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Determination and potential importance of diterpene
(kaur-16-ene) emitted from dominant coniferous trees in Japan

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Abstract

Reactive volatile organic compounds (VOCs) are known to affect atmospheric chemistry. Biogenic VOCs (BVOCs) have a significant impact on regional air quality due to their large emission rates and high reactivities. Diterpenes (most particularly, kaur-16-ene) were detected in all of the 205 enclosure air samples collected over multiple seasons at two different sites from *Cryptomeria japonica* and *Chamaecyparis obtusa* trees, the dominant coniferous trees in Japan,. The emission rate of kaur-16-ene, was determined to be from 0.01 to 7.1 $\mu\text{g dwg}^{-1} \text{h}^{-1}$ (average: 0.61 $\mu\text{g dwg}^{-1} \text{h}^{-1}$) employing branch enclosure measurements using adsorbent sampling followed by solid phase - liquid extraction techniques. The emission rate was comparable to that of monoterpenes, which is known major BVOC emissions, collected from the same branches. In addition, total emission of kaur-16-ene at 30 °C was estimated to exceed that of total anthropogenic VOC emissions.

Keywords: biogenic volatile organic compound; kaur-16-ene; coniferous tree; biogenic emission; atmosphere; diterpene

1. Introduction

Reactive volatile organic compounds (VOCs) have several important impacts on atmospheric chemistry and physics (Andreae and Crutzen, 1997; Chameides et al., 1988). For example, VOCs are a precursor to the production of tropospheric ozone, an effective greenhouse gas, which is toxic at sufficiently high concentrations (Mustafa, 1990). Reactive VOCs at high concentrations may also decrease atmospheric concentrations of the hydroxyl radical (OH), a strong oxidant and major sink of less reactive greenhouse gases, such as methane (Kesselmeier et al., 2000). VOCs may also form secondary organic aerosols, which have direct and indirect effects on the earth's radiation budget (Kavouras et al., 1998).

VOCs are known to have both anthropogenic and biogenic sources. Biogenic VOCs (BVOCs) are mainly emitted by terrestrial plants and are estimated to contribute approximately 90% of total VOC emission on a global scale (Guenther et al., 2006). BVOCs are often unsaturated hydrocarbons, such as isoprene (C_5H_8), monoterpenes ($C_{10}H_{16}$) and sesquiterpenes ($C_{15}H_{24}$), and oxygenated species such as methanol, 2-methy-3-butene-2-ol (methylbutenol) and others. However, it is suggested that there are still unmeasured constituents in the atmosphere (Di Carlo et al., 2004).

Coupled measurements of OH radical reactivity and VOC concentrations revealed that there is still a significant quantity of unmeasured reactant which cannot be detected by present analytical techniques (Sinha et al., 2010; Di Carlo et al., 2004). Studies revealed that there is a significant deviation between the observed modeled (Hallquist et al., 2009; Beerling et al., 2007). These findings suggest the existence of unknown source(s) of reactive VOCs. On the other hand, a study of BVOC emissions from

vegetation showed good agreement between OH reactivity and VOC concentration measurements (Kim et al., 2011). The composition of emitted BVOCs is species-dependent. Unidentified BVOC emissions from some species of vegetation may be significant.

Diterpenes, a group of unsaturated biogenic hydrocarbons containing 20 carbon and 32 hydrogen atoms, have previously been identified in leaf oils and resins but are not thought to be emitted from the vegetation because of their low volatility (Lee et al., 2009; Keeling and Bohlmann, 2006; Kato, 2005; Cool et al., 1998; Yasue et al., 1987; von Rudloff, 1975; Appleton et al., 1970, 1968; Nakatsuka et al., 1957).

Oxygenated diterpenes, which contain one or more oxygen atoms, have also been identified in leaf oils. Thermal desorption is the most commonly used techniques for BVOC measurements but may be unsuitable for the detection of high molecular weight compounds, such as diterpenes, because less volatile compounds cannot be efficiently desorbed from the collection tubes. Consequently, these compounds may have been overlooked in previous studies.

In this study, we focused on heavier BVOC (diterpenes) and applied the solid adsorption - liquid extraction analytical technique on the adsorbent samples collected from branch enclosures (Matsunaga et al., 2011, 2008). The technique enabled the determination of emission rates of the heavier BVOCs. kaur-16-ene and other compounds (likely to be diterpenes) were detected in all of the emission samples collected from *C. japonica* and *C. obtusa* over multiple seasons at two different sites. These two native tree species are the most dominant trees in Japan accounting for approximately 60% of total leaf mass in Japanese forests (Matsunaga, 2011). The emission rate of diterpene was observed to

be comparable to that of monoterpenes which is commonly known to be typical BVOCs. Here, we present the first observation of significant emissions of biogenic diterpene.

2. Experiment

2.1. Site description and field campaign

Seven field campaigns have been conducted at two sites, Tanashi Experimental Station of the Tokyo University Forests (35°44'21"N, 139°32'18"E) in Tokyo and Miyazaki Research Forest of Kyushu University (32°21'23"N, 131°6'2"E) in Shiiba, Miyazaki, Japan. Both of the research forests include mature *C. japonica* and *C. obtusa* trees. The campaigns were conducted in January, August, and November of 2009 and in April of 2010 at Tanashi and in May, August, and November of 2010 at Shiiba. Each sampling period was 5 days.

2.2. Branch enclosure system

Leaves were sampled at branch ends about 5-15 nodes from the trunk and were located at the top of the canopy approximately 10-15 m above the ground level. Tree ages of *C. japonica* at Tanashi and Shiiba were 54 and 56 years, respectively, and for *C. obtusa*, at Tanashi and Shiiba were 33 and 56 years, respectively.

The branches were enclosed with a 15 L Teflon® bag (transparent to more than 95% of PAR). The enclosure samples were collected from the branch of *C. japonica* at canopy top and 5 m (3 m for *C. obtusa*) below the top within the same tree. The bags were installed at least 24 hours prior to the first sample collection to avoid artifacts from disturbances on installation of the bag. The air inside the bag was ventilated with the preconditioned air, which did not contain VOCs or oxidants at significant

concentrations, at a flow rate of 4-5 L min⁻¹, to avoid water condensation and excessive temperature increase in the bag (Figure 1). The bags were installed onto the branches on the first day of the campaign and kept on the branches until the sampling period ended 5 days later. The air was supplied from a commercial compressed air cylinder and cleaned by an activated charcoal trap. The flow rate was set by a mass flow controller. Air entering the bag was supplied through a Teflon[®] tube (6 mm diameters) ring (roughly 200 mm in diameter) with approximately 20 holes. Adsorption of the diterpene onto the surface inside of the enclosure has been examined by the analysis of inside air of the bag immediately after removal of the branch. The amount of diterpene adsorbed onto the surface was found to be around 0.1% of the amount of target compound collected within one hour of sampling time after refilling the bag with the same zero air.

The preconditioned air was compressed from the atmosphere and the CO₂ concentrations are assumed to remain at average ambient level. The maximum relative humidity in the bag was approximately 70-75% in the daytime. Water condensation was observed when the air flow rate was below 4 L min⁻¹. Both leaf and enclosure temperatures were measured in campaigns at Tanashi; there was no significant difference. The enclosure temperature at Shiiba was assumed to be same as leaf temperature. The enclosure temperature was recorded every 2 minutes by a data logger equipped with a Teflon[®] coated thermo sensor (RS-12, Especmic, Ohguchi, Niwa, Aichi, Japan). Branches were harvested after the completion of each field campaign and dried at 60 °C for 48 hours to determine dry weight of the leaves. For *C. japonica*, the dry weight was determined for whole branches harvested because stems also have green needle leaves. Therefore, the dry weight for *C. japonica* includes stem, bark and leaves. Samples were collected from different branches of the same individuals during the

sampling periods in every season.

2.3. Sample collection

Diterpene samples were collected from the bag enclosure described in 2.2. Monoterpenes and sesquiterpenes, which are C₁₀ and C₁₅ BVOCs, respectively, were also collected from the same bag and analyzed to confirm any disturbance (i.e., extraordinarily high concentrations from damage to storage reservoirs of the BVOCs). Mono-, sesqui- and diterpene samples were collected from bag enclosures same branches throughout the campaigns. Detailed description of the sampling procedure is in Tani et al. (2008) and (2002).

The diterpene samples were collected into a 6.35 mm (1/4 inches) o.d. glass tube filled with approximately 60 mg of HayeSep Q (Hayes Separations, Inc., Bandera TX, U.S.A.). Sample flow rate was controlled at 200 ml min⁻¹ by a mass flow controller exhausting to a pump. The collection time ranged from 0.5 to 2 hours. Diterpenes collected on the adsorbent were extracted with approximately 2 ml of hexane (Special grade, Wako chemical, Chuo-Ku, Osaka, Japan) at the site. The extracts were collected into 2 ml glass vials (Supelco, Bellefonte PA, U.S.A.) directly from the sampling tube and stored in a freezer (approximately -15 °C), at the site. The samples were transported to the laboratory by a commercial refrigerated transport service, which kept the packages at -18 °C before further procedures were performed. Details of the sampling procedure are described in Matsunaga et al. (2011), (2009) and (2008).

2.4. GC-MS analysis

A one micro-liter of internal standard solution (20-100 ng μl⁻¹ of

cyclopentadecane in hexane) was added into the extracts prior to a concentration procedure. The extracts were evaporated to approximately 5-10 μl by a gentle argon flow. The internal standard solution was prepared for each field campaign. One μl of the concentrated extract containing the internal standard was injected into the split-splitless injector of the gas chromatograph (GC; Agilent 7890A GC, Agilent Technologies Inc., Santa Clara CA, U.S.A.) connected to a mass spectrometer (MS; Agilent 5975C MSD). Diterpenes were separated using a fused silica capillary column (HP-5ms, 30 m \times 0.25 mm i.d., film thickness of 0.25 μm ; Agilent Technologies Inc.) and helium carrier gas. The injector (300 $^{\circ}\text{C}$) was equipped with a deactivated quartz insert tube and operated in the pulsed splitless mode. GC oven temperatures were programmed to start at 60 $^{\circ}\text{C}$ and increase to 150 $^{\circ}\text{C}$ at 2.5 $^{\circ}\text{C min}^{-1}$, then raised to 300 $^{\circ}\text{C}$ at 30 $^{\circ}\text{C min}^{-1}$ and held for 10 minutes. The identification of kaur-16-ene was made comparing its GC retention time and mass spectra with those of the authentic standard (Figure 2 and Figure 3). The authentic standard was purchased from OlChemIm Ltd., Olomouc, Czech Republic. Kaur-16-ene and an unknown diterpene, likely to be hibaene, were detected as the two most abundant peaks of diterpenes (Figure 2).

Although the analytical technique cannot distinguish stereo-isomers compounds produced in leaves (Cool et al., 1998; Perry and Weavers, 1985; Railton et al., 1984), the kaur-16-ene detected in this study is most likely to be ent-kaurene. The emission rate of kaur-16-ene was estimated to be comparable to total monoterpene emission, a group of commonly emitted BVOCs, and also to total anthropogenic VOC emission in Japan in summer.

Kaur-16-ene was quantified using the MS in the selected ion monitoring mode

(SIM). The amount of the kaur-16-ene was determined by comparing response intensity (peak area on a chromatogram for the selected ions) of the internal standard and the target compounds. The response of the kaur-16-ene was adjusted based on the ratio of the response for internal standard to kaur-16-ene (different SIM ions were selected for kaur-16-ene and the standard). The ratio was determined by analyzing authentic standard for kaur-16-ene and internal standard (cyclopentadecane) purchased from Wako chemical and Sigma-Aldrich (Co., St. Louis MO, U.S.A.). The amount of the standard was gravimetrically determined. The precision of the whole analytical procedure was around 5%. Detection limit was approximately $0.1 \text{ ng g}^{-1} \text{ h}^{-1}$ (depending on the leaf mass enclosed in the bag and the sampling time).

2.5. Calculation for the emission rate of kaur-16-ene

The emission rate was determined based on the equation 1 below.

$$E = \frac{F_{air} \times (M - M_{blank})}{F_{samp} \times t_{samp} \times W_{leaf}} \quad (1)$$

where, E and M are the emission rate in $\mu\text{g g}^{-1} \text{ h}^{-1}$ and determined mass of kaur-16-ene by the GC-MS analysis in μg , respectively. F_{air} , M_{blank} , F_{samp} , t_{samp} and W_{leaf} are flow rate of the purified air supplied to the bag in ml min^{-1} , mass of kaur-16-ene in the blank sample in μg adjusted for the actual sampling time, sample flow rate, sampling time in hour and dry weight of the leaf in g, respectively. M_{blank} for kaur-16-ene was below the detection limit through all field campaigns. The blank samples were collected from the enclosure bag that was used for the sampling after removing the branch. The blank samples were collected from the bags used in the campaign at the end of each sampling period.

2.6 Measurement for the ambient air

Six ambient air samples were also collected to confirm the existence of diterpenes in the atmosphere and were collected at around noontime and through a night on November 3, 2010 at Shiiba, Miyazaki using the same sampling tubes as the tubes used for the enclosure measurements. The ambient samples were collected on the canopy top without oxidant scrubber to avoid adsorption of diterpenes onto the scrubber.

2.7 Calculation of the basal emission rates

Basal emission rate is defined to be an emission rate of BVOC, at the standard condition of 30 °C and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (Photosynthetic Photon Flux Density, Guenther et al., 2006, 1993). Figure 4a and 4b present observed relationships between the inside air temperature of the enclosure bag and the logarithm of the measured emission rate of kaur-16-ene from *C. japonica* and *C. obtusa* observed at Tanashi and Shiiba, respectively. Plot for *C. obtusa* was only for data obtained at Shiiba due to insufficient number of samples at Tanashi. Assuming an exponential temperature dependence of the emission of kaur-16-ene, the emission rate can be estimated according to the G93 algorithm (equation 2).

$$E = E_s \exp\{\beta(T - T_s)\} \quad (2)$$

$$\ln E = \beta(T - T_s) + \ln E_s \quad (3)$$

where, E presents measured emission rate. T and T_s presents measured temperature and the standard temperature (30 °C), respectively. An empirical coefficient β can be calculated as the slope of the linear regression for the plot and quantifies the intensity of

temperature dependence (equation 3). Natural logarithm of the basal emission rate E_s is the intercept at y-axis (Guenther et al., 1993).

2.8 Estimate of atmospheric concentration of kaur-16-ene based on a canopy model.

The atmospheric concentration of kaur-16-ene was estimated using a simple model and compared with the measured ambient concentration. The model calculates the concentration based on the emission rate per unit ground area, vertical convection and chemical loss process of the kaur-16-ene.

Leaf mass for *C. japonica* and *C. obtusa* per unit ground area at the site were estimated based on their trunk volume for the tree species in the research forest. Percentages of leaves and trunk portion in whole tree weight were estimated based on Mitscherlich function for each tree species (Iehara et al., 2004). Tree ages and trunk volumes, which are required for the calculation, were obtained from prefectural record for trunk volume of the tree species prepared by Miyazaki Prefectural Office (Shiiba site is included in Miyazaki Prefecture). Trunk volumes were converted to trunk weight based on dry densities for each species (Nojiri et al., 2009). Leaf weight for the species were finally estimated based on the trunk weight, percentages of leaves and trunk in weight and the Mitscherlich function. Because the Shiiba research forest contains many tree species other than *C. japonica* and *C. obtusa* and is surrounded by private forest and cropland. Leaf weights of *C. japonica* and *C. obtusa* per unit ground area of 4 km radius circular surrounding the sampling site were estimated at 0.015-1.2 and 0.024-1.1 kg m⁻², respectively. The leaf weights over Japan were also estimated by the same procedure and data sources.

The emission rate of kaur-16-ene at the site was estimated to be from 0.003 to 0.19

mg m⁻² h⁻¹ within the period of 11:00 - 13:00 on 3 November based on the basal emission rates (8.7 and 1.0 µg g⁻¹ h⁻¹ for *C. japonica* and *C. obtusa*, respectively) and obtained coefficient β of 0.24 K⁻¹, leaf mass and the ambient temperature.

To calculate vertical convection and atmospheric loss processes of kaur-16-ene, a simple canopy environment model was used to calculate the ambient concentration of kaur-16-ene. The canopy environment model, which considers emission of kaur-16-ene and its vertical transport, was constructed based on the equation 4 below.

$$\frac{\partial C}{\partial t} = E - (k_{OH}[OH] + k_{O_3}[O_3])C + \frac{\partial}{\partial z} \left(k_z \frac{\partial C}{\partial z} \right) \quad (4)$$

where, C , k_{OH} , k_{O_3} and k_z present concentration of kaur-16-ene, reaction rate constant of kaur-16-ene with OH radical, with ozone and vertical convection coefficient, respectively.

Only oxidation losses in reaction with OH radical and ozone were considered in the canopy environment model. The reaction rate constants of kaur-16-ene, k_{OH} and k_{O_3} , were estimated to be 7.2×10^{-11} and 1.2×10^{-17} molecules⁻¹ cm³ s⁻¹ by AOPWIN version 1.92a produced by Environmental Protection Agency in the United States, respectively. The values are similar to those of longifolene, which is a sesquiterpene with one C=C double bond, of 5.95×10^{-11} for OH reaction (Peeters et al., 2007) and 0.7×10^{-17} for ozone reaction (Shu and Atkinson, 1994). The diterpene kaur-16-ene also has one C=C double bond; therefore, the reaction rates are assumed to be slightly larger than those of longifolene.

Vertical mixing coefficient was assumed to be varying from 0.1 to 10 m² s⁻¹. During the November (autumn in Japan) experiment at Shiiba, a remote mountain area,

the maximum ambient concentrations of OH and ozone were assumed to be $0.5 - 5 \times 10^6$ molecules cm^{-3} (Creasey et al., 2001) and 30 ppbv (7.8×10^{11} molecules cm^{-3} , Sunwoo et al., 1994), respectively.

3. Results and discussion

3.1 Basal emission rate of kaur-16-ene

Diterpenes were detected in the ambient samples and all of the 205 enclosure samples collected from multiple individuals of *C. japonica* and *C. obtusa* at two different sites over the seasons. Only kaur-16-ene was identified and quantified in the diterpenes using an authentic standard. Temporal variations of measured emission rates of kaur-16-ene at Shiiba over three months are presented in Figure 5 a-c.

To estimate the regional emission rate, the basal emission rate of kaur-16-ene was calculated. The calculated E_s and β over the seven campaigns are presented in Table 1. Mono- and sesquiterpenes were also measured in all campaigns as well as diterpene samples. As shown in Table 1, the basal emission rate E_s of kaur-16-ene was significantly higher than those of monoterpenes, a more commonly measured BVOC, measured from the same branches. Sesquiterpenes were detected only from *C. japonica*. The averaged basal emission rate of total sesquiterpenes from *C. japonica* was 2.9 and 7.1 $\mu\text{g g}^{-1} \text{h}^{-1}$ at Tanashi and Shiiba, respectively, while that of kaur-16-ene was determined to be 2.8 and 8.7 $\mu\text{g g}^{-1} \text{h}^{-1}$. As shown in Table 2, measured emission rates of kaur-16-ene were also found to be significant compare to those of monoterpenes over the seasons.

Although there may be another factor which controls the emission (e.g. light

intensity), we concluded that temperature is the most effective controlling factor of the emission of kaur-16-ene. Some samples were collected from shade leaves (5 m and 3 m below the canopy top for *C. japonica* and *C. obtusa*, respectively.), however, there were no significant differences in the basal emissions between sun and shade leaves. The basal emissions of kaur-16-ene from *C. japonica* were 2.8 and 2.0 $\mu\text{g g}^{-1} \text{h}^{-1}$ at sun exposed and shaded branches (at approximately 5 m below the canopy), respectively. Therefore, the light intensity is likely not an important factor to control the emission of kaur-16-ene from *C. japonica*.

The basal emission rate of kaur-16-ene did not show significant seasonal variation. As mentioned above, basal emission rate is a normalized emission rate and is regarded to be a constant in most emission models because it is a normalized emission rate at a set of standard conditions. However, recent studies report that the basal emission often has a seasonal variation (Geron and Arnts, 2010). Matsunaga et al. (2011) also reports that the basal emission rates of monoterpenes and sesquiterpenes emitted from *C. japonica* change with season. However, the basal emission rate of kaur-16-ene emitted from *C. japonica* and *C. obtusa* did not clearly change with seasons. This may suggest that diterpenes are produced for other purposes or are produced through different processes than mono- and sesquiterpenes.

3.2 Evaluation of the emission rate measured with the enclosure

The ambient concentration of kaur-16-ene under the canopy was estimated to be from 0.001 to 0.61 ppbv in the period. Averaged leaf weight densities in the research forest are 0.015 (*C. japonica*) and 0.024 kg m^{-2} (*C. obtusa*). Area ratio between the research forest and private forest around the site is 633:4391. Therefore, assuming all of

private forests around the site are planted only by *C. japonica* and *C. obtusa*, averaged leaf weights per area for the species were estimated to be 1.1 and 0.9 kg m⁻² for *C. japonica* and *C. obtusa*, respectively, within a 4 km radius surrounding the site. The mean concentration estimated from the model (with 1.1 kg m⁻² *C. japonica* and 0.9 kg m⁻² *C. obtusa*) leaf weights, OH and ozone concentrations of 1.0 × 10⁶ molecules cm⁻³ of OH and 30 ppbv, and k_z of 5.0 m² s⁻¹) was 0.11 ppbv within the period when the ambient samples were collected (approximately 11:00-13:00 on November 3, 2010).

The estimated concentration varied largely. This is caused by uncertainties on the atmospheric condition and large variation in estimated emission rate (see details for section 2.8). Vertical mixing coefficient (varied 0.1 - 10 m² s⁻¹) and oxidant concentrations (only for maximum OH concentration varied in the range of 0.5 - 5 × 10⁶ molecules cm⁻³) caused a factor of 6.7 and 1.4 of difference, respectively, while the emission rate can vary from 0.003 to 0.19 mg m⁻² h⁻¹ (a factor of 63) at the ambient temperature of 11-12 °C. The emission rates were adjusted to emission rate at the observed ambient temperature using the equation 2. Because the land cover is heterogeneous around the sampling site, the leaf mass densities of those two species can largely vary (refer section 2.8) so that the atmospheric concentration of the kaur-16-ene can also vary with wind direction at the site.

The observed atmospheric concentration of kaur-16-ene (0.02-0.04 ppbv) was, with the previous assumptions, at the low end of the range within estimated range (0.001-0.61 ppbv, 0.11 ppbv of mean value). The low atmospheric concentration of kaur-16-ene, despite of high emission rate, may have resulted in the overlooking of the compound in previous studies.

3.3 Estimate of the kaur-16-ene emission over Japan

The total emission rate of kaur-16-ene over Japan at 30, 15 and 5 °C was based on leaf mass calculated from trunk volumes for *C. japonica* and *C. obtusa* (Matsunaga 2011). As shown in Figure 6, the emission rate of kaur-16-ene over Japan was estimated to be 2.1, 17 and 380 ton h⁻¹ at 5, 15 and 30 °C, respectively, while the emission rate of total monoterpenes (molecular weight; MW: 136) in Japan were estimated to be 21 tons h⁻¹ at 5 °C, 56 tons h⁻¹ at 15 °C and 250 tons h⁻¹ at 30 °C by the emission model MEGAN (Model of Emissions of Gases and Aerosols from Nature) (Guenther et al., 2006). Anthropogenic VOC emission does contrastively not significantly vary with temperature (Kannari et al., 2008) while BVOC tends to largely change with temperature. It appears that the emission rate of kaur-16-ene estimated at 30 °C, which is typical ambient temperature in summer, exceeds the total emission rate of anthropogenic VOCs in Japan (220 ton h⁻¹, Kannari et al. 2008). Considering the result, kaur-16-ene may be regarded as a new and important atmospheric constituent that has a remarkable influence on the air quality in Japan

4. Summary

A significant emission of diterpenes, which has not been previously recognized to be emitted into the atmosphere, has been determined employing both enclosure and ambient air measurements. The emissions were observed throughout year from two tree species which are the two dominant tree species in Japan. Their atmospheric reactivity is expected to be high due to their unsaturated structure and high molecular weight of molecules which contain many hydrogen atoms. In addition, the aerosol formation yield by the oxidation of diterpenes is also expected to be high due to their high molecular

weight and reactivity. Considering the comparable emission rate to other classes of BVOCs such as monoterpenes and sesquiterpenes, which are known to be major BVOC constituents, impacts of the diterpene emissions may be important in atmospheric chemistry and physics.

Samples also contained other diterpene-like compounds, which have not been identified by the authentic standards. One abundant unknown compound in the samples seemed to be hibaene which is a diterpene also known as a constituent of coniferous leaves (Yatagai et al., 1985; Nagahama and Tajima, 1996). The mass spectrum obtained in this study was similar to that in NIST library. The hibaene-like compound was detected in the samples at similar intensities with kaur-16-ene. Therefore, total diterpene emission may be larger than presented in this study. Also, to confirm the importance of diterpene emissions at larger scales, other conifers such as *Abies*, *Larix*, *Picea* and *Pinus* spp., which are common at the global scale, should be investigated for the diterpene emissions in the near future.

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References

- Andreae, M. O. and Crutzen, P. J., 1997. Atmospheric aerosols: Biogeochemical sources and role in atmospheric chemistry. *Science* 276, 1052–1058.
- Appleton, R. A. et al., 1968. The alternative occurrence of (-)-Kaurene or (+)-Phyllocladene in the leaves of *Cryptomeria japonica* D. Don. *Phytochemistry* 7, 135-137.
- Appleton, R. A. et al., 1970. The diterpenes from the leaves of *Cryptomeria japonica*, *Phytochemistry* 9, 581-583.
- Beerling, D. J. et al., 2007. Critical issues in trace gas biogeochemistry and global change. *Philos. T. Roy. Soc. A* 365, 1629-1642.
- Chameides, W. L. et al., 1988. The role of biogenic hydrocarbons in urban smog: Atlanta as a case study. *Science* 241, 1473–1475.
- Cool, L. G. et al., 1998. Foliage terpenoids of Chinese *Cupressus* species. *Biochem. Syst. Ecol.* 26, 899-913.
- Creasey, D. J. et al., 2001. OH and HO₂ measurements in a forested region of north-western Greece. *Atmos. Environ.* 35, 4713-4724.
- Di Carlo, P. et al., 2004. Missing OH Reactivity in a Forest: Evidence for Unknown Reactive Biogenic VOCs. *Science* 304, 722-725.
- Geron, C. D. and Arnts, R. R., 2010. Seasonal monoterpene and sesquiterpene emissions from *Pinus taeda* and *Pinus virginiana*. *Atmos. Environ.* 44, 4240-4251.
- Guenther, A. B. et al., 1993. Isoprene and monoterpene emission rate variability: model

- evaluations and sensitivity analyses. *J. Geophys. Res.* 98, 12609-12617.
- Guenther, A. et al., 2006. Estimates of global terrestrial isoprene emissions using MEGAN (Model of Emissions of Gases and Aerosols from Nature). *Atmos. Chem. Phys.* 6, 3181-3210.
- Hallquist, M. et al., 2009. The formation, properties and impact of secondary organic aerosol: current and emerging issues. *Atmos. Chem. Phys.* 9, 5155–5236.
- Iehara, T. et al., 2004. Improvement on estimate of CO₂ balance within forest and ocean, in: Forestry and Forest Products Research Institute. (Eds.), Project Research Report for Grant-in-Aid, Evaluation for mechanism of reservoir and balance of CO₂ in forest. Forestry and Forest Products Research Institute, Tsukuba, pp. 82-85. (in Japanese)
- Kannari, A. et al., 2008. MICS-Asia II: An inter-comparison study of emission inventories for the Japan region. *Atmos. Environ.* 42, 3584-3591.
- Kato, K., 2005. Factors enabling *Epinotia granitalis* (Lepidoptera: Tortricidae) overwintered larvae to escape from oleoresin mortality in *Cryptomeria japonica* trees in comparison with *Semanotus japonicus* (Coleoptera: Cerambycidae). *J. Forest Res.-Jpn.* 10, doi: 10.1007/s10310-004-0132-9, 205-210.
- Kavouras, I. G. et al., 1998. Formation of atmospheric particles from organic acids produced by forests. *Nature* 395, 683-686.
- Keeling, C. I. and Bohlmann, J., 2006. Diterpene resin acids in conifers. *Phytochemistry* 67, 2415–2423.

- Kesselmeier, J. et al., 2000. Atmospheric volatile organic compounds (VOC) at a remote tropical forest site in central Amazonia. *Atmos. Environ.* 34, 4063–4072.
- Kim, B. et al., 2011. Contributions of primary and secondary biogenic VOC to total OH reactivity during the CABINEX (Community Atmosphere-Biosphere INteractions Experiments)-09 field campaign. *Atmos. Chem. Phys.* 11, 8613-8623.
- Lee, J.-H. et al., 2009. Comparison of chemical compositions and antimicrobial activities of essential oils from three conifer trees; *Pinus densiflora*, *Cryptomeria japonica* and *Chamaecyparis obtusa*. *J. Microbiol. Biotechn.* 19, doi: 10.4014/jmb.0803.191, 391-396.
- Matsunaga, S. N. et al., 2008. Emission of sunscreen salicylic esters from desert vegetation and their contribution to aerosol formation. *Atmos. Chem. Phys.* 8, 7367-7371.
- Matsunaga, S. N. et al., 2009. Leaf level emission measurement of sesquiterpenes and oxygenated sesquiterpenes from desert shrubs and temperate forest trees using a liquid extraction technique. *Geochem. J.* 43, 179-189.
- Matsunaga, S. N. et al., 2011. Monoterpene and sesquiterpene emissions from Sugi (*Cryptomeria japonica*) based on a branch enclosure measurements. *Atmos. Pol. Res.* 2, doi: 10.5094/APR.2011.003, 16-23.
- Matsunaga, S. N., 2011. Biogenic VOC emission inventory, in: Japan Auto-Oil Program (JATOP) Research Report FY2010, Japan Petroleum Energy Center, Tokyo, pp. 233-255. (in Japanese)
- Mochizuki, T. et al., 2011. Factors affecting monoterpene emission from

- Chamaecyparis obtusa*. *Geochem. J.* 45, e15-e22.
- Mustafa, M. G., 1990. Biochemical basis of ozone toxicity. *Free Radical Bio. Med.* 9, doi:10.1016/0891-5849(90)90035-H, 245-265.
- Nagahama, S. and Tajima, M., 1996. Diterpene Hydrocarbons from the leaf oil of *Thujopsis dolabrata* var. *dolabrata*. *Biochem. Syst. Ecol.* 24, 49-52.
- Nakatsuka, T. et al., 1957. Terpenoids. II Composition of the essential oil from leaves of “Sugi” (*Cryptomeria japonica* D. Don). *Mokuzai Gakkaishi* 3, 206-208.
- Nojiri, Y. et al., 2009. Land use, land used change and forestry, in: Greenhouse Gas Inventory Office of Japan, Center for Global Environmental Research, National Institute for Environmental Studies. (Eds.), National Greenhouse Gas Inventory Report in Japan (2009). National Institute for Environmental Studies, Tsukuba, pp. 7-10.
- Peeters, J. et al., 2007. Structure-activity relationship for the addition of OH to (poly) alkenes: Site-specific and total rate constants. *J. Phys. Chem. A* 111, 1618-1631.
- Perry N. B. and Weavers, R. T., 1985. Foliage diterpenes of *Dacrydium Intermedium*: identification, variation and biosynthesis. *Phytochemistry* 24, 2899-2904.
- Railton, I. D. et al., 1984. Ent-kaurene synthesis in chloroplasts from higher plants. *Phytochemistry* 23, 1261-1267.
- Sinha, V. et al., 2010. OH reactivity measurement within a boreal forest: Evidence for unknown reactive emissions. *Environ. Sci. Technol.* 44, 6614-6620.
- Sunwoo, Y. et al., 1994. Characteristics of background ozone in Japan. *Atmos. Environ.*

28, 25-37.

Tani, A. et al., 2002. Monoterpene fluxes measured above a Japanese red pine forest at Oshiba plateau, Japan. *Atmos. Environ.* 36, 3391-3402.

Tani, A. and Kawawata, Y., 2008. Isoprene emission from the major native *Quercus* spp. in Japan. *Atmos. Environ.* 42, 4540-4550.

von Rudloff, E., 1975. Volatile Leaf Oil Analysis in Chemosystematic Studies of North American Conifers. *Biochem. Syst. Ecol.* 2, 131-167.

Yasue, M. et al., 1987. Geographical differentiation of natural *Cryptomeria* stands analyzed by diterpene hydrocarbon constituents of individual trees. *J. Jpn. Forest Soc.* 69, 152-156.

Yatagai, M. et al., 1985. Terpenes of leaf oils from *Cupressaceae*. *Biochem. Syst. Ecol.* 13, 377-385.

Figure captions

Figure 1 An overview for the branch enclosure sampling system.

Figure 2 Typical gas chromatogram of samples collected from *C. japonica*.

Figure 3 Structure and typical sample mass spectrum of kaur-16-ene, analysis of the authentic standard, and NIST library.

Figure 4 A liner relationship between the temperature and natural logarithm for the measured emission rate of kaur-16-ene collected from *C. japonica* at (a) Tanashi and (b) Shiiba.

Figure 5 Temporal variation of the emission rate of kaur-16-ene at Shiiba. Error bars indicate 10% of uncertainties of the emission rates.

Figure 6 Estimated emission rates of kaur-16-ene and monoterpenes at 5, 15 and 30 °C over Japan. The emission rate of total anthropogenic VOC (220 tons h⁻¹, Kannari et al. 2008) is also indicated with a gray line.

Figure 1 Matsunaga et al.

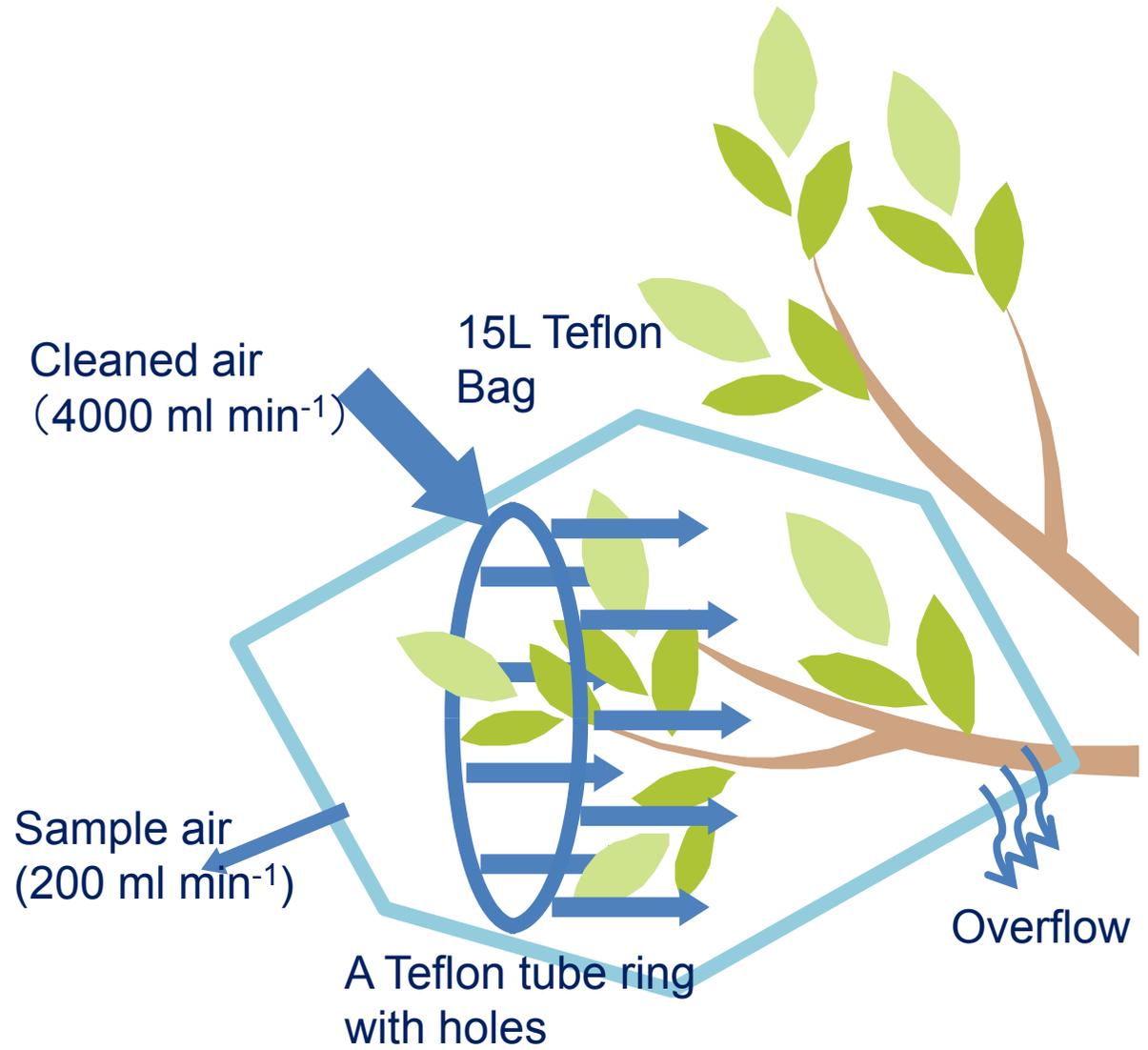


Figure 2 Matsunaga et al.

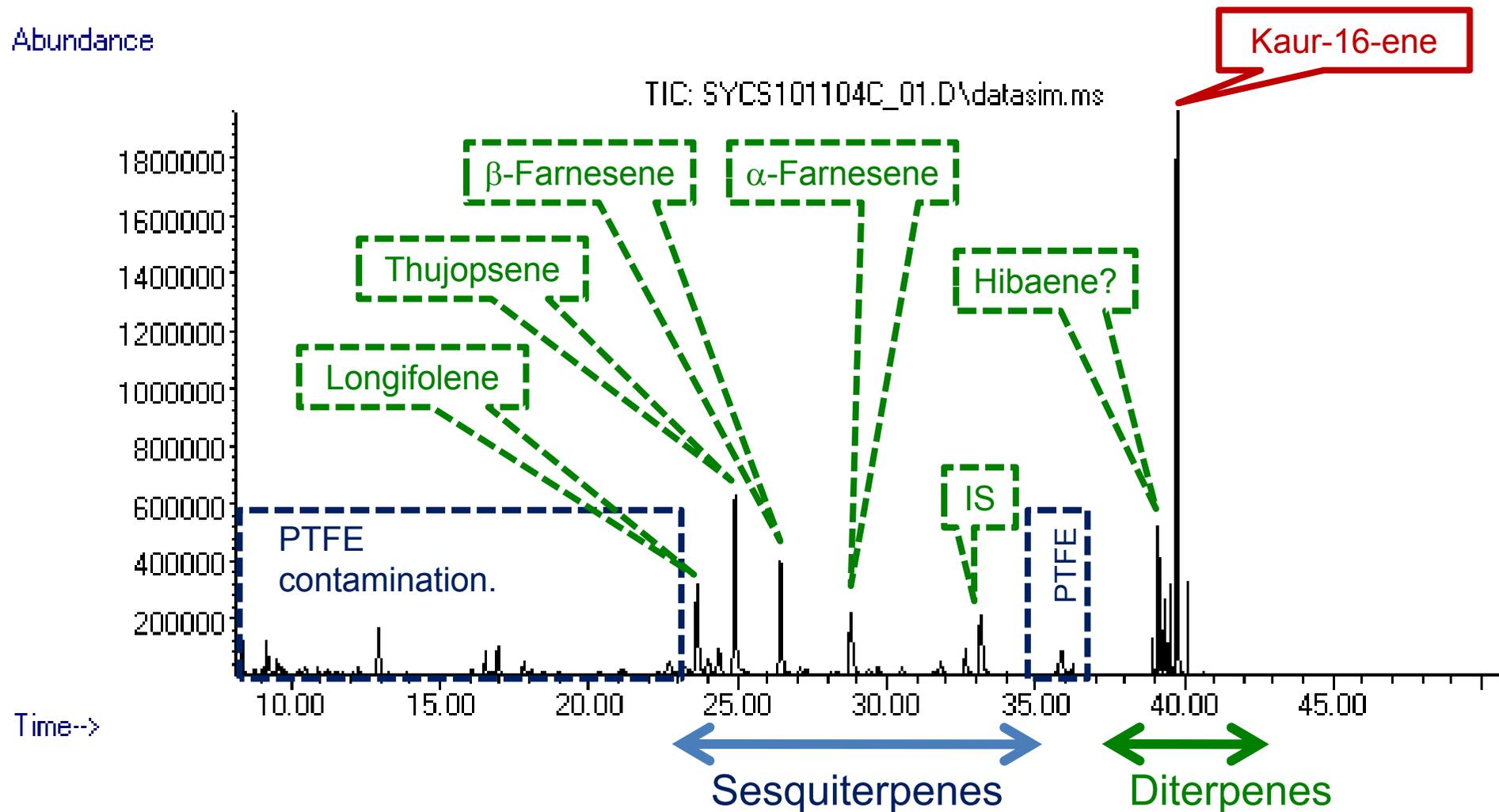


Figure 3 Matsunaga et al.

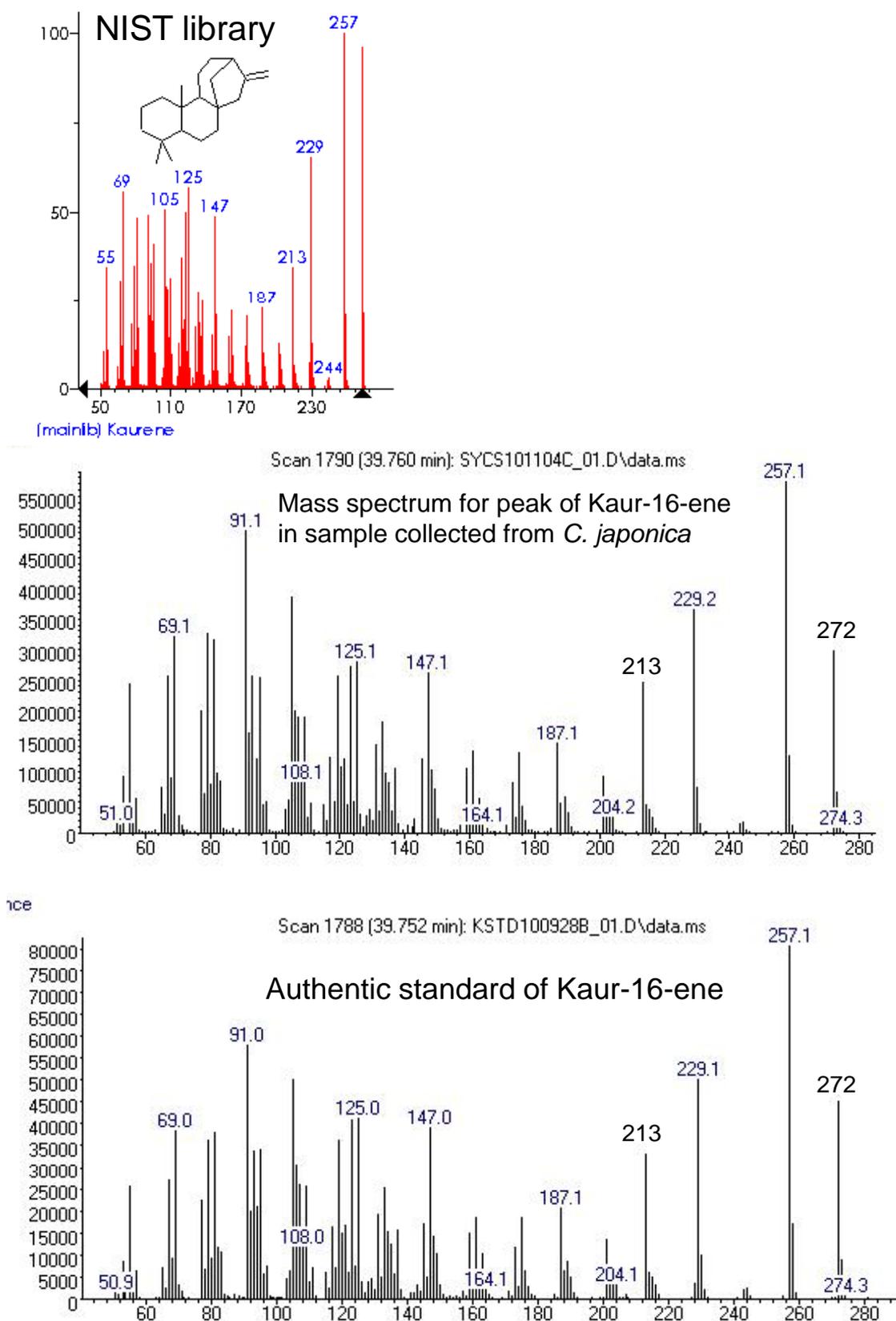


Figure 4 Matsunaga et al.

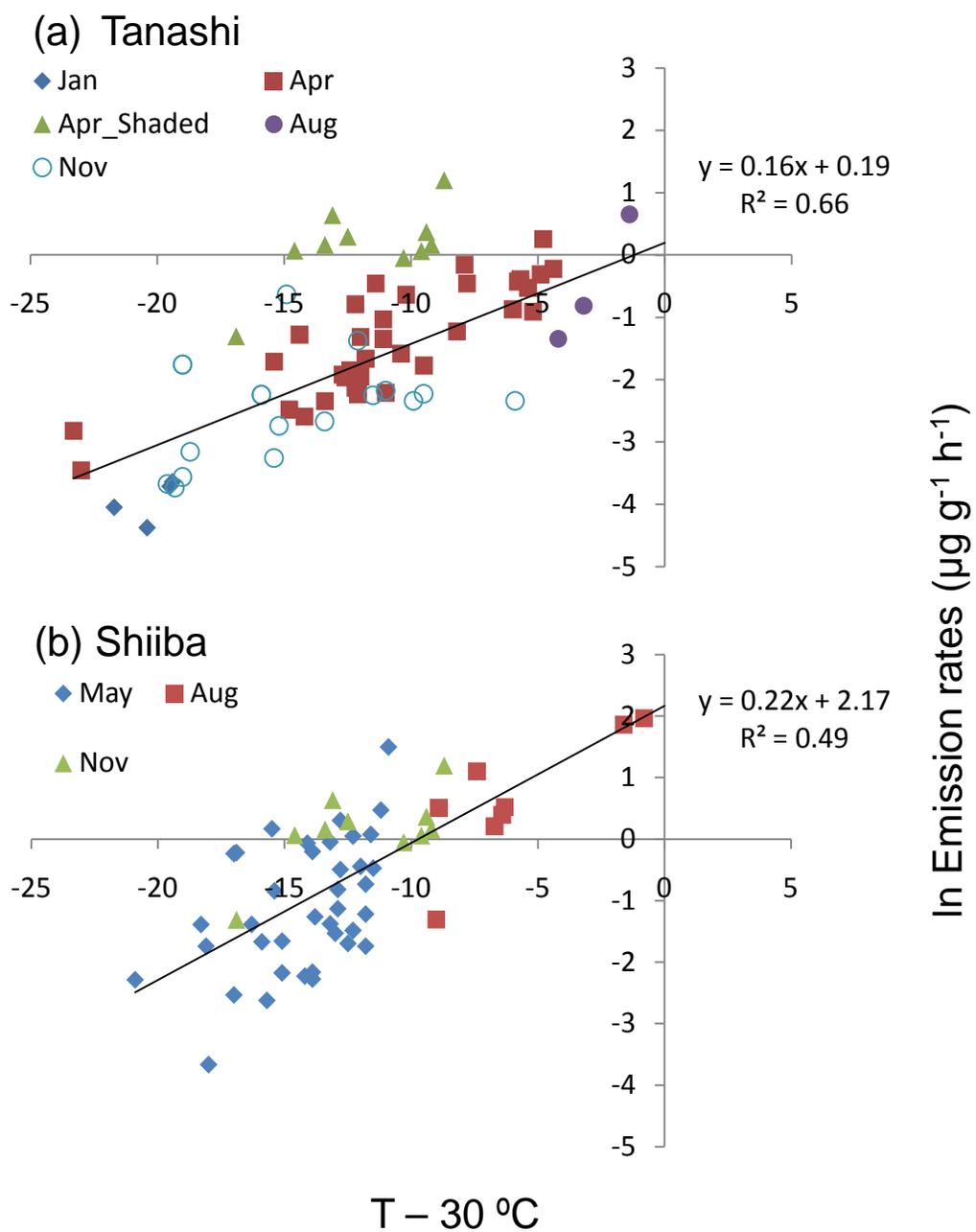


Figure 5 Matsunaga et al.

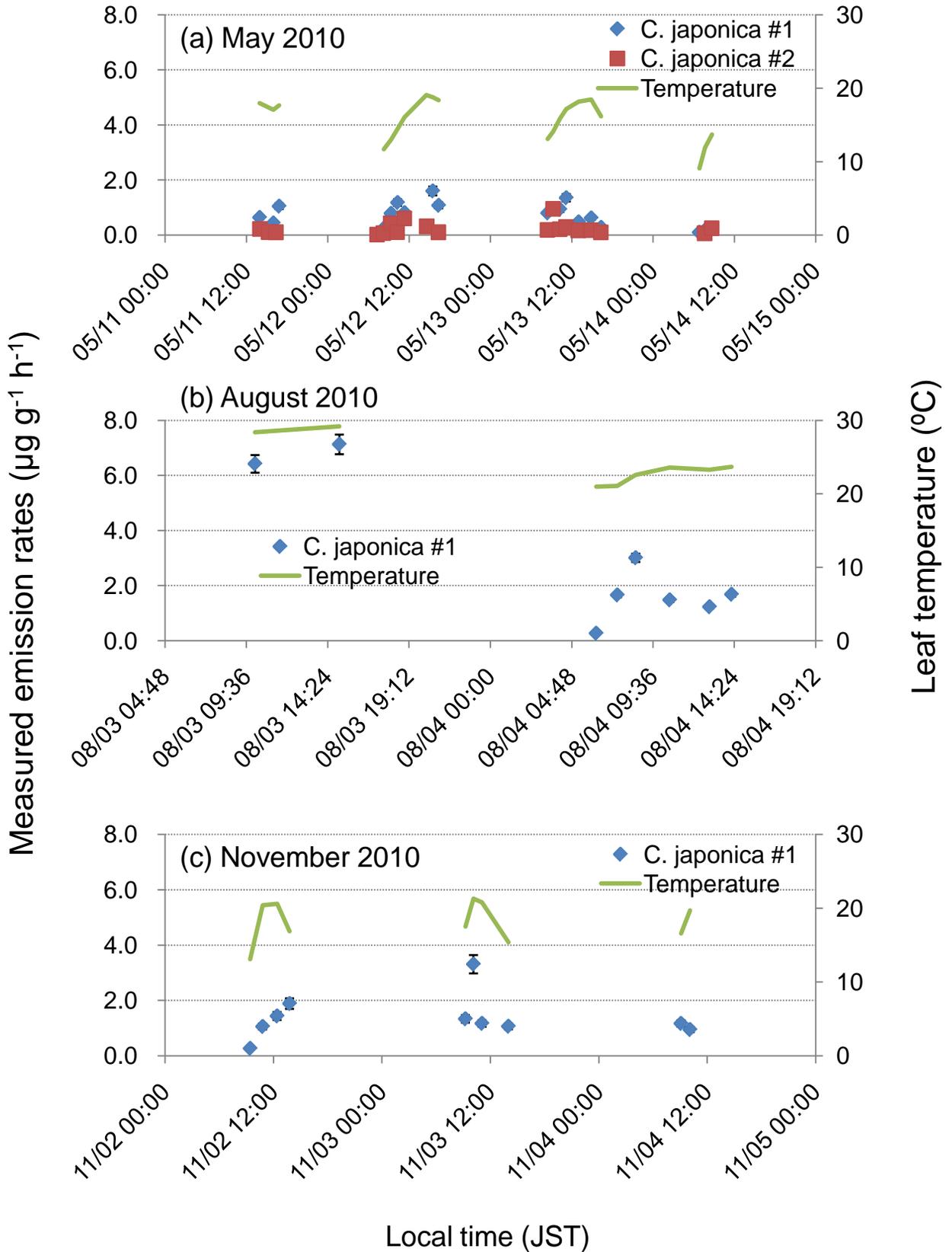


Figure 6 Matsunaga et al.

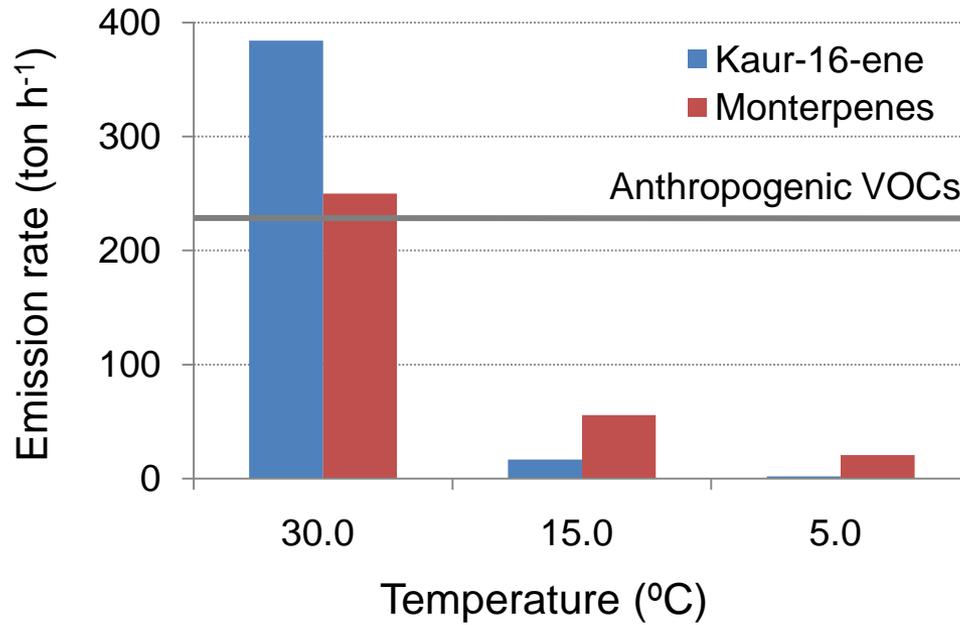


Table 1 Calculated basal emission E_s of Kaurene and total monoterpenes in $\mu\text{g dwg}^{-1} \text{h}^{-1}$, coefficient β and correlation coefficient obtained from seven field measurements conducted at two sites.

	Tree species	number of individuals	number of samples	E_s	β	r^2
Tanashi, Tokyo, 2009-2010 ¹						
Kaur-16-ene	<i>C. japonica</i>	3	72	1.05	0.15	0.61
	<i>C. japonica</i> ²			2.79	0.21	
	<i>C. obtusa</i>	1	17	2.20	0.21	0.50
Shiiba, Miyazaki, 2010 ³						
Kaur-16-ene	<i>C. japonica</i>	2	57	8.74	0.22	0.49
	<i>C. obtusa</i>	2	59	0.99	0.20	0.53

¹ The measurements were conducted on January, August, November of 2009 and April 2010.

² Averaged E_s calculated assuming β of 0.21 by the equation of $E_s = E / \exp(\beta(T - T_s))$.

³ The measurements were conducted on May, August and November of 2010.

Table 2 Averaged measured emission rates of kaur-16-ene and monoterpenes in $\mu\text{g g}^{-1}$ h^{-1} at Tanashi and Shiiba over the seasons.

		January	April ¹	August	November	
Tanashi	<i>C. japonica</i>	Kaur-16-ene	0.02	0.33	0.27	0.12
		Monoterpenes ²	0.04	0.15	0.02	0.10
	<i>C. obtusa</i>	Kaur-16-ene			0.03	0.14
		Monoterpenes ³	0.50	0.07	0.16	0.10
Shiiba	<i>C. japonica</i>	Kaur-16-ene		0.57	1.6	1.1
		Monoterpenes		0.13	0.24	0.06
	<i>C. obtusa</i>	Kaur-16-ene		0.02	0.53	0.12
		Monoterpenes		0.07	0.19	0.03

1: May at Shiiba

2: Matsunaga et al., 2011

3: Mochizuki et al., 2011