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Ethanol and acetone from Douglas-fir roots stressed by *Phellinus sulphurascens* infection: Implications for detecting diseased trees and for beetle host selection

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ABSTRACT

Phellinus sulphurascens (previously the Douglas-fir form of *Phellinus weirii*) is an important native pathogen causing laminated root rot in forests of western North America. Visual crown symptoms, or attacks by bark or ambrosia beetles appear only during advanced stages of the disease with extensive infection in the lower bole. Ethanol synthesis is one of many physiological responses in tree tissues stressed by pathogens. Ethanol, acetone and other volatiles from root tissues of healthy and diseased trees were analyzed using headspace gas chromatography. Xylem and phloem from 20 diseased trees at two western Oregon sites contained higher concentrations of ethanol, acetone, or other headspace volatiles than 20 healthy trees on one or more dates in September, November, or the following May. Root cross-sections from eight diseased trees were sampled along perpendicular transects and found to contain extremely variable ethanol concentrations, with highest xylem quantities in a 0–2 cm zone outside the infection boundary and lowest amounts inside the infection. Acetone concentrations were the opposite. Logistic regression models were built and tested to determine which volatiles could predict diseased trees. A model using xylem ethanol concentrations as the only parameter was selected and validated with measurements from 80 trees on the edges of *P. sulphurascens* infection centers at two different western Oregon sites. This model successfully predicted trees with laminated root rot (78% overall correct classification and 68% for known diseased trees), but worked best for those with infections observed in both root cores and the root collar (100% correct). Early detection of *P. sulphurascens* infected trees remains a challenge. Our ethanol analysis method is useful for research, but provides limited benefits for identifying individual *P. sulphurascens* hazard trees, or for extensive ground surveys in the forest. Whether ethanol is released to the atmosphere in sufficient quantities to confirm infection before the late appearance of crown symptoms, or bark beetles remains unknown. If it is, then development of sensors capable of tree side detection requiring minimal tissue sampling would be useful in managing this disease. We also propose a mechanism for how ethanol with host monoterpenes could play a central role in pioneering bark beetle primary host selection of trees infected with this pathogen.

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1. Introduction

When trees are subjected to stress from various biotic or abiotic agents one of their many physiological responses may be ethanol synthesis if the stressed cells experience impaired aerobic respiration as shown when tissues are deprived of oxygen (Kelsey et al., 1998a; Joseph and Kelsey, 2004). This allows the cells to survive for brief periods until their O_2 supply is restored, and if not restored they die. Ethanol accumulation is dependent on the duration and rates of synthesis (Kelsey et al., 2011), its subsequent dissipation by diffusion as shown by adding it to tissues (Kelsey et al., 2013), movement in the transpiration stream (Kreuzwieser et al., 1999; Cojocariu et al., 2004), metabolism in non-stressed cells to other cellular components (MacDonald and Kimmerer, 1993), direct release to the atmosphere (MacDonald and Kimmerer, 1993; Kreuzwieser et al., 1999; Rottenberger et al., 2008; Ranger et al., 2013), or conversion to acetaldehyde that is released to the atmosphere (Cojocariu et al., 2004).

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Pathogens may be the most common biotic stress agents to induce ethanol synthesis in trees. For example, boles of lodgepole pine, *Pinus contorta* Douglas ex Loudon, with active decay fungi emit 2.4 times more ethanol to the atmosphere than those without the fungi (Gara et al., 1993). Elevated ethanol concentrations occur in stem tissues near the root collar of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco., infected with *Leptographium wageneri* var. *pseudotsugae* Harrington & Cobb, the cause of black stain root disease (Kelsey and Joseph, 1998). This was also observed in stem tissues of ponderosa pine, *Pinus ponderosa* Lawson & C. Lawson, infected with *L. wageneri* var. *ponderosum* (T.C. Harr. & F.W. Cobb) T.C. Harr. & F.W. Cobb, or its combination with *Heterobasidion irregular* Garbelotto & Otrosina, the cause of Heterobasidion root disease of pine (Kelsey et al., 1998b, 2006).

Ethanol can also accumulate within the boundaries of cankers caused by *Phytophthora ramorum* Werres, De Cock & Man in't Veld, the microbe responsible for sudden oak death, on the stems of coast live oak, *Quercus agrifolia* Nee, whereas concentrations in tissues outside the cankers and in adjacent healthy trees remain low (Kelsey et al., 2013). In the absence of any abiotic stressors, especially flooding as it induces synthesis in the roots and transport into the bole (Kreuzwieser et al., 1999; Cojocariu et al., 2004; Ranger et al., 2013), elevated ethanol concentrations in a tree can be a strong indicator of infection. However, it is important to note that low ethanol concentrations do not explicitly confirm the tree is disease free because of the many interacting factors influencing the rates of synthesis and subsequent dissipation. If ethanol escapes from stressed trees into the atmosphere in sufficient quantities and duration it can function, usually in combination with other volatiles released from the tree, as a signal that attracts various bark or ambrosia beetles to land and attack (Kelsey and Joseph, 2001, 2003; Kelsey et al., 2013, 2014; Ranger et al., 2013).

Other compounds occur with ethanol in the headspace analysis of tree tissues and their concentration changes associated with pathogen infections may also function as biomarkers for detecting disease. Acetaldehyde, acetone, methanol, and propanol were all more strongly correlated than ethanol with the severity of black stain and Heterobasidion root diseases in ponderosa pine (Kelsey et al., 1998b). Acetaldehyde was selected as the single best predictor of black stain disease, followed by acetone. In a related study, 284 ponderosa pine trees were randomly selected in stands where crown symptoms were unreliable for identifying diseased trees (Kelsey et al., 2006). In this case, sapwood acetone concentrations were selected as the best chemical headspace markers for predicting root disease. We suspected ethanol, or one of the other headspace compounds might also serve as a useful marker for earlier and more accurate detection of trees with laminated root rot caused by *Phellinus sulphurascens* Pilát (previously the Douglas-fir form of *P. weirii*).

P. sulphurascens infects various conifer hosts, with Douglas-fir, true firs and mountain hemlock being most susceptible (Thies and Sturrock, 1995). It spreads across root contacts between healthy and diseased trees or stumps, with ectotrophic mycelium transfers considered more important than endotrophic transfers (Bloomberg and Reynolds, 1982). Because of root-to-root spread, multiple trees often die near one another creating gaps in the forest canopy with standing dead and fallen trees. These gaps are easily recognized signatures of this pathogen that slowly expand outward in an uneven radial pattern at a rate of less than 50 cm per year (Nelson and Hartman, 1975; McCauley and Cook, 1980). However, some diseased trees also occur sporadically throughout a stand, unassociated with gaps. The infection mechanism for these trees is yet unknown and their detection is challenging (Thies and Nelson, 1997).

Distinct visual symptoms often do not appear in the crowns of infected trees (Wallis and Reynolds, 1965; Bloomberg and Wallis, 1979; Wallis and Bloomberg, 1981; Thies, 1983), and they are typically not attacked by bark beetles (Buckland et al., 1954; Lane and Goheen, 1979; Goheen and Hansen, 1993), until the infections reach advanced stages in the bole. Reduced annual height growth can be an early crown symptom for *P. sulphurascens* (Bloomberg and Wallis, 1979), but this change may be gradual, or variable, and difficult to recognize when an infected tree is among healthy ones whose height growth may also be impacted by competition, water deficits, or other stresses. Trees with healthy appearing crowns and no evidence of beetle colonization may be windthrown during storms exposing the broken roots weakened by decay. These trees are hazardous when growing in campgrounds, along road right-of-ways, or near homes. Their detection and removal is critical. Because of its combined economic and ecological importance in Washington forests, *P. sulphurascens* was recently chosen over other root pathogens as best suited for focused research directed toward improving applied management options (Cook et al., 2013). Their report cites methods for detecting infected stands and conducting ground surveys, but emphasizes the importance for further research to improve early detection, identification accuracy, and cost-effectiveness.

Objectives for the study we report here were to: (1) determine whether roots of Douglas-fir stressed by *P. sulphurascens* infections synthesize and accumulate higher ethanol concentrations than roots from uninfected trees as observed for other pathogens; (2) determine if there are differences in the quantities of acetaldehyde, acetone, methanol, or propanol detected during headspace analysis of ethanol between roots of infected and uninfected trees; and (3) attempt to develop a predictive model using these compounds to help identify trees infected with *P. sulphurascens* before the disease reaches an advanced stage.

2. Methods and materials

2.1. Study sites and tree selection

Four sites were utilized in this study; sites 1 and 2 were used to gather data for model building, while sites 3 and 4 were used to gather data for testing the model. Trees selected for model development were from two Douglas-fir stands near Philomath, Oregon. Site 1 was at 44.475461°, −123.430978° (202 m elev.) and site 2 at 44.545358°, −123.497525° (268 m elev.). At each site 10 tentatively diseased trees were selected and tagged as encountered when they fit the selection criteria of (1) being located on the perimeter of a *P. sulphurascens* infection center; (2) being a dominant or co-dominant tree; (3) having *P. sulphurascens* ectotrophic mycelium present on the bark surface of one or more partially excavated roots; and (4) having no evidence of bark or ambrosia beetle attacks. Visible crown symptoms for these trees were variable with some suggesting advanced infections. Rot or the characteristic stain from infection (Thies and Sturrock, 1995) was detected in at least one root from 13 of the 20 trees considered diseased. Ten putatively healthy trees were also selected and tagged as encountered when they fit the selection criteria of (1) being located well beyond root contact with trees on the infection center perimeter; (2) being a dominant or co-dominant tree; (3) having no ectotrophic mycelium on the bark surface of one or more partially excavated roots; and (4) having no evidence of bark or ambrosia beetle attacks. No rot or stain was observed in their sample cores on any dates. As described later, the phloem and xylem from root cores of these trees were analyzed for headspace volatile concentrations and the values were used to build a model for predicting whether trees at the sites below were likely infected with this pathogen.