NRC Research Press

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Ethanol accumulation during severe drought may signal tree vulnerability to detection and attack by bark beetles

Rick G. Kelsey, D. Gallego, F.J. Sánchez-García, and J.A. Pajares

Abstract: Tree mortality from temperature-driven drought is occurring in forests around the world, often in conjunction with bark beetle outbreaks when carbon allocation to tree defense declines. Physiological metrics for detecting stressed trees with enhanced vulnerability prior to bark beetle attacks remain elusive. Ethanol, water, monoterpene concentrations, and composition were examined in the phloem and sapwood of drought-stressed Aleppo pine (*Pinus halepensis* Mill.) freshly attacked by mature Mediterranean pine shoot beetles (*Tomicus destruens* (Wollaston, 1865)) and in neighboring unattacked trees. The attacked trees were more water-stressed and contained, on average, 2.1 and 2.4 times more ethanol in the phloem and sapwood, respectively, than the neighboring attack-free trees. This response is consistent with the known attraction of *T. destruens* to ethanol. Most monoterpene concentrations in the phloem, but not sapwood, were greater in tissues of attacked trees, whereas compositional differences were minor between the two tree groups for both tissues. Tissue water content explained much of the variation in phloem monoterpene concentrations, which increased as water in the phloem declined, suggesting that higher constitutive quantities existed in the more stressed trees prior to the attacks. Monoterpenes may have contributed to host tree selection by *T. destruens*, but their potential influence is considered less important than that of ethanol based on beetle responses to these compounds in previous trapping studies. This is the first report of elevated ethanol concentrations in tissues of trees experiencing natural drought stress and suggests that ethanol measurements in severely water-stressed trees may allow early detection of those most vulnerable to bark beetle attack.

Key words: water stress, ethanol, *Tomicus destruens*, *Pinus halepensis*, host selection.

Résumé : La mortalité chez les arbres due à la sécheresse causée par la température se manifeste dans les forêts partout dans le monde, souvent conjointement avec les épidémies de scolytes, lorsque la quantité de carbone alloué aux mécanismes de défense diminue. Les métriques physiologiques qui permettraient de détecter les arbres stressés les plus vulnérables avant qu'ils soient attaqués par les scolytes demeurent nébuleuses. La composition et les concentrations en éthanol, eau et monoterpènes ont été examinées dans le phloème et le bois d'aubier de pins d'Alep (*Pinus halepensis* Mill.) stressés par la sécheresse qui venaient juste de subir l'attaque d'hylésines du pin (*Tomicus destruens* Wollaston, 1865) matures en Méditerranée et d'arbres voisins qui n'avaient pas été attaqués. Le stress hydrique était plus prononcé chez les arbres attaqués qui contenaient en moyenne respectivement 2,1 et 2,4 fois plus d'éthanol dans le phloème et le bois d'aubier que les arbres voisins qui n'avaient pas été attaqués. Cette réaction est cohérente avec l'attraction qu'exerce l'éthanol sur *T. destruens*. La concentration de la plupart des monoterpènes dans le phloème, mais pas dans le bois d'aubier, était plus élevée dans les tissus des arbres attaqués tandis que les différences de composition en monoterpènes entre les deux groupes d'arbres étaient mineures quel que soit le tissu. La teneur en eau des tissus expliquait la majeure partie de la variation de la concentration des monoterpènes présents dans le phloème laquelle augmentait à mesure que la teneur en eau du phloème diminuait, ce qui indique qu'une plus grande quantité de monoterpènes constitutifs étaient présents dans les arbres plus stressés avant les attaques. Les monoterpènes peuvent avoir contribué au choix des arbres hôtes par *T. destruens* mais on considère que leur influence potentielle est moins importante que celle de l'éthanol sur la base des réactions de l'insecte à ces composés dans le cadre de précédentes études de piégeage. Il s'agit de la première mention de concentrations élevées d'éthanol dans les tissus des arbres qui subissent un stress dû à une sécheresse naturelle. Ces résultats indiquent qu'il serait peut-être possible de détecter précocement les individus les plus vulnérables à une attaque de scolytes en mesurant la quantité d'éthanol présente chez les arbres soumis à un stress hydrique sévère. [Traduit par la Rédaction]

Mots-clés : stress hydrique, éthanol, *Tomicus destruens*, *Pinus halepensis*, choix de l'hôte.

Introduction

Drought stress from temperature-driven increases in water deficits, alone or in combination with biotic agents, is contributing to increased mortality rates at various scales in forests around the world (Allen et al. 2010; Williams et al. 2013). Drought-related mortality results from hydraulic failure, carbon starvation, or biotic agents involving complex, interdependent interactions among these processes prior to death (McDowell et al. 2008; Sala et al. 2012; McDowell 2011; Plaut et al. 2012; Adams et al. 2013; Anderegg and Anderegg 2013). Water stress limits hydraulic functions, which can impair the production, transport, or availability of nonstructural carbohydrates (NSC) needed to sustain critical metabolic processes, including the hydraulic functions (McDowell et al. 2011). However, these stressed trees are often attacked or

Received 17 October 2013. Accepted 19 February 2014.

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killed by insects or pathogens when the reduced availability of NSC limits their production of defense compounds to protect against these biotic agents (Mattson and Haack 1987; Herms and Mattson 1992; Desprez-Loustau et al. 2006; Allen et al. 2010; McDowell 2011; McDowell et al. 2011; Gaylord et al. 2013). For example, *Pinus ponderosa* Douglas ex C. Lawson that survive both drought and bark beetle attacks have invested more of their internal carbon resources into chemical defense by producing larger resin ducts, at higher density, and covering a larger area of the current growth ring than those trees that died (Kane and Kolb 2010). Water-stressed *Pinus edulis* Engelm. experienced greater bark beetle attack densities and cumulative mortality than the less stressed trees. They also produced fewer resin ducts, at lower densities, that covered a smaller portion of the growth ring area than in less stressed trees, although the differences in resin duct parameters did not become evident until the third year of water stress (Gaylord et al. 2013).

Drought duration and intensity will influence how the NSC are distributed between growth and defense (Herms and Mattson 1992; McDowell et al. 2008, 2011; McDowell 2011), but measurements of changes in defense to detect and predict the vulnerability of water-stressed trees to bark beetle attack remain elusive (Gaylord et al. 2007, 2013). In *P. edulis*, twig resin flow did not differ between trees that were attacked by twig beetles (*Pityophthorus opaculus* LeConte, 1878) and those that remained unattacked (Gaylord et al. 2013). Water potentials and other hydraulic metrics are accurate measures for monitoring the severity of tree water stress (Breshears et al. 2009; Klein et al. 2011; McDowell et al. 2008; Plaut et al. 2012; Anderegg and Anderegg 2013), but they have limitations for predicting the trees' vulnerability to bark beetles, except at a coarse level. For example, *P. edulis* experienced eight or more months of continuous water stress, during a 10-month drought in the southwestern US before *Ips confusus* (LeConte) initiated their attacks (Breshears et al. 2009), and in a subsequent study, no differences in water potentials were detected between the *I. confusus* attacked and unattacked trees (Gaylord et al. 2013). Reduced growth from drought, high stand density, or their combination is another parameter that can be used to predict stands or trees at risk of attack, as shown for outbreaks of the roundheaded pine beetle (*Dendroctonus adjunctus* Blandford) in *P. ponderosa* (Negrón 1997; Negrón et al. 2000; Fischer et al. 2010). However, growth may be reduced for many years before the trees are attacked. Thus, there remains a need for other parameters that can help detect when tree vulnerability to bark beetle attack changes in response to water stress.

Ethanol synthesis and accumulation is another physiological response of tree tissues subjected to water stress, but the process is poorly understood and information is derived primarily from experimentally induced stress rather than natural drought. Under greenhouse conditions, wilted *Betula papyrifera* Marsh. leaves with a water potential of –2.5 MPa contained 296 nmol ethanol·g dry mass^{-1}, whereas less stressed leaves at –1.3 MPa contained none (Kimmerer and Kozlowski 1982). About three weeks after breaking the water column in individual branches on mature Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), ethanol concentrations increased 182 times in the current-year needles and over 1000 times in woody tissues compared with the untreated controls (Kelsey and Joseph 2001). Heitz et al. (2005) stopped water flow in the stem of a single *Picea abies* (L.) Karst. in mid-August by severing sapwood tracheids, and ethanol was detected in volatile emissions trapped at the bark surface 23 days later. Manter and Kelsey (2008) report ethanol concentrations 1.7 to 2.8 times higher in the phloem and 4.1 to 6.2 times higher in the sapwood of three severely water-stressed (predawn Ψ, –3 to –4 MPa) conifer species than in the corresponding tissues from seedling that received high and low water treatments (Ψ, –0.48 or –0.85 MPa, respectively). Stem tissues from stressed *P. menziesii*, the least drought-tolerant species, contained the highest quantities of ethanol, while the same tissues from stressed *P. ponderosa*, the most drought-tolerant species, had the lowest ethanol levels (Manter and Kelsey 2008).

Ethanol has no role in tree defense, but it does function as a kairomone that attracts various bark and ambrosia beetle species (Joseph et al. 2001; Kelsey and Joseph 2001, 2003; Coyle et al. 2005; Gallego et al. 2008; Miller and Rabaglia 2009; Ranger et al. 2011; Kelsey et al. 2013). In addition, the release of ethanol in combination with host terpenoids or pheromones, or all three together, can synergize the attraction of various beetle species (Pitman et al. 1975; Schroeder and Lindelöw 1989; Gallego et al. 2008; Miller and Rabaglia 2009; Lingren et al. 2012). With the potential for ethanol to accumulate in water-stressed trees and the likelihood of increased frequency and intensity of drought in various regions of the world (Sheffield and Wood 2008), it is important to know if increases in ethanol correspond with changes in tree vulnerability to attack by bark beetles.

The Mediterranean pine shoot beetle (*Tomicus destruens* (Wollaston, 1865)) is a circum-Mediterranean and Macaronesian bark beetle (Wood and Bright 1992), occurring also in the mild northern coast of Spain. It is associated with declining Mediterranean pine forests where trees weakened by extended drought are more vulnerable to attack than less stressed individuals (Nanni and Tiberi 1997; Branco et al. 2010). Host species typically include *Pinus halepensis* Mill., *Pinus pinaster* Aiton, *Pinus pinea* L., and *Pinus brutia* Ten., and occasionally, it attacks plantations of introduced Monterey pine (*Pinus radiata* D. Don) in Spain (Gallego et al. 2004; Horn et al. 2006). In autumn, reproductively mature adults emerge and disperse, after an extended period of summer maturation feeding in shoots, to attack and colonize stems of stressed or dying trees and recently cut stumps or logs (Gil and Pajares 1986), which also accumulate ethanol (von Sydow and Birgersson 1997; Kelsey and Joseph 1999). Gallego et al. (2008) found that traps baited with (–)-α-pinene in autumn and winter attracted mature male and female *T. destruens*, but the numbers were generally low at release rates ranging from 30 to 900 mg·day^{-1}. When they released ethanol with α-pinene, the trap catches increased, and synergistic beetle responses were detected at 300 mg α-pinene·day^{-1} with 450, 900, 1350, or 1800 mg ethanol·day^{-1}. However, traps baited with ethanol alone (1350 mg·day^{-1}) also attracted a high number of mature beetles that were not statistically different from the best α-pinene–ethanol blend. This pointed to ethanol as a main kairomonal signal for this species. Recently, Martin et al. (2013) confirmed this high beetle response to ethanol, with increasing numbers captured at release rates of 400, 800, and 2000 mg·day^{-1} and then declining at 3000 and 4000 mg·day^{-1}. However, the combined release of 300 mg·day^{-1} of α-pinene did not resulted in increased catches for any of the ethanol rates tested.

From this information, we hypothesized that ethanol accumulates in the stems of drought-stressed Mediterranean pines in sufficient quantities to influence host tree selection by mature *T. destruens*. Temperatures and drought have increased in the Mediterranean over the last century, with further increases expected in this region and other parts of the world with future climate change (Alpert et al. 2008; Sheffield and Wood 2008). Much of the Iberian Peninsula was under the influence of drought in 2011 and 2012, creating suitable conditions for testing our hypothesis. The main objective of this project was to determine whether *P. halepensis* trees freshly attacked by mature *T. destruens* in the fall, after an extended drought, contained higher ethanol concentrations than neighboring trees that remained attack free. The second objective was to examine the monoterpene concentrations in the same trees.

Materials and methods

Study site and tree sampling

The study site was located southwest of Puerto Lumbreras, Murcia (SE Spain, 37.53401°N, –1.90097°W, elevation 930 m) in a plan-

tation of *P. halepensis* of about 40 years age growing on hilly terrain (20%–30% slope). The soils are siliceous Palaeozoic (lithosol type), bed rock schist. Precipitation values for this area covering the previous decade were obtained from the Murcian Agrometeorological network (SIAM; http://siam.imida.es), Pozohiguera weather station, located approximately16.3 km southeast of our site, but 600 m lower in elevation. Our study site would have received more precipitation during each rainfall event, but the weather station values still provide a reasonable indication of the precipitation shortfall in the immediate area.

Seven trees with fresh *Tomicus destruens* attacks, estimated at 1 to 3 days old, were selected for sampling as they were encountered in the plantation on 18 October 2012. The nearest tree of similar diameter that was not attacked was also selected for sampling to minimize size (11.2 and 11.4 cm diameter for attacked and unattacked trees, respectively; $F_{[1,12]} = 0.02$, $P = 0.879$) and microenvironment differences between the two groups. Two new gallery entrance holes, without regard to aspect, were selected on the stems of six attacked trees at 30 to 154 cm above the soil surface. Just one gallery hole was selected on a seventh tree. At each gallery, one tissue core was removed with an increment borer (5 mm diameter × 2.0 cm sapwood depth) at 5 cm above, below, right, and left of the entrance hole for a total of four. Each neighboring, unattacked tree was sampled at the same aspect and distance from the soil surface. After discarding the outer bark, each core was sealed in a vial with PTFE-lined caps (Supleco, Bellefante, Pennsylvania, USA) and immediately frozen with dry ice for transport to the laboratory where they were stored frozen. On the following day, the phloem and sapwood were separated and sealed in preweighed, 20 mL headspace vials (PerkinElmer, Waltham, Massachusetts, USA), with a PTFE-lined silicone septa (Agilent Technologies, Santa Clara, California, USA) inside an aluminum crimp cap (PerkinElmer). Sealed vials were held on ice until all were processed and then were heated at 102° for 40 min to prevent further ethanol synthesis. Room-temperature vials were reweighed to provide tissue fresh mass.

Quantifying ethanol, monoterpenes, and water content

Headspace analysis was performed with a PerkinElmer Turbomatrix 110 headspace autosampler attached to a PerkinElmer Autosystem XL gas chromatograph (GC) with an FID detector. The injector and detector were set at 200 °C and 250 °C, respectively. Temperature settings on the headspace autosampler were 110 °C, 102 °C, and 100 °C for the transfer line, needle, and vial oven, respectively. Vials were heated for 30 min, with a 4.0 min preinjection pressurization, 0.04 min injection, and 0.1 min needle withdrawal time. The column used was a DBWAX, 30 m × 0.32 mm i.d., with 0.25 μm film thickness (J&W Scientific from Agilent). The column oven was held at 50 °C for 2.4 min, then increased to 120 °C at 45 °C·min^{-1} with a 0.5 min hold, and then returned to 50 °C where it remained for a total run time of 7.46 min. Helium was the carrier gas, with 128.9 kPa pressure at the autosampler and a constant column flow of 2.0 mL·min^{-1} throughout the run.

For ethanol analysis, one of the two beetle gallery holes from each attacked tree was randomly selected, and the phloem and sapwood tissues from the associated cores were separately analyzed by the multiple headspace technique. In this procedure, each sample is analyzed as described above, immediately vented (20 s), cooled to room temperature, and then analyzed a second time. This process accounts for the tissue matrix influence on the headspace concentrations. Tissue samples from the same stem positions on the nonattacked trees were analyzed in the same way. Ethanol concentrations were determined by the external standard method with four vials each containing 5 μL of an aqueous ethanol solution (prepared with deionized water and 100% ethanol; Pharmco-Aaper, Shelbyville, Kentucky, USA) representing one level of a standard curve analyzed with each set of samples. Ethanol concentrations were normalized to micrograms per gram fresh mass because of its strong bond with water, which more accurately represents what beetles encounter when chewing into the tissue.

Monoterpene concentrations were analyzed in tissue samples associated with the remaining beetle gallery hole from each tree by the static headspace technique, where the sample is analyzed just once as described. This was necessary because of the larger sapwood sample and its high α-pinene content. These values still accurately represent the relative concentrations, but without tissue matrix adjustments. Terpenes were quantified by the external standard method using relative response factors (RRF) to ethanol, allowing their mass to be determined from an ethanol standard curve. Separate RRFs were determined for each terpene (diluted in methanol) by comparing their responses with ethanol (diluted in water) using solutions containing known weights of each component. A four-level standard curve (each vial containing 5 μL of an aqueous ethanol standard) was analyzed with each set of phloem samples, while a three-level curve was used for each set of sapwood samples. The monoterpenes were identified by comparison of their retention times with those of known standards in the same manner as others (Tognetti et al. 1997; Michelozzi et al. 2008). Their concentrations were also normalized to micrograms per gram fresh mass for consistency with ethanol.

Tissue dry mass and water content were determined after headspace analysis by drying each sample at 102 °C for 16 h, cooling to room temperature in a desiccator box, and reweighing. The water content is calculated as grams of water per gram dry mass.

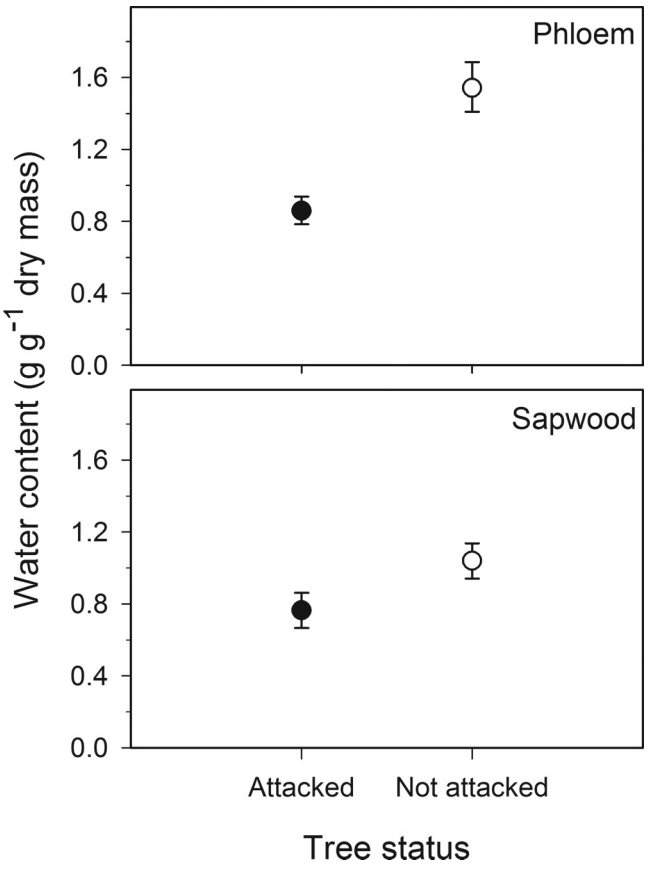
Data analysis

The phloem and sapwood data were analyzed separately by ANOVA with 14 trees, seven attacked by *T. destruens* and seven nearby trees that were not attacked (MIXED procedure SAS 9.3; SAS Institute Inc. 2010). Ethanol was quantified in the phloem and sapwood of all 14 trees (including the two with one core only), whereas monoterpenes were measured in both tissues of 12 trees (only trees with two cores). The response variables compared between the attacked and unattacked trees were the mean ethanol concentrations among the four cores analyzed for ethanol at each gallery hole (hereafter referred to as the ethanol concentration), mean individual and total monoterpene concentrations, and mean percent individual compound composition across all four cores at each gallery hole analyzed for terpenes, mean water content across all eight cores from a tree, and stem diameters. Regression analysis (GLM procedure SAS 9.3; SAS Institute Inc. 2010) was used to evaluate the relationship of ethanol and monoterpene concentrations with tissue water contents. During analysis, model assumptions of normality and equal variance were checked using normal probability and residuals plots (residuals vs. fitted values), respectively. Data sets out of compliance were natural logarithm transformed to meet these assumptions and then analyzed. Transformed means and 95% confidence intervals (CI) were back-transformed for presentation. Differences were considered statistically significant at $\alpha = 0.05$.

Results

The attacked trees experienced greater water stress than the unattacked trees (Fig. 1). Their phloem water content was 0.86 g·g dry mass^{-1} (95% CI, 0.49–0.63), or 55.8% of the amount in the unattacked trees ($F_{[1,12]} = 104.11$, $P < 0.001$), whereas their sapwood contained 0.76 g·g dry mass^{-1} (95% CI, 0.64–0.83) of water, which was 73.4% of the amount in trees remaining attack free ($F_{[1,12]} = 18.86$, $P = 0.001$). The cumulative precipitation during the preceding 12-month period (October 2011 – September 2012, excluding the 1-day 118.0 mm rain event on 28 September) was only 125.2 mm compared with 262.1, 377.2, and 367.2 mm for the same periods from the previous 3 years, respectively, or a 52.2%–66.8% reduction.

Fig. 1. Water content (95% CI) in the phloem and sapwood of *Pinus halepensis* attacked by *Tomicus destruens* compared with adjacent trees that were not attacked. Values are statistically different at $P < 0.001$ in both graphs.

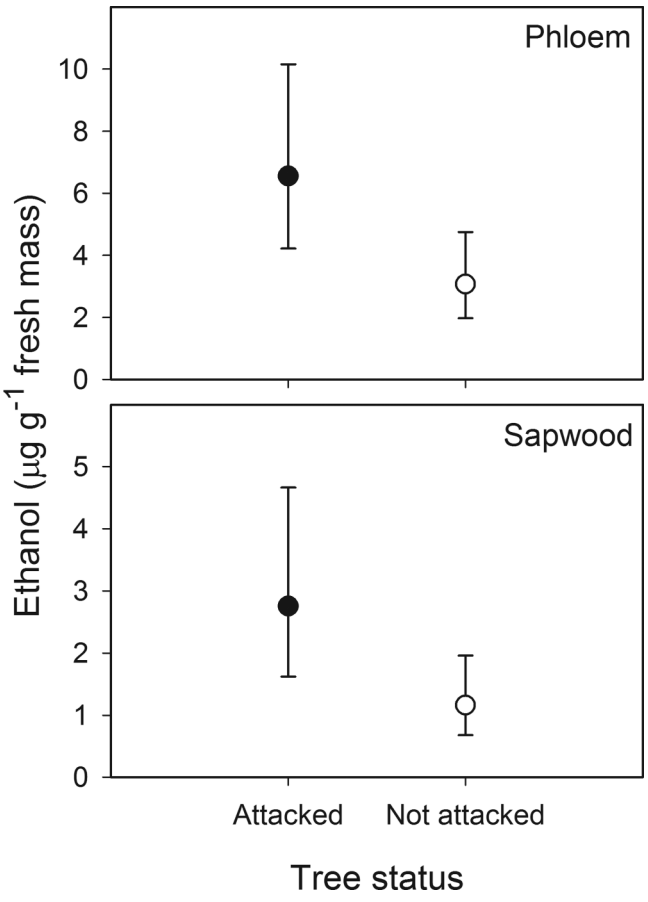


Ethanol concentrations were greater in the tissues of attacked trees than in those remaining attack free (Fig. 2). The phloem in attacked trees contained 6.6 μg ethanol·g fresh mass^{-1}, which was 2.1 (95% CI, 1.1 to 4.0) times the amount in the phloem of trees without attacks ($F_{[1,12]} = 7.10$, $P = 0.021$). Sapwood ethanol concentrations were about half of the amount in the phloem (Fig. 2), but the same pattern persisted, with attacked trees containing 2.8 μg ethanol·g fresh mass^{-1}, which was 2.4 (95% CI, 1.1 to 5.0) times greater than in the trees remaining attack free ($F_{[1,12]} = 6.35$, $P = 0.027$). Ethanol concentrations were negatively related to the tissue water contents in both phloem ($F_{[1,12]} = 7.91$, $P = 0.016$) and sapwood ($F_{[1,12]} = 8.62$, $P = 0.013$), with $r^2 = 0.397$ and 0.418, respectively.

α-Pinene was the dominant monoterpene in both phloem and sapwood (Table 1), with concentrations 2.2 (95% CI, 1.6–3.0) times higher in the phloem of attacked trees than those free from *T. destruens* ($F_{[1,10]} = 30.56$, $P < 0.001$). Concentrations of all of the minor monoterpenes were also significantly higher in the phloem of attacked trees, except for Δ-3-carene and cineole (Table 1). Regression analysis of phloem monoterpene concentrations indicated that the totals and all individual compounds were negatively related to the tissue water contents (all P values ≤ 0.002), except 3-carene and cineole, with r^2 values ranging from a low of 0.638 for myrcene to a high of 0.851 for limonene. The percentage composition of the phloem monoterpenes all differed by less than 1.0% between the two tree groups, but β-pinene and myrcene were still statistically greater and Δ-3-carene lower in the attacked trees (Table 1).

Sapwood concentrations of α-pinene and most of the minor monoterpenes in trees attacked by *T. destruens* did not differ statistically from the concentrations in trees free of beetles, except for β-pinene and Δ-3-carene (Table 1). β-Pinene was the only sapwood monoterpene with concentrations related (negatively) to the tissue water contents ($P = 0.037$), with $r^2 = 0.367$. Composition of the monoterpenes in the sapwood varied less than 1% between the two groups of trees for all compounds, yet most were statistically different, except for α-pinene and cineole (Table 1).

Fig. 2. Ethanol concentrations (95% CI) in the phloem and sapwood of *Pinus halepensis* attacked by *Tomicus destruens* compared with neighboring trees that were not attacked. Phloem values are statistically different at $P = 0.021$, and sapwood values are statistically different at $P = 0.027$. Note differences in the *y*-axes scales. Magnitudes of the back-transformed CIs are indicative of the variation about the means but are not accurate measures for identifying statistical differences between them.



Discussion

The trees attacked by *T. destruens* were more water-stressed than the neighboring trees not attacked, as indicated by their phloem and sapwood water contents, in the same way that water content reflected severe water stress in seedling of three other conifers (Manter and Kelsey 2008). Prior to sampling in October, there were 18 consecutive months (excluding the 1-day rainfall event at the end of September 2012) in which the monthly cumulative precipitation near our study site ranged from 0 to 26.5 mm (mean 11.1 mm), far short of the 40 mm needed to fully recharge stem water potential to its maximum level in deep-rooted, water-stressed *P. halepensis* (Baquedano and Castillo 2007). Trees experiencing greater water stress may have been shallower rooted, facing greater competition from their neighbors, or a combination of both. To our knowledge, the trees were not infected with root pathogens, as root disease could enhance their water stress by reducing water transport (Joseph et al. 1998) and also increase

Table 1. Monoterpene concentrations and percentage composition in the phloem and sapwood of *Pinus halepensis* trees attacked and not attacked by *Tomicus destruens*.

	Phloem[a]						Sapwood[b]					
	Concentration (μg·g fresh mass^{-1})			Composition (%)			Concentration (μg·g fresh mass^{-1})			Composition (%)		
Compound	Attacked	Not attacked	*P*	Attacked	Not attacked	*P*	Attacked	Not attacked	*P*	Attacked	Not attacked	*P*
Unknown	0.7 (0.6–0.8)	0.3 (0.2–0.4)	<0.001	0.28 (0.25–0.32)	0.26 (0.23–0.30)	0.338	1.9 (1.7–2.1)	1.6 (1.3–1.8)	0.053	0.30 (0.28–0.31)	0.27 (0.25–0.28)	0.006
α-Pinene	224.5 (179.6–280.6)	102.6 (82.1–128.2)	<0.001	94.7 (93.8–95.6)	94.8 (94.0–95.7)	0.784	604.9 (521.4–701.6)	546.5 (471.1–634.0)	0.307	94.3 (93.1–95.5)	94.2 (93.0–95.4)	0.895
Camphene	2.4 (2.0–2.8)	0.9 (0.6–1.3)	<0.001	0.96 (0.8–1.1)	0.86 (0.7–1.0)	0.231	6.8 (5.8–7.8)	5.5 (4.5–6.5)	0.070	1.05 (1.00–1.11)	0.93 (0.87–0.98)	0.005
β-Pinene	2.2 (1.9–2.5)	0.8 (0.5–1.1)	<0.001	0.9 (0.8–1.1)	0.7 (0.6–0.8)	0.006	6.9 (5.9–8.0)	5.2 (4.2–6.3)	0.026	1.07 (0.99–1.14)	0.88 (0.80–0.95)	0.003
Δ-3-Carene	0.5 (0.2–1.1)	1.0 0.4–2.3)	0.217	0.2 (0.1–0.5)	0.9 (0.4–2.1)	0.022	1.0 (0.4–2.5)	6.2 (2.5–15.3)	0.011	0.2 (0.1–0.4)	1.1 (0.5–2.4)	0.005
Myrcene	4.4 (2.9–6.8)	1.2 (0.8–1.8)	<0.001	1.9 (1.4–2.7)	1.1 (0.8–1.5)	0.017	15.4 (8.8–22.0)	8.7 (2.1–15.2)	0.138	2.1 (1.5–3.1)	1.3 (0.9–1.8)	0.049
Limonene	1.7 (1.4–2.0)	0.7 (0.6–0.8)	<0.001	0.7 (0.6–0.8)	0.6 (0.55-.67)	0.073	4.6 (3.8–5.7)	3.5 (2.8–4.3)	0.054	0.7 (0.66–0.76)	0.6 (0.55–0.65)	0.004
Cineole	0.23 (0.16–0.33)	0.16 (0.11–0.23)	0.228	0.10 (0.08–0.14)	0.15 (0.11–0.19)	0.066	0.7 (0.6–0.8)	0.6 (0.4–0.7)	0.126	0.11 (0.10–0.12)	0.10 0.09–0.11)	0.061
Total	236.7 (189.5–295.7)	108.1 (86.6–135.1)	<0.001				647.9 (549.9–746.0)	589.1 (491.0–687.1)	0.367			

Note: For all analyses, df = 1,10. Values in parentheses are 95% confidence interval (CI).

[a]All phloem concentrations and percent compositions are back-transformed means and CIs, except for the concentrations of the unknown, camphene, and β-pinene.

[b]Sapwood concentrations of α-pinene, Δ-3-carene, and limonene and all percent compositions except camphene, β-pinene, and limonene are back-transformed means and CIs.

their ethanol concentrations (Kelsey et al. 1998; Kelsey and Joseph 1998).

The phloem and sapwood of trees attacked by *T. destruens* contained higher ethanol concentrations than those not attacked. All evidence indicates that ethanol accumulates in response to severe water stress without the presence of beetles (Kimmerer and Kozlowski 1982; Kelsey and Joseph, 2001; Heitz et al. 2005; Manter and Kelsey 2008), therefore it was not induced by the attacks, and the concentration differences between the two tree groups existed when *T. destruens* were selecting host trees. Gallego et al. (2008) detected synergistic *T. destruens* responses when the release ratios of ethanol to α-pinene ranged from 1.5 to 6, with ethanol release rates from 0.45 to 1.8 g·day^{-1}. However, Martin et al. (2013) did not observe this synergistic effect across similar ethanol–α-pinene release ratios when ethanol release ranged from 1.6 to 4 g·day^{-1}. Optimal attraction was found when 2 g ethanol·day^{-1} alone was released. We did not measure release rates, but the beetles' selection of host trees with higher concentrations of ethanol is consistent with their responses to traps baited with ethanol.

Monoterpene concentrations in the phloem probably reflect constitutive quantities present during *T. destruens* host selection. Wound induction of resin synthesis is common in pines (Franceschi et al. 2005) but does not occur in all species, as shown for *P. ponderosa* (Fischer et al. 2010; Gaylord et al. 2011), and has not been closely examined in *P. halepensis*, to our knowledge. Regression analysis indicated a negative relationship between the phloem monoterpene concentrations and tissue water content that explained 63.7% to 85.1% of the variation for total monoterpenes and six of the eight individual compounds. Because differences in phloem water content between the two tree groups developed prior to the beetle attacks and water loss explained most of the variation in monoterpene concentrations, it then seems likely that the quantities of terpenes measured reflect the constitutive levels present during beetle host selection. Any increased biosynthesis associated with stress prior to attack, or induction after beetle attack, or their combination would have been a minor contributor to the measured variation. Sapwood of the attacked trees also contained higher concentrations of most monoterpenes than that of the unattacked trees, but few were statistically higher, and there was no indication of a concentration effect from water loss, except for β-pinene and this accounted for only 36.7% of its variation. So, here again, the amounts measured in the two tree groups are probably close to constitutive levels.

Monoterpene composition in the phloem and sapwood differed by less than 1% for all compounds between the more water-stressed, attacked trees and the less stressed, unattacked trees. This indicates that neither water stress prior to attacks nor any wound induction following the attacks influenced the monoterpene composition of the tissues, so it would not have contributed to beetle host selection. Others have reported similar results for *P. halepensis*. Tognetti et al. (1997) found no clear relationship between the variability of cortical terpenoid composition and other water relations or hydraulic architectural parameters measured in adequately watered seedling or those exposed to cyclical drought stress. Similarly, the percentage monoterpene composition in mature cortex and needles of older *P. halepensis* remained stable over three growing seasons, including those periods with seasonal drought (Michelozzi et al. 2008).

Although constitutive monoterpene concentrations may have been higher in the phloem of the more water-stressed trees, as suggested above, their influence on beetle host selection may have been limited. α-Pinene is active on *T. destruens* antennae (Faccoli et al. 2008) and is the most abundant *P. halepensis* resin component, but when released alone in traps, it is not a strong attractant for *T. destruens*, and doubling or tripling the release rates does not increase the beetle response (Gallego et al. 2008). When released in combination with ethanol, beetle catches often increase over α-pinene alone, depending on release rates and ratios, but the beetles may or may not respond synergistically in comparison to their attraction to ethanol alone (Gallego et al. 2008; Martin et al. 2013). Myrcene and limonene, both minor components in *P. halepensis*, might also influence *T. destruens* host se-

lection as they are both active on *T. destruens* antennae (Faccoli et al. 2008). However, traps releasing myrcene alone caught very low numbers of *T. destruens* (similar to or less than α-pinene alone), and it did not synergize the beetles response to ethanol (authors' unpublished data). Limonene alone caught 2.5 times more beetles than myrcene but did not synergize ethanol. In *P. contorta* var. *latifolia*, myrcene constitutes only 2.6% and 1.1% of the bole and foliage volatiles, respectively (Pureswaran et al. 2004), but is the strongest monoterpene synergist to *Dendroctonus ponderosae* Hopkins aggregation pheromones (Pureswaran and Borden 2005). This is probably not the case for *T. destruens* as an effective aggregation pheromone has never been verified for this species (Faccoli et al. (2008) and references therein). Although there is some uncertainty for the constitutive monoterpene concentrations between the two groups of stressed trees prior to the beetle attacks, evidence from trapping studies suggests that even if the more water-stressed trees did contain higher quantities, their influence on beetle host selection in this study was likely not as critical as the ethanol.

Ethanol accumulation in the more water-stressed trees during this natural drought is consistent with those studies in which water stress was experimentally imposed at an accelerated pace (Kimmerer and Kozlowski 1982; Kelsey and Joseph 2001; Heitz et al 2005; Manter and Kelsey 2008). Regression analysis indicated that ethanol concentrations in the phloem and sapwood were also negatively related to the tissue water content, but it explained only 39.7% and 41.8%, respectively, of the ethanol variation, a much smaller portion than for the monoterpenes. This may result from multiple factors influencing the ethanol levels. Because of strong hydrogen bonding between water and ethanol, water lost from stem tissues directly to the atmosphere will carry some ethanol with it, limiting concentration increases, if any, and suggesting that other mechanisms contribute to higher ethanol levels in the more water-stressed trees. We speculate that this results from increased ethanol synthesis and slower dissipation–metabolism as a consequence of more depressed sap flow in trees with greater water stress. *Pinus halepensis* is an isohydric species that responds to drying soil and atmosphere by reducing stomatal conductance and transpiration to maintain constant leaf water potential, resulting in less sap flow as water stress increases (Klein et al. 2011). Less sap flow reduces the delivery of dissolved O_2 to the living xylem and nearby cambium (Eklund, 2000; Gansert 2003; Mancuso and Marras 2003; Spicer and Holbrook 2005), increasing their likelihood of becoming hypoxic and inducing ethanol synthesis (Vartapetian 2006). Radial intercellular gas exchange through lenticels, the other O_2 supply route for stem tissues (Gansert 2003; Mancuso and Marras 2003; Spicer and Holbrook 2005), may not be impaired in tissues with lower water contents (Sorz and Hietz 2006). Tissue respiration rates also play a key role in depleting tissue O_2 supplies (Gansert 2003; Spicer and Holbrook 2005) but are unlikely to deplete O_2 levels faster in the more severely stressed trees because severe water stress typically reduces respiration rates (Saveyn et al. 2007; Atkin and Macherel 2009; McDowell et al. 2011). Eklund (2000) reports lower sapwood O_2 levels in water-stressed *Picea abies* (≤1% mole fraction) than in untreated controls (1%–3%) or those receiving irrigation (~5%). In addition, cambium ethanol concentrations increased within 6 h of placing *Populus deltoides* Bartr. logs with no sap flow into atmospheres with ≤5% O_2 (MacDonald and Kimmerer 1991).

Slower ethanol dissipation and metabolism as a result of reduced sap flow may allow tissue ethanol concentrations to remain higher for longer periods in more severely water-stressed trees. Water in sapwood functions as a diffusion sink for ethanol generated in living xylem, in the adjacent cambium, or in the phloem. Upon entering tracheids, it moves upwards in the transpirational stream, usually at rates much faster than diffusion. Along this route, it continues to diffuse into live cells in the stem or leaves, where it will be metabolized into various primary cellular constituents in the presence of adequate O_2 (MacDonald and Kimmerer 1993), or it is metabolized into acetaldehyde and released through leaves to the atmosphere (Kreuzwieser et al. 1999; Cojocariu et al. 2004). Some ethanol also can escape through leaf stomata, but the amounts are generally small (MacDonald and Kimmerer 1993; Kreuzwieser et al. 1999). Decreased sap flow will slow or stop this ethanol movement and associated metabolism. Ethanol can still diffuse, but this slower movement will allow it to remain at higher concentrations for longer periods in cells or tissues of origin. Thus, we suspect that a combination of more ethanol synthesis and impaired dissipation are the key factors causing ethanol concentrations to increase in those trees with greater water stress. Diminished sap flow is a suspected contributor to ethanol accumulation within cankers on diseased *Quercus agrifolia* Nee (Kelsey et al. 2013). Given that all trees can produce ethanol under anaerobic stress, the potential exists for similar responses in other species, but the concentrations of ethanol that they generate under the same conditions may differ substantially (Manter and Kelsey 2008).

Initiation of ethanol synthesis by heat damage to cellular membranes has also been suspected in severely water-stressed tree branches and in small stems of conifer seedlings with impaired water transport (Kelsey and Joseph 2001; Manter and Kelsey 2008). Heat injury induced ethanol synthesis has been demonstrated with herbaceous plant tissue (Anderson 1994; Forney and Jordan 1998) and stems of *P. ponderosa* (Kelsey and Joseph 2003), but this mechanism is probably not involved in the stems here.

At our study site in 2012, the *T. destruens* began attacking *P. halepensis* at the end of September, after a heavy 1-day rain event. In more typical years, *T. destruens* attacks usually start later, after autumnal rainfall occurs and the seasonal drought is over, at the end of October, November, or December. Rainfall following drought may somehow influence the release of ethanol from stressed *P. halepensis* into the atmosphere, attracting *T. destruens* to the more stressed trees with impaired defenses. At our site, it is unknown when the ethanol began to accumulate or how much earlier in the year the higher concentrations were detectable in the more severely water-stressed trees. Kelsey and Joseph (2001) observed bark beetles initiating their attacks on *P. menziesii* branches with high ethanol concentrations between 12 to 24 days after breaking the water column, a treatment causing more rapid and severe water stress than likely occurred here.

Whether measurements of ethanol concentrations in severely drought-stressed trees can be used to predict their vulnerability to bark beetle attacks remains to be determined, and its utility will depend on how far in advance of an attack the changes in ethanol can be detected. Phloem provides a better ethanol signal than sapwood, but many questions remain regarding how species, season, drought duration, time of day, or location on the tree influence its accumulation. Trapping ethanol as it volatilizes to the atmosphere might also work, but if these releases are readily detectable by beetles, there may be minimal time to respond before they arrive. Many bark beetles are not attracted to ethanol, but if its accumulation during drought is associated strongly enough with physiological changes impairing chemical defenses, then it could still function as a valuable biomarker for monitoring the trees vulnerability to beetle attacks.

Acknowledgements

The authors thank Doug Westlind for assistance with statistical analyses. They also thank Ariel Muldoon, Pilar González-Hernández, and Tom Kolb for their reviews of an earlier version of this manuscript. The use of trade, firm, or corporation names is for information and convenience of the reader and does not constitute an official endorsement or approval by the U.S. Department of Agriculture.

References

Adams, H.D., Germino, M.J., Breshears, D.D., Barron-Gafford, G.A., Guardiola-Claramonte, M., Zou, C.B., and Huxman, T.E. 2013. Nonstructural leaf carbohydrate dynamics of *Pinus edulis* during drought-induced tree mortality reveal role for carbon metabolism in mortality mechanism. New Phytol. **197**: 1142–1151. doi:10.1111/nph.12102.

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.-H., Allard, G., Running, S.W., Semerci, A., and Cobb, N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. For. Ecol. Manage. **259**: 660–684. doi:10.1016/j.foreco.2009.09.001.

Alpert, P., Krichak, S.O., Shafir, H., Haim, D., and Osetinsky, I. 2008. Climatic trends to extremes employing regional modeling and statistical interpretation over the E. Mediterranean. Global Planet. Change, **63**: 163–170. doi:10.1016/j.gloplacha.2008.03.003.

Anderegg, W.R.L., and Anderegg, L.D.L. 2013. Hydraulic and carbohydrate changes in experimental drought induced mortality of saplings in two conifer species. Tree Physiol. **33**: 252–260. doi:10.1093/treephys/tpt016.

Anderson, J.A. 1994. Production of methanol from heat-stressed pepper and corn leaf disks. J. Am. Soc. Hortic. Sci. **119**: 468–472.

Atkin, O.K., and Macherel, D. 2009. The crucial role of plant mitochondria in orchestrating drought tolerance. Ann. Bot. **103**: 581–597. doi:10.1093/aob/mcn094.

Baquedano, F.J., and Castillo, F.J. 2007. Drought tolerance in the Mediterranean species *Quercus coccifera*, *Quercus ilex*, *Pinus halepensis*, and *Juniperus phoenicea*. Phytosynthetica, **45**: 229–238. doi:10.1007/s11099-007-0037-x.

Branco, M., Pereira, J.S., Mateus, E., Tavares, C., and Paiva, M.R. 2010. Water stress affects *Tomicus destruens* host pine preference and performance during the shoot feeding phase. Ann. For. Sci. **67**: article 608. doi:10.1051/forest/201021.

Breshears, D.D., Myers, O.B., Meyer, C.W., Barnes, F.J., Zou, C.B., Allen, C.D., McDowell, N.G., and Pockman, W.T. 2009. Tree die-off in response to global change-type drought: mortality insights from a decade of plant water potential measurements. Front. Ecol. Environ. **7**: 185–189. doi:10.1890/080016.

Cojocariu, C., Kreuzwieser, J., and Rennenberg, H. 2004. Correlation of short-chained carbonyls emitted from *Picea abies* with physiological and environmental parameters. New Phytol. **162**: 717–727. doi:10.1111/j.1469-8137.2004.01061.x.

Coyle, D.R., Booth, D.C., and Wallace, M.S. 2005. Ambrosia beetle (Coleoptera: Scolytidae) species, flight, and attack on living eastern cottonwood trees. J. Econ. Entomol. **98**: 2049–2057. doi:10.1603/0022-0493-98.6.2049.

Desprez-Loustau, M.-L., Marçais, B., Nageleisen, L.-M., Piou, D., and Vannini, A. 2006. Interactive effects of drought and pathogens in forest trees. Ann. For. Sci. **63**: 597–612. doi:10.1051/forest:2006040.

Eklund, L. 2000. Internal oxygen levels decrease during the growing season and with increasing stem height. Trees, **14**: 177–180. doi:10.1007/PL00009761.

Faccoli, M., Anfora, G., and Tasin, M. 2008. Responses of the Mediterranean pine shoot beetle *Tomicus destruens* (Wollaston) to pine shoot and bark volatiles. J. Chem. Ecol. **34**: 1162–1169. doi:10.1007/s10886-008-9503-6.

Fischer, M.J., Waring, K.M., Hofstetter, R.W., and Kolb, T.E. 2010. Ponderosa pine characteristics associated with attack by the roundheaded pine beetle. For. Sci. **56**: 473–483.

Forney, C.F., and Jordan, M.A. 1998. Induction of volatile compounds in broccoli by postharvest hot-water dips. J. Agric. Food Chem. **46**: 5295–5301. doi:10.1021/jf980443a.

Franceschi, V.R., Krokene, P., Christiansen, E., and Kreking, T. 2005. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytol. **167**: 353–376. doi:10.1111/j.1469-8137.2005.01436.x.

Gallego, D., Cánovas, F., Esteve, M., and Galian, J. 2004. Descriptive biogeography of *Tomicus* (Coleoptera: Scolytidae) species in Spain. J. Biogeogr. **31**: 2011–2024. doi:10.1111/j.1365-2699.2004.01131.x.

Gallego, D., Galián, J., Diez, J.J., and Pajares, J.A. 2008. Kairomonal responses of *Tomicus destruens* (Col., Scolytidae) to host volatiles α-pinene and ethanol. J. Appl. Entomol. **132**: 654–662. doi:10.1111/j.1439-0418.2008.01304.x.

Gansert, D. 2003. Xylem sap flow as a major pathway for oxygen supply to the sapwood of birch (*Betula pubescens* Ehr.). Plant Cell Environ. **26**: 1803–1814. doi:10.1046/j.1365-3040.2003.01097.x.

Gaylord, M.L., Kolb, T.E., Wallin, K.F., and Wagner, M.R. 2007. Seasonal dynamics of tree growth, physiology, and resin defense in a northern Arizona ponderosa pine forest. Can. J. For. Res. **37**(7): 1173–1183. doi:10.1139/X06-309.

Gaylord, M.L., Hofstetter, R.W., Kolb, T.E., and Wagner, M.R. 2011. Limited response of ponderosa pine bole defenses to wounding and fungi. Tree Physiol. **31**: 428–437. doi:10.1093/treephys/tpr025.

Gaylord, M.L., Kolb, T.E., Pockman, W.T., Plaut, J.A., Yepez, E.A., Macalady, A.K, Pangle, R.E., and McDowell, N.G. 2013. Drought predisposes piñon–juniper woodlands to insect attacks and mortality. New Phytol. **198**: 567–578. doi:10.1111/nph.12174.

Gil, L., and Pajares, J.A. 1986. Los escolítidos de las coníferas de la península Ibérica. INIA, Madrid, Spain.

Heitz, P., Baier, P., Offenthaler, I., Führer, E., Rosner, S., and Richter, H. 2005. Tree temperature, volatile organic emissions, and primary attraction of bark beetles. Phyton, **45**: 341–354.

Herms, D.A., and Mattson, W.J. 1992. The dilemma of plants: to grow or defend. Quart. Rev. Biol. **67**: 283–335. doi:10.1086/417659.

Horn, A., Roux-Morabito, G., Lieutier, F., and Kerdelhue, C. 2006. Phylogeographic structure and past history of the circum-Mediterranen species *Tomicus destruens* Woll. (Coleoptera: Scolytinae). Mol. Ecol. **15**: 1603–1615. doi:10.1111/j.1365-294X.2006.02872.x.

Joseph, G., Kelsey, R.G., and Thies, W.G. 1998. Hydraulic conductivity in roots of ponderosa pine infected with black-stain (*Leptographium wageneri*) or annosus (*Heterobasidion annosum*) root disease. Tree Physiol.**18**: 333–339. doi:10.1093/treephys/18.5.333.

Joseph, G., Kelsey, R.G., Peck, R.W., and Niwa, C.G. 2001. Response of some scolytids and their predators to ethanol and 4-allylanisole in pine forests of central Oregon. J. Chem. Ecol. **27**:697–715. doi:10.1023/A:1010345817756.

Kane, J., and Kolb, T.E. 2010. Importance of resin ducts in reducing ponderosa pine mortality from bark beetle attacks. Oecologia, **164**: 601–609. doi:10.1007/s00442-010-1683-4.

Kelsey, R.G., and Joseph, G. 1998. Ethanol in Douglas-fir with black-stain root disease (*Leptographium wageneri*). Can. J. For. Res. **28**: 1207–1212. doi:10.1139/x98-087.

Kelsey, R.G., and Joseph, G. 1999. Ethanol and ambrosia beetles in Douglas fir logs exposed or protected from rain. J. Chem. Ecol. **25**: 2793–2809. doi:10.1023/A:1020859726152.

Kelsey, R.G., and Joseph, G. 2001. Attraction of *Scolytus unispinosus* bark beetles to ethanol in water-stressed Douglas-fir branches. For. Ecol. Manage. **144**: 229–238. doi:10.1016/S0378-1127(00)00387-X.

Kelsey, R.G., and Joseph, G. 2003. Ethanol in ponderosa pine as an indicator of physiological injury from fire and its relationship to secondary beetles. Can. J. For. Res. **33**(5): 870–884. doi:10.1139/x03-007.

Kelsey, R.G., Joseph, G., and Thies, W.G. 1998. Sapwood and crown symptoms in ponderosa pine infected with black-stain and annosum root disease. For. Ecol. Manage. **111**: 181–191. doi:10.1016/S0378-1127(98)00332-6.

Kelsey, R.G., Beh, M.M., Shaw, D.C., and Manter, D.K. 2013. Ethanol attracts scolytid beetles to *Phytophthora ramorum* cankers on coast live oak. J. Chem. Ecol. **39**: 494–506. doi:10.1007/s10886-013-0271-6.

Kimmerer, T.W., and Kozlowski, T.T. 1982. Ethylene, ethane, acetaldehyde, and ethanol production by plants under stress. Plant Physiol. **69**: 840–847. doi:10.1104/pp.69.4.840.

Klein, T., Cohen, S., and Yakir, D. 2011. Hydraulic adjustments underlying drought resistance of *Pinus halepensis*. Tree Physiol. **31**: 637–648. doi:10.1093/treephys/tpr047.

Kreuzwieser, J., Scheerer, U., and Rennenberg, H. 1999. Metabolic origin of acetaldehyde emitted by poplar (*Populus tremula* × *P. alba*) trees. J. Exp. Bot. **50**: 757–765. doi:10.1093/jxb/50.335.757.

Lingren, B.S., Miller, D.R., and LaFontaine, J.P. 2012. MCOL, frontalin and ethanol: a potential operational trap lure for Douglas-fir beetle in British Columbia. J. Entomol. Soc. B.C. **109**: 71–73.

MacDonald, R.C., and Kimmerer, T.W. 1991. Ethanol in the stems of trees. Physiol. Plant. **82**: 582–588. doi:10.1111/j.1399-3054.1991.tb02950.x.

MacDonald, R.C., and Kimmerer, T.W. 1993. Metabolism of transpired ethanol by eastern cottonwood (*Populus deltoides* Bartr.). Plant Physiol. **102**: 173–179. doi:10.1104/pp.102.1.173.

Mancuso, S., and Marras, A.M. 2003. Different pathways of the oxygen supply in the sapwood of young *Olea europaea* trees. Planta, **216**: 1028–1033.

Manter, D.K., and Kelsey, R.G. 2008. Ethanol accumulation in drought-stressed conifer seedlings. Int. J. Plant Sci. **169**: 361–369. doi:10.1086/526462.

Martin, A.B., Etxebeste, I., Pérez, G., Álvarez, G., Alves, F., Sánchez, E., and Pajares, J.A. 2013. Respuesta de *Tomicus destruens* y *Tomicus piniperda* (Col.: Scolytinae) a compuestos cairomonales de sus hospedantes. *In* Proceedings of the 6th Spanish Forest Congress, Vitoria, Spain, 10–14 June 2013, Paper 6CFE01-417.

Mattson, W.J., and Haack, R.A. 1987. Role of drought in outbreaks of plant-eating insects. Bioscience, **37**: 110–118. doi:10.2307/1310365.

McDowell, N.G. 2011. Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. Plant Physiol. **155**: 1051–1059. doi:10.1104/pp.110.170704.

McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G., and Yepez, E.A. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytol. **178**: 719–739. doi:10.1111/j.1469-8137.2008.02436.x.

McDowell, N.G., Beerling, D.J., Breshears, D.D., Fisher, R.A., Raffa, K.F., and Stitt, M. 2011. The interdependence of mechanisms underlying climate-driven vegetation mortality. Trends Ecol. Evol. **26**: 523–532. doi:10.1016/j.tree.2011.06.003.

Michelozzi, M., Tognetti, R., Maggino, F., and Radicati, M. 2008. Seasonal variations in monoterpene profiles and ecophysiological traits in Mediterranean pine species of group "halepensis". iForest, **1**: 65–74. doi:10.3832/ifor0206-0010065.

Miller, D.R., and Rabaglia, R.J. 2009. Ethanol and (–)-α-pinene: attractant kairomones for bark and ambrosia beetles in the southeastern US. J. Chem. Ecol. **35**: 435–448. doi:10.1007/s10886-009-9613-9.

Nanni, C., and Tiberi, R. 1997. *Tomicus destruens* (Wallaston): biology and behavior in central Italy. *In* Proceedings: Integrating Cultural Tactics into the Management of Bark Beetle and Reforestation Pests. *Edited by* J.C. Gregiore, A.M. Liebhold, F.M. Stephen, K.R. Day, and S.M. Salom. USDA For. Serv. Gen. Tech. Rep. NE-236. pp. 131–134.

Negrón, J. 1997. Estimating probabilities of infestation and extent of damage by the roundheaded pine beetle in ponderosa pine in the Sacramento Mountains, New Mexico. Can. J. For. Res. **27**(12): 1936–1945. doi:10.1139/x97-158.

Negrón, J.F., Wilson, J.L., and Anhold, J.A. 2000. Stand conditions associated with roundheaded pine beetle (Coleoptera: Scolytidae) infestations in Arizona and Utah. Environ. Entomol. **29**: 20–27. doi:10.1603/0046-225X-29.1.20.

Pitman, G.B., Hedden, R.L., and Gara, R.I. 1975. Synergistic effects of ethyl alcohol on the aggregation of *Dendroctonus pseudotsugae* (Coleoptera: Scolytidae) in response to pheromones. Z. Angew. Entomol. **78**: 203–208.

Plaut, J.A., Yepez, E.A., Hill, J., Pangle, R., Sperry, J.S., Pockman, W.T., and McDowell, N.G. 2012. Hydraulic limits preceding mortality in a piñon–juniper woodland under experimental drought. Plant Cell Environ. **35**: 1601–1617. doi:10.1111/j.1365-3040.2012.02512.x.

Pureswaran, D.S., and Borden, J.H. 2005. Primary attraction and kairomonal host discrimination in three species of *Dendroctonus* (Coleoptera: Scolytidae). Agric. For. Entomol. **7**: 219–230. doi:10.1111/j.1461-9555.2005.00264.x.

Pureswaran, D.S., Gries, R., and Borden, J.H. 2004. Quantitative variation in monoterpenes in four species of conifers. Biochem. Syst. Ecol. **32**: 1109–1136. doi:10.1016/j.bse.2004.04.006.

Ranger, C.M., Reding, M.E., Gandhi, K.J.K., Oliver, J.B., Schulty, P.B., Cañas, L., and Herms, D.A. 2011. Species dependent influence of (–)-α-pinene on attraction of ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) to ethanol-baited traps in nursery agroecosystems. J. Econ. Entomol. **104**: 574–579. doi:10.1603/EC10243.

Sala, A., Woodruff, D.R., and Meinzer, F.C. 2012. Carbon dynamics in trees: feast or famine? Tree Physiol. **32**: 764–775. doi:10.1093/treephys/tpr143.

SAS Institute Inc. 2010. SAS/STAT® 9.3. User's guide. SAS Institute Inc., Cary, North Carolina.

Saveyn, A., Steppe, K., and Lemeur, R. 2007. Drought and the diurnal patterns of stem CO_2 efflux and xylem CO_2 concentration in young oak (*Quercus robur*). Tree Physiol. **27**: 365–374. doi:10.1093/treephys/27.3.365.

Schroeder, L.M., and Lindelöw, Å. 1989. Attraction of scolytids and associated beetles by different absolute amounts and proportions of α-pinene and ethanol. J. Chem. Ecol. **15**: 807–817. doi:10.1007/BF01015179.

Sheffield, J., and Wood, E.F. 2008. Projected changes in drought occurrence under future global warming from multi-model, multi-scenario, IPCC AR4 simulations. Clim. Dyn. **31**: 79–105. doi:10.1007/s00382-007-0340-z.

Sorz, J., and Hietz, P. 2006. Gas diffusion through wood: implications for oxygen supply. Trees, **20**: 34–41. doi:10.1007/s00468-005-0010-x.

Spicer, R., and Holbrook, N.M. 2005. Within-stem oxygen concentration and sap flow in four temperate tree species: does long-lived xylem parenchyma experience hypoxia? Plant Cell Environ. **28**: 192–201. doi:10.1111/j.1365-3040.2004.01262.x.

Tognetti, R., Michelozzi, M., and Giovannelli, A. 1997. Geographical variation in water relations, hydraulic architecture and terpene composition of Aleppo pine seedlings from Italian provenances. Tree Physiol. **17**: 241–250. doi:10.1093/treephys/17.4.241.

Vartapetian, B.B. 2006. Plant anaerobic stress as a novel trend in ecological physiology, biochemistry, and molecular biology: 2. Further development of the problem. Russ. J. Plant Physiol. **53**: 711–738. doi:10.1134/S102144370606001X.

von Sydow, F., and Birgersson, G. 1997. Conifer stump condition and pine weevil (*Hylobius abietis*) reproduction. Can. J. For. Res. **27**(8): 1254–1262. doi:10.1139/x97-089.

Williams, A.P., Allen, C.D., Macalady, A.K., Griffin, D., Woodhouse, C.A., Meko, D.M., Swetnam, T.W., Rauscher, S.A., Seager, R., Grissino-Mayer, H.D., Dean, J.S., Cook, E.R., Gangodagamage, C., Cai, M., and McDowell, N.G. 2013. Temperature as a potent driver of regional forest drought stress and tree mortality. Nat. Clim. Change, **3**: 292–297. doi:10.1038/nclimate1693.

Wood, S.L., and Bright, D.E. 1992. A catalog of Scolytidae and Platypodidae (Coleoptera). Part 2. Taxonomic index. Great Basin Nat. Mem. 13.