Measurement of volatile terpene emissions in 70 dominant vascular plant species in Hawaii: aliens emit more than natives

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ABSTRACT

Aim Alien plant invasion is prominent in the Hawaiian Islands. There are many factors involved in invader success. To date, there is a general lack of information about one of them, which we aim to study here: the terpene emission capacity of both Hawaiian native and alien plants.

Location Oahu (Hawaii).

Methods We screened 35 alien and 35 native dominant plant species on Oahu Island for monoterpene emissions. The emission rates were measured from field-grown plants under standardized conditions of temperature and quantum flux density in the laboratory.

Results The emission rates of total terpenes ranged from 0 \( \mu g \cdot g^{-1} \cdot h^{-1} \) to 55 \( \mu g \cdot g^{-1} \cdot h^{-1} \), and altogether 15 different terpenes were emitted in detectable amounts by the overall set of species. A phylogenetic signal was observed for total terpene emissions. Total terpene emission rates were higher in aliens than in native species (12.8 ± 2.0 vs. 7.6 ± 1.9 \( \mu g \cdot g^{-1} \cdot h^{-1} \), respectively).

Main conclusions The greater terpene emission capacity may confer protection against multiple stresses and may partly account for the success of the invasive species, and may make invasive species more competitive in response to new global change-driven combined stresses. These results are consistent with aliens coming from very diverse ecosystems with generally higher biotic and abiotic stress pressures, and having higher nutrient concentrations. On the contrary, these results are not consistent with the ‘excess carbon’ hypotheses. These results indicate changes in vegetation terpene emissions brought about by alien plant invasions.

Keywords Alien species, \( \alpha \)-pinene, Hawaiian Islands, monoterpenes, nitrogen, phosphorus, sesquiterpenes, species invasions, terpene emission.

INTRODUCTION

The success of invasive plants is an important component of current global change (Mooney & Hobbs, 2000). Chemical factors such as chemical defences can be involved in the competition between alien and native plant species. Among plant chemical defences, a group of the carbon-based secondary compounds, the terpenes, has been shown to have direct and indirect roles in protecting plants against herbivory (Llusia & Penuelas, 2001; Peñuelas & Llusià, 2004; Munné-Bosch & Hilker, 2006), as well as in contributing to the plant’s defence strategies against thermal damage (Sharkey & Singsaas, 1995; Copolovici et al., 2005; Peñuelas et al., 2005). Terpenes may also act as antioxidants, protecting the plant membranes against peroxidation and reactive oxygen species such as singlet oxygen (Loreto & Velikova, 2001; Peñuelas & Llusia, 2002; Loreto et al., 2004; Munné-Bosch et al., 2004). All these and other defensive and protective properties of terpenes (Peñuelas & Munné-Bosch, 2005) imply that differences in terpene emission potentials between alien and native plants might be an additional factor in invasion success.

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Although few studies have compared the terpene emissions of native and alien species, some studies show higher terpene emissions in aliens than in co-occurring native species. For example, the presence of bioactive volatile terpenoids has been suggested to play an important role in the establishment and proliferation of *Artemisia vulgaris* in an introduced habitat (Barney et al., 2005). Khanh et al. (2008) have also reported that the parasitic weed *Cuscuta hygrophila* has strong allelopathic potential, exerting a strong inhibitory effect on the growth of native paddy weeds and that volatile terpenoids are some of the substances responsible for this effect. Other studies suggest, however, that monoterpenes released by invasive species would have little direct activity in their volatile gaseous state and instead would have stronger activity in the soil matrix (Barney et al., 2009).

Tropical island ecosystems appear to be particularly sensitive to alien species invasions. Experimental evidence suggests that the high resource availability and the poor ability of native species to capture these resources, makes island communities vulnerable to the establishment and spread of alien species (Allison & Vitousek, 2004). The Hawaiian Islands, one of the most isolated spots on Earth (Vitousek & Walker, 1989), are especially vulnerable to invasions by non-indigenous species (Harrington & Ewel, 1997). Hawaiian native ecosystems and plant species are strongly affected by alien plant species (Mack & D’Antonio, 2003; Hughes & Denslow, 2005; Hughes & Uowolo, 2006). In the Hawaiian Islands, around 861 flowering plant species (47% of the total Hawaiian angiosperm flora) are naturalized alien species (Wagner et al., 1999). As a result, approximately 25% of the Hawaiian native flora, 90% of which is endemic, have been listed as threatened or endangered.

Invasive plants can change N and P cycles (Ehrenfeld, 2003; Ashton et al., 2005), and this has been observed to occur in the Hawaiian Islands as well (Allison & Vitousek, 2004; Hughes & Denslow, 2005). In particular, higher nutrient capture and use by invasive species has been demonstrated (Ashton et al., 2005; Hughes & Denslow, 2005; Dassonville et al., 2007; Siemann & Rogers, 2007; Peñuelas et al., 2009). Changes in the nutrient availability and use affect terpene production. Higher nitrogen availability is usually expected to be translated into higher terpene production and emission, as a result of increased carbon fixation and activity of the limiting enzymes (Harley et al., 1994; Litvak et al., 1996; King et al., 2004). However, recent studies have observed a decrease in terpene emissions in *Phragmites australis* at high levels of phosphorus supply (Fares et al., 2008). Moreover, since emissions protect against stress, a strong expression of the capacity for emission is expected to make the plants more competitive in stressful environments. This is in line with the opportunistic emissions hypothesis (Peñuelas & Llusia, 2004; Owen & Peñuelas, 2005). However, a negative relationship has been found between the concentration of N and P (extractable phosphorus) and terpene emissions in *Pinus halepensis* (Blanch et al., 2007); but no relationship has also been reported (Heyworth et al., 1998; Ormeno et al., 2007). In fact, a lower production of terpenes as carbon-based secondary compounds (CBS) under higher nutrient availabilities can be expected from the CBS source–sink or ‘excess carbon’ hypotheses. When fixed CO₂ is in ‘excess’ because it cannot be processed for growth, i.e. when carbon sources exceed carbon sinks, these hypotheses expect a higher allocation to defensive and storage CBS (Loomis, 1932; Bryant et al., 1983; Herms & Mattson, 1992; Peñuelas & Estiarte, 1998).

In the Hawaiian Islands, as far as we know, only certain weed species have been analysed for terpene emissions (Komai & Tang, 1989), and no comprehensive comparison between native and alien species has been conducted. In this study, we screened a total of 70 dominant species, 35 natives and 35 aliens for leaf volatile terpene emissions in Oahu, Hawaii. Our specific aims were: (1) To screen the terpene emission in the Hawaiian flora, including the terpene emission of several species not previously analysed, and capture the dominant species in the ecosystems analysed. (2) To test which of the proposed hypotheses for the terpene emission rates fit better the results of the screening analyses, namely either that aliens emit more terpenes as they are new arrivals, whereas natives may have reduced or lost the trait in these isolated spots with fewer biotic and abiotic pressures, and/or that aliens emit more as they have higher concentrations of nutrients and enzymes, or that aliens emit fewer terpenes because they have higher nutrient concentrations and growth and a lower allocation to carbon-based secondary compounds. (3) To test whether the difference between aliens and natives is caused by taxonomic relatedness (species phylogeny). (4) To learn whether communities at different elevations dominated by different sets of species have different capacities for terpene emission. (5) To relate the emission rates to chemical and physiological variables such as nutrient concentrations, photosynthetic capacity or leaf mass per area (LMA).

**MATERIALS AND METHODS**

**Field sites**

The study was conducted in May 2007 in Oahu, the third largest of the Hawaiian Islands. As is typical of the larger Hawaiian Islands, the climate is characterized by very steep rainfall gradients over short distances (Muller-Dombois & Fosberg, 1998). Lowlands on the leeward side have a pronounced dry summer season, while precipitation is distributed almost uniformly in lowland and mountain rain forests. Due to the oceanic tropical climate, temperature oscillations are small with winters having temperatures on average 2–3 °C cooler than summers. As large differences in the composition of native and alien vegetation occur in response to rainfall gradients, four sites with distinct precipitation regimes were selected for plant sampling in the leeward lowlands of Oahu and the leeward side of the Koolau Mountains (Table 1). For a detailed description of the study sites and their soil characteristics, see Peñuelas et al. (2009).

**Plant sampling and site climate**

The twigs for gas-exchange measurements were sampled during the morning between 08:00 and 12:00 h. Due to difficult topog-
Tertiary growth forms were drought-deciduous (some of the alien species, mostly legumes, collected in the dry species (Fig. 1). All native species sampled were evergreen, but sampled in the four sites, including 35 native and 35 alien species (Table 1). A total of 70 species, including all dominant species, were

Table 1 Description of the study sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>Coordinates altitude (m)</th>
<th>Average ± SD†</th>
<th>Average ± SD precipitation (mm)</th>
<th>Average ± SD annual temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St Louis Heights</td>
<td>21°18’ N, 157°48’ W</td>
<td>170 ± 70</td>
<td>1430 ± 210</td>
<td>18.7 ± 0.5 26.9 ± 0.5 13 0 13</td>
</tr>
<tr>
<td>Hahaione Valley</td>
<td>21°19’ N, 157°43’ W</td>
<td>390 ± 140</td>
<td>1268 ± 22</td>
<td>17.1 ± 0.6 25.7 ± 0.5 11 2 9</td>
</tr>
<tr>
<td>Tantalus</td>
<td>21°20’ N, 157°49’ W</td>
<td>441 ± 24</td>
<td>3670 ± 440</td>
<td>16.2 ± 0.6 24.1 ± 0.6 20 12 8</td>
</tr>
<tr>
<td>Wiliwilinui</td>
<td>21°19’ N, 157°45’ W</td>
<td>660 ± 120</td>
<td>2100 ± 150</td>
<td>15.2 ± 0.9 23.8 ± 0.8 26 21 5</td>
</tr>
</tbody>
</table>

†Averages are based on the number of species sampled and species-specific locations. In statistical analyses, exact species-specific environmental data were used. n, number of samples; N, native; A, alien.

...phy, gas-exchange measurements were conducted in the laboratory using cut twigs, as is common in plant gas-exchange studies (Larcher, 1963; Ludlow, 1991; Kull & Niinemets, 1998). Plant sampling for the gas exchange follows the protocol of Niinemets et al. (2009). The twigs were cut under water and retained in water in plastic bags with wet filter paper to stop transpiration until transportation to the laboratory. We standardized the sampling by choosing new well-developed leaves as much as possible. In the laboratory, the twigs were re-cut under water and stabilized at room temperature (23–25 °C) in dim light as recommended in previous studies (Niinemets et al. 2005, 2009). The measurements were conducted at 30 °C and 1000 μmol m⁻² s⁻¹ photosynthetic photon flux density (PPFD) within 2 days of collection. All leaf gas-exchange characteristics and foliage volatile organic emission rates were stable during this period. As previous studies have demonstrated, this stabilization period is needed to achieve high stomatal openness and get maximum and stable values for the net foliage assimilation rate (Niinemets et al. 2009). Control experiments demonstrated that stomatal conductance and photosynthesis immediately after shoot cutting were low, but recovered to maximum values after overnight stabilization. For each species, three individual plants were sampled. All the measurements were conducted in similar manner in natives and in aliens, in order to compare them. However, transferring these data to the field should be done with caution. See Peñuelas et al. (2009) for details on plant sampling and leaf economics analyses.

Species coordinates and sampling altitude were noted in each site, and this information was used to link species locations to specific soil types and to derive location-specific climatic data. We used ArcGIS 9.1 to determine long-term average monthly and annual precipitation, and average, maximum and minimum temperatures from high-resolution climatic grids (Giambelluca et al., 1986; Giambelluca & Nullet, 1991).

**Study species**

A total of 70 species, including all dominant species, were sampled in the four sites, including 35 native and 35 alien species (Fig. 1). All native species sampled were evergreen, but some of the alien species, mostly legumes, collected in the dry sites, were drought-deciduous (*Desmodium incanum, Falcataria moluccana, Senna surattensis, Tabebuia rosea*) or semi-deciduous (*Haematoxylon campechianum, Leucaena leucocephala*). Out of the 70 studied species, 42 were trees, 22 shrubs, 4 woody vines to shrubs, 1 herb to shrubs, and 1 mistletoe (*Korthalsella complanata*). For a detailed description of the plant growth forms, characteristic ecological distribution of the species and exact altitude of sampling see Peñuelas et al. (2009).

**C, N and P analyses**

Leaf samples were dried in an oven at 70 °C to a constant weight, and thereafter pulverized with a Cyclotec 1093 sample mill (Foss Tecator, Höganas, Sweden). In all cases, the sample mill was washed with double-distilled water after grinding each sample to avoid contamination.

For the estimation of P concentration, the samples of 100 mg of pulverized leaf dry mass were solubilized with an acid mixture of HNO₃ (60%) and HClO₄ (60%) (143255 and 141054, respectively, purissimum, Panreac, Barcelona) (2:1) in a microwave oven (Samsung, TDS, Seoul, South Korea) using Oak Ridge 50-ml Teflon centrifuge tubes (Nalge Nunc International, Rochester, NY, USA). Two millilitres of the mixed acid solution per 100 mg of dry biomass was added to each sample. After digestion, the solutions were brought to 10 ml of final volume with distilled water. The P concentrations were measured with by inductively coupled plasma mass spectroscopy (ICP-MS; Agilent, model 7500CE, Tokyo, Japan). Standard certified biomass (DC73351, poplar leaf, purchased from China National Analysis Center for Iron and Steel) as well as two blanks (2 ml of acid mixture without a sample) were also analysed to assess the accuracy of digestion and the analytical procedures.

For the determination of C and N concentration, 1–2 mg of fine-sieved biomass plus 2 mg of V₂O₅ as oxidant were used. The C and N biomass concentrations were analysed by gas chromatography after combustion in oxygen with a Thermo Electron gas chromatograph (model NA 2100, C. E. Instruments, Thermo Electron, Milan, Italy).

**Measurements of leaf terpene emissions**

Three different plants were sampled for each of the species studied. Terpene sampling was conducted using a gas exchange
formations as checked against trapped standards (water. In these tubes, terpenes did not undergo chemical trans-
vices of activated carbon minimized sample displacement by Qmax air sampling pump (Supelco). The hydrophobic proper-
separated by plugs of quartz wool. Samples were taken using a Carboxen 1003, and Carbopack Y (Supelco, Bellefonte, PA)
diameter) manually filled with terpene adsorbents Carbopack B,
pumped through a glass tube (8 cm long and 0.3 cm internal
change cuvette of 25 cm². Exhaust air from the cuvette was
with a stream of purified helium. The sampling time was
10 min, and the flow varied between 470 and 500 ml min⁻¹ depending on the glass tube adsorbent and quartz wool packing.
The trapping and desorption efficiency of liquid and volatilized
standards such as α-pinene, β-pinene or limonene was 99%.
Blank air sampling on tubes was conducted for 10 min imme-
diately before and after each measurement without the plants in the
cuvettes. The glass tubes were stored in a portable fridge at
−28 °C until the analysis. Analyses of the replicate samples
immediately and after 6 months’ storage indicated no detectable
changes in terpene concentrations after storage of the tubes. In
calculations of the terpene emission rates, terpene contents in
the blank samples measured without the plants were subtracted from the samples measured with the plants.

Terpene analyses were performed by a gas chromatography–
mass spectrometry (GC-MS) system (Hewlett Packard HP59822B, Palo Alto, CA, USA). The monoterpenes trapped on
the tubes were processed with an automatic sample processor
(Combi PAL, Focus-Atas GL International, Veldhoven, The
Netherlands) and desorbed using an OPTIC3 injector (Atas GL
International) into a 30 m × 0.25 mm × 0.25 μm film thickness capillary column (HP-5, Crosslinked 5% pHe ME Silicone,

Figure 1 Phylogenetic tree of the woody plant species studied (in four sites as indicated on the map), obtained from the phylomatic program (Webb & Donoghue, 2005). The scale depicts millions of years.
Supelco). The injector temperature (60 °C) was increased at 16 °C s^-1 to 300 °C. The injected sample was cryofocused at –20 °C for 2 min. After this time, the cryotrap was heated rapidly to 250 °C. Helium flow was 0.7 ml min^-1. Total run time was 23 min and the solvent delay was 4 min. After the sample injection, the initial temperature (40 °C) was increased at 30 °C min^-1 up to 60 °C, and thereafter at 10 °C min^-1 up to 150 °C. This temperature was maintained for 3 min, and thereafter increased at 70 °C min^-1 up to 250 °C, and maintained for another 5 min. Helium flow was 1 ml min^-1.

The identification of monoterpenes was conducted by comparing the retention times with standards from Fluka (Buchs, Switzerland), and the fractionation mass spectra with standards, literature spectra, GCD Chemstation G1074A HP and the mass spectra library wiley7n. Terpene concentrations were determined from calibration curves. The calibration curves for common monoterpenes, α-pinene, Δ^3-carene, β-pinene, β-myrcene, p-cymene, limonene and sabine, and common sesquiterpenes such as α-humulene were determined once every five analyses using four different terpene concentrations. The calibration curves were always highly significant ($r^2 > 0.99$ for the relationships between the signal and terpene concentration). The other monoterpenes and sesquiterpenes were calibrated using these calibration curves of the most common mono- and sesquiterpenes. The most abundant terpenes had very similar sensitivity with differences among the calibration factors of less than 5%. The quantification of the peaks was conducted using the fractionation product with mass 93.

Statistical analyses

The program PHYLOMATIC (Webb & Donoghue, 2005) was used to build a phylogenetic tree for the species studied (Fig. 1). The phylogeny was transformed into a pdtree document of the phylogenetic distances with the pdtree 5.0 module (University of California, Riverside, CA). Then, the pddist module (University of Wisconsin, Madison, WI) was used to create the distance matrices in ASC format. The phylogenetic signal (Blomberg & Garland, 2002) was calculated for all the leaf variables analysed employing MATLAB 7.6.0 with the physig module developed by Blomberg et al. (2003). A k statistic was calculated which indicates the amount of signal in the emission trait relative to what would be expected for the specified phylogenetic tree (topology and branch lengths) given a Brownian motion model of evolution. If $k = 1$, then the specific emission trait has exactly the amount of signal expected for the given phylogenetic tree, whereas values greater than 1 indicate more signal than expected and values less than 1 indicate less signal than the expected. To determine whether the observed phylogenetic signal was statistically significant, the actual data was compared with the values obtained after the data had been permuted randomly across the tips of the tree without the phylogenetic relationships. With this aim, 1000 random datasets were simulated under the Brownian motion assumption (Garland et al., 1993; Blomberg et al., 2003). Thus, the $k$ statistic and the probability of error in rejecting the phylogenetic signal ($P$) were determined according to Blomberg et al. (2003). Thereafter the variables with $P > 0.10$ were analysed by an ordinary generalized linear model (GLM) without the phylogenetic distances matrix, using MATLAB 7.6.0 with the regressionv2 module (Lavin et al., 2008). The variables with $0.10 > P > 0.05$ were analysed by an ordinary GLM without and with a phylogenetic distance matrix, and the model with the lower Akaike information criterion (AIC) was selected. Finally, the variables with a $P < 0.05$ were directly analysed by a GLM using a phylogenetic distance tree matrix.

The altitude above sea level of each species collection was significantly correlated with all the main climatic variables of each site (total annual precipitation, the precipitation of the three driest months, mean annual temperature, annual mean of the daily minimum temperatures, annual mean of the daily maximum temperatures – data not shown). Because of these strong covariations, altitude was used as a covariate in all the GLMs to take into account the effects that climate could have on the leaf variables analysed.

To examine all the environmental, phylogenetic and species origin effects on total emitted terpenes, we conducted a GLM with site (four different sample sites), species origin (native and alien) and soil type (five different soil types) as independent categorical variables, and altitude as independent continuous variable; in the case of variables with a significant phylogenetic signal, phylogenetic distances were also included as a continuous independent factor. Correlation analyses were also conducted between terpene emissions and leaf chemical, anatomical and physiological traits, and climate variables. To conduct these analyses we used MATLAB 7.6.0 with the regressionv2 module (Lavin et al., 2008).

RESULTS

Terpene emissions in relation to species phylogeny and origin

The emission rates of total terpenes ranged from 0 μg g^-1 h^-1 in Trema orientalis and Scaevola gaudichaudiana to 55 μg g^-1 h^-1 in Elaeocarpus bifidus. Almost all the species studied (68 out of 70) emitted at least one terpene compound (Table 2 and Appendices S1–S3 in Supporting Information) under the sampling conditions of this study. Phylogenetic signal (i.e. resemblance between closely related species) among the 15 different terpenes detected was only observed in the case of α-pinene (Table 3), which was the monoterpene emitted by more species and with higher emission rates. The other 14 terpenes did not exhibit significant phylogenetic signal (Table 3). However, the total terpene emissions (the sum of emissions of all terpenes), dominated by α-pinene emissions, did show a significant phylogenetic signal (Table 3).

The emissions of α-pinene were more than twice as high in alien species (8.2 ± 1.6 μg g^-1 h^-1) compared with native species (3.5 ± 1.1 μg g^-1 h^-1) ($P < 0.05$, Table 3). Overall, the total terpene emission rates were also greater ($P < 0.05$) in alien species (13.3 ± 2.0 μg g^-1 h^-1) than in native species (8.7 ± 1.9 μg g^-1 h^-1) (Fig. 2), although the variability was high.
Table 2 Monoterpenes and sesquiterpene emission rates (µg g⁻¹ h⁻¹) for the species studied.

<table>
<thead>
<tr>
<th>Plant type</th>
<th>Alien species</th>
<th>Total monoterpenes</th>
<th>Total sesquiterpenes</th>
<th>Plant type</th>
<th>Native species</th>
<th>Total monoterpenes</th>
<th>Total sesquiterpenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shrub</td>
<td>Ageratina adenophora</td>
<td>24.35 ± 13.05</td>
<td>0.81 ± 0.57</td>
<td>Tree</td>
<td>Acacia koa</td>
<td>4.04 ± 0.01</td>
<td>n.d.</td>
</tr>
<tr>
<td>Shrub</td>
<td>Ardisia crenata</td>
<td>3.60 ± 0.96</td>
<td>0.18 ± 0.12</td>
<td>Shrub</td>
<td>Alyxia stellata</td>
<td>15.02 ± 5.55</td>
<td>0.77 ± 0.46</td>
</tr>
<tr>
<td>Tree</td>
<td>Bischiera javanica</td>
<td>7.78 ± 1.10</td>
<td>0.04 ± 0.02</td>
<td>Tree</td>
<td>Antidesma platypellum</td>
<td>2.72 ± 1.29</td>
<td>0.28 ± 0.13</td>
</tr>
<tr>
<td>Shrub</td>
<td>Buddleja asiatica</td>
<td>4.42 ± 0.43</td>
<td>1.86 ± 0.84</td>
<td>Tree</td>
<td>Bollea elatior</td>
<td>5.95 ± 3.98</td>
<td>n.d.</td>
</tr>
<tr>
<td>Shrub</td>
<td>Campanula retusa</td>
<td>0.60 ± 0.19</td>
<td>0.03 ± 0.02</td>
<td>Shrub</td>
<td>Boussaisia arguta</td>
<td>0.38 ± 0.31</td>
<td>n.d.</td>
</tr>
<tr>
<td>Shrub</td>
<td>Cestrum nocturnum</td>
<td>10.18 ± 3.88</td>
<td>0.09 ± 0.05</td>
<td>Tree</td>
<td>Clermontia oblongifolia</td>
<td>2.16 ± 0.56</td>
<td>0.59 ± 0.13</td>
</tr>
<tr>
<td>Tree</td>
<td>Citharexylum caudatum</td>
<td>0.47 ± 0.12</td>
<td>n.d.</td>
<td>Shrub</td>
<td>Coprosma longifolia</td>
<td>0.88</td>
<td>0.02</td>
</tr>
<tr>
<td>Shrub</td>
<td>Clerodendrum macrostegium</td>
<td>5.67 ± 3.03</td>
<td>n.d.</td>
<td>Shrub</td>
<td>Cyanea angustifolia</td>
<td>31.43 ± 2.13</td>
<td>n.d.</td>
</tr>
<tr>
<td>Shrub</td>
<td>Clidemia hirta</td>
<td>8.35 ± 2.67</td>
<td>n.d.</td>
<td>Tree</td>
<td>Diospyros sandwicensis</td>
<td>3.43 ± 1.07</td>
<td>0.06 ± 0.04</td>
</tr>
<tr>
<td>Shrub</td>
<td>Desmodium incanum</td>
<td>11.66 ± 3.13</td>
<td>0.13 ± 0.09</td>
<td>Tree</td>
<td>Baeoaarpus hulderi</td>
<td>50.92</td>
<td>4.35</td>
</tr>
<tr>
<td>Tree</td>
<td>Eucalyptus robusta</td>
<td>11.81 ± 3.88</td>
<td>n.d.</td>
<td>Liana</td>
<td>Freycinetia arborea</td>
<td>0.83 ± 0.12</td>
<td>n.d.</td>
</tr>
<tr>
<td>Tree</td>
<td>Emataxylum campechianum</td>
<td>53.86 ± 9.98</td>
<td>0.02 ± 0.02</td>
<td>Tree</td>
<td>Hedyotis acuminata</td>
<td>18.83</td>
<td>0.95</td>
</tr>
<tr>
<td>Tree</td>
<td>Helicarpus americanus</td>
<td>38.31 ± 0.37</td>
<td>1.94 ± 0.10</td>
<td>Parasitic shrub</td>
<td>Korthalsella complanata</td>
<td>13.09 ± 8.14</td>
<td>0.13 ± 0.11</td>
</tr>
<tr>
<td>Liana</td>
<td>Jasminum fluminense</td>
<td>10.02 ± 7.02</td>
<td>n.d.</td>
<td>Tree</td>
<td>Melicope dusifolia</td>
<td>0.64 ± 0.36</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Tree</td>
<td>Leucena leucocephala</td>
<td>19.98 ± 11.83</td>
<td>n.d.</td>
<td>Shrub</td>
<td>Melicope peduncularis</td>
<td>20.03 ± 11.82</td>
<td>1.50 ± 0.61</td>
</tr>
<tr>
<td>Tree</td>
<td>Mangifera indica</td>
<td>1.17</td>
<td>0.32</td>
<td>Tree</td>
<td>Metrosideros macropus</td>
<td>0.98</td>
<td>0.1</td>
</tr>
<tr>
<td>Shrub</td>
<td>Malacacta quinquenervia</td>
<td>29.42</td>
<td>3.36</td>
<td>Tree</td>
<td>Metrosideros polymorpha</td>
<td>2.66</td>
<td>0.5</td>
</tr>
<tr>
<td>Shrub</td>
<td>Murraya paniculata</td>
<td>14.58 ± 6.21</td>
<td>0.13 ± 0.09</td>
<td>Shrub</td>
<td>Metrosideros rugosa</td>
<td>2.38 ± 0.73</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Shrub</td>
<td>Odhna thomassiana</td>
<td>34.48 ± 0.78</td>
<td>4.11 ± 1.81</td>
<td>Shrub</td>
<td>Metrosideros tremuloides</td>
<td>9.96</td>
<td>2.82</td>
</tr>
<tr>
<td>Liana</td>
<td>Passiflora suberosa</td>
<td>22.70 ± 11.37</td>
<td>0.40 ± 0.15</td>
<td>Tree</td>
<td>Myrriina leptisiana</td>
<td>0.42 ± 0.30</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>Liana</td>
<td>Persoa americana</td>
<td>11.79 ± 3.41</td>
<td>0.06 ± 0.05</td>
<td>Tree</td>
<td>Myrriina sandwicensis</td>
<td>1.31 ± 0.54</td>
<td>10.43 ± 5.42</td>
</tr>
<tr>
<td>Tree</td>
<td>Pimenta dioica</td>
<td>7.41 ± 3.06</td>
<td>0.23 ± 0.10</td>
<td>Shrub</td>
<td>Osteomeles anthophiloides</td>
<td>3.50 ± 1.64</td>
<td>1.49 ± 0.10</td>
</tr>
<tr>
<td>Tree</td>
<td>Psidium cattleianum</td>
<td>4.33</td>
<td>0.98</td>
<td>Tree</td>
<td>Pipturus albidus</td>
<td>2.66 ± 1.73</td>
<td>9.71 ± 6.59</td>
</tr>
<tr>
<td>Tree</td>
<td>Psidium guajava</td>
<td>1.37 ± 0.12</td>
<td>n.d.</td>
<td>Tree</td>
<td>Psinum umbelifera</td>
<td>1.89 ± 1.11</td>
<td>0.18 ± 0.13</td>
</tr>
<tr>
<td>Shrub</td>
<td>Rubus rosifolius</td>
<td>3.72 ± 0.80</td>
<td>0.13 ± 0.03</td>
<td>Tree</td>
<td>Poteria sandwicensis</td>
<td>0.81 ± 0.16</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>Tree</td>
<td>Schefflera actinophylia</td>
<td>3.05 ± 1.20</td>
<td>1.18 ± 0.46</td>
<td>Tree</td>
<td>Psychotria marianiana</td>
<td>2.37 ± 1.68</td>
<td>0.12 ± 0.09</td>
</tr>
<tr>
<td>Shrub</td>
<td>Semna suntellensis</td>
<td>0.49 ± 0.20</td>
<td>n.d.</td>
<td>Tree</td>
<td>Santalum freycinetianum</td>
<td>13.85 ± 5.66</td>
<td>0.30 ± 0.09</td>
</tr>
<tr>
<td>Forb</td>
<td>Stachytarpheta capensis</td>
<td>4.45 ± 1.84</td>
<td>0.59 ± 0.23</td>
<td>Shrub</td>
<td>Scaevola gaudichaudiana</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Tree</td>
<td>Strychnium cunisi</td>
<td>8.24 ± 2.09</td>
<td>0.53 ± 0.06</td>
<td>Liana</td>
<td>Smilax melatomifolia</td>
<td>1.18 ± 0.33</td>
<td>n.d.</td>
</tr>
<tr>
<td>Tree</td>
<td>Tabebuia rosea</td>
<td>0.21 ± 0.15</td>
<td>0.07 ± 0.05</td>
<td>Shrub</td>
<td>Vaccinium calycinum</td>
<td>1.58 ± 0.51</td>
<td>n.d.</td>
</tr>
<tr>
<td>Tree</td>
<td>Trema orientalis</td>
<td>n.d.</td>
<td>n.d.</td>
<td>Tree</td>
<td>Wikstroemia ohlensii</td>
<td>3.88 ± 0.62</td>
<td>0.58 ± 0.38</td>
</tr>
</tbody>
</table>

n.d., not detected.

Values are mean ± SEM.
Terpene emissions of invasive plants

Table 3 Terpene emission rates (mean ± SE, μg g⁻¹ h⁻¹) depending on the origin.

<table>
<thead>
<tr>
<th>Emitted terpene</th>
<th>Native</th>
<th>Alien</th>
<th>P-value</th>
<th>Phylogenetic fingerprinting</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Pinene</td>
<td>3.55 ± 1.11</td>
<td>8.22 ± 1.65</td>
<td>0.025</td>
<td>P = 0.009</td>
</tr>
<tr>
<td>α-Thujene</td>
<td>0.52 ± 0.3</td>
<td>0.13 ± 0.05</td>
<td>0.32</td>
<td>P = 0.398</td>
</tr>
<tr>
<td>Camphene</td>
<td>0.62 ± 0.46</td>
<td>0.24 ± 0.06</td>
<td>0.90</td>
<td>P = 0.701</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>0.38 ± 0.16</td>
<td>1.10 ± 0.39</td>
<td>0.27</td>
<td>P = 0.084</td>
</tr>
<tr>
<td>Δ²-Carene</td>
<td>0.38 ± 0.13</td>
<td>0.561 ± 0.24</td>
<td>0.79</td>
<td>P = 0.382</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>0.28 ± 0.07</td>
<td>0.20 ± 0.09</td>
<td>0.90</td>
<td>P = 0.218</td>
</tr>
<tr>
<td>Limonene</td>
<td>0.65 ± 0.22</td>
<td>0.90 ± 0.15</td>
<td>0.097</td>
<td>P = 0.047</td>
</tr>
<tr>
<td>γ-Terpine</td>
<td>0.07 ± 0.02</td>
<td>0.10 ± 0.02</td>
<td>0.59</td>
<td>P = 0.871</td>
</tr>
<tr>
<td>p-Cymene</td>
<td>0.11 ± 0.04</td>
<td>0.14 ± 0.04</td>
<td>0.71</td>
<td>P = 0.200</td>
</tr>
<tr>
<td>p-Mentha-1,5,8</td>
<td>Δ1-triene</td>
<td>0.98 ± 0.39</td>
<td>0.99 ± 0.54</td>
<td>0.63</td>
</tr>
<tr>
<td>Junipene</td>
<td>0.68 ± 0.23</td>
<td>0.40 ± 0.13</td>
<td>0.26</td>
<td>P = 0.995</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>0.28 ± 0.23</td>
<td>0.05 ± 0.02</td>
<td>0.10</td>
<td>P = 0.691</td>
</tr>
<tr>
<td>α-Humulene</td>
<td>0.09 ± 0.06</td>
<td>0.03 ± 0.14</td>
<td>0.51</td>
<td>P = 0.986</td>
</tr>
<tr>
<td>α-Copaene</td>
<td>0.02 ± 0.02</td>
<td>0.02 ± 0.01</td>
<td>0.97</td>
<td>P = 0.421</td>
</tr>
</tbody>
</table>

The significance of origin and phylogeny in the general linear model Y = site + origin + soil type + altitude (covariate, in the variables that were correlated significantly with this variable) + phylogeny (covariate) are also shown. The k statistic indicates the amount of signal in a trait relative to what would be expected for the specified phylogenetic tree. Significant (and marginally significant) differences are highlighted in bold.

with several strong and very weak emitters present among aliens and natives. No different pattern was found for any of the different types of monoterpenes (Fig. 2), but sesquiterpene emissions were not significantly different between alien and native species (Fig. 2).

Terpene emissions versus foliage C, N and P leaf concentrations

There was no significant relationship between total leaf terpene emissions and P concentration in the species studied (R = 0.01, P = 0.94). α-Pinene and total terpene emissions were marginally and positively correlated with leaf N concentration (R = 0.23, P = 0.08 and R = 0.22, P = 0.07, respectively), but this correlation was not significant for alien and native species separately. The ratios between total leaf terpene emissions and leaf N concentrations, and total leaf terpene emissions and leaf P concentrations were not significantly different between the alien and native plants (Table 3). The higher total terpene emissions together with the higher leaf N concentrations and the marginally higher leaf P concentrations in alien than in native species (data not shown) implied no changes in the ratio between total leaf terpene emissions and leaf N and P concentrations.

Terpene emissions relationships with altitude, climate and leaf traits

The emissions of α-pinene, total terpenes, total monoterpenes and cyclic monoterpenes were negatively correlated with altitude (Table 4). Terpene emissions were not correlated with any of the precipitation estimates (total annual precipitation, average precipitation of the three driest months, data not shown). α-Pinene, β-pinene, total terpene, total monoterpene, cyclic monoterpenes and aromatic monoterpenes were positively correlated with mean annual temperature of the sampling site (Table 4). No other significant correlations were detected between terpene emissions and leaf nutrient concentrations, LMA or photosynthetic capacity (data not shown).

DISCUSSION

To the best of our knowledge, this is the first study of terpene emissions from a large number of Hawaiian plant species, providing the emission potentials not only for native species but...
also for a large number of aliens which have not previously been studied. In general, most of the species studied belong to families and orders in which many species emitting terpenes have been reported in the literature. However, of our study species only five, *Casuarina equisetifolia*, *Grevillea robusta*, *Melaleuca quinquenervia*, *Lantana camara* and *Persea americana*, had already been identified as terpene emitters (Rasmussen, 1978; Winer *et al*., 1998; Klinger *et al*., 1998). Thus the majority of terpene-producing species studied here are new additions to the literature (Table 2). Similar to results from screening studies in Mediterranean species (Owen *et al*., 1997; Lluïà & Peñuelas, 2000), most or almost all species emitted terpenes in this study (68 out of 70), even if the emissions were minute (for 9 species out of the 70, total terpene emission rates were below 1 μg g⁻¹ h⁻¹).

The key finding of this study, apart from the screening results, is that the average total monoterpene emission rate was 69% higher in alien than in native species. Given that terpenes most probably enhance the abiotic and biotic stress tolerance of the plant (Vickers *et al*., 2009), the invasive success of these alien plants can be partly explained by their higher terpene emission rates. Moreover, under novel combinations of stresses brought about by global change, aliens with greater emissions are expected to perform better.

The higher emission rates in alien plants are consistent with the hypothesis that aliens should have higher emission rates than natives, as the latter may have reduced their emission traits in these isolated areas where there are fewer biotic and abiotic pressures. The result also concords with the hypothesis that aliens emit more because they have higher concentrations of nutrients and enzymes (Harley *et al*., 1994; Litvak *et al*., 1996).

In contrast, the results do not support the ‘excess carbon’ hypotheses (Loomis, 1932; Bryant *et al*., 1983; Herm & Mattson, 1992; Peñuelas & Estiarte, 1998), as alien species were the species with higher terpene emissions in spite of also being the species with higher nitrogen concentrations. This result agrees with recent studies that show that the production of carbon-based terpenes does not follow the ‘carbon-nutrient balance’ or ‘excess carbon’ hypotheses because the synthesis and emission rates of many leaf monoterpenes are related to active photosynthesis and vigorous growth (King *et al*., 2004). However, there are also other studies that have reported higher terpene production at sites with low resource availability (Moore *et al*., 2004), in accordance with the ‘carbon-nutrient’ balance hypotheses, and there are still others finding no clear link between soil nutrient availability, growth and terpene production (Ormeño *et al*., 2008).

High terpene production suggests that alien plants can have a greater investment in lower-cost leaf chemical defences that have less or no effect on specialist herbivores (Landau *et al*., 1994) but are more effective against generalist herbivores. In fact, in the classical plant defence classification between qualitative and quantitative
quantitative defences (Coley et al., 1985), terpenes were placed in the qualitative defence group. This should be in accordance with recent modifications in the development of the increased competitive ability (EICA) hypothesis (Müller-Schärer et al., 2004) that proposes that invasion success is related to the production of less costly qualitative defences, such as terpenes, that may be more toxic to generalist herbivores (Joshi & Vrieling, 2005; Stastny et al., 2005).

The emission rate of the most frequently and abundantly emitted monoterpenes, \( \alpha \)-pinene, had a phylogenetic signal, i.e. there was a significant resemblance in \( \alpha \)-pinene emission rates among the most closely related species. Although the linkage between terpene emission and phylogeny has not been investigated in detail, a clear relation between the plant family and individual molecular terpene emission rates has been observed in a review of the floral scent of some plant families (Knudsen et al., 2006), and the capacity to emit several volatile compounds has been used as a basis for studying taxonomic relationships in oaks (Loreto et al., 1998). However, in the present study, many of the native plant species from families harbouring many strong emitters such as Myrtaceae (Metrosideros macropus or Metrosideros rugosa) had very low terpene emissions. Overall, these data demonstrate that the lower emission potentials in the native species compared with the alien species were not due to a lack of genera and families characteristically having high emissions, but in fact reflect the lower emission potential in natives relative to aliens within given phylogenetic lines.

The decrease in terpene emission with altitude was a consequence of the higher percentage of native species with lower terpene emission potentials at higher altitudes. Although alien species prevalently populate the stations characterized by warmer temperatures, the measurements were conducted at the same standard temperature and are therefore estimating emission rate capacities, not actual emission rates. Terpene emissions were positively related to the temperature of the sampling sites, in another indication of the effect of temperature history (Sharkey et al., 1999; Blanch et al. 2010). However, they were not correlated with precipitation, nor with leaf economic spectrum traits such as nutrient concentrations, LMA or photosynthetic capacity (data not shown; see data on these ecophysiological variables in Peñuelas et al., 2009).

Different terpene emission rates among alien and native plants could have several key consequences for the environment in the short and long term. Volatile organic compounds (VOCs) have an important role in atmospheric chemistry (Singh & Zimmerman, 1992; Peñuelas & LLüsia, 2003) and particularly in the development of aerosols and ozone (Andreae & Crutzen, 1997). Went (1960) recognized that foliar emissions of VOCs could have a significant impact on tropospheric chemistry by influencing the processes controlling the formation of atmospheric haze. In addition, aerosol production reduces atmospheric transmittance directly, and also indirectly, through the formation of cloud condensation nuclei (Huff Hartz et al., 2005; Engelhart et al., 2008). Therefore, VOC emissions can have significant consequences for climate at regional to global scales (Kulmala et al., 2004). Thus, any difference in the emission characteristics between native and alien species implies that any change in aerosol coverage from native- to alien-dominated ecosystems can alter whole ecosystem terpene emission scenario and thereby the atmospheric quality and climate. This in turn can either facilitate or constrain the invasive success of aliens in future climates.

Conclusions and final remarks

Most plant species (68 out of 70) in our study at Oahu, Hawaii, emitted terpenes under our sampling conditions. We have described the terpene emission rates of 70 species, of which 65 have not been reported before to be terpene emitters. Alien species emitted 69% more monoterpenes than native species. Terpene emission had a clear phylogenetic signal for the most common and abundant terpene compound, \( \alpha \)-pinene, but not for the others.

A greater capacity for terpene emissions that can protect plants against multiple environmental stresses may partially account for the invasive success of alien species in Hawaii. From an environmental perspective, plant invasion success in Oahu may involve an increase in the emission of the most abundantly emitted terpenes and of total terpenes. Thus these results indicate a possible change in patterns of terpene emissions brought about by aggressive alien plant invasion. This could affect both the chemical ecology of invaded ecosystems and the atmospheric chemistry and climate above them (Peñuelas & LLüsia, 2003). Since plant invasion success is an emerging global phenomenon, future studies should take into account the possible effects of the success of alien warm-climate plants world-wide with the concomitant impact on total VOC emission, atmospheric chemistry and climate.

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**SUPPORTING INFORMATION**

Additional Supporting Information can be found in the on-line version of this article:

**Appendix S1** Monoterpene emission rates for the species studied.

**Appendix S2** Sesquiterpene emission rates for each species studied.

**Appendix S3** Terpene emission rates depending on the sampling site and soil type, and results for the fixed factors of the general linear model.

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**BIOSKETCHES**

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