

Science

FINDINGS

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issue one hundred seventy three / may 2015

“Science affects the way we think together.”

Lewis Thomas

What Do Cocktail Parties and Stressed Trees Have in Common? Plenty of Alcohol!



Rick Kelsey

Tree tissues produce ethanol when under many kinds of stress. Here, a researcher collects a core sample from a fire-scorched ponderosa pine.

“Here’s to alcohol, the cause of—and the answer to—all of life’s problems.”
—Matt Groening

All living things respond to stress. Humans produce adrenalin—the fight or flight hormone—and cortisol, which helps keep us fully alert and able to respond to stress for a prolonged period. When trees are stressed, they often produce ethanol. The stress may come from drought, disease, fire, or flooding, but the response mechanism is often the same. Over time,

some insects have developed the ability to detect ethanol, reading it as a signal that the tree is vulnerable and a prime target for attack.

Rick Kelsey, a research forester with the U.S. Forest Service Pacific Northwest Research Station has been studying trees’ response to stress for the past 25 years. “My work has been focused on understanding the commonality and interactions of these stress agents,” says Kelsey. “I’m slowly unraveling how the various stressors impact the trees, and this has provided the clues needed to understand their linkages with one another.”

IN SUMMARY

Stress in trees is caused by disturbances such as fire, flood, disease, or insect infestations. A single stressor may not be enough to kill a tree, but a combination can be deadly.

Tree tissues produce and accumulate ethanol in response to many stressors. Ethanol provides the stressed tissues with an emergency energy source when their normal source of energy from aerobic respiration is impaired by stressors. Many insects, including various bark and ambrosia beetles, can detect ethanol. If the stressed tree releases enough ethanol into the atmosphere, it serves as a signal, attracting the beetles and stimulating an attack.

Rick Kelsey, a research forester with the U.S. Forest Service Pacific Northwest Research Station, has spent his career studying the interplay between tree stress and ethanol production. His findings reveal the key role that ethanol plays in tree stress physiology and disturbance ecology.

Most recently, he and colleagues in Spain found that severely drought-stressed pine attacked by bark beetles contained more ethanol than their un-attacked neighbors.

Knowing the connections among stress, ethanol production, and insect host-tree selection may eventually help foresters identify stressed trees that are vulnerable to insect attack and develop remedial measures to help the trees survive.

When he started working on this topic, it was known that trees stressed by one agent such as fire, drought, or disease were more vulnerable to other stress agents, especially insects. But the reasons for this were not known. “Each of these stress agents impacted the trees by different mechanisms, and yet the outcome was generally the same: greater vulnerability to insect attacks. Why? How? It was a big mystery then, but less so now, at least for some insects,” Kelsey says.

Even though stressors affect trees in different ways (limited water during drought, heat from fire, cell damage by pathogens, etc.), there is a point at the cellular level where regardless of the stressor, the result is impaired aerobic respiration within the tree.

Respiration uses oxygen and sugars to generate energy and release carbon dioxide and water. If a tree doesn’t get enough oxygen, or cells are damaged and respiration can’t occur, then the cells quickly start to disintegrate. When animal muscles, including humans, run low on oxygen during periods of moderate or strenuous use, they synthesize and accumulate lactic acid until oxygen levels are restored. When tree tissues are stressed by lack of oxygen, they may start producing lactic acid, but then quickly switch to ethanol. This is an age-old metabolic pathway that has been maintained in tree tissues for millennia. It allows the tree’s cells to produce just enough energy to survive.

“It’s like a backup generator in a hospital,” Kelsey says. “It can’t go on forever, but it allows the cell tissues to endure short periods of low, or no oxygen.”

FOUNDATIONAL KNOWLEDGE

Early studies of ethanol in trees focused on the response of roots to flooding. Then in 1982, experiments with seedlings demonstrated that other stressors could cause tree tissues to accumulate ethanol. These responses were observed under carefully controlled greenhouse conditions. Kelsey explains, “It was unknown whether these same responses occurred in mature trees growing in their natural environments. And, if they did, what were the ecological consequences?”

Kelsey focused on answering these questions. Initially he spent a lot of time refining research methods and tools. One of his advancements was adapting instruments used by criminologists for measuring blood alcohol levels. He found that a method called headspace gas chromatography could be used to measure alcohol in tree tissues just as easily as in blood samples. To do so, scientists need a tissue sample, which for trees is obtained by using an increment borer. Back in the lab, the tissue is placed in a vial and heated. Then

KEY FINDINGS	
<ul style="list-style-type: none"> • When under certain kinds of stress, trees may produce ethanol. Stressors stimulating this response include severe drought, pathogens, fire, and flooding, among others. If the ethanol accumulates and escapes into the atmosphere, some insects can sense this as a signal from a weakened tree and attack it. 	
<ul style="list-style-type: none"> • In a recent study in Spain, researchers found that drought-stressed Aleppo pines (<i>Pinus halepensis</i>) attacked by Mediterranean pine shoot beetles contained higher ethanol concentrations than nearby trees that were not attacked by the beetles. In a previous study, Douglas-fir (<i>Pseudotsuga menziesii</i>), lodgepole pine (<i>Pinus contorta</i>), and ponderosa pine (<i>Pinus ponderosa</i>) seedlings also accumulated ethanol in their tissues when severely water stressed. 	
<ul style="list-style-type: none"> • Tree tissues stressed by heat during fire accumulate ethanol rapidly, and those containing the most ethanol attracted more scolytid beetles than trees with lower levels of ethanol. 	
<ul style="list-style-type: none"> • Cankers caused by the sudden oak death pathogen on the trunks of coast live oak (<i>Quercus agrifolia</i>) contain more ethanol than tissues outside the cankers. Scolytid beetles detect the ethanol and attack the cankers. The beetle tunnels and ensuing decay increase stem fracturing and shorten the tree’s life. 	

Synthesized ethanol moves into the tree’s internal water pool, and may move rapidly up the stem in the sapwood water column that supplies water to the needles or leaves. This keeps low ethanol concentrations inside the trees and allows healthy trees to survive brief periods of stress. However, if the ethanol accumulates and then escapes from the tree, it serves as a signal to insects that the tree is stressed.

Through co-evolution, some bark- and wood-boring beetles (scolytids) have developed an

ability to detect ethanol and use it as a primary attractant to find a stressed host. This is typically the behavior of nonaggressive beetle species that colonize weakened, dying, or recently dead trees and not the aggressive beetles, such as the mountain pine beetle and others that attack and kill more vigorous trees. The ethanol-sensitive insects are opportunists: they use ethanol to find weakened trees that may not be capable of producing as many defensive chemicals, such as oleoresins, to keep them away.



Rick Kelsey

The phloem and sapwood from this increment core will be immediately frozen and returned to the laboratory where its ethanol content will be measured and compared to samples from trees not burned.

an air sample is taken from the vial and the volatile compounds within it are analyzed. This method allows many samples to be processed quickly, accurately, and with minimal preparation.

Purpose of PNW Science Findings

To provide scientific information to people who make and influence decisions about managing land.

PNW Science Findings is published monthly by:

Pacific Northwest Research Station
USDA Forest Service
P.O. Box 3890
Portland, Oregon 97208

Send new subscriptions and change of address information to:

pnw_pnwpubs@fs.fed.us

Rhonda Mazza, editor; rmazza@fs.fed.us

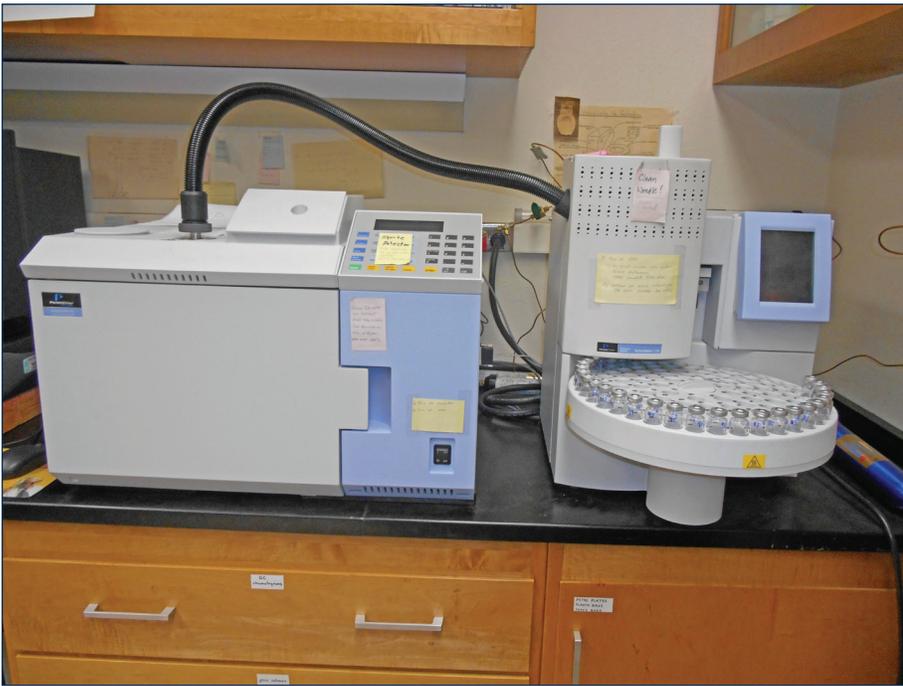
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Rick Kelsey

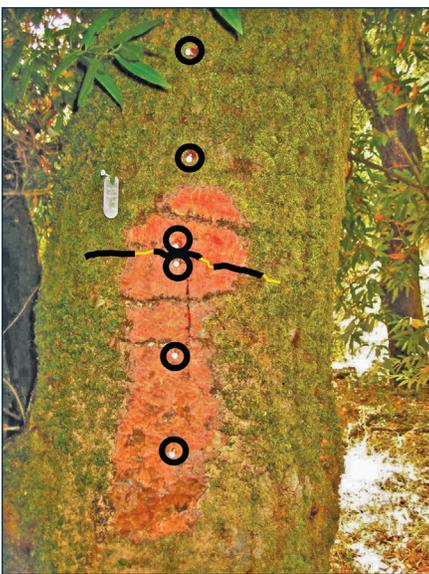
Tree tissues are placed into vials and analyzed for ethanol by using the headspace autosampler (right) and gas chromatograph (left).

Kelsey applied this technique to a study in 2003 that examined ethanol levels in fire-damaged trees. He measured the ethanol and water content in ponderosa pines 2 weeks after a wildfire and found that the quantity of ethanol increased with each level of injury. Trees that were severely scorched contained as much as 53 times more ethanol in their phloem and sapwood than pines that were not burned. The heat-stressed trees also attracted

substantially more beetles than unburned trees the following spring.

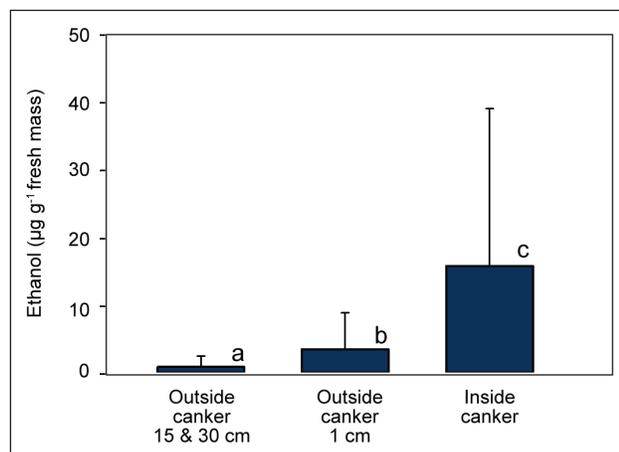
Kelsey and his research team have also documented that disease can trigger ethanol production. In 2013 they analyzed the sapwood of coastal live oak trees in California that were infected with *Phytophthora ramorum*, the plant pathogen that causes sudden oak death. The disease has killed millions of oak trees since the mid-1990s, according to the California Oak Mortality Task Force.

The pathogen also causes twig and foliar diseases in numerous other plant species, including California bay laurel (*Umbellularia californica*), Douglas-fir, and coast redwoods (*Sequoia sempervirens*). The few control



Rick Kelsey

This coast live oak tree is infected with *Phytophthora ramorum*, which causes sudden oak death. Researchers took core samples at varying distances from the upper border (dashed line) of a large basal canker and analyzed the ethanol content in each sample.



Median sapwood ethanol concentrations inside and outside *Phytophthora ramorum* cankers on coast live oak (\pm 95% confidence interval). Medians with different letters are significantly different at $P < 0.05$.

mechanisms that exist for the disease rely on early detection and proper disposal of infected plant material.

One symptom of the disease in coast live oak is the presence of bleeding cankers on the tree's trunk. Kelsey's team found that trees with large basal cankers had high levels of ethanol, and that the cankers themselves attracted bark and ambrosia beetles. As in other trees stressed by drought or fire, the beetles smell the ethanol and invade the tree, which contributes to branch or stem failure, and hasten its death.

Kelsey points out, however, that not all pathogens lead to increased ethanol production. Swiss needle cast, a fungal disease affecting Douglas-fir in the Pacific Northwest, causes trees to shed their needles prematurely, resulting in sparse crowns and reduced growth. But Douglas-fir infected with Swiss needle cast do not accumulate ethanol in their stems. "The dogma that if you have stress, you're going to have a beetle attack is not necessarily true," Kelsey says. "If ethanol is present in excessive amounts, you know there's a potential problem. But if there's no ethanol, you don't always know for sure that you *don't* have a problem," he explains.

Kelsey has conducted several studies exploring the connection between ethanol production and drought stress. The first was a controlled experiment using ponderosa pine, lodgepole pine, and Douglas-fir seedlings. The three species were chosen for their relative drought tolerance: ponderosa pine is the most tolerant, followed by lodgepole pine, while Douglas-fir is the least tolerant of the three.

The seedlings were planted under controlled conditions in a greenhouse at Oregon State University so the scientists could carefully regulate the amount of water the seedlings received. They induced water stress in the trees, and each species reacted differently according to their innate levels of drought tolerance. Ponderosa pine produced the least ethanol while the Douglas-fir seedlings produced the most. Kelsey hypothesized that the ethanol was a result of heat injury brought on by each tree's waning ability to cool itself when it started running low on water. Whether ethanol accumulates in mature trees of these species during severe, natural drought remains to be demonstrated.

In 2012, near the end of a severe drought in Spain, Kelsey and colleagues gathered samples

from water-stressed Aleppo pines in an effort to link drought with ethanol production and beetle host selection. They examined levels of ethanol, water, and hydrocarbons in the phloem and sapwood of trees freshly attacked by Mediterranean pine shoot beetles. They knew from previous experiments that these beetles were attracted to traps baited with ethanol.

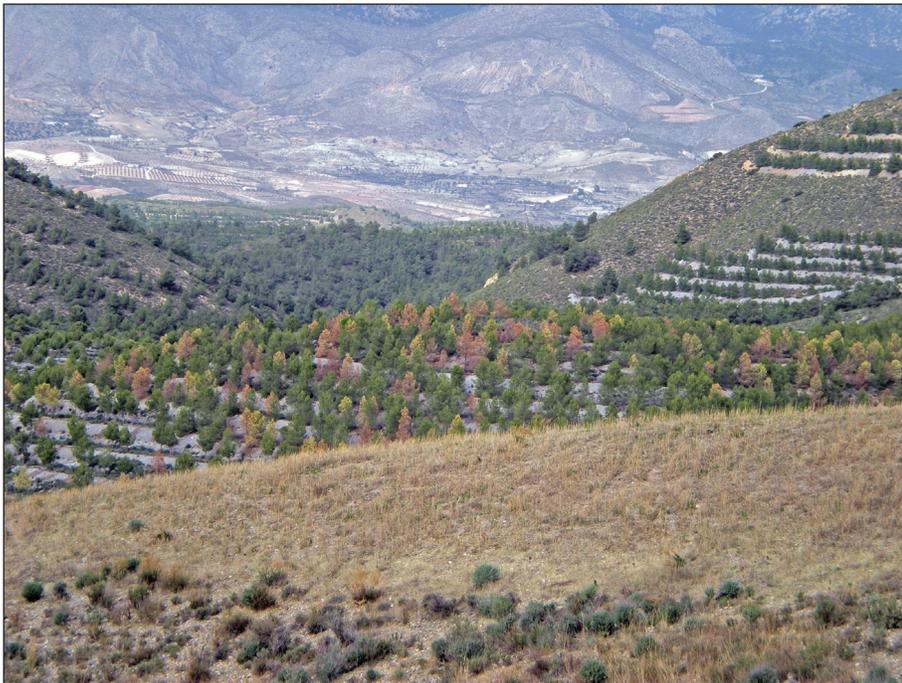
The scientists also took samples from neighboring trees that had not been attacked by the beetles. They found that the attacked trees were more water stressed and contained more than twice the amount of ethanol as those not attacked. Kelsey and his colleagues hypothesize that reduced sap flow in the tissues of severely water-stressed trees slowed the

dissipation and metabolism of ethanol, resulting in higher concentrations within the trees for longer periods.

Interestingly, the beetles didn't seem to notice these pines until after a day of heavy rain in September. The rain was unusually early: typically the beetles start attacking the Aleppo pines later in the fall when the seasonal drought ends sometime in October, November, or December. Kelsey conjectures that the rainfall may somehow influence the release of ethanol from the stressed trees, which then attracts the beetles.

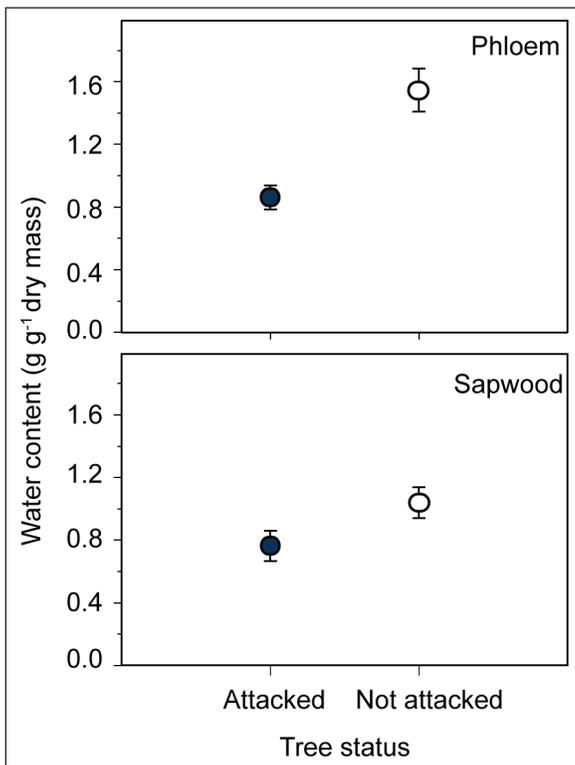
This study in Spain is the first to show a direct correlation between drought-stressed trees and ethanol accumulation. "It suggests that measuring the ethanol in severely water-stressed trees may allow early detection of those most vulnerable to bark beetle attack, but a lot more work is required to confirm this assumption," Kelsey says.

For example, when does ethanol begin to accumulate in drought-stressed trees? Is there a measurable threshold? How far in advance of an attack can the ethanol be detected? Do all tree species show the same response? Answering these types of questions would go a long way toward developing a management tool using ethanol measurements.

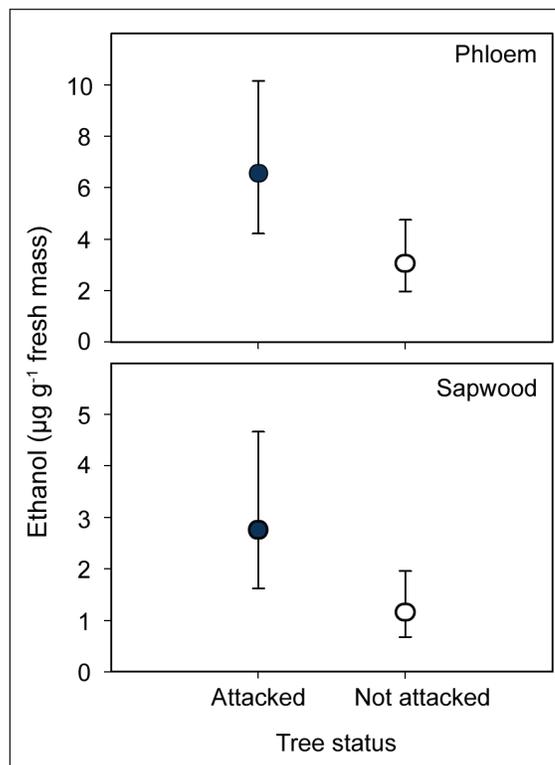


Diego Gallego

Drought-stressed pine in Spain under attack by bark beetles. Scientists found a correlation between drought-stressed Aleppo pine and ethanol accumulation.



Water content (95% confidence interval) in Aleppo pine attacked by Mediterranean pine shoot beetles compared with adjacent trees that were not attacked. Values are statistically different at $P < 0.001$ in both graphs.



Ethanol (95% confidence interval) concentrations in the phloem and sapwood of Aleppo pine attacked by Mediterranean pine shoot beetles, compared with neighboring trees that were not attacked. Values are statistically different at $P \leq 0.029$ for both tissues.

A CUE FOR MANAGEMENT?

“The findings from these various studies shed light on the chemical ecology behind stress-related bark beetle attacks and lay the groundwork for future efforts to manage stressed trees,” Kelsey explains. They provide a mechanistic explanation of how stress agents interact to injure or kill a tree. This knowledge may become increasingly useful to forest managers in the coming decades as the world’s climate continues to change and stresses such as drought and fire become more common.

Ethanol monitoring could potentially quantify the levels of stress that individual trees or stands are experiencing. Foresters could then target appropriate treatments where they would have the greatest impact and likelihood of success. However, currently there is no easy way to find stressed trees that are producing and accumulating ethanol. But just as technological advances made alcohol breathalyzer tests routine, new technological advancements may make ethanol monitoring in trees just as common. For example, scientists at the Massachusetts Institute of Technology recently developed sensors that can detect various volatile compounds by using a cell phone application. Feasibly, a handheld device might

 LAND MANAGEMENT IMPLICATIONS 
<ul style="list-style-type: none">• Maximizing tree health and vigor is essential for maintaining resilient forest ecosystems and strong resistance to stress and disturbance. Ethanol monitoring someday may be used to quantify the levels of stress that individual trees or stands are experiencing.
<ul style="list-style-type: none">• Identifying trees or stands under the most stress would allow managers to implement treatments in areas where they would have the greatest impact and likelihood of success.
<ul style="list-style-type: none">• Tree improvement through molecular technology may focus on modifying the physiological stress response in tree tissues to minimize the accumulation of ethanol without reducing its ability to help tree tissue survive periods of stress.

be developed for forest health specialists to take into the woods and monitor tree stress.

Down the road, the basic science that Kelsey and his colleagues have conducted could also contribute to tree improvement program where geneticists use molecular technology to modify the physiological stress response in tree tissues. Molecular biologists might develop trees that accumulate minimal ethanol, thus dimming the signals to ethanol-sensing beetles, but without reducing the ability of ethanol to help tree tissue survive periods of stress.

This is foundational knowledge about tree physiology and response to stress, essential for

developing remedial treatments for stressed trees. “We’re unlocking the interactions among these stress agents,” Kelsey explains. “There is no longer as much mystery surrounding tree stress, and that’s going to help us understand possible ways to use this information for better forest management.”

Mystery creates wonder and wonder is the basis of man's desire to understand.

—Neil Armstrong

FOR FURTHER READING

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SCIENTIST PROFILE



RICK KELSEY is a research forester with the Pacific Northwest Research Station. His research focuses on understanding the complex interactions between trees

and the biotic (insects, pathogens) and abiotic (fire, drought, etc.) agents that cause them stress. His work is unraveling the mystery of why trees stressed by one agent, such as fire or a pathogen, become more susceptible to other agents, such as insects. His other research is directed toward discovering compounds in tree tissues with biological activity and understanding their ecological importance. Kelsey received his Ph.D. in forestry from the University of Montana.

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