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VOLATILE METABOLITES PRODUCED BY TWO FUNGAL SPECIES CULTIVATED ON BUILDING MATERIALS

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Abstract—Two fungal species commonly found in indoor environments, *Penicillium commune* and *Paecilomyces variotii*, were cultivated on pine wood and on a combination of gypsum board and mineral wool. Air from the cultures was adsorbed on Tenax TA and analysed using thermal desorption–cold trap injection gas chromatography. Identification of the produced volatile metabolites was performed by mass spectrometry. The majority of the compounds produced were alcohols, ketones, ethers and terpenoid compounds. Commonly produced metabolites were 2-methyl-1-propanol, 3-methyl-1-butanol, 1-hexanol, 2-heptanone, 2-pentanone and 2,5-dimethyl-furan. The production was highly influenced by both medium and species.

INTRODUCTION

Excessive growth of micro-organisms is one reason often mentioned in relation to health problems in buildings, the so-called sick building syndrome. Both fungi and bacteria are reported as being the cause of bad health (Morey *et al.*, 1984; Platt *et al.*, 1989; Reynolds *et al.*, 1990; McJilton *et al.*, 1990; Binne, 1991; Koskinen *et al.*, 1994). The problems are usually linked with dampness, which favours growth of micro-organisms.

The traditional approach for measuring the growth of micro-organisms is determination of airborne spore levels. These methods work well in many cases when the spore source is in direct contract with the open air as, for example, in forage storehouses. Various investigations have shown, however, that there is often no significant difference between airborne spore levels in affected and non-affected buildings (Strachan *et al.*, 1990; Flannigan *et al.*, 1991; Nevelainen *et al.*, 1991; Blomquist and Andersson, 1994). The micro-organisms may grow within walls or ceilings, and the fungal spores are too large to diffuse through such materials. An alternative method consists of measuring microbially produced volatiles. These compounds can diffuse through building materials (Ström *et al.*, 1994) and would, therefore, reach the indoor air. This approach has been suggested by Sunesson *et al.* (1995b), Miller *et al.* (1988) and Ström *et al.* (1994). Investigations in buildings performed by Miller *et al.* (1988) and Ström *et al.* (1994) showed higher levels of

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compounds considered to have microbial origin in problem buildings than in reference buildings.

Some micro-organisms found in buildings are able to produce highly toxic metabolites. A review of such metabolites and their sources is given by Flannigan *et al.* (1991). Some microbially produced volatiles have an unpleasant odour (Kaminski *et al.*, 1974; Collins, 1976; Kikuchi *et al.*, 1981; Karahadian *et al.*, 1985a,b; Harris *et al.*, 1986; Börjesson, 1993; Larsen and Frisvad, 1994), which can cause both physical and psychical discomfort to people who stay in the building. Both the odorous and the odourless compounds produced may include substances with adverse health effects. The effect of long-term exposure to combinations of such compounds is not known, but a harmful effect cannot be ruled out despite the low concentrations.

The production of metabolites from micro-organisms depends on several factors. Sufficiently high humidity is necessary for growth, and thereby for metabolite production. The nature of the metabolites produced depends mainly on species and medium. In a study of metabolites from fungal species grown on various grains and grain-based agar media, Börjesson *et al.* (1993) reported that the production of volatiles depended more on fungal species than on media. Norrman (1971a,b) stated that metabolite production by *Dipodascus aggregatus* was dependent on both the carbon and the nitrogen sources of the media. Terpene production by *Ceratocystis* species has also been shown to be highly dependent on the carbon source (Sprecher and Hanssen, 1982). In an earlier investigation of metabolites produced by five common indoor air fungi cultivated on two different media, we found some metabolites produced by several species and on both media, but a majority of the identified volatiles were produced by only one species and on one medium only (Sunesson *et al.*, 1995b). The dependence on medium was also shown in a study of the influence of several factors on metabolite production by *Streptomyces albidoflavus*.

The vital importance of medium for metabolite production makes cultivation on building materials necessary in order to provide an indication of which metabolites may be expected in buildings. In this study, two frequently found indoor air fungi (Samson *et al.*, 1994), *Penicillium commune* and *Paecilomyces variotii*, were cultivated on common building materials—pine wood and a combination of gypsum board and mineral wool. The objective was to study which metabolites were produced and to ascertain whether any metabolites were commonly produced. It was also of interest to note similarities and differences in metabolite production on building materials, compared with production in the earlier study when the same species were grown on artificial media (Sunesson *et al.*, 1995b).

MATERIALS AND METHODS

Fungal cultures

The following strains were used: *Penicillium commune* Pitt and *Paecilomyces variotii* Bain. The strains were obtained from Pegasus Lab AB, Uppsala, Sweden.

Building materials for cultivation

The fungi were cultivated on pine wood and on a combination of gypsum board and mineral wool. All building materials were bought from retailers. The planed pine

wood, 15 mm thick, was sawn into pieces, 15 x 100 mm, put into autoclavable bags and autoclaved at 125°C for 15 min. The gypsum board (Norgips, Norway), 12 mm thick, was cut into 15 x 100 mm-pieces and sterilized as above. The mineral wool (Rockwool, Sweden) was torn in 1 cm layers and put into the culture flasks so that the bottom of each flask as covered with mineral wool. The culture flasks were autoclaved as above. The gypsum board used in the study was chosen from the middle of a pile of boards in an unopened package. The mineral wool was also taken from an unopened package.

Preparation of spore suspension and inoculation

The fungi were cultivated on malt extract agar for 10 days. [The malt extract agar was prepared by adding 20 g of malt extract (Difco laboratories) and 15 g of agar (Oxoid, Bacterial Agar No. 1) to 1 l. demineralized water, stirred at 85°C for 1.5 h and autoclaved at 125°C for 15 min.] Ten millilitres of sterile 0.05% Tween-80 (Kebo Lab AB) was added to each culture, followed by smooth shaking and isolation of the suspension. The spore concentration was determined by counting in a Bürker 0.100 mm x 0.04/0.0025 mm^2 counting chamber, and adjusted to 1.0×10^6 spores/ml. For each of the two species, the following experiments were performed: (i) with pine wood, eight pieces per cultivation flask were inoculated with 0.20 ml spore suspension per piece and the suspension was spread over the surface using a sterile platinum needle. The pieces of wood were placed with the inoculated side upwards in the cultivation flasks using a sterile pair of tweezers, after which 40 ml sterile-filtered water was added to provide a humid germination environment; (ii) the pieces of gypsum board were inoculated with 0.10 ml spore suspension on each paper side of the pieces. The pieces were put with one of the inoculated paper sides downwards on the sterile, human mineral wool in the cultivation flasks, using a sterile pair of tweezers. Eight pieces of gypsum board were put into each cultivation flask, and 20 ml sterile-filtered water was added.

For each series of experiments, two cultivation flasks were inoculated and one served as a blank. This makes a total of two pine wood blanks and two gypsum board–mineral wool blanks. The blanks were treated as above, but with sterile 0.05% Tween-80 instead of the spore suspensions.

Cultivation and sampling equipment

The cultivation was performed in 2-l. culture flasks made of glass, with glass adapters for air inlet and outlet. The equipment has previously been described by Sunesson *et al.* (1995b). Cleaned, humidified air with a flow of 30 ± 2 ml min^{-1} was constantly led through the flasks. The cultivation was performed at a constant room temperature of 21°C.

Sampling of volatile metabolites

The volatiles released by the cultures were sampled on Tenax TA (Chrompack) (Sunesson *et al.*, 1995a). Samples were taken on days 1, 3, 5, 7, 10, 14, 21, 28 up until 56 days after inoculation. The sampling time was 60 min, and the sampling flow 30 ± 2 ml min^{-1}. Three samples were taken from each cultivation flask on every sampling occasion, two for analysis using flame ionization detection and one for mass-spectrometric determination.

Analysis and identification

The samples were purged with 100 ml helium (100 ml min^{-1} for 1 min) before analysis, in order to remove water from the adsorbent. The volatile metabolites trapped on the adsorbent were analysed using thermal desorption–cold trap injection gas chromatography. For determination by flame ionization detection, an ATD 400 thermal desorption injector (Perkin-Elmer) was used, and for mass-spectrometric identification the tubes were desorbed in a Chrompack 16200 thermal desorption cold trap injector. The gas-chromatographic separations were performed on HP 5890 gas chromatographs with fused silica columns (HP Ultra 2, 50 m × 0.2 mm i.d. coated with cross-linked 5% phenylmethylsilicone, film thickness 0.33 μm). The data from the flame ionization detection (FID) analyses were recorded, integrated and quantified by Millennium® Chromatography Manager (Waters) run on a Digital 486 computer. The mass-spectrometric identifications were performed using a Finnigan INCOS 500 mass spectrometer, in electron impact mode at an electron energy of 70 eV. When available, reference compounds were used to ensure the identities of the detected compounds.

The analysed metabolites were quantified as equivalents of toluene. Reference values were obtained by injecting a standard mixture containing toluene (Merck, *p.a.*) and dimethyl disulphide (Janssen, *p.a.*) in methanol (Merck, *p.a.*) onto the adsorbent tubes. Using a gas-tight syringe (Hamilton 7000.5 KH), 0.30 μl was applied onto the silanized glass wool preceding the adsorbent, and 100 ml helium (AGA, 100 ml min^{-1} for 1 min) was blown through the tube in order to transfer the substances to the adsorbent and for removal of most of the solvent (De Bortoli *et al.*, 1992). Flame ionization detection was used for the quantification. Dimethyl disulphide was used for quantification of the sulphur-containing compounds, because of the low detector sensitivity towards those compounds.

Reference compounds

The reference compounds used for identification were as follows.

Hydrocarbons: cyclohexane (Merck, *p.a.*), decane (PolyScience Corporation, *p.a.*), ethylbenzene (Fluka, >99%), toluene (Merck, *p.a.*), *m*-xylene (Aldrich, 98%), *o*-xylene (Aldrich, 97%), *p*-xylene (Aldrich, 99%).

Alcohols: 1-decanol (EGA-Chemie, 99%), 2-ethyl-1-hexanol (Fluka, pract.), 1-hexanol (Kebo, puriss), 2-methyl-1-butanol (Aldrich, 99%), 3-methyl-1-butanol (Baker, 98%), 2-methyl-1-propanol (Sigma-Aldrich, 99.5%), 1-nonanol (Aldrich, 98%), 1-octen-3-ol (Aldrich, 98%), phenol (Merck, *p.a.*).

Aldehydes: benzaldehyde (Kebo, puriss), decanal (Aldrich, 98%), furfural (Merck, *p.a.*), heptanal (Aldrich, 95%), hexanal (Aldrich, 99%), 3-methylbutanal (Kebo, purum), nonanal (Aldrich, 95%), octanal (Aldrich, 99%), pentanal (Merck, zur Synthese).

Ketones: acetone (Merck, *p.a.*), acetophenone (Hopkins & Williams Ltd, >95%), 2-butanone (Merck, >99%), 2-heptanone (Aldrich, 98%), 2-hexanone (Aldrich, 98%), 3-methyl-2-pentanone (Aldrich, 99%), 3-octanone (Aldrich, 99%), 2-pentanone (Aldrich, 97%).

Ethers: 2,5-dimethylfuran [synthesized as in Sunesson *et al.* (1995b), >98%], 3-methylanisole (Aldrich, 99%), 3-methylfuran (Tokyo Kasei Organic Chemicals, 99%).

Esters: methyl acetate (Aldrich, 99%), propyl acetate (Theodor Schuchardt, für Chromatographie).

Sulphur compounds: dimethyl disulphide (Janssen, *p.a.*), dimethyl trisulphide (Oxford Chemicals Ltd, *p.a.*).

Terpenes and terpene derivatives: geosmin [synthesized according to Hansson and Carlson (1990) and Hansson *et al.* (1990) >98%], R(+)-limonene (Fluka, 98%), S(−)-limonene (Fluka, 97%), α-pinene (Aldrich, 98%), β-pinene (Aldrich, 98%).

RESULTS

Metabolites were detected from both species and on both media; the volatiles produced are summarized in Table 1. The background emission from the building materials is presented in Table 2. Gas-chromatograms of air samples from the cultures and material blanks are shown in Figs 1 and 2.

Several compounds were produced by both species when cultivated on pine wood. On gypsum board–mineral wool only 2-methyl-1-propanol was found from both species. Sesquiterpenoid compounds were the main products from both species on this medium. On pine wood, no terpenoid compounds produced by the fungi could be detected.

Penicillium cummune produced 3-methyl-1-butanol, 2-methyl-1-butanol, 2-methyl-1-propanol, 2-butanone and 1-methoxy-3-methylbutane on both media. On pine wood, alcohols were the dominating compounds, and geosmin was the main product in cultivation on gypsum board–mineral wool. Consequently, the cutures on gypsum board–mineral wool had a strong earthy smell.

Paecilomyces variotii produced mainly ketones when cultured on pine wood. The production was most predominant in the beginning of the cultivation. Terpenes were the main compounds from the gypsum board–mineral wool cultures. Two $C_{13}H_{20}$ compounds with mass spectra showing terpenoid structures, a $C_{10}H_{16}$ monoterpene, two $C_{15}H_{24}$ sesquiterpenes and two terpene alcohols were produced. No metabolites were produced on either media.

The majority of the emission compounds from pine wood are terpenes and terpene derivatives, of which only a few could be identified. Only monoterpenoid structures were found—many of the gas-chromatic peaks not identified had mass spectra indicating monoterpenols ($C_{10}H_{16}O$ and $C_{10}H_{18}O$). Pine wood is known to emit a variety of terpenoid compounds (von Schantz and Hiltunen, 1983; Imamura, 1989). The gypsum board–mineral wool medium emitted mainly hydrocarbons, alcohols, aldehydes and two esters. Dimethyl disulphide was found in trace amounts. The background emission from the combined gypsum board–mineral wool medium was the same as the background from the gypsum board used solely in another study (Sunesson *et al.*, submitted).

The time dependence of the production of some of the major metabolites produced is shown in Figs 3 and 4. The relative standard deviations of the amounts produced were generally below 30% ($n=4$). Production from *Paecilomyces variotii* was generally higher during the first half of the cultivation period. *Penicillium commune* produced many compounds in increasing amounts during the cultivation time studied.

Table 1. Production of volatiles from *Penicillium commune* and *Paecilomyces variotii* on gypsum board–mineral wool (G) and pine wood (W). The amounts of metabolites are indicated as toluene equivalents (except for the sulphur compounds, see 'Analysis and identification' in text) when determined with flame ionization detection: G++ and W++ are used for metabolite production in amounts corresponding to >50 ng toluene h^{-1}, G+ and W+ for 10–50 ng h^{-1}, G and W for 1–10 ng h^{-1} and G− and W− for <1 ng h^{-1}. Substances identified with reference compounds are marked R. For substances whose identities are indicated by the mass-spectrometric library the reported purity and fit values are given. Metabolites produced by the fungi also on MEA and/or DG18 (Sunesson *et al.*, 1995b) are marked with an asterisk

No.	Compounds	*Pe. commune*	*Pa. variotii*	Purity/fit or reference compound	Also found on MEA and/or DG18
	Hydrocarbons				
1	1-(1,1-Dimethylethyl)-4-ethylbenzene		G	873/981	
	Alcohols				
2	2-Ethyl-1-hexanol	G		R	
3	1-Hexanol	W++	W++	R	
4	2-Methyl-1-butanol	G+, W		R	*
5	3-Methyl-1-butanol	G, W+	W	R	*
6	2-Methyl-1-propanol	G+, W+	G	R	*
7	1-Octen-3-ol	G		R	
	Ketones				
8	Acetone		W	R	*
9	2-Butanone	G, W		R	*
10	2-Heptanone	W+	W++	R	
11	2-Hexanone		W+	R	
12	3-Methyl-2-pentanone		W	R	
13	3-Octanone	G		R	
14	2-Pentanone	W++	W+	R	
	Ethers				
15	2,5-Dimethylfuran	W	W	R	*
16	3-Methylfuran		G−	R	*
17	3-Methylanisole	G+		R	*
18	1-Methoxy-3-methylbutane	G, W		822/951	
19	2,3,5-Trimethylfuran		G−	889/979	
	Esters				
20	Methyl acetate	W		R	*
21	Propyl acetate		W	R	
	Sulphur compounds				
22	Dimethyl disulphide	G		R	*
23	Dimethyl trisulphide	G−		R	
	Terpenes and terpene derivatives				
24	Geosmin	G++		R	*
25	1-Methoxy-4-(1-methylethyl)-benzene	G+		860/957	
26	Monoterpene $C_{10}H_{16}$		G		
27	Monoterpenol $C_{10}H_{16}O$		G−		
28	Terpenoid compound $C_{13}H_{20}$		G		
29	Terpenoid compound $C_{13}H_{20}$		G+		
30	Sesquiterpene $C_{15}H_{24}$		G		
31	Sesquiterpene $C_{15}H_{24}$		G		
32	Sesquiterpenol $C_{15}H_{24}O$		G		

Table 2. Background emission from the building materials used as media. Presence of a compound is marked with an X under the respective medium. The degree of identification (reference compound or mass-spectrometric library) is given as in Table 1. Numbers are as in Table 1

No.	Compound	Pine wood	Gypsum board–mineral wool	Purity/fit or reference compound
	Hydrocarbons			
33	Cyclohexane		X	R
34	Decane		X	R
35	Ethylbenzene		X	R
36	Toluene	X	X	R
37	Xylene		X	R
	Alcohols			
38	1-Decanol		X	R
2	2-Ethyl-1-hexanol		X	R
39	2-(1-Methylethyl)-phenol		X	845/997
40	4-(1-Methylethyl)-phenol		X	662/958
41	1-Nonanol		X	R
42	Phenol		X	R
	Aldehydes			
43	Benzaldehyde		X	R
44	Decanal		X	R
45	Furfural	X		R
46	Heptanal		X	R
47	Hexanal	X	X	R
48	3-Methylbutanal		X	R
49	Nonanal		X	R
50	Octanal		X	R
51	Pentanal	X		R
	Terpenes and terpene derivatives			
52	Limonene	X		R
53	α-Pinene	X		R
54	β-Pinene	X		R
	Others			
55	Acetophenone		X	R
22	Dimethyl disulphide		X	R
56	5-Ethyldihydro-2(3H)-furanone		X	832/905
57	2-Methyl-propanoic acid, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester		X	743/964
58	2-Methyl-propanoic acid, 3-hydroxy-2,4,4-trimethylpentyl ester		X	797/974

DISCUSSION

Although the two fungi studied here are commonly found in indoor environments, few studies of their production of volatile metabolites are found in the literature (Larsen and Frisvad, 1994; Sunesson *et al.*, 1995b). In accordance with previous studies (Sunesson *et al.*, 1995b), their production of metabolites proved to depend on both fungal species and media, but some metabolites were commonly produced. In this study, 3-methyl-1-butanol and 2-methyl-1-propanol, both frequently reported as fungal metabolites also from other species (Kinderlerer, 1989; Eriksson *et al.*, 1992; Börjesson, 1993; Larsen and Frisvad, 1994; Sunesson *et al.*, 1995b), were produced by *Penicillium commune* on both building materials and by *Paecilomyces variotii* on pine wood and gypsum board–mineral wool,

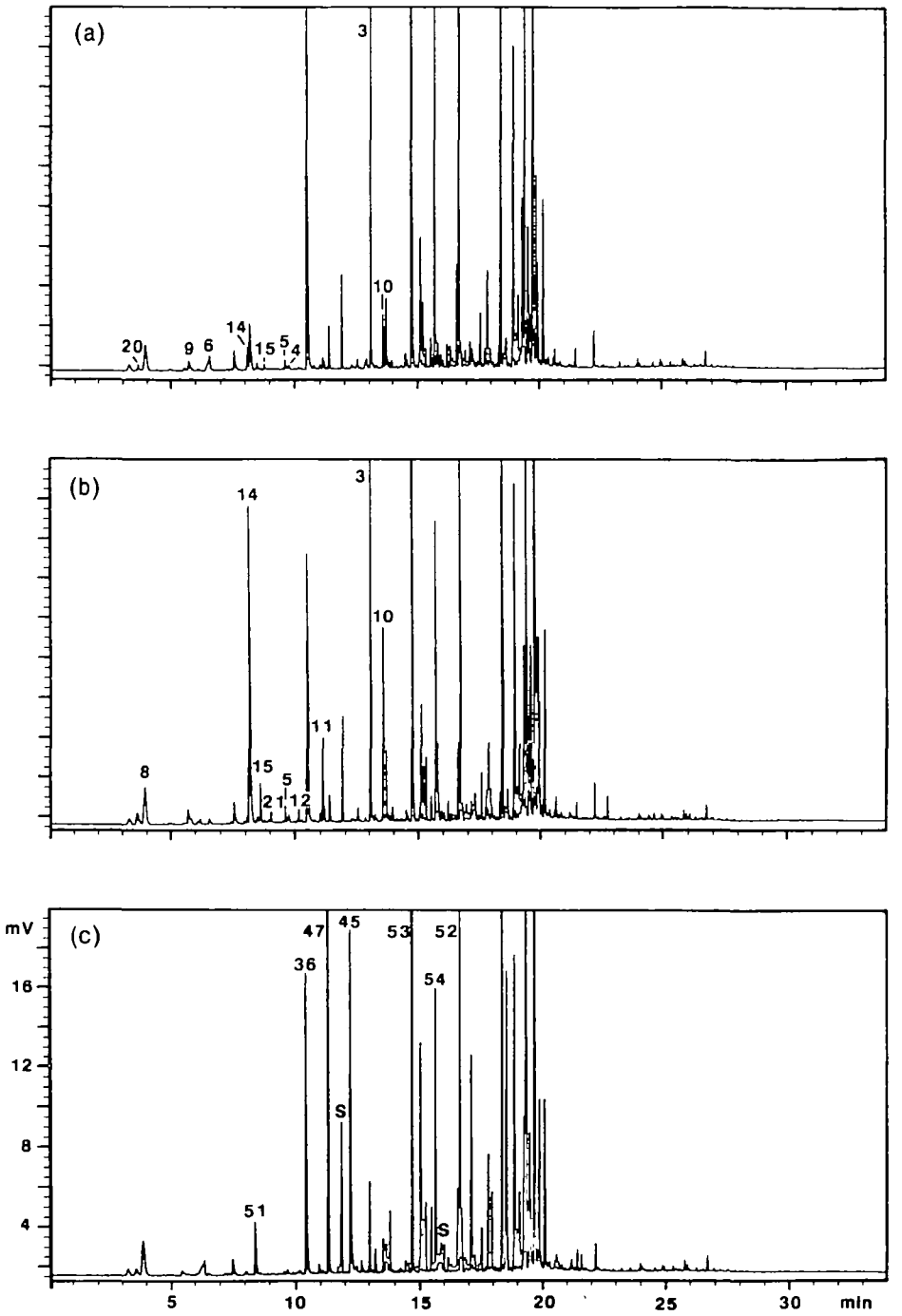

Fig. 1. Gas-chromatograms of volatiles from (a) *Penicillium commune* (day 10) and (b) *Paecilomyces variotii* (day 7) on pine wood. Chromatogram (c) is the pine wood blank at day 7. The numbers identify the compounds as listed in Tables 1 and 2. Compounds marked with S are bleeding from the cold trap of the injector.

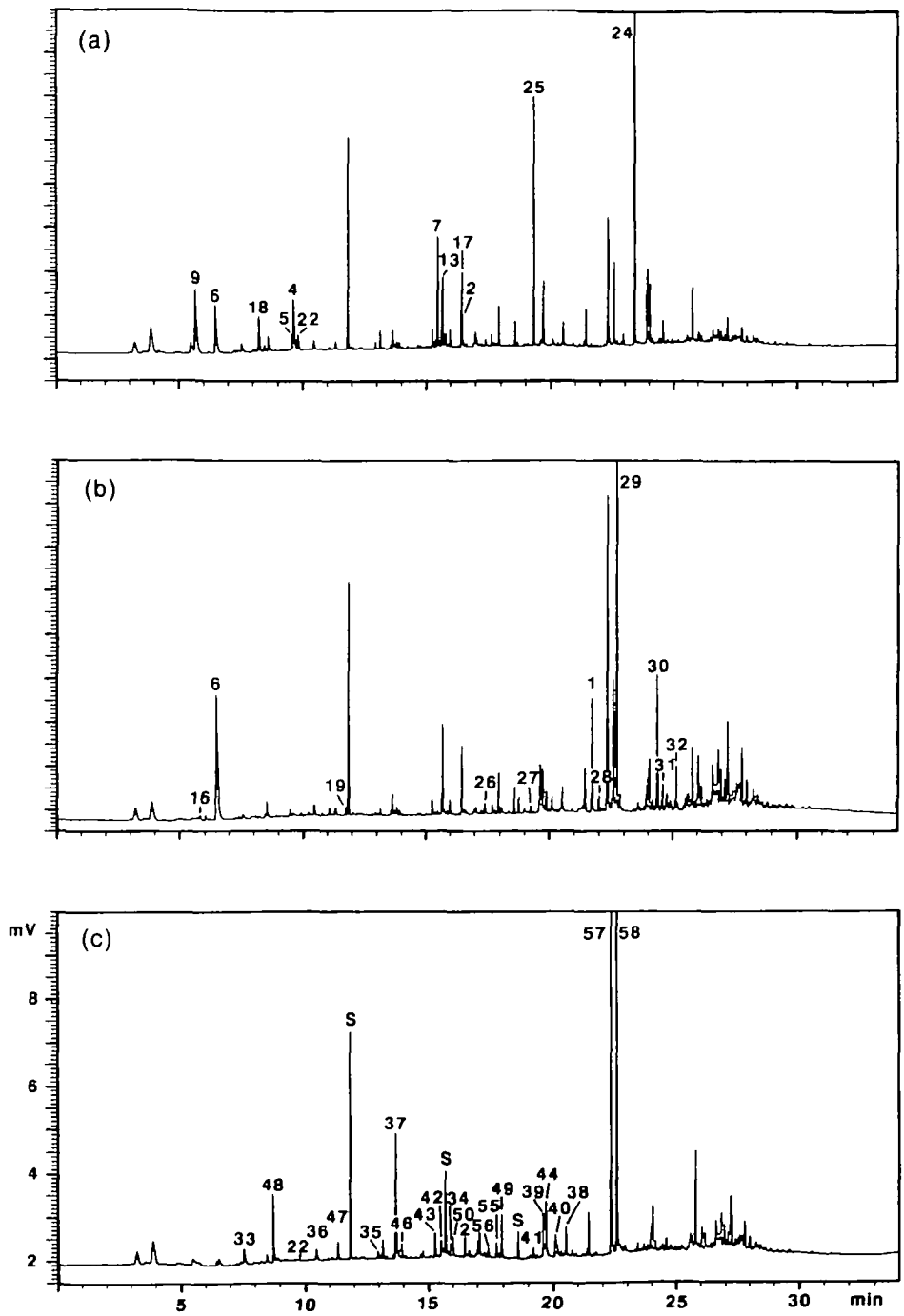

Fig. 2. Gas-chromatograms of volatiles from (a) *Penicillium commune* (day 34) and (b) *Paecilomyces variotii* (day 21) on gypsum board–mineral wool. Chromatogram (c) is the gypsum board–mineral wool blank at day 34. The numbers identify the compounds as listed in Tables 1 and 2. Compounds marked with S are bleeding from the cold trap of the injector.

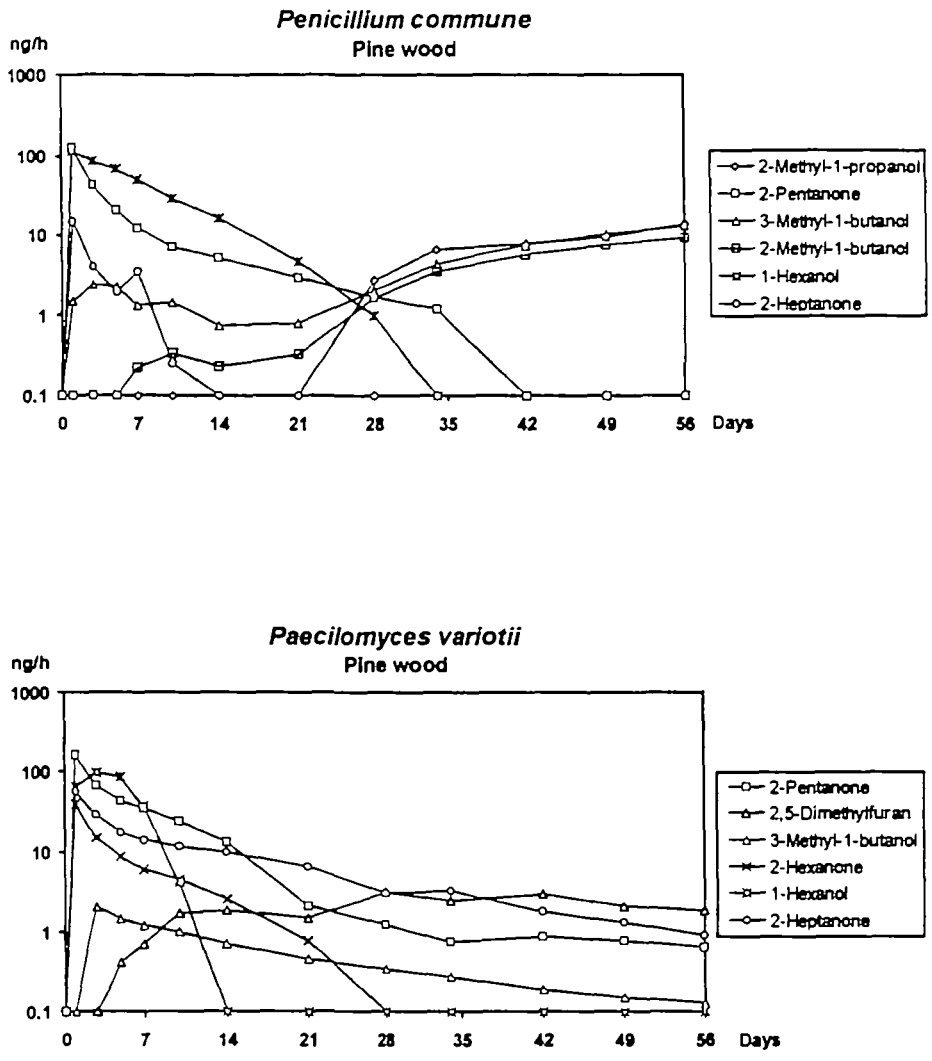

Fig. 3. Time dependence of metabolite production from *Penicillium commune* and *Paecilomyces variotii* on pine wood.

respectively. 1-Hexanol, 2-pentanone, 2-heptanone and 2,5-dimethylfuran, which were identified from the cultures on pine wood, were also produced by both species.

Some other volatiles often reported as being of microbial origin were found also in the study. This concerns, for example, 2-methyl-1-butanol, 1-octen-3-ol, 3-methylfuran, dimethyl disulphide, 2-hexanone, 3-octanone and geosmin (Kaminski *et al.*, 1974; Collins, 1976; Karahadian *et al.*, 1985a,b; Harris *et al.*, 1986; Wasowicz and Kaminski, 1988; Kinderlerer, 1989; Flannigan *et al.*, 1991; Börjesson, 1993; Sunesson *et al.*, 1995b). These substances and the more commonly produced substances mentioned above, would all be of interest as indicators of mould growth in buildings. It is, however, highly important to consider other possible sources for the presence of the compounds in indoor air. Compounds that could be expected to

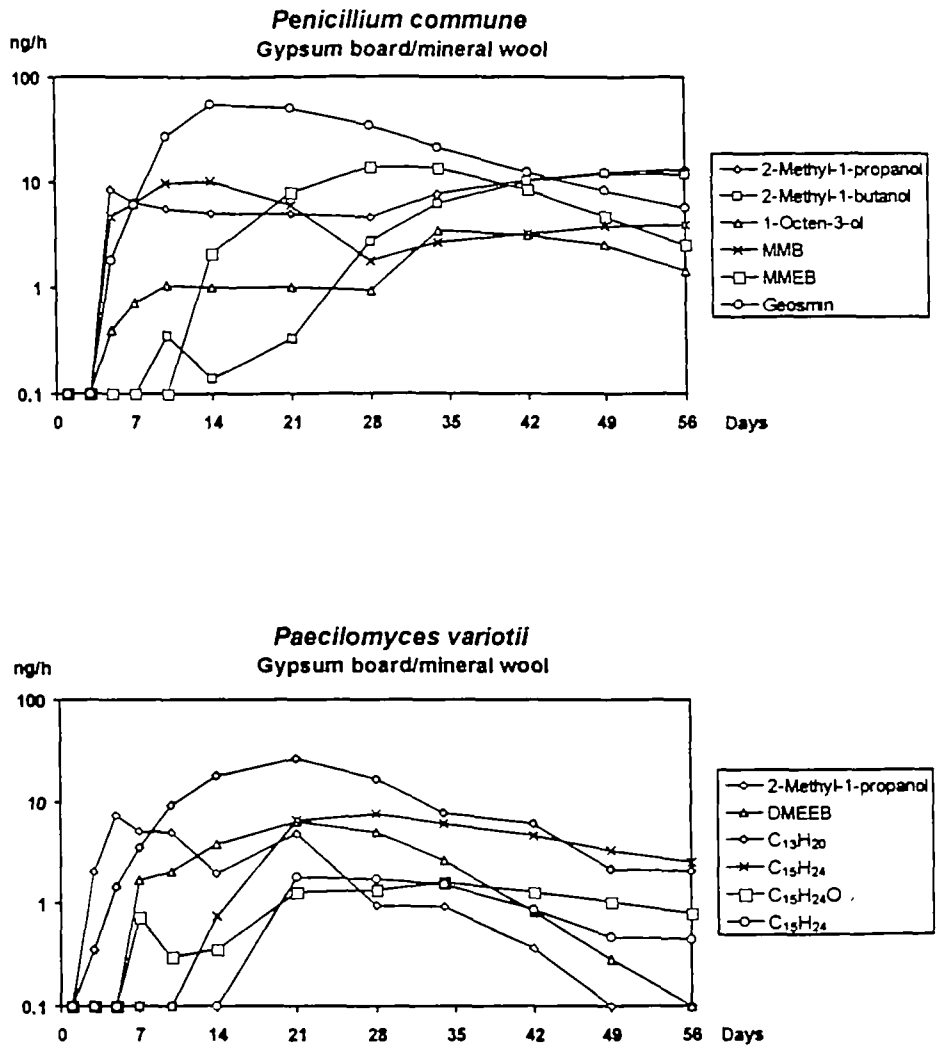

Fig. 4. Time dependence of metabolite production from *Penicillium commune* and *Paecilomyces variotii* on gypsum board–mineral wool. MMB, 1-methoxy-3-methylbutane; MMEB, 1-methoxy-4-(1-methylethyl)-benzene; and DMEEB, 1-(1,1-dimethylethyl)-4-ethylbenzene.

be found, for example, in paint or detergents would only have a limited potential as indicators of microbial growth in buildings. 2-Ethyl-1-hexanol is emitted from the gypsum board and is also produced by *Penicillium commune* on this medium. This alcohol is often mentioned as a sick building compound connected with the disintegration of water-damaged carpets (Andersson *et al.*, 1984), and would therefore not be possible to be linked with microbial activity. Dimethyl disulphide, which is produced by several fungal species and which can also be produced in considerable amounts by *Streptomyces albidoflavus*, is found in low amounts as an emission compound from the gypsum board itself. This makes it difficult to use this compound as a marker for microbial growth, without knowing what levels of

dimethyl disulphide that could be expected in indoor air when gypsum board is used in the walls.

Several of the metabolites produced on the building materials used in this investigation were also found when the species were cultured on malt extract agar (MEA) and dichloran glycerol agar (DG18) in a previous study (Sunesson *et al.*, 1995b), (these compounds are marked with an asterisk in Table 1). 3-Methyl-1-butanol and 2-methyl-1-butanol, the most commonly produced substances in this study, were also produced by both species on both MEA and DG18. Geosmin, which was the major metabolite from *Penicillium commune* on gypsum board–mineral wool, was produced in minor amounts by this species on both of the artificial media and could be considered as a commonly produced metabolite from this species, but no geosmin was detected from the pine wood cultures and it seems that pine wood lacks some necessary precursor for the production of this compound. Larsen and Frisvad (1994) did not detect any geosmin when culturing *Penicillium commune* on Czapek yeast autolysate agar and wallpaper paste agar, but found monoterpenes and 2-methylisoborneol, another earthy-smelling compound. *Paecilomyces variotii* produced mainly sesquiterpenes on gypsum board–mineral wool, which is in accordance with the findings obtained when cultivation was performed on both MEA and DG18 (Sunesson *et al.*, 1995b). It is, however, impossible to determine whether some structures are the same in the studies, because of the high similarities of mass spctra for terpenoid compounds and because of thermal breakdown of terpenoid structures, especially when sampling on Tenax GR (Sunesson *et al.*, 1995a), which was used as adsorbent in the study on MEA and DG18. α-Curcumene, which was identified from the species when cultivated on DG18, is not found in this study. 2-Hexanone and 2-heptanone, which were used as microbial markers by both Miller *et al.* (1988) and Ström *et al.* (1994), are found from the cultures on pine wood. None of these compounds were detected when culturing on the artificial media. Methyl ketones are generally known to be produced by fungi and are the main contributors to the odour of blue cheeses (Kinderlerer, 1989; Gallois and Langlois, 1990).

The terpenoid compounds produced by *Paecilomyces variotii* could not be identified because of lack of reference substances. Although the mass-spectrometric library suggests structures for these compounds as well, the mass spectra for terpenes with the same molecular composition are usually very similar. Most often, several different structures have spectra which would give about the same purity and fit values, and it would therefore be doubtful to suggest a probable identity without any other indications of the structure. These compounds are therefore only given with their probable molecular composition in Table 1. In addition, a large number of terpene structures emitted from pine wood could not be identified because of the reasons given above.

Some compounds in the media are consumed during the fungal growth. This concerns, for example, hexanal and furfural from pine wood (Fig. 1), and 3-methylbutanal and xylene from gypsum board–mineral wool (Fig. 2). This is in accordance with our other studies of metabolite production from micro-organisms (Sunesson *et al.*, 1995b).

As seen in Fig. 3, the amounts of 1-hexanol, 2-pentanone and 2-heptanone, which are produced by both species on pine wood, decline rapidly. When growth

occurs in a building, this could mean that these substances are found only in the beginning of microbial growth. These substances might therefore have a more limited potential as microbial markers than compounds produced in more constant amounts during the growth period studied.

The dependence on species and media complicates the selection of suitable compounds for use as chemical markers for excessive growth of micro-organisms in buildings. It is obviously not possible to use only a few metabolites as indicators of mould and/or bacterial growth and to expect that they will provide a reliable assessment of the quality of the indoor air. This applies particularly to situations when increased levels of the compounds chosen as microbial markers not are found. Selection of marker substances places a limitation on the possible number of substances for study, and it cannot be ruled out that some species can grow and produce metabolites that are not detected as microbial volatiles. It is, therefore, highly important to run indoor air samples in such a way that mass-spectrometric identification of all sampled compounds is possible and not, for example, by selected ion monitoring, to restrict the analysis to only a few tracer compounds.

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REFERENCES

Andersson, B., Andersson, K. and Nilsson, C.-A. (1984) Mass spectrometric identification of 2-ethylhexanol in indoor air: recovery studies by charcoal sampling and gas chromatographic analysis at the micrograms per cubic metre level. *J. Chromatogr.* **291**, 257–263.

Binnie, P. W. H. (1991) Biological pollutants in the indoor environment. In *Indoor Air Pollution* (Edited by Kay, J. G., Keller, G. E. and Miller, J. F.), Chap. 3, pp. 13–24. Lewis Publishers, Chelsea, Michigan.

Blomquist, G. and Andersson, B. (1994) Measurements of microorganisms in non-industrial indoor environments in northern Sweden. In *Health Implications of Fungi in Indoor Environments* (Edited by Samson, R. A., Flannigan, B., Flannigan, M. E., Verhoeff, A. P., Adan, O. C. G. and Hoekstra, E. S.), pp. 39–47. Elsevier, Amsterdam.

Börjesson, T. (1993) Volatile fungal metabolites as indicators of mould growth in stored cereals. Dissertation, report 55. Swedish University of Agricultural Sciences, Uppsala.

Börjesson, T., Stöllman, U. and Schnürer, J. (1993) Off-odorous compounds produced by molds on oatmeal agar: identification and relations to other growth characteristics. *J. Agric. Food Chem.* **41**, 2104–2111.

Collins, R. P. (1976) Terpenes and odoriferous materials from microorganisms. *Lloydia* **39**, 20–24.

De Bortoli, M., Knöppel, H., Pecchio, E., Schauenburg, H. and Vissers, H. (1992) Comparison of Tenax and Carbotrap for VOC sampling in indoor air. *Indoor Air* **2**, 216–224.

Eriksson, C. E., Kaminski, E., Adamek, P. and Börjesson, T. (1992) Volatile compounds and off-flavours produced by microorganisms in cereals. In *Off-flavours in Foods and Beverages* (Edited by Charalambous, G.), pp. 37–56. Elsevier, Amsterdam.

Flannigan, B., McCabe, E. M. and McGarry, F. (1991) Allergenic and toxigenic micro-organisms in houses. *J. appl. Bacteriol. Symp. Suppl.* **70**, 61S–73S.

Gallois, A. and Langlois, D. (1990) New results in the volatile odorous compounds of French cheeses. *Lait* **70**, 89–106.

Hansson, L. and Carlson, R. (1990) Synthesis of (±)-geosmin. Part 2. A one-pot four-step conversion of 1,4a-dimethyl-1α,8aα-epoxyperhydronaphthalen-2-one into (±)-geosmin. *Acta Chem. Scand.* **44**, 1042–1045.

Hansson, L., Carlson, R. and Sjöberg, A.-L. (1990) Synthesis of (±)-geosmin. Part 1. On the synthesis and epoxidation of 1,4a-dimethyl-4,4a,5,6,7,8-hexahydronaphthalen-2(3H)-one. *Acta Chem. Scand.* **44**, 1036–1041.

Harris, N. D., Karahadian, C. and Lindsay, R. C. (1986) Musty aroma compounds produced by selected molds and actinomycetes on agar and whole wheat bread. *J. Food Prot.* **49**, 964–970.

Imamura, H. (1989) Contribution of extractives to wood properties. In *Natural Products of Woody Plants II* (Edited by Rowe, J. W.), pp. 843–860. Springer, Berlin.

Kaminski, E., Stawicki, S. and Wasowicz, E. (1974) Volatile flavour compounds produced by mold of *Aspergillus, Penicillium* and *Fungi imperfecti*. *Appl. Microbiol.* **27**, 1001–1004.

Karahadian, C., Josephson, D. B. and Lindsay, R. C. (1985a) Contribution of *Penicillium* sp. to the flavours of Brie and Camembert cheese. *J. Dairy Sci.* **68**, 1865–1877.

Karahadian, C., Josephson, D. B. and Lindsay, R. C. (1985b) Volatile compounds from *Penicillium* sp. contributing musty-earthy notes to Brie and Camembert cheese. *J. Agric. Food Chem.* **33**, 339–343.

Kikuchi, T., Shigetoshi, K., Suehara, H., Nishi, A. and Tsubaki, K. (1981) Odorous metabolites of a fungus, *Chaetomium globosum* Kinze ex Fr. Identification of geosmin, a musty-smelling compound. *Chem. Pharm. Bull.* **29**, 1782–1784.

Kinderlerer, J. L. (1989) Volatile metabolites of filamentous fungi and their role in food flavour. *J. appl. Bacteriol. Symp. Suppl.* **67**, 133S–144S.

Koskinen, O., Husman, T., Hyvärinen, A., Reponen, T., Ruuskanen, J. and Nevalainen. A. (1994) Respiratory symptoms and infections in mouldy dwellings and day-care centres. In *Health Implications of Fungi in Indoor Environments* (Edited by Samson, R. A., Flannigan, B., Flannigan, M. E., Verhoeff, A. P., Adan, O. C. G. and Hoekstra, E. S.), pp. 211–214. Elsevier, Amsterdam.

Larsen, T. O. and Frisvad, J. C. (1994) A simple method for collection of volatile metabolites from fungi based on diffusive sampling from Petri dishes. *J. Microbiol. Meth.* **19**, 297–305.

McJilton, C. E., Reynolds, S. J., Streifel, A. J. and Pearson, R. L. (1990) Bacteria and indoor odor problems—three case studies. *Am. ind. Hyg. Ass. J.* **51**, 545–549.

Miller, J. D., Laflamme, A. M., Sobol, Y., Lafontaine, P and Greenhalgh, R. (1988) Fungi and fungal products in some Canadian houses. *Int. Biodeterior.* **24**, 103–120.

Morey, P. R., Hodgson, M. J., Sorenson, W. G., Kullman, G. J., Rhodes, W. W. and Visvesvara, G. S. (1984) Environmental studies in moldy office buildings: biological agents, sources and preventive measures. *Ann. Am. Conf. Gov. ind. Hyg.* **10**, 21–35.

Nevalainen, A., Pasanen, A.-L., Niininen, M., Reponen, T., Kalliokoski, P. and Jantunen, M. J. (1991) The indoor air quality in Finnish homes with mold problems. *Environ. Int.* **17**, 145–162.

Norrman, J. (1971a) A gas chromatographic investigation of the influence of different carbon sources on the production of volatile compounds by *Dipodascus aggregatus*. *Archs Microbiol.* **75**, 145–162.

Norrman, J. (1971b) The influence of different nitrogen sources on the production of volatile compounds by *Dipodascus aggregatus*. *Archs Microbiol.* **80**, 338–350.

Platt, S. D., Martin, C. J., Hunt, S. M. and Lewis, C. W. (1989) Damp housing, mould growth, and symptomatic health state. *Br. Med. J.* **298**, 1673–1678.

Reynolds, S. J., Streifel, A. J. and McJilton, C. E. (1990) Elevated airborne concentrations of fungi in residential and office environments. *Am. ind. Hyg. Ass. J.* **51**, 601–604.

Samson, R. A., Flannigan, B., Flannigan, M. E., Verhoeff, A. P., Adan, O. C. G. and Hoekstra, E. S. (Editors) (1994) *Health Implications of Fungi in Indoor Environments*. Elsevier, Amsterdam.

Sprecher, E. and Hanssen, H.-P. (1982) Influence of strain specificity and culture conditions on terpene production by fungi. *Planta Medica* **44**, 41–43.

Strachan, D. P., Flannigan, B., McCabe, E. M. and McGarry, F. (1990) Quantitification of airborne molds in the homes of children with and without wheeze. *Thorax* **45**, 382–387.

Strõm, G., West, J., Wessén, B. and Palmgren, U. (1994) Quantitative analysis of microbial volatiles in damp Swedish houses. In *Health Implications of Fungi in Indoor Environments* (Edited by Samson, R. A., Flannigan, B., Flannigan, M. E., Verhoeff, A. P., Adan, O. C. G. and Hoekstra, E. S.), pp. 291–305. Elsevier, Amsterdam.

Sunesson, A.-L., Nilsson, C.-A. and Andersson, B. (1995a) Evaluation of adsorbents for sampling and analysis of microbial volatiles using thermal desorption-gas chromatography. *J. Chromatogr.* **A699**, 203–214.

Sunesson, A.-L., Vaes, W. H. J., Nilsson, C.-A., Blomquist, G., Andersson, B. and Carlson, R. (1995b) Identification of volatile metabolites from five fungal species cultivated on two media. *Appl. Environ. Microbiol.* **61**, 2911–2918.

von Schantz, M. and Hiltunen, R. (1983) Coniferae (Nadelbäume). *Commentationes Physico-Math* **57**. The Finnish Society of Science and Letters, Helsink. (In German.)

Wasowicz, E. and Kaminski, E. (1988) Volatile compounds of sound and musty wheat grains. *Chem. Mikrobiol. Technol. Lebensm.* **11**, 161–168.