Plant Physiology and Biochemistry 142 (2019) 405–414

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Seasonal variations in emission rates and composition of terpenoids emitted from *Chamaecyparis formosensis* (Cupressaceae) of different ages

Ying-Ju Chen[a,b], Chun-Ya Lin[a], Huai-Wan Hsu[b], Chen-Ying Yeh[b], Yu-Han Chen[a], Ting-Feng Yeh[a,**], Shang-Tzen Chang[a,*]

[a] School of Forest and Resource Conservation, National Taiwan University, Taipei, 10617, Taiwan
[b] Division of Forest Chemistry, Taiwan Forestry Research Institute, Taipei, 10070, Taiwan

ARTICLE INFO

Keywords:
Chamaecyparis formosensis
Terpenoids
Emission rate
Seasonal variation
Tree age

ABSTRACT

Chamaecyparis formosensis (Cupressaceae) is among the most precious endemic conifers in Taiwan. Field study was conducted on seasonal variations in emission rates and compositions of terpenoids from this tree species of two different ages. A total of 21 terpenoids were detected, of which there were 13 monoterpenoids (MTs), 4 sesquiterpenoids (STs), and 4 diterpenoids (DTs). MTs dominated the emissions in both saplings and adult trees and produced more than 80% of terpene emissions. Contrasting seasonal pattern between saplings and adult trees was found. Total actual emissions from saplings were higher in cold seasons (range, 64.40 ± 13.18 to 140.74 ± 18.90 $ng\,g^{-1}\,h^{-1}$) than in warm seasons (range, 55.63 ± 15.84 to 63.48 ± 11.85 $ng\,g^{-1}\,h^{-1}$). Photosynthetically active radiation (PAR) was found to be the most important factor affecting terpene emissions from saplings. On the contrary, higher emissions were found in warm seasons for adult trees (range, 101.49 ± 12.29 to 181.35 ± 80.15 $ng\,g^{-1}\,h^{-1}$), and the emissions were mainly in response to temperature. Some compounds in *C. formosensis* of both ages (e.g., β-myrcene, α-terpinene, *trans*-β-ocimene, terpinen-4-ol, α-cedrene and *trans*-β-farnesene) showed comparably higher contents in cold seasons. Results presented here provide important fundamental information for better understanding of forest bathing and estimating air quality in Taiwan.

1. Introduction

Terpenoids are one of the major group of biogenic volatile organic compounds (BVOCs) emitted by vegetation into the atmosphere. They not only play an important role in ecological relationships with other plants and animals (Vivaldo et al., 2017; Llusia et al., 2013), but also have significant influence on atmospheric chemistry and pose potential threat to air quality. On the other hand, terpenoids are one of the major components of forest aerosols (Cho et al., 2017), also called "phytoncides", and are widely believed to have a positive impact on forest bathers' health by inhaling these naturally occurring phytochemicals (Franco et al., 2017). In view of both positive and negative effects of plant volatiles on human life, careful investigation of their emission behavior and chemical characteristics is necessary for fundamental analysis on photochemical smog and ecotourism. Diverse terpenes might possess different chemotherapeutic potency in forest bathing therapy (Cho et al., 2017). Therefore, the emission inventory of various plants is required for the further development and promotion of forest bathing or ecotourism. Emission rates and composition of BVOCs are two substantial factors governing their total reactivity in the atmosphere (Faiola et al., 2018), resulting in enhanced ozone production and formation of organic aerosols (Matsunaga et al., 2013). Both emission rates and terpene compositions are related to tree species and environmental factors (e.g., temperature and light intensity, etc.), as well as phenology (Fares et al., 2012; Yue et al., 2015) and tree age (Lim et al., 2011). However, there are limited reports describing seasonal variations in chemical compositions of terpene compounds (Matsunaga et al., 2013; Geron and Arnts, 2010), and only scant information on effects of tree age (Kim et al., 2005; Lim et al., 2011) on terpene emissions is available.

Compound-specific emissions vary over the year (Helmig et al., 2013; Mochizuki et al., 2011; Llusia and Peñuelas, 2000); hence, the variations in relative terpene emission are more likely to be tree- or species-dependent (Helmig et al., 2013). To understand better the impact of terpenoids on air quality in local and/or regional scale, and also the potential use as chemotherapeutic agents in different forest types, it

* Corresponding author.
** Corresponding author.
E-mail addresses: stfyeh@ntu.edu.tw (T.-F. Yeh), peter@ntu.edu.tw (S.-T. Chang).

https://doi.org/10.1016/j.plaphy.2019.08.002
Received 23 March 2019; Received in revised form 1 August 2019; Accepted 2 August 2019
Available online 05 August 2019
0981-9428/

is necessary to obtain detailed and complete terpene emission data for major/important tree species in each region. However, reports on volatile emissions from living trees in Taiwan are limited. To date, only two prior studies have examined terpene emissions from trees in Taiwan. Chen et al. (2010) focused on emissions from saplings of *Calocedrus macrolepis* var. *formosana* in winter, while Lin et al. (2015) monitored emissions from adult trees of *Cryptomeria japonica* in fall (September) and winter (December). Extending their scope of research, this study compares terpene emissions from saplings and adult trees of *Chamaecyparis formosensis* in four different seasons.

C. formosensis is an important endemic conifer with a large number of plantations in Taiwan. This precious species is famous for the attractive fragrance and excellent durability of its wood. Numerous studies have demonstrated that terpene-contained essential oil from *C. formosensis* showed good biological activities (i.e., antitermitic activities (Cheng et al., 2007; Hsu et al., 2016), antipathogenic activities (Ho et al., 2012), and antifungal activity (Wang et al., 2005)). The present study measured both actual and basal emissions rates of monoterpenoids (MTs), sesquiterpenoids (STs), and diterpenoids (DTs). The relative contributions of individual compounds to the total emission were compared, and the seasonal variations were investigated. Furthermore, the dominant environmental factor affecting the emission behavior in trees of different ages was identified. Results presented here provide fundamental information for understanding seasonal variations in emission behavior in trees of different ages, and will be helpful in better estimation of air quality to further predict the possible benefits of forest bathing in Taiwan in the future.

2. Results and discussion

2.1. General emission pattern

The relative emission rates (RERs) of different classes of terpenes emitted from saplings and adult trees are shown in Fig. 1. MTs were the dominant terpenoids emitted in all seasons (84.90–94.64% in saplings; 82.20–93.73% in adult trees). STs account for emissions of only 1.82–10.29% in saplings, and 3.30–15.79% in adult trees. The proportion of DTs was the highest in winter, with emission of 4.81% in saplings and 7.06% in adult trees.

Fig. 2 shows RERs of terpenes from *C. formosensis*. The contribution of α-pinene emission (23.17–69.82%) from saplings was predominant in all seasons, followed by that of limonene (0.16–17.55%), β-myrcene (3.43–12.98%) and sabinene (0.71–12.49%) (Fig. 2a; Table S1). In adult trees, α-pinene was also predominant during most of the sampling seasons (38.46–49.16%) except for fall. *trans*-β-Ocimene (28.51%), α-pinene (21.05%) and (*E*,*E*)-α-farnesene (13.73%) accounted for more than 60% of RERs in fall (Fig. 2b; Table S1).

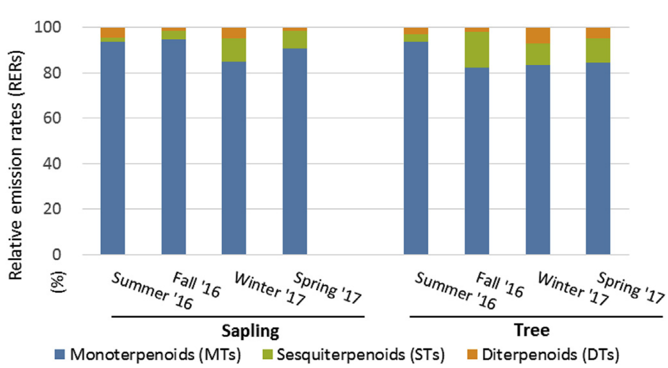

Fig. 1. Relative emission rates (RERs) of different terpene classes from saplings and adult trees in *C. formosensis*.

2.2. Seasonal variation in total actual emission rates (TERs)

To examine the effects of environmental factors on terpene emissions, the seasonal meteorological data were measured and illustrated in Fig. 3. Meteorological data from the experimental site showed high humidity throughout the year, with relative humidity ranging from 85.9 ± 2.1% - 89.8 ± 2.0%. The average air temperature was significantly higher in summer (26.00 ± 0.17 °C) and fall (25.20 ± 0.59 °C) than in spring (20.40 ± 0.63 °C) and winter (14.80 ± 0.50 °C). The PAR did not exhibit a significant seasonal trend, with 50.94 ± 8.30 μmol m^{-2} s^{-1} in summer, 77.48 ± 6.46 μmol m^{-2} s^{-1} in fall, 79.40 ± 8.36 μmol m^{-2} s^{-1} in winter, and 80.26 ± 9.62 μmol m^{-2} s^{-1} in spring.

Except for the saplings, the TERs (Fig. 4) presented here showed no statistically significant difference during the experimental periods. The TERs of saplings in spring (140.74 ± 18.90 ng g^{-1} h^{-1}) were significantly higher than other seasons, followed by winter (64.40 ± 13.18 ng g^{-1} h^{-1}), fall (63.48 ± 11.85 ng g^{-1} h^{-1}) and summer (55.63 ± 15.84 ng g^{-1} h^{-1}) (Fig. 4). The average emission rates in warm seasons (summer and fall) were comparable to those in winter even though the air temperature was significantly higher (Figs. 3 and 4). For adult trees, TERs were in general higher in warm seasons (summer and fall), but decreased in cold seasons (winter and spring), even though there were no significant differences among seasons. TERs of adult trees peaked in summer at 181.35 ± 80.15 ng g^{-1} h^{-1}, followed by that in fall (101.49 ± 12.29 ng g^{-1} h^{-1}), that in spring (40.15 ± 4.69 ng g^{-1} h^{-1}), and the lowest was observed in winter (35.32 ± 6.74 ng g^{-1} h^{-1}).

Multiple regression analysis was performed to determine the effect of different environmental variables (temperature, PAR and humidity) on terpene emissions. The statistics was calculated by summing all measurements in a dataset and dividing them into tertiles by PAR values (lowest tertile, ≤ 20 μmol m^{-2} s^{-1}; middle tertile, 20–80 μmol m^{-2} s^{-1}; highest tertile, ≥ 80 μmol m^{-2} s^{-1}). The relative importance of different variables in the three tertiles are shown as standardized coefficients (std β) (Table 1). As can be seen, the contribution of temperature (std β = 0.058 in lowest tertile; std β = −0.048 in middle tertile) was lower than that of PAR (std β = 0.309 in lowest tertile; std β = 0.498 in middle tertile) in both lowest and middle tertiles of saplings, indicating relatively lower contribution of temperature compared with PAR. On the contrary, for adult trees, temperature had the greatest effect on terpene emissions (std β = 0.425 in lowest tertile; std β = 0.725 in middle tertile) in the lowest or middle tertile, whereas PAR was the relatively most important factor (std β = 0.493) only in the highest tertile.

According to the regression results shown in Table 1, seasonal variations of terpene emissions from adult trees correlated mainly with air temperature. This observation is in agreement with the results of other species, such as *Pinus longaeva*, *P. pungens*, *P. halepensis, Pseudotsuga menziesii, Bupleurum fruticosum* and *Q. sube*, which usually have maximum emission rates during summer (Llusia et al., 2013; Helmig et al., 2013; Pio et al., 2005). The common feature of these species is that they contain a specialized storage, and terpene volatiles could be sequestered in these storage organelles. Some studies have suggested that terpene emissions (especially monoterpene) from storing species seem to depend more on temperature than light intensity (Llusia and Penuelas, 2000; Niinemets and Monson, 2013). *C. formosensis* was found to possess the storage structure (resin ducts) in both saplings and adult trees (Figs. S1a and b). To identify the difference of terpenoid concentration in storage structure between saplings and adult trees, the terpenoid content was measured using the solvent microextraction method modified from Sereshti et al. (2012). The content of the major compound α-pinene (accounting for over 90% of total terpenoids) was significantly higher (Scheffe's test, $p < 0.05$) in adult trees (276.56 ± 14.69 ng g^{-1} leaf dry wt) than in saplings (240.78 ± 21.25 ng g^{-1} leaf dry wt) (Fig. S1c), indicating that

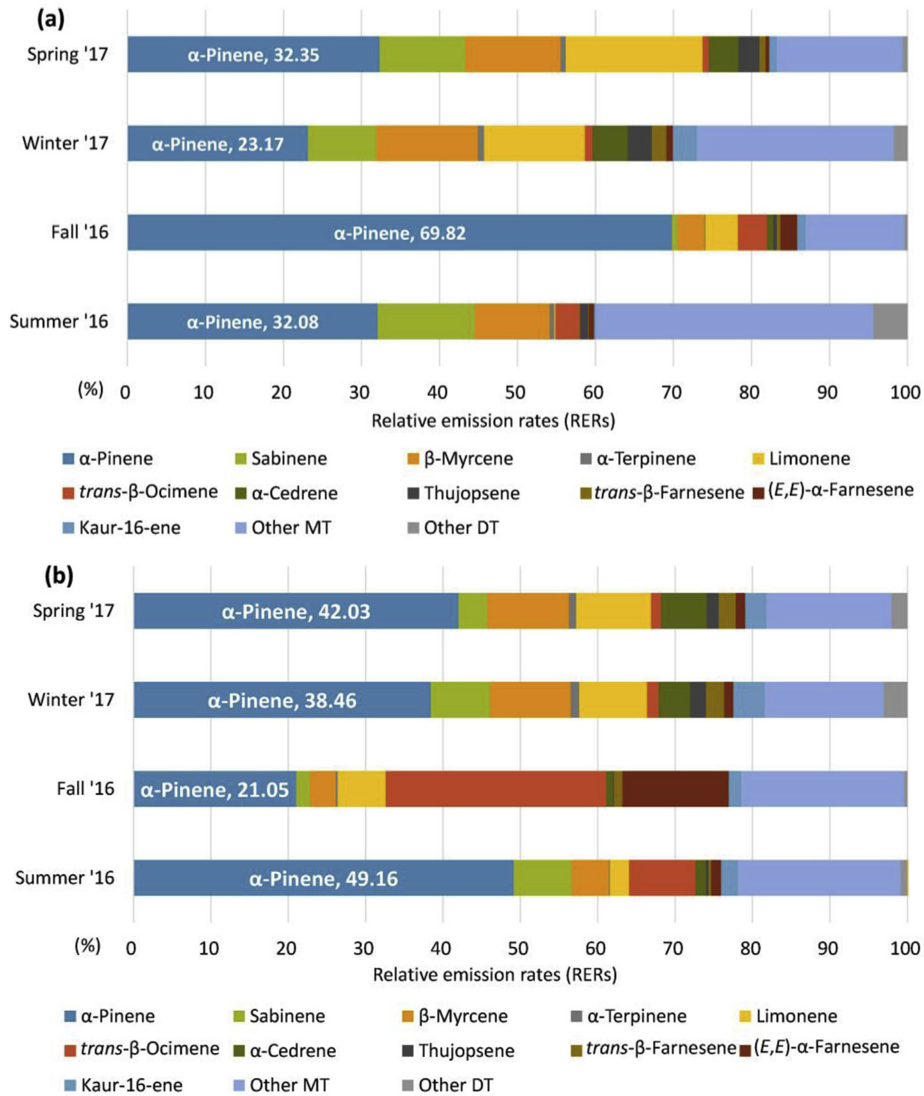

Fig. 2. Relative emission rates (RERs) of major terpenes from saplings (a) and adult trees (b) of *C. formosensis*.

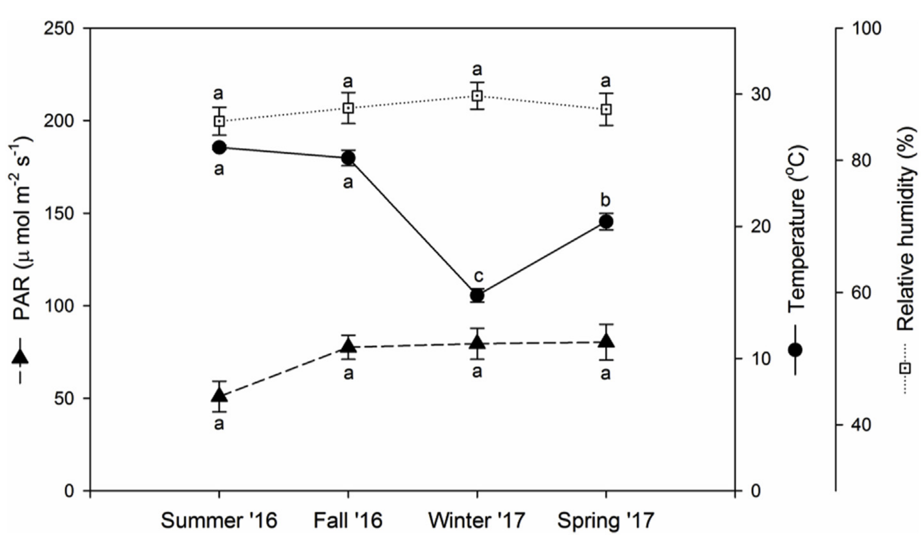

Fig. 3. Seasonal variations in photosynthetically active radiation (PAR), air temperature and relative humidity at the Xinxian Nursery site. Error bar is the standard error of mean (n = 9). Means with the same letter are not significantly different at 5% level by Scheffe's test.

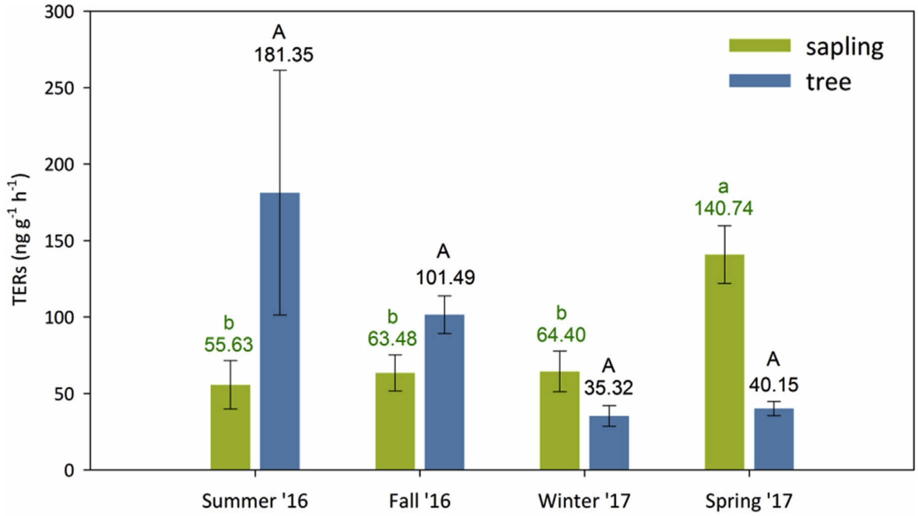

Fig. 4. Total actual emission rates (TERs) in each sampling period from *C. formosensis*. The same upper-case letters represent no significant difference between seasons in saplings and adult trees, respectively. (Mean ± SE, n = 3).

seasonal variations of emission rates from adult trees correlated mainly with air temperature over seasons because of their higher terpenoid content.

On the other hand, multiple regression analysis results indicate that PAR is more influential than temperature in affecting terpene emissions in saplings. In this study, the high emission period of saplings corresponds to bud break and needle elongation (January to April), and this may activate terpene production and elevate TERs. This finding is in line with some previous studies on saplings (Helmig et al., 2013; Geron and Arnts, 2010; Kim et al., 2001; Loreto et al., 2000), which reported an elevation of emission rates at bud break period. Although different species might possess different emission patterns, it seems that saplings of younger age might have higher phenological sensitivity than adult trees. It is thus proposed that the relatively higher emission rates usually act as a protective agent against feeding or against pathogenic attacks when new buds form and new leaves grow (Kim et al., 2005; Geron and Arnts, 2010; Loreto et al., 2000). In addition, leaf development is usually linked with changes in hardening of cuticle, which may alter the diffusive resistance and terpene vapor pressure, thus resulting in seasonal induction of terpene emissions (Llusia and Penuelas, 2000). This fluctuation seems more obvious when new leaves emerge. Furthermore, endogenous parameters such as the activity of some terpene synthases may also be related to the reduced emission in warm seasons (Holzke et al., 2006). The deduced variation of terpene synthase activity is comparable to a recent study on *P. pinaster* and *P. pinea in vitro* cultures where the terpene synthase showed the highest activity at 4 °C and an increased α-pinene production by almost a factor of 2 in comparison to that at 21 °C and 37 °C (Trindade et al., 2016). This may be an explanation for the results reported herein on low emission rates from saplings in summer and fall.

In addition, some studies have reported that substrate-level competition in leaf can significantly modify the environmental responses of isoprene emission (Niinemets et al., 2015). They suggested that isoprene emissions increase in response to increasing light and temperature, and decrease in response to the increases in intercellular CO_2 concentration (Rasulov et al., 2016; Monson et al., 2016). Besides, the effects of different environmental factors in isoprene synthesis and emissions are ultimately regulated by the isoprene synthase activity and the DMADP pool size in plant (Niinemets and Sun, 2015; Monson et al., 2016). As regards the DMADP pool size, they vary with different ages of leaves (Niinemets et al., 2015). The contrasting emission rate in sapling and adult tree observed in this study, might provide a clue to whether the DMADP pool size would be also varied with tree ages, which leads to the change in the response of terpenoids emissions in different environment factors. Further investigations are required to distinguish emission behavior from synthase activities, and to evaluate whether terpene synthase activities of saplings and adult trees were different.

2.3. Effect of season and tree age on total basal emission rates (TEs)

Total Es (TEs) denotes the sum of Es in different classes of terpenoids (including MTs, STs, and DTs). There was no statistically significant difference (t = 0.947, p = 0.358) in average annual TEs between saplings (122.87 ng g^{-1} h^{-1}) and adult trees (182.02 ng g^{-1} h^{-1}) (data not shown), but different seasonal trends were observed between these trees of different ages. The seasonal variations of TEs in saplings were higher during cold seasons (winter and spring) and lower during warm seasons (summer and fall) (Fig. S2). The highest TEs of 182.37 ± 28.44 ng g^{-1} h^{-1} was recorded in spring, followed by 148.99 ± 24.76 ng g^{-1} h^{-1} in winter. The TEs of summer (104.61 ± 9.28 ng g^{-1} h^{-1}) and fall (77.52 ± 6.65 ng g^{-1} h^{-1}) were lower than those in spring and winter. The seasonal pattern of TEs and TERs in adult trees were similar, where emission rates peaked in summer (Fig. S2). The TEs was 3–4 times higher in summer (353.69 ± 139.99 ng g^{-1} h^{-1}) than in other seasons (78.02 ± 4.79–113.84 ± 7.71 ng g^{-1} h^{-1}). Of note is that the decline in TEs in fall (96.68 ± 24.03 ng g^{-1} h^{-1}) was lower than that in winter (113.84 ± 7.71 ng g^{-1} h^{-1}). Previous studies also found reductions of Es in warm seasons in spite of the higher temperatures (Llusia and Penuelas, 2000; Matsunaga et al., 2013), attributing the reduction to high temperatures above the optimum as assumed by the G93 algorithm of Guenther et al. (1993). In this study, the average air temperature in summer (26.00 ± 0.17 °C) was identical to that in fall (25.20 ± 0.59 °C) (Fig. 3), but the TERs in summer was much higher than that in fall (Fig. 4). Again, the average air temperature in winter (14.80 ± 0.50 °C) was much lower than that in spring (20.40 ± 0.63 °C), but the TERs in these two seasons were comparable (Figs. 3 and 4). Different air temperature ranges having approximate TERs might result in such a drastic difference in TEs according to the exponential temperature relationship (Guenther et al., 1993; Geron and Arnts, 2010).

Studies investigating the influence of tree age on terpene emission rates are scarce. Some reported adult trees of *P. pinea*, *C. japonica* and *P. koraiensis* having higher emission rates than their younger counterparts (Street et al., 1997; Kim et al., 2005), but a reverse trend was observed in *P. elliottii* and *C. obtusa* (Kim, 2001; Kim et al., 2005). It was

Table 1
Parameters describing the dependence of total terpene emissions on different environmental variables.

Age	Factors	Low					Medium					High				
		β	SE	std β	p	model statistics	β	SE	std β	p	model statistics	β	SE	std β	p	model statistics
Sapling	TMP	0.002	0.003	0.058	0.356	R^2 = 0.631	−0.001	0.004	−0.048	0.763	R^2 = 0.235	0.017	0.005	0.352	0.002	R^2 = 0.278
	PAR	0.004	0.001	0.309	< 0.001	p = < 0.001	0.001	< 0.001	0.498	< 0.001	p = 0.004	< 0.001	< 0.001	0.276	0.018	p = < 0.001
	HMD	−0.019	0.002	−0.694	< 0.001	F = 88.516	0.002	0.003	0.088	0.531	F = 5.025	0.01	0.022	0.050	0.642	F = 8.620
Tree	TMP	0.052	0.006	0.425	< 0.001	R^2 = 0.333	0.053	0.005	0.725	< 0.001	R^2 = 0.560	0.028	0.006	0.443	< 0.001	R^2 = 0.726
	PAR	0.002	0.001	0.116	0.081	p = < 0.001	< 0.001	< 0.001	0.072	0.265	p = < 0.001	0.001	< 0.001	0.493	< 0.001	p = < 0.001
	HMD	0.014	0.003	0.355	< 0.001	F = 25.588	0.003	0.003	0.072	0.237	F = 51.806	0.207	0.041	0.484	< 0.001	F = 28.203

TMP: temperature; PAR: photosynthetically active radiation; HMD: humidity; β: unstandardized coefficient; std β: standardized coefficient; Low: PAR value ≤ 20 μmol m^{-2} s^{-1}; Medium: PAR value = 20–80 μmol m^{-2} s^{-1}; High: PAR value ≥ 80 μmol m^{-2} s^{-1}.

suggested that difference in biological metabolisms among plant species contributes to difference in emission rates for different tree species and ages (Kim et al., 2005).

2.4. Seasonal variations in basal emission rates of monoterpenoids, sesquiterpenoids and diterpenoids

Fig. 5 shows the seasonal Es of MTs (Fig. 5a), STs (Fig. 5b) and DTs (Fig. 5c) of saplings and adult trees, respectively. Since MTs made up the major portion of TERs, both Es and TEs of MTs displayed similar seasonal variations. In saplings, Es of MTs were higher during spring (170.06 ± 30.84 ng g^{-1} h^{-1}) and winter (114.76 ± 21.81 ng g^{-1} h^{-1}), followed by summer (104.33 ± 11.02 ng g^{-1} h^{-1}) and fall (73.94 ± 7.08 ng g^{-1} h^{-1}). On the other hand, Es of MTs in adult trees showed a seasonal peak in summer (344.99 ± 139.04 ng g^{-1} h^{-1}) (Fig. 5a).

Es of STs from saplings present a seasonal pattern similar to Es of MTs, while Es of STs from adult trees were about 2–3 times higher during fall than in other seasons (Fig. 5b). The differences in seasonal patterns between these trees of two different age groups might be attributed to the differences in biological metabolisms discussed above. Previous researches have found a similar pronounced peak emission of STs in warm seasons and a high-level emission in the month preceding leaf senescence in trees about 4–10 years old (Geron and Arnts, 2010; Helming et al., 2013). It was speculated that the fluctuations in emission of STs might be related to the following reasons: (1) biological function activities or phenology; (2) summertime synthesis and pool accumulation; and (3) their lower volatilities compared with MTs (Matsunaga et al., 2013).

Moreover, Matsunaga (2012) first highly addressed the emissions of biogenic DTs (dominant in kaur-16-ene) from *C. japonica* and *C. obtusa*. They indicated that these compounds might have long been overlooked in considering the low volatility and the difficulty in detection of DTs in winter, where the lowest Es of DTs were observed in winter and did not show significant seasonal variation (Matsunaga et al., 2012). In this study, we also measured the Es of DTs in different seasons. The results showed that these compounds in saplings (54.22 ± 12.99 ng g^{-1} h^{-1}) and adult trees (13.73 ± 6.17 ng g^{-1} h^{-1}) in winter were higher than in other seasons (0.31 ± 0.01–6.90 ± 5.59 ng g^{-1} h^{-1} in saplings; 1.90 ± 1.58–8.18 ± 3.65 ng g^{-1} h^{-1} in adult trees), but only in saplings showed significant difference among seasons (Scheffe's test, $p < 0.05$) (Fig. 5c and Table S2). Furthermore, Matsunaga et al. (2012) have hypothesized that the difference in Es pattern between DTs and MTs/STs may due to the different production purposes or processes of three terpene classes. Although the Es patterns of DTs presented here were higher in winter, which was inconsistent with that from Matsunaga (2012), it would be more rational to deduce that terpene emission was the combined effects of phenology, ontogeny, leaf age and terpene synthesis characteristic of each species. For example, Apple (*Malus domestica*) and Cherry (*Prunus avium*) released high amount of monoterpenes during flower full bloom stage, and then dramatically decreased at fruit-set and ripening stages (Rapparini et al., 2001); apparently, changes in chemical composition were also observed at different developmental stages (Baghi et al., 2012; Bendiabdellah et al., 2013). Moreover, some studies indicate that the production/emission behavior of terpenes is linked to synthase activities depending on plant developmental stage (Holzke et al., 2006; Ivamoto et al., 2017). These seasonal variations of terpene emission may be attributed to ecological functions throughout plant development (Holzke et al., 2006; Rapparini et al., 2001). However, the reports mentioned above were only conducted in specific tree ages; therefore it is difficult to compare these results with contrasting patterns of distinct terpene classes detected in two tree ages. Further studies on physiological and enzymatic activities of different tree ages are required to better investigate the biological role of these different terpene classes. It is worth noting that some compounds in the present study showed remarkable seasonal variations

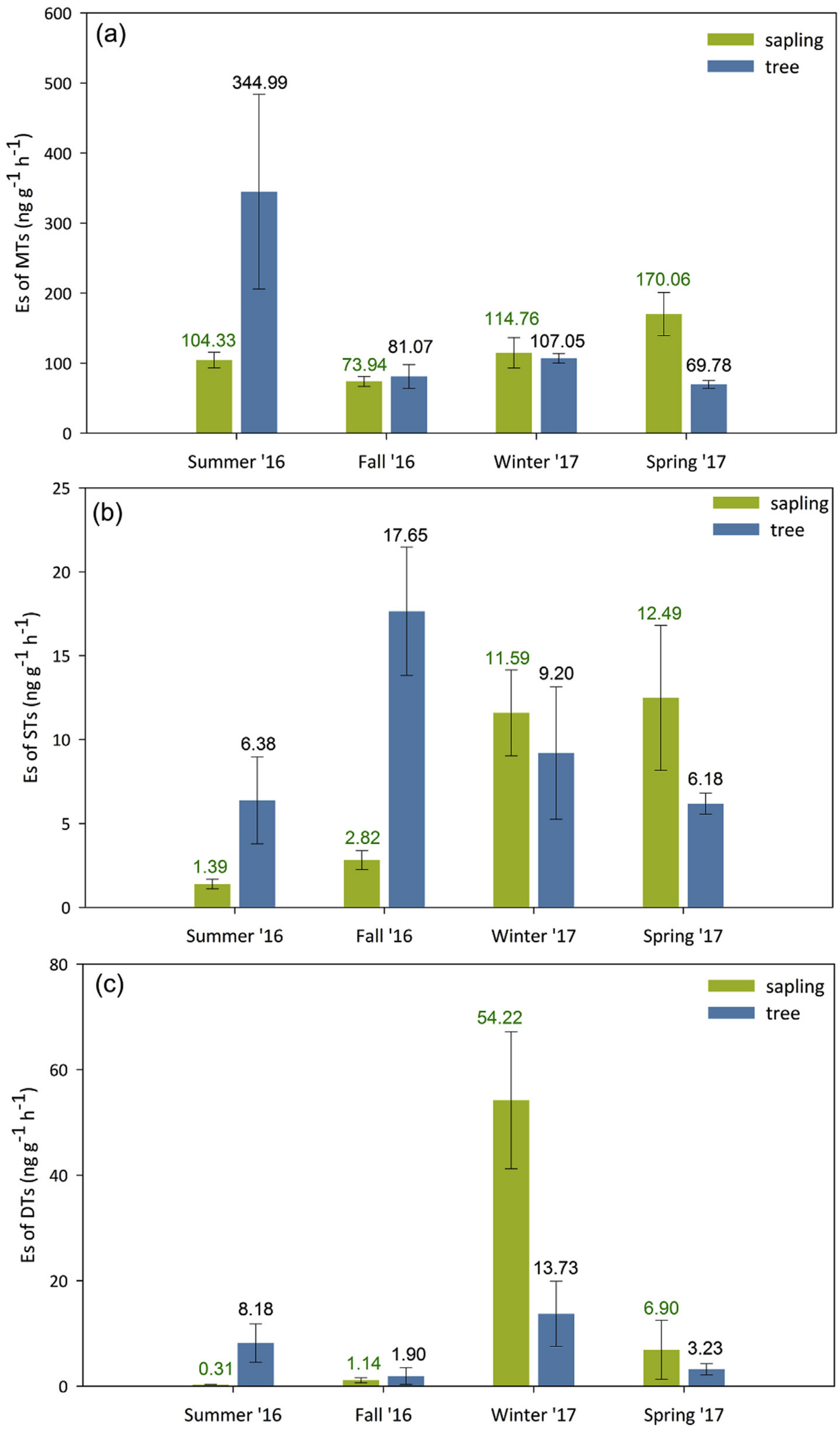

Fig. 5. Basal emission rates (Es) of monoterpenoids (MTs) (a), sesquiterpenoids (STs) (b) and diterpenoids (DTs) (c) averaged in each sampling period from *C. formosensis*. (Mean ± SE, n = 3).

in Es and RERs, and the characteristic variation of individual compounds in different seasons will be discussed in the following.

2.5. Seasonal variation of individual terpenoid emission pattern

The observations of individual emission pattern for compound-specific emissions were found to vary over seasons. Fig. 6 displays the bubble plot showing the relationship of compound (Y-axis) and season (X-axis) with RERs (bubble size) of individual compounds. To investigate the seasonal variations of RERs, a one-way ANOVA was performed to determine whether there were significant differences among the sampling seasons in both saplings and adult trees. The Tukey's post

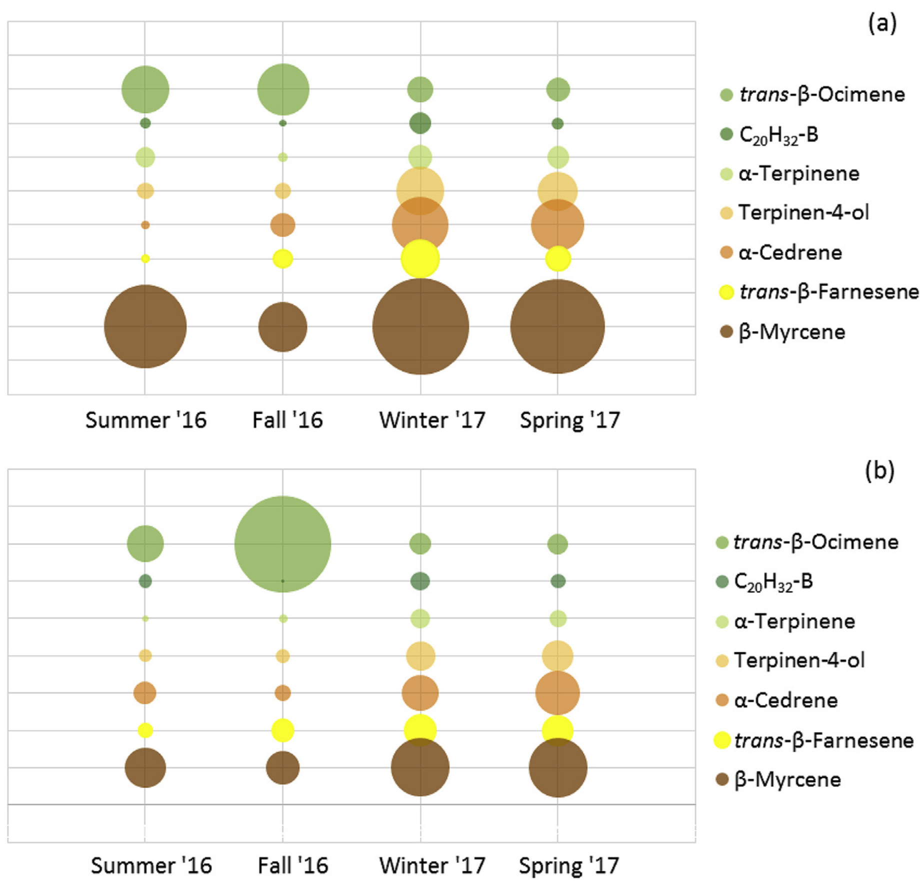

Fig. 6. Bubble plots of the significant seasonal compounds emitted from saplings (a) and adult trees (b) of *C. formosensis* with the different seasons; bubble size represents the RERs of each individual compound.

hoc test showed that MTs β-myrcene, α-terpinene, *trans*-β-ocimene, terpinen-4-ol, STs α-cedrene and *trans*-β-farnesene, and DT $C_{20}H_{32}$–B from both saplings and adult trees displayed significant seasonal variations (Fig. 6; Table S1). It is of interest that, except for *trans*-β-ocimene and DT $C_{20}H_{32}$–B, all other compounds revealed similar trends; i.e., relatively higher content in low-temperature seasons (winter and spring), and relatively lower content in high-temperature seasons (fall and summer). On the contrary, *trans*-β-ocimene, exhibited comparatively higher contribution in warm seasons (Fig. 6). Some previous studies also found seasonal variations in emission ratio for many MTs or STs. In both *C. japonica* and *C. obtusa* adult trees, STs, such as longifolene, thujopsene, α-farnesene, and β-farnesene, showed clear and consistent seasonal variations (Matsunaga et al., 2013), while MTs in *C. obtusa* adult trees, such as sabinene, β-myrcene, *p*-cymene, γ-terpinene, and terpinolene, also showed small seasonal variations (Mochizuki et al., 2011). In addition, Staudt et al. (1997) found an obvious fluctuation of relative terpene emission from *P. pinea* (L.) adult trees, in which the proportion of *trans*-β-myrcene increased while that of limonene decreased from May to August. However, these findings in adult trees were limited. To our best knowledge, no previous works have examined variations of individual compounds in four seasons for both saplings and adult trees. This study is the first to report on different tree ages that both show a constant trend of multiple terpene variations. It is well known that the change in specific emission might be related to the requirements of some physiological process or the protection activity of trees, and/or their adaptation to environmental stress (Jardine et al., 2017; Matsunaga et al., 2013; Helmig et al., 2013). For example, β-ocimene has been demonstrated that it may enhance the thermotolerance of photosynthesis by functioning as antioxidants within plants (Jardine et al., 2017). Whether the variations in ER and RERs of these compounds play a certain role in their physiological or ecological functions warrants further detailed investigation in the future.

Interestingly, it is usually the minor compounds that displayed significant difference between seasons. In contrast, the major individual compounds did not show any statistically significant seasonal fluctuations (Table S1). This finding was in line with observations by Matsunaga et al. (2011). The most abundant terpenes (sabinene, α-terpinene and α-pinene) emitted from *C. japonica* were almost the same over the seasons, while the less abundant terpenes (terpinolene, myrcene, limonene and γ-terpinene) showed significant differences in emission ratio over the year.

Furthermore, the Henry's law constant was another factor affecting the volatility of each individual terpene compound. In this study, larger emission ratios of minor volatile terpenes such as limonene (2850 Pa m^3 mol^{-1}), camphene (1600 Pa m^3 mol^{-1}) and α-terpinene (1960 Pa m^3 mol^{-1}) were observed in spring and winter, while the major volatile terpenes such as α-pinene (13,600 Pa m^3 mol^{-1}), sabinene (6450 Pa m^3 mol^{-1}) and δ-3-carene (13,640 Pa m^3 mol^{-1}) were emitted at a relatively consistent ratio throughout the study period (Table S1). This result echoes previous studies indicating that the most volatile terpenes are more responsive to temperature than the least volatile terpenes (Llusia and Penuelas, 2000).

3. Conclusions

In this study, the terpene emissions from *C. formosensis* trees of two different ages were measured over four seasons using the enclosure technique. MTs were the most dominant terpenoids emitted from both *C. formosensis* saplings and adult trees. The seasonal variations in TERs, TEs, and Es of MTs showed opposite trends between saplings and adult trees. Terpene emissions from adult trees correlated mainly with air temperature and were higher in warm seasons, while terpenoids from saplings were primarily affected by PAR and peaked in spring and winter. Some less volatile (low Henry's law value) MTs showed relatively higher content at low-temperature seasons in both saplings and adult trees compared with other terpenoids.

4. Materials and methods

4.1. Experimental site

This field study was conducted at the Xinxian Nursery in north Taiwan (24°50′27.2″N, 121°32′1.5″E), at an elevation of 341 m above mean sea level (a.m.s.l.). Three well-grown 5-year-old saplings (50–70 cm in height) and three 20-year-old adult trees (around 10 m in height) of *C. formosensis* were randomly selected from the same nursery. They grew close to each other in similar soil conditions, and received similar PAR. Sampling of terpenoids was conducted in situ over four seasons (summer, July 2016; fall, October 2016; winter, February 2017; and spring, April 2017). In each sampling of terpenoids from the same season, the emission rates were measured continuously for 3 days of the same specimen.

4.2. Sampling procedure

Dynamic branch enclosures were used for sampling terpenoids. Branches were carefully enclosed within the 1-L glass chamber (160 mm wide, ψ = 90 mm) and were allowed to acclimate overnight prior to the first sample collection to minimize irritation-induced emissions. Saplings and adult trees were enclosed in separate chambers and sampled simultaneously. The incoming air was purified by the zero air generator (75-83NA, Parker-Balston, Haverhill, MA, USA) to remove particles and VOCs present in ambient air. Purified air was continuously passed through the enclosure chamber at a constant rate of 0.5 L min^{-1} (regulated by a Burkert 8711 mass flow controller, Burkert Co., Baden-Württemberg, Germany). A Teflon tubing with O.D. of 6.35 mm extended from the enclosure chamber to an automated adsorbent cartridge sampler. Volatile emissions from the enclosed branch were collected into the stainless steel adsorbent cartridge filled with 200 mg Tenax TA (mesh 60/80, Supelco, Bellofonte, PA, USA). The adsorbent were installed in a modified automated sampler (STS-25®; PerkinElmer, USA) equipped with a Gilian® LFS-1130 pump (Sensidyne LP, USA). The sampling air then flowed through the tube at 150 mL min^{-1} for 60 min, representing a sampled volume of 9 L. To monitor dynamic changes in emission rates, sampling for a period of 60 min took place every hour during daytime (4:00–16:00) and every 2 h during nighttime (18:00–04:00), giving a total of 17 sampling points during a 24-h period per individual specimen. The 17 sampling points were averaged and each individual specimen had 3 daily average per season. The 3 daily average were averaged for each individual adult tree and sapling. Three adult trees and three saplings were used as replicate for each tree age. The ambient/inside chamber temperature and relative humidity were recorded with WatchDog B-series button loggers (Spectrum Technologies, Aurora, Illinois), and the photosynthetically active radiation (PAR) was monitored by a data logger (LI-COR 1400, LI-COR®, NE, USA). Each meteorological data was recorded every 10 min, 9 daily average data were averaged and represent the mean value of each season. After the sampling procedure, the leaves enclosed in the chamber were harvested to obtain its oven-dried weight (g). The actual emission rates (ER; ng g^{-1} h^{-1}) of terpenoids were determined according to the following equation (Ortega and Helmig, 2008).

$$ER = C \times Q \times m_{dry}^{-1} \quad (1)$$

Where C denotes the concentration (ng L^{-1}) of interested compound adsorbed in the sample tube, Q represents the flow rate (L h^{-1}) of the purified air into the chamber, and m denotes dry weight (g) of leaves.

4.3. Analytical methods

Terpenoids were identified and quantified with a gas chromatography mass spectrometer (GC-MS) (Clarus 600 GC-MS system, PerkinElmer Instruments, USA) equipped with a thermal desorption system (Turbo Matrix ATD, PerkinElmer Instruments, USA). Before desorption, sample tubes were purged with pure nitrogen for 1 min at ambient temperature to remove excess humidity, and a fixed amount of gaseous internal standard (chlorobenzene d5) was automatically introduced onto the sample tube for internal standard calibration. During the first desorption step, sampling tubes were desorbed at 240 °C for 20 min at 30 mL min^{-1} onto a Tenax® TA 60/80 packed cold trap (PerkinElmer) at −30 °C. The second desorption involved heating at a rate of 40 °C s^{-1} to a final temperature of 280 °C for 12 min. Separation of terpenoids was achieved using a fused silica capillary column (DB-5ms, length 30 m, i.d. 0.25 mm, film 0.25 μm). Initial oven temperature column was from 50 °C to 90 °C at 5 °C min^{-1}, to 160 °C at 3 °C min^{-1}, to 280 °C at 35 °C min^{-1}, and maintained at 280 °C for 5 min. The flow rate of He (carrier gas) was 1 mL min^{-1}. The temperature of the GC injector and transfer line were both 250 °C. The MS detector was set up at 230 °C in scan mode with m/z ranging from 40 to 350 amu.

Identification of terpenoids was made by comparing mass spectra and arithmetic index (AI) with mass spectra library and the reference AI (rAI) (Adams, 2007). MS database used included the Wiley/NBS Registry of Mass Spectral Database (version 7) and NIST MS Search (version 2). The spectra of external standards for commercially available compounds were also employed to identify and quantify the chemical composition of terpenoids, including α-pinene (98%, Acros), camphene (96%, ICN), sabinene (92%, SG), β-pinene (98%, Acros), β-myrcene (90%, Sigma), δ-3-carene (90%, Acros), α-terpinene (85%, Acros), limonene (92%, Acros), α-phellandrene (90%, TCI), *trans*-β-ocimene (90%, SAFC), γ-terpinene (98%, Acros), terpinolene (95%, TCI), terpinen-4-ol (97%, Acros), α-cedrene (99%, Aldrich), thujopsene (97%, Fluka) and *trans*-β-farnesene (90%, ChromaDex). In addition, kaur-16-ene was collected and purified from the extract of *Cryptomeria japonica* leaves using HPLC in the laboratory. Standards in methanolic solution were injected in adsorbent cartridges in helium stream. The compound α-phellandrene was used for quantifying β-phellandrene which did not have a pure standard available. For the same reason, *trans*-β-farnesene was used for quantifying sesquiterpene (*E*,*E*)-α-farnesene, and kaur-16-ene for diterpenes.

4.4. Emission rates

Basal emission rates (Es), defined as normalized emission rates at a specific temperature, were calculated using the G93 algorithm [$ER = Es \exp^{\beta (T-Ts)}$] (Guenther et al., 1993; Matsunaga et al., 2011), where ER denotes the actual emission rates, β represents the dependence of ER on temperature, T and Ts denote the measured temperature inside chamber and standard temperature (30 °C), respectively. Es represents the estimated ER at standard temperature. Relative emission rates (RERs) denote the contributions of different terpenoids to the total actual emission rates (TERs).

4.5. Statistical analyses

All statistical analyses (ANOVAs with a Scheffe's test, and regression models) were conducted using the SPSS program package (SPSS 17.0). The effects of season on ER, Es, and RERs in trees of different ages were examined using analysis of variance (ANOVA). Given that Scheffe's test examined all possible comparisons and would be helpful to observe any possible contrast, Scheffe's test was used to evaluate the statistical significance of ER and Es among seasons. Tukey's test was chosen to compare means of RER because of the equal sample sizes, and it was conservative with respect to Type I error. The significance level for these tests was set at the 5% level. The relationships of environmental factors including temperature, light intensity, and humidity with terpene emission rates were assessed using multiple regression analysis.

Contributions

YJC, TFY and STC designed the study. YJC, CYL, HWH and CYY performed the research. YJC analyzed the data, and YJC, CYL, YHC, TFY and STC wrote the manuscript. All the authors reviewed the manuscript.

Acknowledgements

This work was supported by the Ministry of Science and Technology, Taiwan under grant no. MOST 103-2313-B-002-005-MY3. The authors would like to express special thanks to the Dongshih Forest District Office and Dr. Han-Ming Yu of Taiwan Forestry Research Institute for providing the saplings, to Dr. Cheng-Kuen Ho, Mr. Kuo-Chang Chen, Mr. Hsin-Te Lin, Mr. Yuan-Shang Lin, Mr. Fu-Ching Ma, Mr. De-Zhong Su and Mr. Chih-Tsen Wu of Taiwan Forestry Research Institute for their support during measurements, and to Dr. Ya-Lun Huang for her technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.08.002.

References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography - Mass Spectrometry, fourth ed. Allured Publ. Corporation, Carol Stream (Ill.).

Baghi, R., Helmig, D., Guenther, A., Duhl, T., Daly, R., 2012. Contribution of flowering trees to urban atmospheric biogenic volatile organic compound emissions. Biogeosciences 9 (10), 3777–3785. https://doi.org/10.5194/bg-9-3777-2012.

Bendiabdellah, A., Dib, M.E., Meliani, N., Muselli, A., Nassim, D., Tabti, B., Costa, J., 2013. Antibacterial activity of *Daucus crinitus* essential oils along the vegetative life of the plant. J. Chem 2013: Article ID 149502. https://doi.org/10.1155/2013/149502.

Chen, Y.J., Cheng, S.S., Chang, S.T., 2010. Monitoring the emission of volatile organic compounds from the leaves of *Calocedrus macrolepis* var. *formosana* using solid-phase micro-extraction. J. Wood Sci. 56, 140–147. https://doi.org/10.1007/s10086-009-1071-z.

Cheng, S.S., Chang, H.T., Wu, C.L., Chang, S.T., 2007. Anti-termitic activities of essential oils from coniferous trees against *Coptotermes formosanus*. Bioresour. Technol. 98 (2), 456–459. https://doi.org/10.1016/j.biortech.2006.01.006.

Cho, K.S., Lim, Y.R., Lee, K., Lee, J., Lee, J.H., Lee, I.S., 2017. Terpenes from forests and human health. Toxicol. Res. 33 (2), 97–106. https://doi.org/10.5487/tr.2017.33.2.097.

Faiola, C., Buchholz, A., Kari, E., Yli-Pirilä, P., Holopainen, J., Kivimäenpää, M., Miettinen, P., Worsnop, D.R., Lehtinen, K.E.J., Guenther, A., Virtanen, A., 2018. Terpene composition complexity controls secondary organic aerosol yields from Scots pine volatile emissions. Sci. Rep. 8, 3053. https://doi.org/10.1038/s41598-018-21045-1.

Fares, S., Park, J.H., Gentner, D.R., Weber, R., Ormeno, E., Karlik, J., Goldstein, A.H., 2012. Seasonal cycles of biogenic volatile organic compound fluxes and concentrations in a *California citrus* orchard. Atmos. Chem. Phys. 12, 9865–9880. https://doi.org/10.5194/acp-12-9865-2012.

Franco, L.S., Shanahan, D.F., Fuller, R.A., 2017. A review of the benefits of nature experiences: more than meets the eye. Int. J. Environ. Res. Public Health 14, 864. https://doi.org/10.3390/ijerph14080864.

Geron, C.D., Arnts, R.R., 2010. Seasonal monoterpene and sesquiterpene emissions from *Pinus taeda* and *Pinus virginiana*. Atmos. Environ. 44, 4240–4251. https://doi.org/10.1016/j.atmosenv.20https://doi.org/10.06.054.

Guenther, A.B., Zimmerman, P.R., Harley, P.C., Monson, R.K., Fall, R., 1993. Isoprene and monoterpene emission rate variability - model evaluations and sensitivity analyses. J. Geophys. Res.-Atmos. 98, 12609–12617. https://doi.org/10.1029/93JD00527.

Helmig, D., Daly, R.W., Milford, J., Guenther, A., 2013. Seasonal trends of biogenic terpene emissions. Chemosphere 93, 35–46. https://doi.org/10.1016/j.chemosphere.2013.04.058.

Ho, C.L., Hua, K.F., Hsu, K.P., Wang, E.I.C., Su, Y.C., 2012. Composition and anti-pathogenic activities of the twig essential oil of *Chamaecyparis formosensis* from Taiwan. Nat. Prod. Commun. 7 (7), 933–936. https://doi.org/10.1177/1934578X1200700734.

Holzke, C., Hoffmann, T., Jaeger, L., Koppmann, R., Zimmer, W., 2006. Diurnal and seasonal variation of monoterpene and sesquiterpene emissions from Scots pine (*Pinus sylvestris* L.). Atmos. Environ. 40, 3174–3185. https://doi.org/10.1016/j.atmosenv.2006.01.039.

Hsu, C.Y., Lin, C.Y., Chang, S.T., 2016. Antitermitic activities of wood essential oil and its constituents from *Chamaecyparis formosensis*. Wood Sci. Technol. 50 (4), 663–676. https://doi.org/10.1007/s00226-016-0811-7.

Ivamoto, S.T., Sakuray, L.M., Ferreira, L.P., Kitzberger, C.S.G., Scholz, M.B.S., Pot, D., Leroy, T., Vieira, L.G.E., Domingues, D.S., Pereira, L.F.P., 2017. Diterpenes biochemical profile and transcriptional analysis of cytochrome P450s genes in leaves, roots, flowers, and during *Coffea arabica* L. fruit development. Plant Physiol. Biochem. 111, 340–347. https://doi.org/10.1016/j.plaphy.2016.12.004.

Jardine, K.J., Jardine, A.B., Holm, J.A., Lombardozzi, D.L., Negron-Juarez, R.I., Martin, S.T., Beller, H.R., Gimenez, B.O., Higuchi, N., Chambers, J.Q., 2017. Monoterpene "thermometer' of tropical forest-atmosphere response to climate warming. Plant Cell Environ. 40, 441–452. https://doi.org/10.1111/pce.12879.

Kim, J.C., 2001. Factors controlling natural VOC emissions in a southeastern US pine forest. Atmos. Environ. 35, 3279–3292. https://doi.org/10.1016/S1352-2310(00)00522-7.

Kim, J.C., Kim, K.J., Kim, D.S., Han, J.S., 2005. Seasonal variations of monoterpene emissions from coniferous trees of different ages in Korea. Chemosphere 59, 1685–1696. https://doi.org/10.1016/j.chemosphere.2004.10.048.

Lim, Y.J., Armendariz, A., Son, Y.S., Kim, J.C., 2011. Seasonal variations of isoprene emissions from five oak tree species in East Asia. Atmos. Environ. 45, 2202–2210. https://doi.org/10.1016/j.atmosenv.2011.01.066.

Lin, C.Y., Chang, T.C., Chen, Y.H., Chen, Y.J., Cheng, S.S., Chang, S.T., 2015. Monitoring the dynamic emission of biogenic volatile organic compounds from *Cryptomeria japonica* by enclosure measurement. Atmos. Environ. 122, 163–170. https://doi.org/10.1016/j.atmosenv.2015.09.046.

Llusia, J., Peñuelas, J., Guenther, A., Rapparini, F., 2013. Seasonal variations in terpene emission factors of dominant species in four ecosystems in NE Spain. Atmos. Environ. 70, 149–158. https://doi.org/10.1016/j.atmosenv.2013.01.005.

Llusia, J., Penuelas, J., 2000. Seasonal patterns of terpene content and emission from seven Mediterranean woody species in field conditions. Am. J. Bot. 87, 133–140. https://doi.org/10.2307/2656691.

Loreto, F., Nascetti, P., Graverini, A., Mannozzi, M., 2000. Emission and content of monoterpenes in intact and wounded needles of the Mediterranean Pine, *Pinus pinea*. Funct. Ecol. 14 (5), 589–595. https://doi.org/10.1046/j.1365-2435.2000.00457.x.

Matsunaga, S.N., Chatani, S., Nakatsuka, S., Kusumoto, D., Kubota, K., Utsumi, Y., Enoki, T., Tani, A., Hiura, T., 2012. Determination and potential importance of diterpene (kaur-16-ene) emitted from dominant coniferous trees in Japan. Chemosphere 87, 886–893. https://doi.org/10.1016/j.chemosphere.2012.01.040.

Matsunaga, S.N., Mochizuki, T., Ohno, T., Endo, Y., Kusumoto, D., Tani, A., 2011. Monoterpene and sesquiterpene emissions from Sugi (*Cryptomeria japonica*) based on a branch enclosure measurements. Atmos. Pollut. Res. 2, 16–23. https://doi.org/10.5094/APR.2011.003.

Matsunaga, S.N., Niwa, S., Mochizuki, T., Tani, A., Kusumoto, D., Utsumi, Y., Enoki, T., Hiura, T., 2013. Seasonal variation in basal emission rates and composition of mono- and sesquiterpenes emitted from dominant conifers in Japan. Atmos. Environ. 69, 124–130. https://doi.org/10.1016/j.atmosenv.2012.12.004.

Mochizuki, T., Endo, Y., Matsunaga, S., Chang, J., Ge, Y., Huang, C.A., Tani, A., 2011. Factors affecting monoterpene emission from *Chamaecyparis obtusa*. Geochem. J. 45, E15–E22. https://doi.org/10.2343/geochemj.1.0130.

Monson, R.K., Neice, A.A., Trahan, N.A., Shiach, I., McCorkel, J.T., Moore, D.J.P., 2016. Interactions between temperature and intercellular CO_2 concentration in controlling leaf isoprene emission rates. Plant Cell Environ. 39 (11), 2404–2413. https://doi.org/10.1111/pce.12787.

Niinemets, U., Monson, R.K., 2013. Controls and Models of Tree Volatile Organic Compound Emissions. Springer, Dordrecht, Netherlands. https://doi.org/10.1007/978-94-007-6606-8.

Niinemets, U., Sun, Z.H., 2015. How light, temperature, and measurement and growth $[CO_2]$ interactively control isoprene emission in hybrid aspen. J. Exp. Bot. 66 (3), 841–851. https://doi.org/10.1093/jxb/eru443.

Niinemets, U., Sun, Z.H., Talts, E., 2015. Controls of the quantum yield and saturation light of isoprene emission in different-aged aspen leaves. Plant Cell Environ. 38 (12), 2707–2720. https://doi.org/10.1111/pce.12582.

Ortega, J., Helmig, D., 2008. Approaches for quantifying reactive and low-volatility biogenic organic compound emissions by vegetation enclosure techniques - Part A. Chemosphere 72 (3), 343–364. https://doi.org/10.1016/j.chemosphere.2007.11.020.

Pio, C.A., Silva, P.A., Cerqueira, M.A., Nunes, T.V., 2005. Diurnal and seasonal emissions of volatile organic compounds from cork oak (*Quercus suber*) trees. Atmos. Environ. 39, 1817–1827. https://doi.org/10.1016/j.atmosenv.2004.11.018.

Rapparini, F., Baraldi, R., Facini, O., 2001. Seasonal variation of monoterpene emission from *Malus domestica* and *Prunus avium*. Phytochemistry 57 (5), 681–687. https://doi.org/10.1016/S0031-9422(01)00124-8.

Rasulov, B., Talts, E., Niinemets, U., 2016. Spectacular oscillations in plant isoprene emission under transient conditions explain the enigmatic CO_2 response. Plant Physiol. 172 (4), 2275–2285. https://doi.org/10.1104/pp.16.01002.

Sereshti, H., Rohanifar, A., Bakhtiari, S., Samadi, S., 2012. Bifunctional ultrasound assisted extraction and determination of *Elettaria cardamomum* Maton essential oil. J. Chromatogr. A 1238, 46–53. https://doi.org/10.1016/j.chroma.2012.03.061.

Staudt, M., Bertin, N., Hansen, U., Seufert, G., Ciccioli, P., Foster, P., Frenzel, B., Fugit, J.L., 1997. Seasonal and diurnal patterns of monoterpene emissions from *Pinus pinea* (L.) under field conditions. Atmos. Environ. 31, 145–156. https://doi.org/10.1016/S1352-2310(97)00081-2.

Street, R.A., Owen, S., Duckham, S.C., Boissard, C., Hewitt, C.N., 1997. Effect of habitat and age on variations in volatile organic compound (VOC) emissions from *Quercus ilex* and *Pinus pinea*. Atmos. Environ. 31, 89–100. https://doi.org/10.1016/S1352-2310(97)00077-0.

Trindade, H., Sena, I., Figueiredo, A.C., 2016. Characterization of α-pinene synthase gene

in *Pinus pinaster* and *P. pinea in vitro* cultures and differential gene expression following *Bursaphelenchus xylophilus* inoculation. Acta Physiol. Plant. 38, 143. https://doi.org/10.1007/s11738-016-2159-x.
Vivaldo, G., Masi, E., Taiti, C., Caldarelli, G., Mancuso, S., 2017. The network of plants volatile organic compounds. Sci. Rep. 7 (11050), 1–18. https://doi.org/10.1038/s41598-017-10975-x.
Wang, S.Y., Wu, C.L., Chug, F.H., Chien, S.C., Kuo, Y.H., Shyur, L.F., Chang, S.T., 2005. Chemical composition and antifungal activity of essential oil isolated from *Chamaecyparis formosensis* Matsum. wood. Holzforschung 59 (3), 295–299. https://doi.org/10.1515/hf.2005.049.
Yue, X., Unger, N., Zheng, Y., 2015. Distinguishing the drivers of trends in land carbon fluxes and plant volatile emissions over the past 3 decades. Atmos. Chem. Phys. 15, 11931–11948. https://doi.org/10.5194/acp-15-11931-2015.