Physiological Stress and Ethanol Accumulation in Tree Stems and Woody Tissues at Sublethal Temperatures from Fire

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The lethal temperature limit is 60 degrees Celsius (°C) for plant tissues, including trees, with lower temperatures causing heat stress. As fire injury increases on tree stems, there is an accompanying rise in tissue ethanol concentrations, physiologically linked to impaired mitochondrial oxidative phosphorylation energy production. We theorize that sublethal tissue temperatures of 30°C to 60°C cause physiological changes to (a) oxygen supply, (b) membrane function, or (c) enzyme activity that individually or simultaneously create stress by impairing aerobic respiration and inducing ethanol synthesis. Accumulating ethanol dissipates via diffusion, sapflow, and metabolism, but the ability of these processes to decrease ethanol depends on what temperatures and physiological stress mechanism(s) the tissues and whole trees experience. The synthesis and dissipation interactions determine postfire tissue ethanol concentrations. Wildfire trends positively with temperature and drought, and all are projected to increase in western US forests and elsewhere globally, increasing the importance of understanding tree sublethal heat stress from fire.

Keywords: Fire, heat stress, oxygen, membranes, enzymes

Fire has been a major disturbance agent in western US ecoregions for millennia and is influenced by complex climate, vegetation, topography, and human interactions (Marlon et al. 2012). The number of large wildfires, their severity, and their annual area burned began to increase in the mid-1980s (Littell et al. 2009, Dennison et al. 2014) in response to the rising temperatures and drought that increase fuel aridity (Peterson and Marcinkowski 2014, Abatzoglou and Williams 2016, Littell et al. 2016). Temperatures across the United States are projected to continue increasing in the range of 0.8°C–1.9°C by 2050 and 2.5°C–5.3°C by 2099, depending on greenhouse gas emissions (relative to 1971–2000; Miniat and Peterson 2014). This will be accompanied by more frequent and longer drought in the United States and globally (Miniat and Peterson 2014), including forests that have already experienced drought- and heat-induced tree mortality (Allen et al. 2010). If temperatures in western US ecoregions increase by 1°C, as is projected for midcentury, the median annual area burned could increase more than 200% (relative to 1950–2003; Peterson and Littell 2014). Prescribed fire is also used by government agencies and private landowners to currently burn 0.8 million–1.2 million hectares annually (National Interagency Fire Center 2016) to decrease fuel loads and mitigate wildfire severity, but the future area treated may increase. Clearly, fire will retain its status as a major stress agent, combined with drought, bark beetles, and pathogens, shaping the structure, composition, function, productivity, and sustainability of forest ecosystems through the twenty-first century (Parker et al. 2006).

Fire-injured ponderosa pine (*Pinus ponderosa* Lawson and C. Lawson) stem tissues synthesize and accumulate ethanol, with concentrations increasing as severity of injury to the bole and crown increases (Kelsey and Joseph 2003). In a related study (Kelsey and Westlind 2017), tissues from trees severely injured by wildfire contained higher ethanol concentrations than those from less injured trees in a prescribed fire, a pattern consistent with previous results (Kelsey and Joseph 2003). Information from these two studies, in conjunction with results from other stress studies relevant to ethanol synthesis, has been considered here to explore the physiological changes responsible for stress in the cells and tissues of tree stems and other woody tissues that reach sublethal temperatures of 30°C–60°C when heated by fire. This model provides a framework for further research

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and experimental testing to evaluate the validity of these proposed processes. Although the model was developed using ponderosa pine, we suspect that these physiological mechanisms apply to most tree species. In addition, ethanol is a key factor in some postburn ecological relationships. When ethanol is released to the atmosphere in combination with host oleoresin monoterpenes, the mixture functions as a primary attractant during host selection by pioneering individuals of some bark beetle species that typically colonize stressed trees and that may vector pathogens to those surviving their burn injuries.

Ethanol links to stress

Ethanol synthesis is physiologically linked to aerobic respiration. Briefly, aerobic respiration involves oxidative phosphorylation in mitochondria (figure 1a) to produce adenosine triphosphate (ATP) energy for all cellular processes, including metabolism, catabolism, and the maintenance of membrane structures and functions (Gibbs and Greenway 2003, Greenway and Gibbs 2003, Felle 2005, Bailey-Serres and Voesenek 2008). It requires two primary substrates: oxygen (O_2) and pyruvate supplied by carbohydrate breakdown via glycolysis that also produces a small amount of ATP. When O_2 supplies are adequate, the tissues metabolize pyruvate by oxidative phosphorylation, but if O_2 concentrations drop to low levels (hypoxia) or are completely depleted (anoxia), then cytoplasmic pH drops, thereby increasing the activity of the two fermentation enzymes (pyruvate decarboxylase and alcohol dehydrogenase) that metabolize accumulating pyruvate to ethanol in the cell's cytoplasm (Gibbs and Greenway 2003, Greenway and Gibbs 2003, Bailey-Serres and Voesenek 2008). Eliminating tree-tissue O_2 supplies (figure 1b) by placement in a nitrogen atmosphere (Kelsey et al. 1998) or submersion in water (Kreuzwieser et al. 2000) demonstrates this rapid ethanol induction. Ethanol synthesis allows glycolysis to continue producing the small quantities of ATP needed to maintain cell-membrane integrity and minimal functions until O_2 levels are replenished and aerobic respiration resumes. If glycolysis or ethanol synthesis stops before aerobic respiration resumes, then membrane functions stop, accompanied by uncontrollable acidosis and cell death (Gibbs and Greenway 2003, Greenway and Gibbs 2003, Felle 2005, Ishizawa 2014). Therefore, ethanol synthesis and accumulation are physiological indicators of a stress agent affecting cellular energy via oxidative phosphorylation.

Aside from heat, other types of stress inducing tree tissues to synthesize and accumulate ethanol include flooding (Kreuzwieser et al. 2000), pathogens (Kelsey et al. 2013), severe water deficits and drought (Kimmerer and Kozlowski 1982, Kelsey et al. 2014), crushing (Kimmerer and Kozlowski 1982), air pollutants (Kimmerer and Kozlowski 1982), mechanical injury to crowns (Sjödin et al. 1989), or basal-stem severing with ethanol accumulating in dying stumps and logs (Kelsey 1994, Kelsey and Joseph 1999).

Woody-tissue temperatures from fire. The lethal temperature limit for most plant tissues, including trees, is widely recognized as 1 minute at 60°C (Agee 1993, Dickinson and Johnson 2004, Michaletz and Johnson 2007). Mortality of 10%, 50%, and 90% was observed for 2- to 4-week-old ponderosa-pine seedlings after 5 minutes of lower stem exposure to 51.2°C, 56.3°C, and 59.0°C, respectively (Seidel 1986). The same level of mortality in that study was detected at lower temperatures (48.8°C, 52.0°C, and 53.5°C, respectively) by extending stem treatment time to 60 minutes. This demonstrates the temperature–time, or dose-interaction, phenomenon, suggesting that physiological changes occur at lower temperatures but require more time to reach a nonrecoverable, lethal threshold. This may also reflect discrepancies in using temperature to measure heat rather than heat energy that is transferred into woody plant tissues from external heat sources such as fire, primarily by conduction (Michaletz and Johnson 2007, Kremens et al. 2010). Heat energy and temperature are not equivalent measures; heat energy can be transferred without a temperature change (Michaletz and Johnson 2007). Although there is a need for improved instruments and methods to measure the heat-energy transfer between fire and vegetation (Kremens et al. 2010), temperature is the common metric for heat in physiological studies, and that information is used here to understand the physiological changes in tissues heated by fire.

During a fire, the bark influences woody-tissue temperatures and heat stress, with the thickness having more impact than the tree species producing it (Dickinson and Johnson 2004, Michaletz and Johnson 2007). For example, ponderosa pine cambium reaches 60°C in 8 to 17 minutes with 1- to 2-cm bark thickness and 17 to 167 minutes with 2- to 3-cm-thick bark heated on the outside to 400°C (van Mantgem and Schwartz 2003). The thermal diffusivity of bark and underlying tissues, or the ratio of their thermal conductivity to volumetric heat capacity, determines their rates of temperature change during a fire (Dickinson and Johnson 2004, Michaletz and Johnson 2007). When an area of bark surface is heated, it produces a temperature gradient radiating three-dimensionally through the underlying live stem tissues (Chatziefstratiou et al. 2013), exposing various portions of its volume to 30°C–60°C temperatures. Once the external heat source is gone, the rates and duration of tissue cooling will depend on surrounding tissue temperatures and their thermal diffusivity, including bark. The cooling of heated stem vascular cambium in various tree species took multiple times longer to reach ambient than it did to heat (Chatziefstratiou et al. 2013), and this can greatly extend live tissues' exposure times to temperatures causing stress.

Defining woody-tissue heat stress

Heat stress and heat tolerance, with or without drought, are crucially important to crop production and the world's food supply (Wahid et al. 2007). *Heat stress* for plants or their tissues is often defined as "the rise in temperature beyond a threshold level for a period of time sufficient to

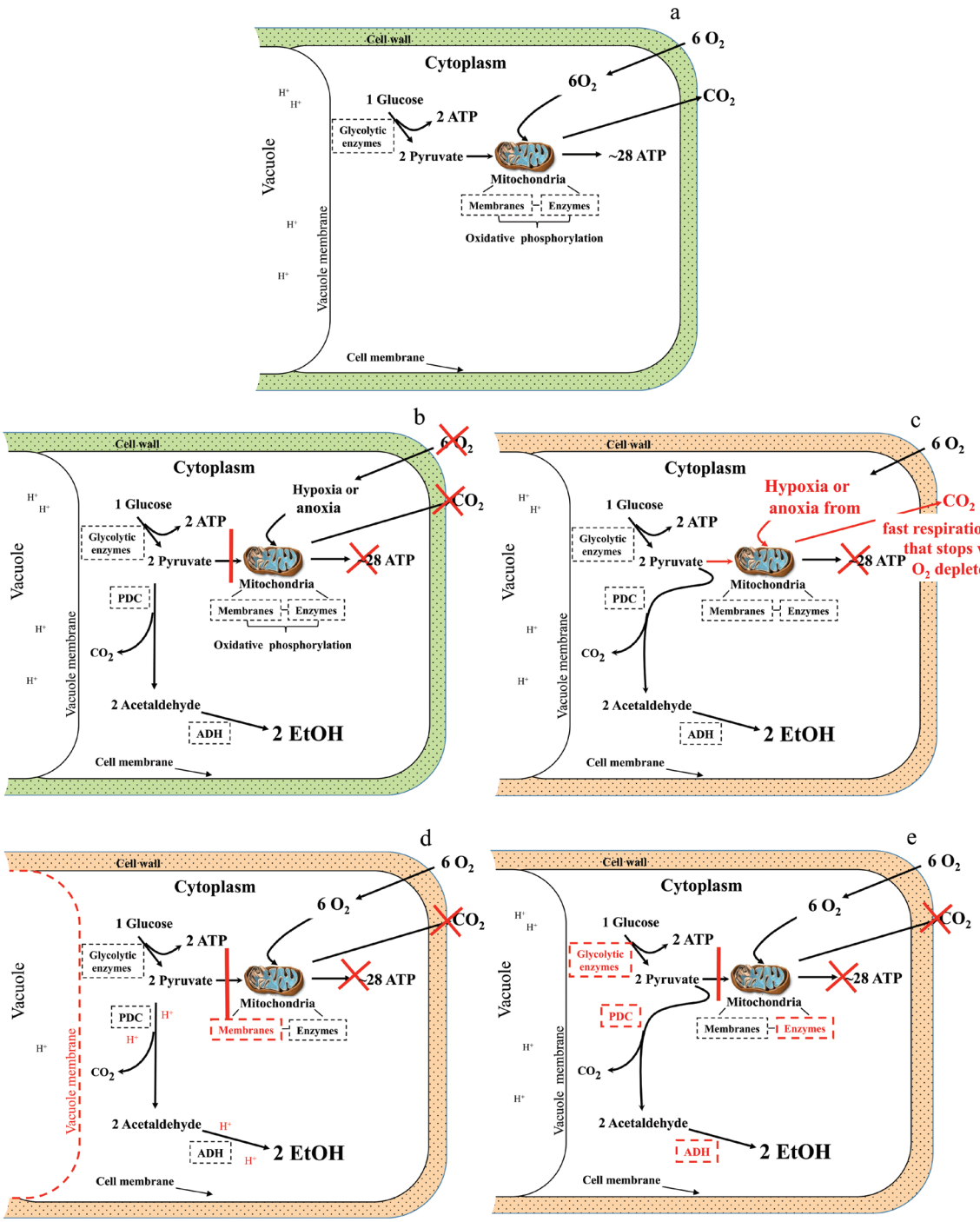

Figure 1. Proposed physiological mechanisms for heat stress in tree-stem tissues below 60°C: (a) Nonstressed cell oxidative phosphorylation with an ample O_2 supply at ambient temperature; (b) induced ethanol synthesis by hypoxia or anoxia resulting from the blockage of cells' external O_2 supply at ambient temperature, as in flooded roots or tissues placed in a nitrogen atmosphere; (c) O_2-supply heat stress, caused by elevated temperatures increasing respiration rates fast enough to deplete internal cell-O_2 supplies to hypoxic or anoxic levels long enough to induce ethanol synthesis; (d) membrane-function heat stress, caused by heat impairing the functions of mitochondrial membranes, vacuole membranes, or both simultaneously with protons leaking through damaged membranes, dropping cytoplasm pH (H^+) and increasing PDC and ADH enzyme activities, thereby contributing to ethanol synthesis; and (e) enzyme-activity heat stress, in which enzymes' activities rise with temperature to a peak rate and then decline at temperatures above optimum and eventually may be denatured. Temperature-mediated enzyme activity contributes to both O_2-supply heat stress and membrane-function heat stress, as well as rates of ethanol synthesis, as we discussed further in the text. Abbreviations: PDC, pyruvate decarboxylase; ADH, alcohol dehydrogenase.

cause irreversible damage to growth and development," and a transient temperature increase in the range of 10°C–15°C above ambient is considered sufficient to cause heat shock or heat stress (Wahid et al. 2007). The transient heating of woody tissues during a fire exposes cells, varying portions of tissues, or whole plants to temperatures much higher than 10°C–15°C above ambient (Hare 1965, Chatziefstratiou et al. 2013). On the basis of the links of ethanol to the physiological processes described above and the heat-stress mechanism proposed below, we define *sublethal heat stress* in tree stems and woody tissues as exposure to temperatures high enough and for sufficient time to impair mitochondrial oxidative phosphorylation energy production or other crucial physiological processes (Wahid et al. 2007) that may or may not be directly reversible but are sufficiently recoverable to prevent death, with variable impacts on metabolic functions, growth, or development. Lethal heat stress of 60°C or greater is the same, except some physiological threshold(s) is exceeded that is nonreversible or nonrecoverable and results in death.

Physiological stress mechanisms and ethanol synthesis from heat

In this section, tissue-temperature ranges are assigned to each physiological stress mechanism but are only guidelines that likely vary by tree species, tissue type, and seasonal physiological status, among many other factors. Whether one mechanism dominates or they occur simultaneously will depend on the temperature, its duration, and its rate of change, in combination with other physiological parameters. When woody-tissue temperatures rise to 30°C–60°C in response to heat-energy transfer from fire, it may cause one or more of the following physiological responses:

Oxygen supply stress, approximately 30°C–40°C. This process begins when tissue temperatures approach or exceed 30°C and increase respiration rates sufficiently (Ryan 1990, Stockfors and Linder 1998) to consume O_2 faster than it can be supplied, creating hypoxic or anoxic conditions to induce ethanol synthesis (figure 1c). Tree woody tissues, particularly stems and roots, are more likely to experience this type of heat stress than well-aerated foliage are. Live cells deep within woody tissues are particularly vulnerable because they can experience low O_2 levels even at ambient temperatures, especially during periods of rapid growth when meristematic tissues, such as cambium, rapidly consume O_2 (Hook and Brown 1972, Eklund 1990, 2000). This stress mechanism dominates at lower temperatures as long as O_2 remains the parameter limiting aerobic respiration. Physiological damage in cells subjected to O_2-supply stress is limited over short durations, so upon returning to postfire ambient temperatures and O_2 supplies, aerobic respiration restarts, and ethanol synthesis stops. An O_2-supply deficit from heat was demonstrated to induce ethanol synthesis in Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco, stem segments sampled in December while incubated in open vials at 30°C for 5.5 hours, but not for stem segments in May from the same trees and under the same conditions (Joseph and Kelsey 2004). A seasonally slower gas exchange rate with the atmosphere was considered a factor limiting tissue O_2 supplies in December compared with those in May (Stockfors and Linder 1998, Joseph and Kelsey 2004).

Membrane function stress, approximately 40°C–50°C. This mechanism occurs when tissues reach temperatures that destabilize cellular membranes (figure 1d) enough to impair their permeability, functions, or cause irreversible structural damage (Grigorova et al. 2012). When plant membranes are affected by heat, there is an accompanying electrolyte, or ion leakage, a response used in studying heat tolerance or stress in crop plants (Wahid et al. 2007). Electrolytes may be released from cellular organelles with heat-damaged membranes, such as vacuoles storing acidic constituents (Shen et al. 2013, Ishizawa 2014), and especially the central vacuole(s) enclosed by the tonoplast membrane (Daniell et al. 1969, Weigel 1983). Electrolyte leakage in leaves and shoots of three-year-old olive (*Olea europaea* L.) trees begins about 40°C–45°C depending on the cultivar (Mancuso and Azzarello 2002). In two holly (*Ilex* L.) species and their hybrid, 45°C–50°C is the temperature at which leakage begins in roots, whereas 50°C–55°C is the temperature for leaves (Rute 1993).

Membrane-function stress can affect ethanol synthesis in two ways. First, when heat destabilizes mitochondrial membranes, aerobic respiration rates decline (Lin and Markhart 1990, Balk et al. 1999), at some point inducing ethanol synthesis. Second, leakage of acidic constituents into cytoplasm causes the pH to drop into a range enhancing the activity of pyruvate decarboxylase and alcohol dehydrogenase, the two fermentation enzymes controlling ethanol synthesis (Greenway and Gibbs 2003, Ishizawa 2014). Membrane-function stress does not depend on tissue O_2 supplies. Rapidly heated tissues experience membrane-function stress (Anderson 1994) and may induce ethanol synthesis before depleting their O_2 supplies. Alternatively, in slowly heated tissues, the membrane impairment or damage may occur after respiration has depleted O_2 supplies and induced ethanol synthesis. This might have no immediate impact for ongoing ethanol production, but it could affect synthesis after tissues return to ambient temperatures and O_2 supplies, because the recovery of membrane functions from moderate to severe damage may be slow or never occur (Lin and Markhart 1990). The permanent loss of some aerobic respiratory capacity may allow a proportionate level of ethanol synthesis to continue after heat injury, provided that the fermentation enzymes remain functional. But subsequent ethanol synthesis will vary with ambient temperature until membranes are repaired or replaced or its synthesis is limited by some crucial metabolic components, such as available carbohydrate substrates, pH, etc. Ethanol synthesis postheat stress was demonstrated in apples, *Malus sylvestris* (L.) Mill., exposed to 46°C for 0, 4, 8, or 12 hours

and then stored at 20°C, as was indicated by increasing concentrations over a 4-day period (Song et al. 2001). Apples heated similarly in a related study were stored at 0°C posttreatment, and the ethanol continued to increase over a 2- to 3-month period (Fan et al. 2005), with some generating higher concentrations than those stored for 4 days at 20°C. In both experiments, the final concentrations depended on the cultivars and their 46°C exposure time.

Enzyme activity stress, 30°C–60°C. This occurs across the entire sublethal temperature range and is not as uniquely discernable as the other two mechanisms, in part because they are linked (figure 1e). Enzymes control cellular processes, including glycolysis, respiration, and ethanol synthesis. The activities of all enzymes rise with temperature to a maximum and then decline (Elias et al. 2014), but the optimum temperature can vary among enzymes. Any loss in activity at temperatures exceeding an enzyme's thermal peak may be reversible when temperatures drop, provided they are not permanently denatured. O_2-supply stress typically occurs when glycolysis and aerobic respiration rates increase as a consequence of the temperature-enhanced activities of their regulating enzymes. The rates of either process drop when temperature exceeds the maximum activity of any of their enzymes. Membrane-function and enzyme-activity stress mechanisms are conjoined because the enzymes controlling oxidative phosphorylation are in the mitochondria (Bailey-Serres and Voesenek 2008), and temperatures that compromise the membranes also affect the enzymatic activity. Once ethanol is induced, its synthesis rate depends on the thermal impacts on glycolysis and the fermentation enzyme activities in the cytoplasm. Any lost enzyme activity from thermal denaturation will remain when tissues return to ambient postfire temperatures and O_2 supplies until new enzymes are produced. Extensive enzyme degradation will result in cell death. The accumulation of temperature-related physical and physiological impacts on tissues reaching 50°C–55°C increases their likelihood of death, whereas 55°C–60°C is the range in which thermal death commences quickly—as has been reported for ponderosa pine (Seidel 1986)—and all enzyme-regulated activities, including ethanol synthesis, stop.

Ethanol synthesis in tissues with minimal or no direct heat

The mechanisms above occur in cells and tissues directly exposed to heat. There is another mechanism in fire-injured trees that can cause cells not directly heat stressed or minimally heat stressed to synthesize ethanol. This may occur in response to severely impaired water conductivity, when nearly all foliage has been scorched or consumed (Kelsey and Joseph 2003) or high vapor-pressure deficits during the fire cause embolisms in branch xylem (Kavanagh et al. 2010) or any other heat damage to xylem conductivity (Michaletz et al. 2012). The transpiration water stream from roots in adequately aerated soils carries dissolved O_2 to the stem's live secondary xylem, cambial cells, and, at times, inner phloem. In some tree species or seasons, this route may provide more O_2 to these tissues than radial diffusion with the atmosphere (Hook and Brown 1972, Gansert 2003, Mancuso and Marras 2003). When sapflow is impaired, the supply of dissolved O_2 to internal stem tissues declines. Tissues may become sufficiently hypoxic or anoxic to induce ethanol synthesis if respiration rates deplete O_2 supplied by radial gas exchange with the atmosphere. We suspect that this may explain ethanol's accumulation in *P. sylvestris* L. stems after sapflow was impaired by cutting off an upper portion of crown (Sjödin et al. 1989). This likely adds some ethanol to tissues in trees with 100% crown scorch (Kelsey and Westlind 2017), but if stem tissues have been sufficiently heat stressed, it is difficult to determine the extent of this contribution.

Ethanol synthesis response to other physiological factors

Seasonal differences in stem-tissue respiration rates and fermentation enzyme activities likely contribute to differences in ethanol accumulation for trees stressed at different times of the year. Stem cambial growth in spring and early summer increases the metabolically active cell volume beneath a unit area of bark compared with trees in fall with older, mature tissues. Consequently, at a given temperature, stems in spring have faster respiration rates than stems in fall (Ryan 1990, Stockfors and Linder 1998), creating a higher O_2 demand and seasonally lower O_2 levels in spring tissues (Eklund 1990, 2000). Upon heating, tissues in spring could more rapidly deplete O_2 levels, especially if they are already seasonally low, inducing ethanol synthesis more quickly than in trees in fall. In addition, tissues in spring have greater activities of the two enzymes regulating ethanol synthesis (Kimmerer and Stringer 1988), allowing faster synthesis rates for trees in spring after induction by the heat-stress mechanisms above.

As fire raises stem temperatures, the phloem or cambium will initially have high ethanol concentrations because they are the first affected by external heat (van Mantgem and Schwartz 2003, Chatziefstratiou et al. 2013), they have greater fermentation enzyme activity (Kimmerer and Stringer 1988), and they can produce more ethanol than xylem when stressed (Kelsey et al. 1998). But there are numerous mitigating dissipation processes influencing all tissue concentrations, so phloem may not always retain the highest quantities (Kelsey and Westlind 2017).

Ethanol dissipation and heat-stress mechanisms

As ethanol accumulates after induction, it immediately begins to dissipate via interactions of three processes: (1) diffusion, (2) sapflow, and (3) metabolism (figure 2). All are affected differently by the heat-stress mechanism the tissues and whole-tree experience.

Diffusion. Initially, ethanol moves by diffusion from areas of high to low concentrations (figure 2), as has been shown by infusing sapwood in a segment of tree stem without sapflow and trapping its release on the bark surface (Kelsey et al.

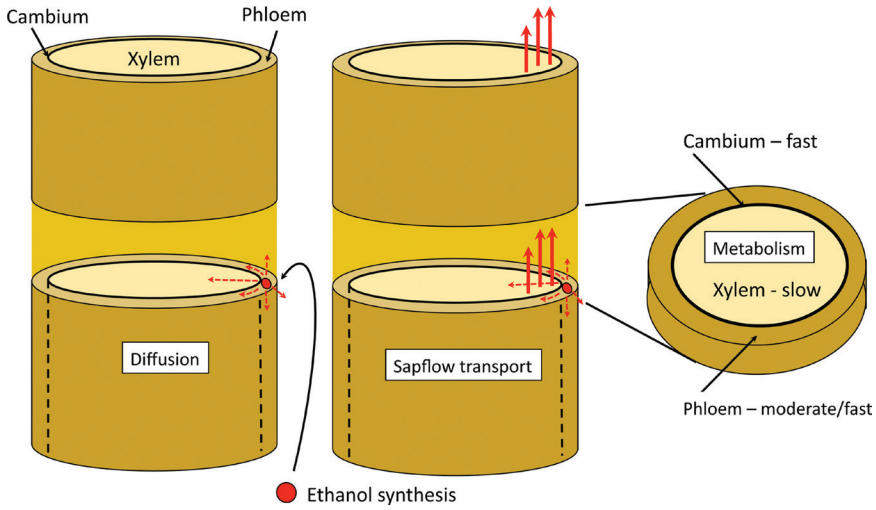

Figure 2. Ethanol dissipation mechanisms: diffusion, sapflow, and metabolism with relative rates at ambient conditions. Ethanol is metabolized by alcohol dehydrogenase to acetaldehyde (Zanon et al. 2007), which is converted by aldehyde dehydrogenase to acetate, which is converted by acetyl-CoA synthase into acetyl-CoA (MacDonald and Kimmerer 1993, Gass et al. 2005). The latter can enter the tricarboxylic acid or glyoxylate cycles or be used to synthesize lipids depending on heat damage to membranes and enzymes.

2013). Ethanol moves unimpeded through cell membranes because it is nonionic and hydrogen-bonds strongly to water (Fileti et al. 2004). But it also hydrogen-bonds to cell-wall polymers and can penetrate into cellulose (Stamm and Tarkow 1950, Mantanis et al. 1995), slowing its diffusion rates and temporarily protecting some from metabolism. The capacity of diffusion to dissipate ethanol from sites of synthesis is influenced by ethanol sinks and their strengths, tissue water content, and temperature. Surrounding tissues function as sinks, with their volume, composition, and ethanol contents contributing to sink strength that declines as their ethanol levels rise. The external atmosphere is a limitless sink for outward diffusion, but the length and metabolic activity of the tissues ethanol must pass through to vaporize externally will influence the rate of ethanol decline. Tree diameter influences these sinks in two ways. First, larger trees have greater internal tissue sink volumes for inward diffusion, and second, their outward diffusion paths to the atmosphere are longer, especially when bark thickness increases with diameter, as for ponderosa pine (van Mantgem and Schwartz 2003, Thies et al. 2013). Oak bark fissures, with shorter radial paths and thinner bark, released ethanol infused in the sapwood faster than the adjacent thick bark plate (Kelsey et al. 2013). Ethanol's stem diffusion rate, like O_2 (Sorz and Hietz 2006), may be dependent on the tissues' proportions of water and gas. Ethanol diffusion rates through stem tissues are rather slow (Kelsey et al. 2013) but are influenced by temperature. Postburn ambient temperature likely influences ethanol diffusion more than the transient and relatively short duration of high temperatures associated with the fire. In general, this dissipation mechanism is not influenced greatly by heat stress.

Sapflow, or transpiration stream transport. This mechanism moves ethanol more rapidly than diffusion after it enters functional sapwood tracheids (figure 2), as has been shown by flooding tree roots (Kreuzwieser et al. 2000). Because of its reliance on transpiration water flow, fire's impairment of water conductivity in foliage (Kelsey and Joseph 2003) or through xylem (Kavanagh et al. 2010, Michaletz et al. 2012) can negatively affect this dissipation mechanism. Sapflow movement of ethanol postburn will also be mitigated by changes in soil-water availability in response to seasonal precipitation or drought (Kelsey et al. 2014, 2016). If sapflow keeps ethanol concentrations in sapwood below those in surrounding cells or tissues, then this contributes to a strong sapwood sink strength and helps direct ethanol diffusion inward.

Metabolism. Ethanol levels are also reduced by metabolism (figure 2) into other primary cellular components, organic acids, amino acids, lipids, etc. (MacDonald and Kimmerer 1993) or acetaldehyde (Cojocariu et al. 2004, Tohmura et al. 2012). Metabolism will be active in any cells with sufficient O_2 to avoid heat stress in the 30°C–40°C range, and they may have higher enzyme activity rates than at lower temperatures. Cells experiencing predominately O_2-supply stress with limited injury can presumably return postburn to ambient temperature able to rapidly metabolize any resident ethanol within or any entering by diffusion. In contrast, tissues reaching 40°C to more than 55°C with some portion of their ethanol-metabolizing enzymes thermally deactivated will have slower metabolism after returning to postfire conditions, contributing to higher ethanol levels. Metabolism occurs in cytoplasm, so any ethanol hydrogen bonded to cell-wall polymers on the outside will be protected from immediate conversions. This reservoir probably has more impact on sapwood ethanol, with its high proportion of cell-wall and reduced nonliving-cell volume, than in phloem or cambium. Also, the proportions of phloem cells with full, partial, or no postfire metabolism activity will mitigate the amount of ethanol diffusing radially through them to the atmosphere. Consequently, this dissipation mechanism is the one most directly affected by the fire temperatures and types of heat stress the stem tissues experience.

Conclusions

Tree stems, branches, and roots typically have greater mass and thicker tissues than herbaceous plants, with an outer

Box 1. Heat-stress ethanol in tree stems is a link to additional stress from some bark beetles and pathogens.

Trees with nonlethal heat injuries may be subjected to additional stress from bark-beetle colonization (Parker et al. 2006, Fettig and McKelvey 2014) and symbiotic pathogens they vector into their hosts (Parker et al. 2006, Ploetz et al. 2013). The parameters influencing the bark-beetle host selection of fire-stressed trees have largely been a mystery, but for some, such as the red turpentine beetle (*Dendroctonus valens* LaConte), their initial dispersing pioneers are attracted to ethanol generated by heat stress when released in combination with host-tree monoterpenes (Kelsey and Westlind 2017). Attacks from *D. valens* contribute minimally to postfire mortality (Fettig and McKelvey 2014), but a portion of them vector pathogen spores (Schweigkofler et al. 2005, Taerum et al. 2013), with the potential to affect the health and resilience of those trees surviving both fire injury and beetle colonization. Some *D. valens* offspring emerging from diseased trees will continue vectoring the pathogen(s) into suitable host trees within the burned area or surrounding forest, perpetuating disease.

bark that can provide some protection from heat during a fire, especially those species that have coevolved in and adapted to fire-prone environments. When fire forces tree tissues into the 30°C–60°C range, there are physiological changes causing stress that they may or may not survive depending on exposure duration. Ethanol concentrations increase with injury levels in fire-damaged tree stems. Other studies demonstrate that ethanol induction is initiated by the impaired mitochondrial oxidative phosphorylation that reduces the energy supply required for all cellular processes. This information was used to propose physiological responses involving (a) O_2 supply, (b) membrane function, or (c) enzyme activity that may individually or in combination impair aerobic energy production and induce ethanol synthesis at sublethal temperatures. But ethanol-synthesis rates are also influenced by related physiological factors that can change seasonally, such as enzyme activity levels, among others. In addition to synthesis, tissue ethanol accumulation is dependent on its dissipation by diffusion, sapflow, and metabolism, which are also influenced by the types of heat stress the tissues and whole trees experience. These complex, interactive processes contribute to dynamic and variable ethanol concentrations in tree stems postburn and, although complicated, are useful in understanding sublethal heat stress. For example, tree-stem tissues injured in a June wildfire had ethanol concentrations at three or more orders of magnitude greater than those injured by an October prescribed fire (Kelsey and Westlind 2017). This was attributed primarily to disparate ethanol-synthesis rates caused by seasonal differences in their tissues' physiological status—in conjunction with different primary heat-stress mechanisms, membrane function for wildfire, and O_2 supply for prescribed fire—as a consequence of higher temperatures and longer exposure times for wildfire trees, as was indicated by their associated fire-injury levels. Finally, ethanol not only plays a central role in trees' physiological responses to sublethal temperatures, but it also contributes to their postburn ecological relationships by functioning as a link to some bark-beetle species and pathogens that can create additional stress (box 1).

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