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VOLATILE ORGANIC COMPOUNDS EMITTED FROM BEECH LEAVES

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Key Word Index—*Fagus sylvatica*; Fagaceae; headspace; GC-MS; VOC; monoterpenes; leaf volatiles; quantification.

Abstract—Volatile organic compounds (VOC) from beech (*Fagus sylvatica*) leaves have been studied. Headspace samples were collected in the forest from leaves *in situ*, and were analysed by GC-mass spectrometry. The major volatile constituent was sabinene accompanied by other isoprenoids as well as by (Z)-3-hexenyl acetate and (Z)-3-hexenol. Average emissions of 0.28 μg (g leaf dry wt hr)$^{-1}$, being equivalent to 84 μg (m^2 hr)$^{-1}$, were found in the beech forests studied. These figures indicate about one to two orders of magnitude lower emissions from beech, compared to needle-bearing trees and isoprene-producing broadleaf species. The variation in emissions was explored using multivariate methods, and was found to be clearly correlated to ambient temperature. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

An array of monoterpene hydrocarbons are released from conifers in the northern hemisphere [1–5], the main components being α-pinene, β-pinene, camphene and, in one case, sabinene. Reports regarding volatile organic compounds (VOC) from broadleaf trees are fewer. They are mainly reported to emit considerable amounts of isoprene [6–10]. However, they also contribute monoterpene emissions as shown by Evans *et al.* [8] and the present paper on beech trees.

Naturally produced VOC are known to form photooxidants and O_3 in combination with, for example, nitrogen oxides [11]. Natural terpenes are likely to be important sources of olefinic compounds involved in the formation of phototoxic compounds [11, 12]. As a result of pollution stress, different tree species seem to react by either producing quantitatively changed or qualitatively other monoterpenes [13, 14]. Most changes from the natural VOC composition have potentially negative effects. A change in resin monoterpenes can make conifers more vulnerable to insect-pest attacks, and increased amounts of monoterpenes can be an extra stress as they themselves can act as pollutants. A knowledge of the emission from broadleaf species, in addition to those from conifers, will add basic knowledge about VOC emissions and better help us to understand and estimate the effects of air pollution in forested areas.

The present study concerns VOC emitted from leaves of beech (*Fagus sylvatica* L.) trees. Qualitative and quantitative chemical analyses of the VOC emitted from beech leaves *in situ* was carried out by headspace analysis. These data were compared to similar estimations of emissions from other broadleaf species and from conifers. An extrapolation was made to estimate the total yearly emissions from beech trees in Swiss forests.

RESULTS AND DISCUSSION

All of the major volatile compounds isolated from beech leaves were identified by GC-mass spectrometry. The collection of samples in almost closed plastic bags ensured that the amounts of background volatiles were kept low. Toluene and xylenes were found in most samples, but mostly in low amounts compared to terpenes, and they were not quantified. None of the compounds present in the leaf samples were found in samples of beech tree trunks or branches without leaves.

The average percentages of volatiles in the 39 samples analysed are presented in Table 1. Monoterpene hydrocarbons made up 88.2% of the beech leaf volatiles. The main constituent in all but three samples was sabinene, averaging 74.8%. Other monoterpene hydrocarbons present in considerable amounts were myrcene (2.4%), limonene (3.7%), β-phellandrene (3.8%) and p-cymene (2.0%). The only oxygenated terpenes present were (E)-sabinene hydrate, linalool and 4-terpineol, all being found in small amounts.

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Table 1. Average VOC composition of 39 samples of beech (*F. sylvatica*) leaves collected by headspace adsorption

Compound	Mean (%) ($N = 39$)	
α-Pinene	0.5	(29)*
α-Thujene	0.2	(22)
β-Pinene	0.4	(28)
Sabinene	74.8	(39)
Myrcene	2.4	(30)
α-Terpinene	0.04	(9)
Limonene	3.7	(37)
β-Phellandrene	3.8	(37)
γ-Terpinene	0.2	(16)
(*E*)-Ocimene	0.06	(9)
p-Cymene	2.0	(22)
Terpinolene	<0.01	(2)
4,8-Dimethyl-1,3(*E*), 7-nonatriene	2.4	(26)
(*Z*)-3-Hexenyl acetate	7.5	(31)
(*Z*)-3-Hexenol	0.5	(16)
(*E*)-Sabinene hydrate	0.02	(4)
Linalool	0.2	(12)
Caryophyllene	0.5	(13)
4-Terpineol	<0.01	(3)
Germacrene D	0.08	(6)
(*Z*)(*E*)-α-Farnesene	0.1	(3)
(*E*)(*E*)-α-Farnesene	0.5	(10)
Total	99.9	

*Number of samples where individual compounds were detected.

Some sesquiterpene hydrocarbons were also present in low concentrations. The homomonoterpene 4,8-dimethyl-1,3(*E*), 7-nonatriene was a rather prominent constituent averaging 2.4%. The two fatty acid derivatives (Z)-3-hexenyl acetate and (Z)-3-hexenol were present in many samples, averaging 7.4 and 0.4%, respectively. The pattern of leaf volatiles reported here is similar to that found in a recent study [15], where sabinene and β-pinene were found to be the main constituents of *Fagus* leaf volatiles.

The emissions of beech leaves differ partly from those reported for conifer needles. Needle leaf emissions are mainly dominated by α-pinene, β-pinene, 3-carene and limonene [1–4, 16]; however, sabinene and β-pinene are the main components of *Pseudotsuga menziesii* [5]. In addition to isoprene, known to be emitted from several broadleaf species [6, 8], a range of other compounds, including monoterpenes, have been reported in VOC emissions from leafy trees [1, 8]. Isoprene was not found in any sample in the present study, neither in the samples routinely analysed, where it might have been missed, nor in the few eluates specifically investigated by the use of isopropanol to let isoprene elute before the solvent peak. This indicates that isoprene is not emitted by beech trees. The homomonoterpene mentioned above is a common constituent of flower scents [17] as well as of green plant parts [18]. (Z)-3-Hexenyl acetate and (Z)-3-hexenol are expected to occur in most green plant parts, and have been previously reported from broadleaf trees including *Fagus* [1, 15].

A total of 0.03–70.4 μg of VOC (average 6.7 μg) could be collected from beech leaves over a sampling period of *ca* 3–5 hr. This equals average amounts of 0.13 μg (g leaf fr. wt hr)$^{-1}$ (range 0.001–1.14) or 0.28 μg (g leaf dry wt hr)$^{-1}$. This compares well to the values of 0.2 μg (g leaf dry wt hr)$^{-1}$ reported by König *et al.* [15]. A compilation by Janson [16] showed VOC emissions from some different needle-bearing trees to be 1.9 μg (g leaf dry wt hr)$^{-1}$ (range 0.1–10) on average. The isoprene emission from broadleaf species was typically between 10 and 40 μg (g leaf dry wt hr)$^{-1}$ under high production conditions [8, 9]. Thus, in comparison with beech monoterpenes, the isoprene emissions reported are roughly one to two orders of magnitude larger during peak production.

According to literature reports, a beech forest produces *ca* 3 t of dry leaf litter (ha year)$^{-1}$ [19, 20]. The water content of beech leaves is *ca* 55% [21]. Using these data, the average VOC production in one hectare (ha) of beech forest turns out to be 0.84 g (ha hr)$^{-1}$. Assuming that this average VOC production is maintained for 8 hr per day over a 100 day season, The following yearly emissions are obtained for Switzerland. (1) Based on the figure of 675 g (ha year)$^{-1}$ VOC production and the fact that there is a total of 1 043 900 ha of forest in Switzerland of which some 16.2% is beech forest [22], an amount of 114 t of VOC are emitted from beech forests per year. (2) The second extrapolation is based on the figures of a VOC production of 280 mg (t hr)$^{-1}$ leaf dry weight and the facts that the leaf dry weight is *ca* 1.4% of the above ground wood dry weight [19, 20], the wood having a density of about 550 kg m^{-3} [19, 20] and there are 5.92×10^7 m^3 beech wood present in the Swiss forests [22]. This gives a figure of 103 t of VOC emitted from beech forests per year in Switzerland. By increasing the assumed period of VOC production to 150 days and 12 hr per day, extrapolated yearly emissions of 256 and 232 t, respectively, are obtained. These estimations are of course very rough, but give at least an idea of VOC emissions from beech forests. From the above figures, we can calculate an emission rate of 84 μg (m^2 hr)$^{-1}$ in a beech forest, the minimum and maximum values being 21 and 210 μg (m^2 hr)$^{-1}$ under low and high temperature conditions, respectively (Table 2). This can be compared to 1400–6800 μg (m^2 hr)$^{-1}$ for different needle-bearing trees at different temperatures [12]. The difference in VOC production between beech and needle-bearing trees is larger when calculated per square metre of forest as when calculated per unit of biomass, which is due to the fact that needle forests produce more green biomass per forest area compared to beech forests [20].

Some of the compounds found here are known to be released by plants after being attacked by herbivores [23, 24], e.g. (*E*)-ocimene, 4,8-dimethyl-1,3(*E*), 7-nonatriene, (Z)-3-hexenyl acetate, (Z)-3-hexenol and linalool. These compounds are systemically induced in some plants as defence chemicals in tritrophic ecological interactions. It is not known if the presence of such

Table 2. VOC amounts in ng (g fr. wt hr)$^{-1}$ collected from *F. sylvatica* leaves during low and high temperature conditions, respectively

Compound	10–14° ($N = 4$)	25–29° ($N = 8$)
α-Pinene	0.3	2.4
α-Thujene		1.4
β-Pinene	0.2	2.2
Sabinene	28.6	275
Myrcene	0.3	11.5
α-Terpinene	<0.01	0.2
Limonene	0.9	8.4
β-Phellandrene	0.6	8.8
γ-Terpinene	<0.01	0.5
(*E*)-Ocimene		0.4
p-Cymene	<0.01	1.1
Terpinolene		
4,8-Dimethyl-1,3(*E*), 7-nonatriene	<0.01	1.3
(*Z*)-3-Hexenyl acetate	0.3	1.4
(*Z*)-3-Hexenol	<0.01	0.4
(*E*)-Sabinene hydrate		0.3
Linalool		0.1
Caryophyllene		0.2
4-Terpineol		<0.01
Germacrene D		0.05
(*Z*)(*E*)-α-Farnesene		0.1
(*E*)(*E*)-α-Farnesene		0.6
Total	31.2*	316*

*Significantly different at 5% (Wilcoxon's rank sum test).

compounds in beech leaf emissions indicates a response to herbivory. It is, however, known that several plants can produce homoterpenes, these being potential defence chemicals [18]. When volatiles are collected, as here, from natural leaf samples, there is always some insect or fungal damage present. Thus, some of the chemicals found here might be induced as part of a natural chemical defence in beech trees, but must still be considered as natural VOC emissions.

The variation between samples is large and probably very complex, involving genetic, phenotypic and environmental factors. A principal components analysis was made from a matrix including all 39 samples and 22 variables (chemicals), but no obvious trends could be detected among the values (data not shown). The variable weights, however, were distributed in a pattern possibly reflecting the vapour pressure of individual compounds, ranging from sesquiterpenes, monoterpene alcohols, (Z)-3-hexenyl acetate and (Z)-3-hexenol, the homomonoterpene, to open and cyclic monoterpenes. Vapour pressure has been suggested to be important in regulating VOC emissions [13]. At present, it is not possible to conclude if differences in volatility or in biogenic regulation mechanisms are responsible for the variable weight distribution seen. The known induceable defence chemicals (see above) were also more or less grouped together. Since the samples were collected in the forest, factors like temperature and light intensity more or less co-varied and could not be manipulated. An increase in VOC emission rate was correlated to increased ambient temperature and possibly also to solar irradiation. Roughly, samples collected at temperatures over 25° emitted 10 times more VOC as samples collected at temperatures below 15° (Table 2), the differences being significant at the 5% level (Wilcoxon's rank sum). Temperature correlated changes in VOC emission rates have been reported in previous studies [1, 9]. In addition, there is also a diurnal variation present in VOC emissions of both isoprene- and monoterpene-emitting species [2, 9].

From the present study, it is quite clear that broadleaf species like beech trees emit considerable amounts of VOC, although these are one to two orders of magnitude lower than emissions from conifers and isoprene-producing broadleaf trees. It is likely that the amounts of VOC detected here are to some extent influence by the methods used. For example, the temperature inside the sampling bag increased to above ambient when samples were exposed to the sun, probably increasing the emission rate. On the other hand, adsorption to the surface of the plastic bag in combination with a relatively low sampling flow rate can cause losses of volatiles. The extrapolations of VOC production in beech forests on a yearly basis are naturally very uncertain, but it is still interesting to compare them to those from anthropogenic sources. Anthropogenic VOC emissions in Switzerland are estimated to be 239.5 kt in the year 1995, subdivided into 36.5 kt from transportation, 48.6 kt from household and 154.4 kt from industry (including trade and agriculture) [25]. Natural VOC emissions were recently estimated to be 87 kt per year from Swiss forests [26]. An algorithm for *Fagus* was lacking, but if we assume that beech forests have the same biomass density as oak forests, but emit one to two orders of magnitude less VOC, we arrive at 150–1500 t per year VOC from *Fagus*. This is in the same range as the results from the present study. Although the natural emissions from beech leaves are small compared to anthropogenic VOC emissions, they can be locally important in forested areas due to their reactivity and potential to form phototoxic compounds. It is possible that stress in any form may alter the natural composition of VOC emissions, resulting in a disturbance of the balance between the host tree and its insect pests [14].

EXPERIMENTAL

Volatiles were collected by dynamic headspace trapping, and analysed by GC-MS. In the field, an intact branch with leaves was enclosed in a plastic frying bag (PET). The bag had openings in both ends. One end was gently tied around the branch leaving a small hole for incoming air, the other was tightly closed around an adsorbent filter. The volatile-containing air was pumped through the charcoal adsorbent filter (5 mg) at *ca* 65 ml min^{-1} using a portable electric pump. Blank samples from empty plastic bags were collected in parallel to every second leaf sample. Background samples were also collected from tree trunks and branches without leaves. Samples were typically collected for 4 hr between 11.00 and 15.00 from between 5.1 and 25.6 g leaves. To cover low and high emission conditions, care was taken to collect samples in both sunny and shady locations, as well as during warm and cold weather. After sampling, the leaves were collected and weighed. The charcoal filters were eluted with 20 μl hexane–Me_2CO (5:1). Two samples not considered in Table 1 were eluted with *iso*-PrOH and checked for highly volatile materials (isoprene) by GC-MS. An int. standard (500 ng hexadecane) was added to each sample before analysis by GC or GC-MS. All samples were analysed by GC; DBWax (30 m × 0.32 mm), cold on-column injection, FID 250°, temp. programme 3 min at 50°, $4° min^{-1}$ to 240°. To identify compounds some representative samples were analysed by GC-MS; Finnigan MAT 212, column and temp. programme as above. Identifications by MS were always verified by GC retention data of authentic compounds. The results presented here are based on 39 samples collected between May 25 and 23 Sept. 1994. All samples were collected in forests dominated by beech in the vicinity of Dübendorf, ZH, Switzerland. Principal component analysis was carried out by the Statgraphics Plus Program.

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