

Headspace Volatiles from 52 oak Species Advertise Induction, Species Identity, and Evolution, but not Defense

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Abstract Leaf volatiles convey information about a plant to other organisms in their proximity. Despite increasing interest in understanding the relevance of volatile emissions for particular ecological interactions, there has been relatively little effort to assess generally what information volatile profiles transmit. We surveyed the volatile profiles of wounded and unwounded leaves of 52 oak (*Quercus*) species. We used phylogenetic comparison and multivariate techniques to assess in what circumstances oak individuals advertised their species identity, evolutionary history, direct defenses, or damage. We found that both species identity and evolutionary history were advertised when leaves were wounded, but species could not be differentiated by odor when leaves were not wounded. Various fatty-acid derivative compounds showed the strongest phylogenetic signal suggesting that they may best disclose taxonomic affiliations in oaks. We tested whether oak volatile composition or diversity advertised high defensive investment, but we found no evidence for this. Wounded leaves disclose much about an oak species' identity and taxonomic affiliation, but unwounded leaves do not. This is consistent with the idea

that volatile information is targeted toward natural enemy recruitment.

Keywords VOC · Volatile · *Quercus* · Aposematic · Green leaf volatiles · Macroevolution

Introduction

Plant leaves emit a broad array of volatile compounds that have profound ecological consequences (Dicke and van Loon, 2000; Dicke and Baldwin, 2010; McCormick et al., 2012). Historically, research has focused on identifying specific ecological consequences of particular volatile compounds. For example, many leaf-emitted compounds attract natural enemies of herbivores (Kessler and Baldwin, 2001), coordinate plant defenses (Heil and Silva Bueno, 2007; Karban and Shiojiri, 2009), or attract herbivores themselves (Bruce et al., 2005). Recent studies have found that the same volatile cue can elicit multiple ecological outcomes (Halitschke et al., 2008), which has led to the understanding that plant-emitted volatiles represent public information that may be utilized by friends, foes and even organisms that do not directly interact with the plant (Dicke and van Loon, 2000; Dicke and Baldwin, 2010; McCormick et al., 2012). Viewing plant volatile emissions as a public signal begs two related questions: What information do plant volatile signals actually transmit? What ecological situations might it be beneficial for a plant to advertise this information (for example your identity, health, or physiological status) to the community at large?

The most commonly studied information associated with plant volatile cues relates to herbivore damage (Dicke and van Loon, 2000). Numerous studies have found that herbivores elicit the production of various volatile compounds, and that these compounds attract predators and parasitoids of herbivores (Kessler and Baldwin, 2001) or deter

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herbivores directly (De Moraes et al., 2001). Some herbivore-plant interactions elicit an herbivore-specific volatile profile (Rodríguez-Saona et al., 2003; Delphia et al., 2007), while in other systems various herbivores or mechanical damage elicit similar changes in plant volatile profile (Mithofer et al., 2005; Hare and Sun, 2011). In each of these cases, the information broadcast by the plant is available to both parasitoids that may benefit the plant and to other herbivores that may harm the plant (Halitschke et al., 2008). However, the net effect of information about herbivore damage is thought to benefit the plant in most cases; that is the benefit of alerting parasitoids outweighs the cost of herbivore eavesdropping, as eavesdropping herbivores may simply encounter higher rates of attack (Sabelis et al., 2007; Kessler and Heil, 2011).

It is less clear to what degree plant volatiles reliably advertise their species identity and how this might affect the plant. At a broad taxonomic scale, many plant groups have unique volatile profiles (Harbourne and Turner, 1984). For example, in a survey of volatile compounds from tropical trees, 43 out of 55 often distantly related tree species were identifiable by statistical analysis of volatile profiles (Courtois et al., 2009). It is less clear whether more closely related species, which often co-occur within ecological communities, differ in odor, and whether between-species differences in volatile composition are greater than within-species differences. One reason for species-specificity in volatile constituents might simply be evolutionary conservatism (Pagel and Harvey, 1991)—closely related individuals or species share volatile constituents because of shared ancestry, and distant relatives have divergent odors. This assumption provided the basis for the use of volatile constituents in the now largely displaced field of chemosystematics, though the assumption of evolutionary conservatism of volatile constituents has only rarely been tested explicitly (but see Becerra et al., 2009). Additionally, there are likely situations when actively advertising species identity might be beneficial to the plant. For example, most herbivores feed on relatively few plant species, so a plant may be able to attract the correct natural enemy of its herbivore by disclosing its own identity, although this advertisement might also attract species-specific herbivores themselves. In this case, it would be expected that a plant might advertise its identity only when suffering herbivore attack.

We propose a novel hypothesis that volatile profiles also may contain valid information about the defenses employed by a plant (**the aposematic fragrance hypothesis**). Many animal species employ visual cues such as aposematic coloration to advertise their high level of defense to potential predators (Mallet and Joron, 1999). In a sensory environment dominated by olfactory cues, it would be analogous for well defended plant species to advertise their defense via odor. What aspects of volatile profiles might serve as a signal of plant defense? Previous studies have suggested

that volatile diversity (i.e., the number of compounds comprising an odor) might be important in mediating plant-herbivore interactions (Becerra et al., 2009). Alternatively, particular volatile compositions might be a reliable signal of plant defense. Presently, there are no explicit comparisons of both direct defenses and volatile composition among related plant species, and it is unclear to what degree a plant species' volatile profile discloses its defensive investment. The efficacy of plant volatile constituents as herbivore repellents suggests there may be a defense associated with repellent volatile signals. That defense may be indirect (for example the attraction of natural enemies), but volatile cues also may simply be a valid signal of direct physiological plant defenses.

While there has been considerable interest in the induction of plant volatile compounds by herbivore damage, there have been few comparative studies that relate volatile emissions across multiple species within a taxonomic group to plant defense, induction, or herbivory. In this study, we surveyed the leaf volatile profiles of 52 oak species in a common garden in order to test several related hypotheses about the information contained in leaf odors.

First, we tested whether various volatile constituents changed in response to mechanical leaf damage, as a proxy for general herbivore attack. We chose mechanically damaged plants in order to standardize damage across multiple oak taxa that are variously palatable to most herbivores, with the caveat that other studies have shown that many herbivores elicit species-specific VOC profiles from their hosts (Halitschke et al., 2001; Savchenko et al., 2012). Next, we tested whether the volatile profiles from wounded and unwounded leaves contained information about oak species identity with the expectation that wounded trees may benefit from advertising species identity to parasitoids, but unwounded trees have little reason to do so. Likewise, we mapped oak volatile composition onto a phylogeny and tested whether inter-specific differences in volatile profiles could be attributed to evolutionary conservatism (i.e., shared ancestry) using an estimate of phylogenetic signal, Pagel's lambda (Freckleton et al., 2002). Finally, we tested whether the diversity or chemical composition of volatile profiles was a reliable signal of plant direct defenses.

Methods and Materials

Study Site We assessed the volatile headspace and herbivore damage on 116 mature oak tree individuals from 52 *Quercus* (oak) species at Shields Oaks Grove at UC Davis, a common garden located in the California Central Valley [38.529453°N, 121.763218°W]. The majority of trees at this site are >30 year old. Valley oak (*Q. lobata*) is the single native species surrounding the site. Since their introduction, local herbivores

from *Q. lobata* have colonized the non-native oak species to varying degrees (Pearse and Hipp, 2009).

Volatile Collection Volatile collections were made from 10:00 AM–2:00 PM from April 2010–May 2010. Collections were timed to match each tree's individual leaf phenology, such that collections were made during the phase of late leaf expansion (~2.5 week following bud break). This phenological period was chosen, as young leaves of many plants (Aide, 1993) including oaks (Hunter, 1992) are the stage colonized by herbivorous insects. On each tree, two twigs at a canopy height of 2–4 m were chosen with one twig randomly assigned to a wounding treatment, and each leaf received three lateral stripes of mechanical damage from a fabric pattern wheel on either side parallel to the midvein. After 30 min, a 20×30 cm Teflon bag with two Teflon septa was placed over each branch. The bag was left open for another 30 min on the branch in order to minimize the effects of ephemeral tactile responses of the tree. The bag then was fastened to the twig with binder clip, and a Solid Phase Microextractor (SPME) fiber with polydimethylsiloxane (PDMS) matrix [Supelco Analytical, Bellefonte, PA, USA] was inserted into one of the bag's septa. An additional SPME fiber was attached to the second septum of 1/5 of sampling bags, in order to confirm compound identity on a DB-1 column (described below). On each sampling, date~10 trees were sampled. In addition, on each date, 2 Teflon sampling bags were clipped to an oak branch where they were sealed without any plant, and SPME samples were taken in order to account for background odors. In all cases, after 45 min, the SPME fiber was removed, put on ice, and immediately taken to the US Department of Agriculture, Agricultural Research Service at Albany, CA, USA for gas-chromatographic analysis.

Volatile Analysis and Identification Volatile analysis followed procedures from previous plant emission surveys (Beck et al., 2008) with some additions. All samples were analyzed using one of two HP-6890 gas chromatographs (GC) coupled to an HP-5973 mass selective detectors (MS; Palo Alto, CA, USA). Samples from the collection bag's first septum were separated using a 60 m×0.32 mm i.d.×0.25 μm DB-wax column, and samples from the second septum were analyzed using a 60 m×0.32 mm i.d.×0.25 μm DB-1 column (J&W Scientific, Folsom, CA, USA). Desorbed volatiles were separated using the following methods: DB-wax, injector temperature of 200 °C, splitless mode, inlet temperature of 200 °C, constant flow of 3.0 ml/min, oven settings of initial temperature of 40 °C, ramp 1 at 4 °C/min, final temperature of 200 °C, hold time of 40 min; DB-1, injector temperature of 200 °C, splitless mode, inlet temperature of 200 °C, constant flow of 2.0 ml/min, oven settings of initial temperature of 40 °C,

ramp 1 at 4 °C/min, final temperature of 250 °C, hold time of 30 min.

National Institute of Standards and Technology (NIST) and an internally generated database were used for initial fragmentation pattern identification. The retention indices (RIs) were calculated using a homologous series of *n*-alkanes on DB-Wax and DB-1 columns. Volatile identifications were verified by injection of commercially available or isolated standards and comparison of retention times and fragmentation patterns (Supplementary Table 1). Each identified compound matched the RI of known standards on both DB-Wax and DB-1 runs, and had a similar fragmentation pattern to standards run on both columns. Compounds that could not be identified by this procedure were designated as "tentative" assignments.

Deconvolution of overlapping peaks and initial peak alignment were conducted using AMDIS, Automated Mass Spectral Deconvolution and Identification System (National Institute of Standards and Technology). Data from each sample were manually checked against chromatograms in order to correctly align peaks that were assigned incorrectly in the automated AMDIS analysis. Trace peaks were aligned if their retention time matched that of an authentic standard ±0.05 min and major ions matched fragments of authentic standards. Background and GC contaminants were determined based on presence in bags without leaf material and were removed from further analysis. For each compound, the averaged background profile was subtracted from the sample's compound abundance. As SPME does not readily provide quantitative assessment of compound abundance, but does allow for qualitative between-sample comparisons and determination of "major" vs. "minor" peaks (Romeo, 2009), we rounded each compound's abundance (deconvoluted total ion count- dTIC) to the nearest order of magnitude. A compound was considered absent if its dTIC was less than 1000.

Defensive Investment A principal components axis (PC-1) of 9 leaf traits (leaf toughness, water content, total tannin content, condensed tannin content, phenolic content, trichome abundance, peroxidase activity, specific leaf area, and protein content) was taken from previous work on these same trees (Pearse and Hipp, 2009; Pearse and Baty, 2012). In a recent study, the survivorship of a generalist caterpillar, *Orgyia vetusta*, was greatly reduced on oak leaves with higher defense scores, using the same trait combinations (Pearse, 2011), thus suggesting that this axis is a meaningful measure of general oak defense.

Statistical Analysis—Information Content about Damage and Species Identity We used multivariate redundancy analysis—RDA (Legendre and Legendre, 1998) to determine whether volatile profiles contain information about damage

Table 1 The six most abundant metabolites present in the leaf headspace of 52 species of oaks

RI (DB-wax)	1305	1315	1592	1746	1702	1459	1548	1385	1248
Name	(E)-4,8-dimethyl-1,3,7-nonatriene	(Z)-3-hexenyl acetate	(E)-β-caryophyllene	(E,E)-α-farnesene	germacrene-D	(Z)-3-hexenyl butyrate	linalool	(Z)-3-hexenol	(E)-β-ocimene
Class	FAD	FAD	ST	ST	ST	FAD	MT	FAD	MT
Wounded	uw w	uw w	uw w	uw w	uw w	uw w	uw w	uw w	uw w
<i>Acutissima</i>	++	++	++	+++	+	-	+++	-	++
<i>Agrifolia</i>	++	+++	+++	++	+++	-	+++	-	++
<i>Alba</i>	+++	+++	+++	+++	+++	-	+++	-	+++
<i>Ballota</i>	++	+++	+++	+++	+++	-	+++	-	+++
<i>Berberidifolia</i>	++	+++	+++	+++	+	+	++	++	+++
<i>Brandegeeii</i>	+++	+++	+++	+++	-	++	+++	++	+++
<i>Buckleyi</i>	++	+++	+++	++	+++	-	+++	-	+++
<i>Calliprinos</i>	-	+++	+++	+	++	-	++	+++	+
<i>Candicans</i>	-	+++	+++	-	+++	-	++	++	+++
<i>Castaneifolia</i>	+++	+++	+++	+++	+++	-	+++	+++	++
<i>Cerris</i>	+++	+++	+++	+++	+++	-	+++	+++	++
<i>Chrysolepis</i>	+	+++	+++	+++	+++	-	+++	+++	+++
<i>Coccifera</i>	-	++	+++	-	++	-	++	+++	++
<i>Crassifolia</i>	++	+	+++	-	+++	-	++	+	++
<i>Crassipes</i>	+++	+++	+++	-	+++	-	+++	++	+++
<i>Diversifolia</i>	+	+++	+++	+++	+++	-	++	+++	++
<i>Douglasii</i>	++	+++	+++	+++	++	++	++	+++	+
<i>Engelmannii</i>	++	+++	+++	+++	+++	-	+++	+++	+++
<i>Faginea</i>	++	+++	+++	++	+++	++	+++	+++	++
<i>Fusififormis</i>	++	+	+++	-	+	-	++	+	+++
<i>Gambelii</i>	++	++	+++	+++	+++	-	+++	+++	+++
<i>Garryana</i>	++	+++	+++	+++	++	+++	++	+++	++
<i>Glauca</i>	++	+++	+++	++	+	-	+++	+++	++
<i>Gravesii</i>	+++	+++	+++	+++	+++	-	+++	+++	+++
<i>Greggii</i>	+++	+++	+++	-	+++	-	+++	+++	+++
<i>Grisea</i>	+++	+++	+++	+++	+++	-	+++	+++	+++
<i>Ilex</i>	-	+++	+++	+++	+++	-	+++	+++	++
<i>Infectoria</i>	++	+++	+++	+++	+	-	+++	+++	-
<i>Ithaburensis</i>	+++	+++	+++	++	++	-	+++	+++	+

Table 1 (continued)

RI (DB-wax)	1305	1315	1592	1746	1702	1459	1548	1385	1248
Name	(E)-4,8-dimethyl-1,3,7-nonatriene	(Z)-3-hexenyl acetate	(E)- β -caryophyllene	(E,E)- α -farnesene	germacrene-D	(Z)-3-hexenyl butyrate	linalool	(Z)-3-hexenol	(E)- β -ocimene
Class	FAD	FAD	ST	ST	ST	FAD	MT	FAD	MT
Wounded	uw w	uw w	uw w	uw w	uw w	uw w	uw w	uw w	uw w
Lobata	++	+++	+++	+++	++	++	++	-	+++
Macrocarpa	++	+++	+++	+++	++	-	++	-	++
Margaretiae	+++	+++	+++	+++	+++	-	-	+	+++
Mongolica	+++	+++	+	-	-	++	-	-	+
Muehlenbergii	+++	+++	+++	+++	+++	-	-	-	+++
Oblongifolia	++	+++	+++	-	++	-	-	-	+++
Obusata	-	+++	+++	+++	-	-	-	+	++
Oleoides	++	+++	+++	-	+	+	+++	-	+++
Palmeri	++	+++	+++	+++	+++	++	+++	-	+++
Parvula	+++	+++	+++	+++	+++	+++	+	-	+++
Phillyreoides	+++	+++	+++	+++	+	++	+++	++	+++
Primoides	+++	+++	+++	+++	+++	-	+++	-	+++
Pungens	++	+++	+++	+++	+++	-	-	-	+++
Robur	++	+++	+++	+++	+++	++	+++	-	++
Rugosa	+++	+++	+++	-	+	-	-	-	-
Serrata	++	+++	+++	+++	-	-	++	-	+++
Sinuata	++	+++	+++	-	-	++	-	-	-
Suber	+++	+++	+++	+++	+	++	+++	-	+++
Tomentella	++	+++	+++	-	+++	-	+++	-	+++
Trojana	+++	+++	+++	+++	+++	-	+	+	+++
Turbinella	+++	+++	+++	+++	-	++	-	-	+++
Variabilis	+++	+++	+++	+++	+++	-	-	+	+++
Vaseyana	+++	+++	+++	-	+	-	++	-	+++
Virginiana	N.A.	+++	N.A.	+++	N.A.	N.A.	N.A.	N.A.	+++

Compound classes are fatty acid derivatives (FAD), sesquiterpenes (ST), and monoterpenes (MT). Experimentally wounded leaves (w) generally had a greater abundance of most compounds than unwounded leaves (uw). Compound rough abundance is represented as “+++” major peak, “++” intermediate peak, “+” minor peak, and “-” absent. A complete list of all 119 compounds is given in Supplementary Table 2

and species identity. First, we ran an RDA model with damage and species as constraining factors. Next, we split the dataset into wounded and unwounded samples, and ran an RDA model with species identity as a constraining axis on each dataset in order to ask whether volatile profiles from wounded and unwounded oaks contain information about species identity. The probability of significance of the constraining axis was determined using a permutation test with 10,000 permutations. All redundancy analyses were calculated using the function “rda” in R package *vegan* (Oksanen et al., 2010).

In later analyses, we used PCA to reduce the dimensionality of volatile profiles. Specifically, we converted the data for each sample into a relative abundance profile by normalizing to total volatile abundance. For the total volatile profile, as well as each compound class (monoterpenes, fatty acid derivatives, and sesquiterpenes), we calculated the first principal component axis (PC-1) as a measure of volatile (or volatile class) composition. We also calculated the relative abundance of each compound class.

Inducibility Next, we assessed the inducibility of the three major compound classes of volatile compounds in oaks (monoterpenoids, fatty acid derivatives, and sesquiterpenoids) after mechanical wounding. Using species-level data, we compared compound class abundance with damage as a fixed factor and species identity as a random factor in a mixed model ANOVA, which is comparable to a paired *t*-test.

Phylogenetic Signal and Comparison with Plant Defense In order to assess whether the volatile profiles contained information about taxonomic affiliation of a species, we mapped volatile abundance and composition onto a recent phylogeny of the genus *Quercus* (Pearse and Hipp, 2009; Pearse and Hipp, 2012). From this, we calculated Pagel’s lambda (Pagel and Harvey, 1991), a measure of phylogenetic signal (i.e., clustering of similar traits on closely related species) for each identified compound as well as on measures of volatile profiles. When Pagel’s lambda approaches 1, it suggests that evolutionary conservatism plays a role in the distribution of that volatile compound (or volatile class), and that closely related species will have similar amounts of that volatile or similar profiles (Pagel and Harvey, 1991).

We then compared the plant volatile profile with the defensive investment of each oak species (described above). First, we asked whether plant volatile profiles contained information about defensive investment using RDA analysis as described above, but with leaf defensive investment as the constraining axis. Next, we tested the hypothesis that a greater diversity of volatile compounds reflects a higher investment in leaf defenses using a linear regression of the number of volatile compounds to predict leaf defensive investment.

Table 2 Results of multivariate redundancy analysis (rda) using species identity as a constraining axis of volatile profiles for wounded and unwounded leaves of 52 oak species

Wounded			
<i>Term</i>	<i>df</i>	<i>permutations</i>	<i>Pr (>F)</i>
Species	51	10,000	0
Residual	60		
Non-wounded			
<i>Term</i>	<i>df</i>	<i>permutations</i>	<i>Pr (>F)</i>
Species	51	10,000	0.19
Residual	60		

Species as a significant constraint indicates that volatile profiles contain meaningful information about species identity

All statistics were calculated in R using packages *vegan* and *nlme* (R Core Development Team, 2010; Pinheiro et al., 2009; Oksanen et al., 2010).

Results

Description of Oak Volatile Profiles Gas chromatographic analysis revealed 110 metabolites from the volatile profiles of 52 species of oaks, which could not be readily attributed to background contamination. Of these compounds, 64 were identified and confirmed with validated standards. An additional 20 were identified to compound class (i.e., monoterpene, sesquiterpene, fatty acid derivative, etc.) based on ion fragmentation patterns but did not match known standards. Of all compounds identified to class, 22 were monoterpenes, 34 were sesquiterpenes, 25 were fatty acid derivatives (also referred to as green leaf volatiles), and 3 were from other compound groups (Table 1, Supplementary Table 2). The identity of terpenoid compounds that we observed was

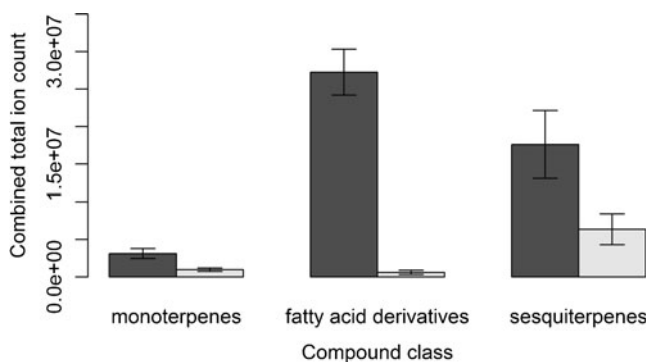


Fig. 1 The induction of the three major volatile classes by mechanical damage on 52 species of oaks. Mechanical damage (dark grey bars) increased the headspace abundance of all three compound classes above undamaged leaves (light grey bars). Species identity was included as a random factor to account for interspecific variation. Data are means \pm SE

Table 3 Phylogenetic signal (Pagel's lambda) as an estimate of evolutionary conservatism of identified volatile compounds from wounded leaves of 52 oak species

Compound	Class	RI	Lambda	P(lambda=1)
(Z)-3-hexenal	FAD	1137	0	<0.001
(E)-2-hexenal	FAD	1213	0	<0.001
n-hexyl acetate	FAD	1270	0.42	0.03
(Z)-4,8-dimethyl-1,3,7-nonatriene	FAD	1272	0.47	0
(E)-4,8-dimethyl-1,3,7-nonatriene	FAD	1305	0.92	0.44
(Z)-3-hexenyl acetate	FAD	1315	0.22	0
NA	FAD	1328	0	<0.001
(E)-2-hexenyl acetate	FAD	1332	0	<0.001
n-hexanol	FAD	1354	0.73	0.03
(E)-3-hexenol	FAD	1364	0	0.07
NA	FAD	1383	0	0.01
(Z)-3-hexenol	FAD	1385	1	1
(Z)-3-hexenyl ester	FAD	1387	0.68	0.02
(Z)-2-hexenol	FAD	1407	0	<0.001
n-hexyl butyrate	FAD	1415	1	1
hexyl-2-methylbutyrate	FAD	1426	1	1
NA	FAD	1431	0	<0.001
hexyl-3-methylbutyrate	MT	1444	1	1
NA	FAD	1452	0	<0.001
(Z)-3-hexenyl butyrate	FAD	1459	1	1
(Z)-3-hexenyl-2-methylbutyrate	FAD	1472	1	1
(E)-2-hexenyl butyrate	FAD	1473	1	1
(Z)-3-hexenyl-3-methylbutyrate	FAD	1487	1	1
(Z)-3-hexenyl pentanoate	FAD	1556	0.48	0.1
NA	FAD	1655	0.55	0.02
α -pinene	MT	1018	0.5	<0.001
α -thujene	MT	1022	0	<0.001
camphene	MT	1059	0	<0.001
β -pinene	MT	1104	0.46	<0.001
Sabinene	MT	1117	0.3	<0.001
Myrcene	MT	1160	0.27	<0.001
α -terpinene	MT	1175	0	<0.001
Limonene	MT	1195	0	<0.001
β -phellandrene	MT	1204	0.29	<0.001
1,8-cineole	MT	1206	0	0.17
(Z)- β -ocimene	MT	1232	0.32	0.18
γ -terpinene	MT	1242	0	<0.001
(E)- β -ocimene	MT	1248	0.62	0
p-cymene	MT	1265	0	<0.001
Terpinolene	MT	1279	0	<0.001
(Z)-linalool-oxide	MT	1470	0	<0.001
Linalool	MT	1548	0.32	0
Linalyl acetate	MT	1554	0	<0.001
4-terpineol	MT	1600	0	<0.001
β -cyclocitral	MT	1615	0	0.01

Table 3 (continued)

Compound	Class	RI	Lambda	P(lambda=1)
α -terpineol	MT	1695	0	<0.001
(E)-geraniol	MT	1849	1	1
Geranyl acetone	MT	1851	0.94	0.65
Isoprene	Other	NA	0	<0.001
Nonanal	Other	1391	0.2	<0.001
Decanal	Other	1495	0.39	<0.001
Undecanal	Other	1600	1	1
Methyl salicylate	Other	1767	0.72	0.14
ar-curcumene	Other	1769	0	<0.001
NA	ST	1395	0.8	0
α -cubebene	ST	1455	0	<0.001
NA	ST	1476	0.66	<0.001
α -copaene	ST	1488	0.03	<0.001
NA	ST	1508	0.45	0.05
Cyperene	ST	1522	0	<0.001
α -gurjunene	ST	1526	0	<0.001
β -cubebene	ST	1535	0.73	0.04
NA	ST	1570	0	<0.001
(E)- α -bergamotene	ST	1582	0	<0.001
β -copaene	ST	1587	0	0.01
(E)- β -caryophyllene	ST	1592	0.24	0
NA	ST	1596	0	<0.001
NA	ST	1636	0	<0.001
Alloaromadendrene	ST	1641	0.8	0.1
α -humulene or (E)- β -farnesene	ST	1663	0	0.01
NA	ST	1671	1	1
NA	ST	1675	0.07	0.22
Germacrene-D	ST	1702	0	0
NA	ST	1706	0.74	0.57
β -selinene	ST	1713	1	1
NA	ST	1715	0	0
NA	ST	1717	0	<0.001
α -selinene	ST	1718	1	1
α -muurolene	ST	1720	0.77	0.53
NA	ST	1723	0	<0.001
Bicyclgermacrene	ST	1728	0.79	0.35
(E,E)- α -farnesene	ST	1746	0.11	0.01
δ -cadinene	ST	1753	0.81	0.56
γ -cadinene	ST	1755	0.62	0.18
NA	ST	1765	0	<0.001
(Z)-nerolidol	ST	2040	0	<0.001

When lambda approaches one, evolutionary conservatism affects the distribution of the compound across oak species. The probability that lambda deviates from 1 (i.e. that other factors beyond phylogenetic conservatism drive the distribution of the compound) was assessed using a likelihood approach

broadly concordant with previous studies of oak volatiles (Csiky and Seufert, 1999; Staudt et al., 2001).

Damage and Species Identity We assessed whether the composition of volatile profiles contained information about species identity and damage using redundancy analysis (Table 2). First, we constructed a redundancy analysis model using species identity and damage as constraining axes. Using a permutation approach to assess significance, both species identity ($P=0.001$) and damage status ($P<0.001$) were significant constraining axes of volatile profile. Next, we split the dataset into wounded and unwounded samples, and we constructed redundancy analysis models for wounded and

unwounded leaves using species identity as a constraining axis. We found that species identity was a significant constraining axis for wounded leaves ($P=0.002$), but not for unwounded leaves ($P=0.181$).

Unsurprisingly, mechanically damaged oak leaves emitted more monoterpenes ($df=1,48$; $F=10.9$, $P=0.002$), sesquiterpenes ($df=1,48$; $F=6.3$, $P=0.015$), and fatty acid derivatives ($df=1,48$; $F=70.1$, $P<0.001$) than undamaged oak leaves (Fig. 1). The amounts of the six most abundant compounds increased with mechanical damage (Table 1).

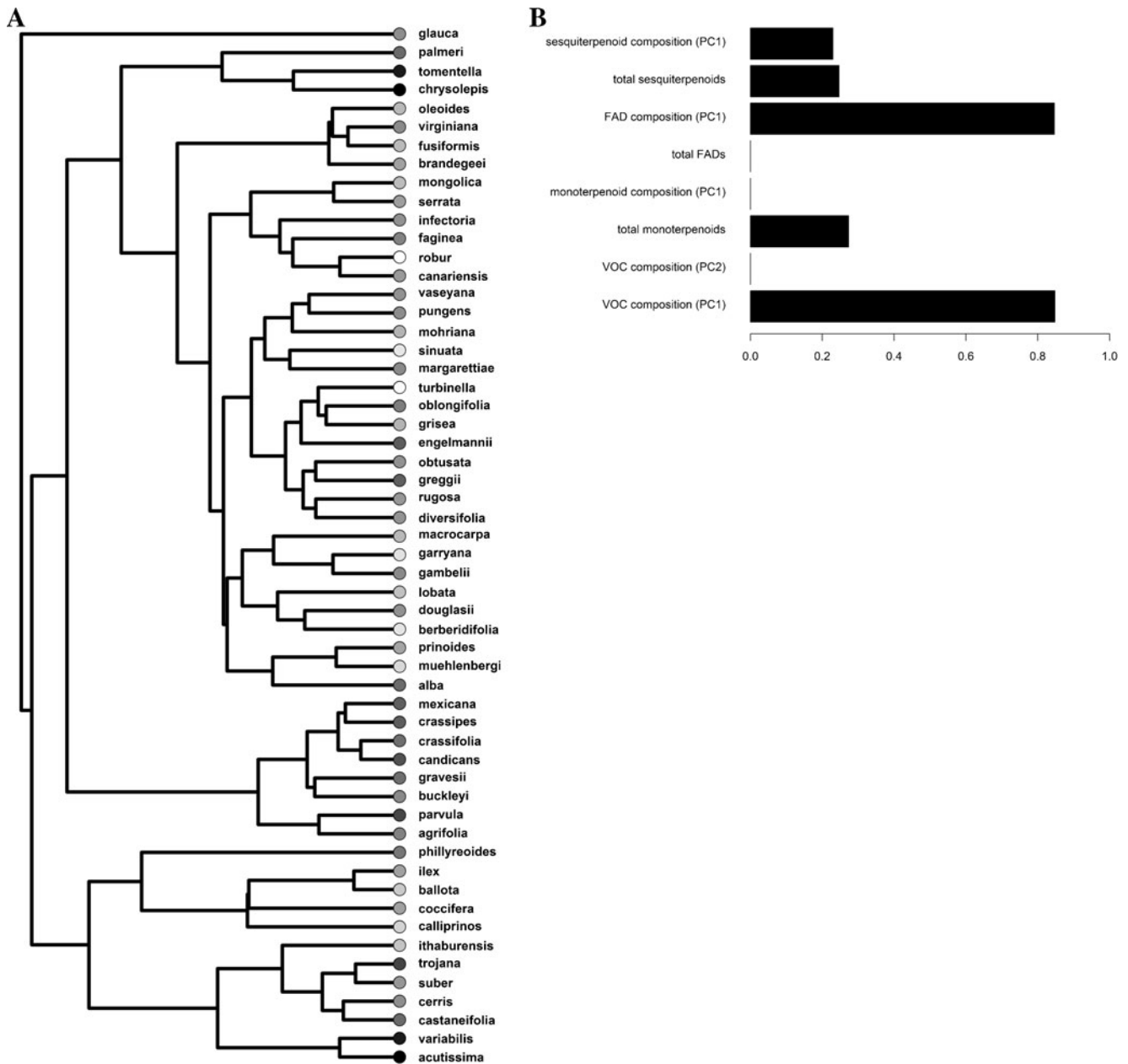


Fig. 2 Phylogenetic pattern of volatile profiles of 52 oak species. **a** The first principal component (PC-1) of total volatile profile mapped onto a phylogeny of the oak genus (*Quercus*). **b** Pagel's lambda, a measure of phylogenetic signal, in the composition (principal component axes) and

total abundance of the three major classes of oak volatiles. When Pagel's lambda approaches one, shared ancestry can explain the distribution of volatile production. When Pagel's lambda approaches zero, volatile production is random with respect to phylogeny

Phylogenetic Signal of Volatile Profile As we found wounded leaves to have species-specific odors (Table 2), we assessed whether wounded leaves of closely related species tended to have similar volatile profiles (Table 3, Fig. 2). We mapped the rounded abundance of each volatile (Table 3), each volatile class, and three principal component axes of volatile composition onto a recent oak phylogeny (Fig. 2a). We then calculated Pagel's lambda (a measure of phylogenetic signal, or clustering of a trait among closely related species, that ranges from 0 non-conserved to 1 highly conserved) for each trait. The first principal component of total volatile composition had a high lambda estimate that did not deviate significantly from 1 (Fig. 2a, b). The high phylogenetic signal for total volatile composition was driven largely by a high phylogenetic signal in the composition of fatty acid derivatives (Fig. 2b). In contrast, the composition of sesquiterpenes and monoterpenes showed low phylogenetic signal. Likewise, the total abundance of fatty acid derivatives showed low phylogenetic signal, and the total abundance of monoterpenes and sesquiterpenes showed an intermediate phylogenetic signal (Fig. 2b). The phylogenetic signal of individual metabolites ranged dramatically (Table 3). Interestingly, several structurally similar fatty acid derivatives including (*Z*)-3-hexenyl butyrate, (*Z*)-3-hexenyl-2-methylbutyrate, and (*E*)-2-hexenyl butyrate all showed high phylogenetic signal in oaks, suggesting that these compounds may underlie phylogenetic differences in odor (Table 3).

Volatiles as a Signal of Defense We tested whether volatile profile contained information about an oak species' defensive investment. We calculated defensive investment as a principal component axis of 9 leaf defensive traits, which, in recent feeding trials, explains a high portion of the variation in the survivorship of a generalist caterpillar (Pearse, 2011). We then constructed a redundancy analysis model of volatile composition with leaf defensive investment as a constraining axis. We found that leaf defensive investment was not a significant constraining axis for oak volatile composition (RDA permutation test: 10,000 permutations, $P=0.498$). Next, we tested the hypothesis that oak species with a higher volatile diversity also invest more in leaf defenses, but we found little support for this (Fig. 3, $r=0.13$ $P=0.33$). Likewise, we found no relationship between total VOC abundance and leaf defenses ($r=-0.01$, $P=0.63$).

Discussion

The volatile profiles of different oak species are distinguishable from one another only when the leaves are wounded (Table 2). This suggests that in an environment in which a tree's leaves are not substantially wounded, it may be possible for trees to escape their species-specific herbivores by

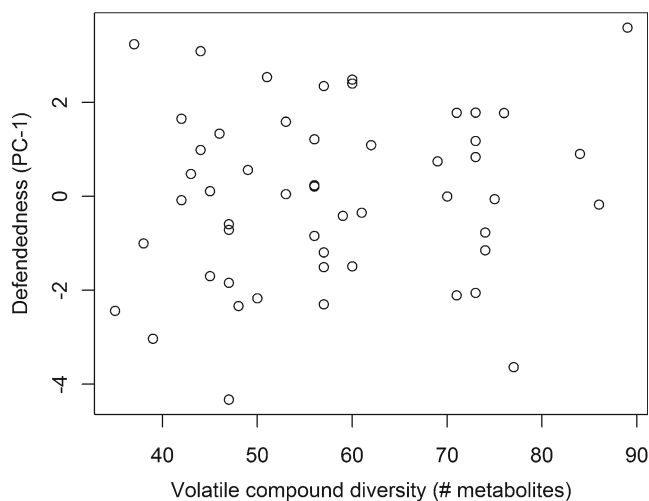


Fig. 3 The relationship between plant defensive investment (the first principal component PC-1 of 9 oak direct defenses) and volatile diversity (number of observed compounds). We found no correlation between volatile diversity and defensive investment

not disclosing their identity. Even though initial colonization by herbivores often takes place on largely undamaged tissue, many studies have not focused on volatile profiles of undamaged tissues, as most volatiles are greatly induced by damage, and undamaged volatile profiles are affected by many collection techniques (e.g., Courtois et al., 2009). Perhaps wounded leaves provide at least some estimate of a plant's sensory environment, as in a natural environment, trees are continuously exposed to low levels of herbivory as well as mechanical abrasion of leaves by wind or non-herbivorous animals. In this study, the "undamaged" leaves that we surveyed also were subject to low-level herbivory from thrips, aphids, and mirids as well as any mechanical disturbances to leaves, which probably represents a more accurate estimate of a tree's real background emissions than either excised leaves or completely clean plants grown in a laboratory. Likewise, patterns of herbivore damage to leaves are far more complex than the mechanical damage used to simulate herbivore damage in this study. In a concurrent survey, we found on average ca. 10 species of herbivores per oak tree (Pearse, unpublished data). Previous studies in other species have shown that herbivores elicit distinct volatile responses from a plant (Delphia et al., 2007), suggesting that mechanical damage may capture only some of the information contained in plant volatile profiles. Future studies might ask explicitly whether volatile profiles are more constrained by plant species identity or the herbivore that is causing the damage. Currently, this study shows that in the absence of leaf damage, all oaks smell alike.

For wounded leaves, we found that the between-species distribution of many oak volatiles could be explained by evolutionary conservatism (Fig. 2). In particular the composition of fatty acid derivative compounds (FAD's) appeared

to most closely mirror evolutionary relationships among oak species (Fig. 2b). This pattern is striking, because FAD's are also the most inducible compound group by mechanical damage (Fig. 1), are highly bioactive in attracting natural enemies of herbivores to plants (Rose et al., 1998), and are emitted only briefly following damage (Turlings et al., 1998). This suggests that volatile profiles advertise the taxonomic status of an oak only briefly after wounding, and that this information likely is perceived by herbivores and their predators alike. Interspecific patterns of terpenoid emission are broadly concordant with past studies of terpenoids in oaks (Loreto et al., 1998; Csiky and Seufert, 1999; Loreto, 2002), but differ in a few key features. Similar to Loreto (2002), we found isoprene emission in the Lobatae, *Quercus* s.s., and Protobalanus groups of oaks, but not in Cerris or Cyclobalanus groups (Supplemental Table 2). The taxonomic pattern of the emission of monoterpene compounds in our survey is more complicated than in previous studies that show clear taxonomic affiliations (Loreto et al., 1998; Csiky and Seufert, 1999). Concordant with these studies, however, the composition of monoterpenes in oaks shows no phylogenetic signal, but the total abundance of monoterpenes shows an intermediate phylogenetic signal (Fig. 2b). Both the abundance and the composition of sesquiterpene compounds (which were the most diverse compound group in our survey) showed only an intermediate phylogenetic signal (Fig. 2b).

We did not find support for the aposematic fragrance hypothesis in oaks. Neither volatile composition nor volatile diversity (Fig. 3) contained any information about an oak species' defensive investment. While we found no support for the aposematic fragrance hypothesis in oaks, it is possible that the fragrances of other plant groups may advertise their defenses. As an analogy, in animals, warning coloration is a common cue in some taxonomic groups, but not others (Mallet and Joron, 1999). Likewise, oak volatile profiles simply may correlate with defenses that were not assayed in this study. For example, in a previous studies, the same suite of oak defenses explained about half of the variation in the survival of a generalist moth on oaks (Pearse, 2011), but explained little variation in herbivore damage to oaks in a common garden (Pearse and Hipp, 2009), so there certainly are other defensive characteristics to oaks that were not surveyed here. Another hypothesis suggests that there might be negative correlations between types of defenses (Agrawal, 2012). In this case, investment in total VOC production as an indirect defense might correlate negatively with other defensive investment. However, we found no such negative correlation in our study.

Viewing plant volatile profiles as public signals offers new insights into the interactions of plants and higher trophic levels. For example, we show that volatile profiles advertise both species identity and evolutionary affiliation

only when leaves are damaged and may be recruiting natural enemies. Surveys of plant volatiles in a field setting are necessary to uncover what plant cues are actually available to higher trophic levels. The volatile detection capabilities of herbivores and natural enemies adds an additional constraint on the information contained in plant volatile profiles, and it would be interesting to observe what information plant volatiles contain within the detection limits of interacting organisms, for example by limiting volatile constituents to those compounds that are physiologically detectable by interacting insects. As an alternative view, recent studies have shown that insects perceive complex volatile mixtures as more than the sum of their parts, where even compounds that individually elicit no behavioral response may modulate the response to other VOC constituents (van Wijk et al., 2010, 2011). Our study suggests that fatty acid derivatives (FADs) may be particularly valid signals of a plant's identity, as the composition of this compound group appears to be taxonomically conserved in the oaks. FADs are also the most inducible compounds in an oak's volatile profile, so taxonomic identity was only advertised immediately following leaf damage. In some situations, oaks advertise their identity, and in other situations they hide their chemical identity.

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