions in *S. sempervirens* stems. The vascular cambium stays viable only under relatively mild heat conditions, with necrosis occurring under 15 minutes at 70°C. It takes much longer than 15 minutes to generate meaningful embolism. For example, the PLC does not reach 50% unless stems are heated at 70°C for more than 30 minutes. Furthermore, a loss in conductivity greater than 88%, which may exceed the safety margin for lethal xylem dysfunction (Delzon and Cochard 2014), is not achieved until the stems are exposed to 100°C for 45 minutes. In our study, any major effects of embolism happen long after cambium necrosis has already occurred. Furthermore, the duration at which *S. sempervirens* xylem becomes substantially compromised by embolism exceeds the 6-15 minute residence time of fire temperatures in the 70 – 100°C range (Fahnestock and Hare 1964, Wotton et al. 2012, Battipaglia et al. 2016).

Although our results suggest that cambium is impacted far before xylem, this does not imply that xylem function is insignificant to post-fire tree health. Fires and environmental conditions are extraordinarily complex, which could lead to more nuanced responses than we observed in a standardized laboratory experiment. While we evenly heated stems, fire severity varies, even in small spatial and temporal scales, due to shifts in fuels (Nolan et al. 2016), topography (Linn et al. 2007, Harris and Taylor 2017), and weather(Thompson and Spies 2009). This likely leads to variation in tissue injury among branches, and especially at the bole of the tree where bark is thicker. Uneven heating of the cambium could allow some portions of the tree to remain viable, allowing the injured portion to recover. Additionally, trees in the field are likely to have partially embolized xylem prior to fire, given the hot and dry conditions that often lead to fire. The effects of fire-induced embolism could be more consequential in trees that already have partially compromised xylem. These considerations highlight some of the complexities that are difficult or impossible to effectively control for in a laboratory study.

The above-ground residence time of a wildfire may be short, but soils may remain hot for some time even after the flames subside. Previous work has shown that smoldering duff results in prolonged heating of the roots and vascular cambium near the base of the tree (Kreye et al. 2020). Indeed, several studies have observed smoldering fires at lethal temperatures regularly lasting over an hour and sometimes more than 5 hours (Ryan and Frandsen 1991, Swezy and Agee 1991, Varner et al. 2009). In this scenario, severe damage to the roots and the root crown will likely supersede the injury to the shoot. Fires vary in duration and intensity, depending on location and climate so we need to appreciate the nuances of fire injury to better understand its effect on cambial function and tree hydraulics.

Post-fire conditions, such as concurrent droughts, may also impact the trees' potential for recovery. We tested one aspect of this by assessing vulnerability to embolism following heat treatments. While there was no significant impact of heat on xylem vulnerability to embolism, we were unable to refill the stems after they had been heated. We suspect this was caused by pit aspiration (Delzon et al. 2010). This limited the vulnerability curve analysis to a subset of functional tracheids, which exhibited the same vulnerability to embolism as the control stems (Fig. 5). If sustained heat damage had only caused conduit warping without impacting refilling (West et al. 2016, Bär et al. 2018), we would have expected the post-heat stems to be more vulnerable to embolism. However, the finding that control and heated stems have similar vulnerability curves, coupled with their 20-40 percent loss of K_{max} (Fig. 6), indicates that heat-induced embolism rather than heat damage to the cell walls is the most immediate and consequential impact suffered by the xylem in *S. sempervirens*. Consequently, reduced absolute conductivity in heated stems may constrain the delivery of water to the canopy, especially during episodes of drought.

Previous studies have recorded mixed results with vulnerability curves. Bär et al. (2018) showed significantly higher (more vulnerable) P_{50}'s in all three species (*Picea abies, Pinus sylvestris, and Fagus sylvatica*) of their heated stems compared to control stems in the lab. They reported similar patterns in those same species in the field, although not as pronounced. Partelli-Feltrin et al. (2021) observed more vulnerable *Pinus ponderosa* saplings 21 months following fire compared to unburned saplings. Lodge et al. (2018) also found *Pinus palustris* to have stems and roots more vulnerable to embolism following mild water bath treatments. Alternatively, Battipaglia et al. (2016) reported no difference in vulnerability between *Pinus pinea* stems exposed to a prescribed burn and control stems. This variation might be due to differences across species, particularly with each species' ability to withstand xylem damage to their cell walls and pit membranes (Pittermann et al. 2010, Jansen et al. 2012). Variation might also be caused by the differences in methodology, including water baths, drying ovens, and fire application. Additionally, insulative properties of bark (Pinard and Huffman 1997) likely affect retention of functional xylem. Further measures of xylem vulnerability across a diversity of species may help explain the variation in hydraulic traits following lab heat treatments and exposure to fire.

Quantifying vascular cambium viability was not a trivial task. For those seeking similar tests of vascular cambium viability in woody stems, we suggest the method described here using Neutral Red. While testing various viability stains, we sought to develop a method to provide a simple binary answer to whether cells were living or not. To this end, Neutral Red staining provides much more resolution than we had originally expected. Neutral Red is a relatively easy stain to prepare and work with, and can be quite useful in assessing tree survival. Often, tree mortality is assessed post-fire by scraping under the bark and judging whether there is a green layer of cambium. While such observations are both convenient and useful, the Neutral Red method offers a more empirically and mechanistically grounded approach to determine tree survival following fire because it captures critical vacuolar function. We tested several other methods before selecting this one. Rhodamine 123 is a fluorescent stain used to test for viability (Johnson et al. 1980). However, when we applied Rhodamine 123 and viewed the section under fluorescence, we observed numerous naturally fluorescing structures in the section. Plant cells are filled with many secondary compounds that naturally fluoresce, making it impossible to distinguish natural fluoresce from the Rhodamine 123 stain. We also tried using Tetrazolium Chloride (TTC), which is commonly used for testing seed and cell culture viability by staining mitochondria in living cells

(Lakon 1949), and has been successfully used by Achchige et al. (2021) on eucalypts. We were able to successfully stain our woody sections, but the stain was sometimes difficult to identify and challenging to quantify because the mitochondria are so small. In contrast, Neutral Red stains vacuoles, which are relatively large, visually striking, and thus easy to quantify in terms of area.

This study highlights the relative susceptibility of vascular cambium viability and xylem function in *S. sempervirens* after heat damage. Further field research assessing both vascular cambium and xylem function can help progress our understanding of the critical physiological components of post-fire forest recovery. Future studies may consider installing thermocouples under the bark in trees prior to prescribed burns to understand the temperatures that trees and their vascular cambium experience during fire. These data would provide ground truthing for our laboratory experiment. Furthermore, our study focused on just one species. In the future, we would like to expand our dataset to include other dominant species across California, including conifers less adapted to fire and angiosperms. Data across a wide range of species could help managers make decisions on prescribed burn conditions and restoration efforts. The data in this study indicates that vascular cambium is more susceptible to heat damage than xylem in distal stems exposed to temperatures characteristic of low severity fires. Since redwoods commonly resprout from the bole after a fire, we conclude that living tissue preservation is more important than xylem function for their survival even in high severity fires. The methods used in this study provide a useful tool for tracking forest recovery.

Data Availability

Data will be made available upon acceptance.

Supplementary Data

Figures S1, S2, and S3 included as separate files.

Conflict of Interest

None declared

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Author Contribution

R.S. and J.P designed the experiment. R.S. executed the experiment and collected the data. R.S. and J.P. interpreted the results and prepared the manuscript.

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Fig. 1 Schematic illustrating different portions of the same stem allocated for each component of analysis.

Fig. 2 Xylem temperature plotted alongside oven temperature during heat treatments. Error bars represent a single standard deviation.

Fig. 3 Examples of tangential cambial sections. **(a)** shows a section that is not stained in Neutral Red. **(b, c)** show a stained section, with **(b)** illustrating how the vacuoles were manually traced.

Fig. 4 Summary of hydraulic data following 70 and 100 C heat treatments. The top panels **(a, b)** show the comparison of K_{leaf} between controls and heated samples. ** denotes $p < 0.01$ and *** denotes $p < 0.001$, as determined by paired t-tests. **(c, d)** show PLC calculated from K_{leaf} measures. P-values were calculated with a single factor ANOVA. Lower case letters in **(c, d)** indicate significant differences between treatments as determined by a Tukey-Kramer post-hoc test.

Fig. 5 Vulnerability curves for each of the four treatments. Black circles and lines indicate control stems. Grey circles and lines indicate heated stems. The curves are fit to the average of the individual samples. Two stems in the 100°C 15 minute sample behaved unusually, in which case the points remain to show variability, but the curve is fitted to the remaining four samples. P values were calculated using a paired t-test.

Fig. 6 Vulnerability curves for each of the four treatments displaying conductivity measures on the y-axis. Black circles and lines indicate control stems. Grey circles and lines indicate heated stems. The curves are fit to the average of the individual samples. Two stems in the 100°C 15 minute sample behaved unusually, in which case the points remain to show variability, but the curve is fitted to the remaining four samples. Dashed red lines indicate the P_{50} and the corresponding conductivity. P values were calculated using a paired t-test.

Fig. 7 Boxplots displaying vascular cambium viability, determined by percent area stained red by Neutral Red. Both 70°C and 100°C treatments are located on the same x axis, indicating an overall increase in extremity. The 70°C, 30 and 45 minute treatments each had one of the eight samples remaining viable, with the remaining seven showing no stain. The p-value was calculated with a single factor ANOVA. Lower case letters indicate significant differences between treatments as determined by a Tukey-Kramer post-hoc test.

Fig. 8 (a) shows an example of a control image. **(b - e)** show 70°C heated for 6 minutes, 70°C heated for 15 minutes, 100°C heated for 6 minutes, and 100°C for 15 minutes, respectively. Panels **(b – e)** illustrate the decline of viable cambium throughout progressive heat treatments. The arrow in **(d)** points to a lone cell stained red. The scale bars are set to 200 μm.

Fig. 9 Overall summary of vascular cambium and hydraulic data. Red boxes display vascular cambium viability and correspond with the left y axis. Blue boxes display PLC and correspond with the right y axis. A single x axis is used for controls, 70°C, and 100°C treatments, and indicates more extreme conditions from left to right. PLC data do not have a control because PLC is calculated using a comparison of a control segment with a heated segment. The 70°C, 30 and 45 minute cambium viability treatments each had one of the eight samples remaining viable, with the remaining seven showing no stain.

Fig. S1 Boxplots displaying the percent of hydraulic conductivity that was lost in stems (n = 8) due to a moderate heat treatment of 100°C for 15 minutes. This pilot experiment compared two methods of preventing water loss through the cut end of the stem, in which we found no significant difference.

Fig. S2 Boxplots displaying the leaf specific conductivity (K_{leaf}) in unheated control stems (n = 8). The paired basal and distal segments were measured with spacing equivalent to the spacing between control and heated segments used in the remainder of the study. The percent difference between the two measurements was minimal (3% ± 7%), which indicates leaf area is appropriate for standardizing conductivity.

Fig. S3 Xylem viewed under a scanning electron microscope at 1,000x and 10,000x magnification. None of the controls or heated stems appeared to have obvious lesions in the tracheid walls or pit membranes.