APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1995, p. 2911–2918 Vol. 61, No. 8
0099-2240/95/$04.00+0

Identification of Volatile Metabolites from Five Fungal Species Cultivated on Two Media

ANNA-LENA SUNESSON,[1] WOUTER H. J. VAES,[1]† CARL-AXEL NILSSON,[1]*
GÖRAN BLOMQUIST,[1] BARBRO ANDERSSON,[1] AND ROLF CARLSON[2]

Analytical Chemistry Division, National Institute of Occupational Health, S-907 13 Umeå, Sweden,[1]
and Section for Chemistry, Institute for Mathematics and Physical Sciences,
University of Tromsö, N-9037 Tromsö, Norway[2]

Received 21 February 1995/Accepted 19 May 1995

Five fungal species, *Aspergillus versicolor*, *Penicillium commune*, *Cladosporium cladosporioides*, *Paecilomyces variotii*, and *Phialophora fastigiata*, were cultivated on two media, malt extract agar and dichloran glycerol agar. Culture flasks provided with inlet and outlet tubes were used and purified, and humidified air was constantly led through the flasks. Air samples from the cultures were sorbed on Tenax GR and analyzed by thermal desorption-gas chromatography. The produced volatile metabolites were analyzed by mass spectrometry. Various hydrocarbons, alcohols, ketones, ethers, esters, sulfur-containing compounds, and terpenes were identified. The most commonly produced substances were 2-methyl-1-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 3-methylfuran, and dimethyl disulfide. The production was highly dependent on both medium and species.

During recent years, an increasing number of people have experienced negative health effects because of their indoor climate. This is usually referred to as the sick building syndrome and is characterized by an increased prevalence of certain nonspecific symptoms in more than 20% of the workforce (21). The most common symptoms are irritation of the eyes and the mucous membranes of the nose and throat, lethargy, and headache. Several scientific disciplines have tried to identify the causes of these symptoms. Combustion by-products, tobacco smoke, asbestos, radon, formaldehyde, growth of microorganisms, and volatile organic compounds are some of the explanations that have been suggested (31).

It is not likely that there is only one explanation for the negative health effects. A combination of several factors or various sources in different buildings is more probably the case. When excessive growth of microorganisms, especially fungi, is suspected to be the cause of poor health, the traditional approach is to measure airborne spore levels. Different investigations have, however, shown that there is often no significant difference in airborne spore levels in affected and unaffected buildings (1, 8, 24, 33). According to Nevalainen et al. (24), airborne spore counts may be low even in rooms where fungal growth is clearly visible, because fungi do not sporulate continuously (15, 28). Growth of microorganisms in buildings may also occur inside floors, walls, etc. The fungal spores are too large to diffuse through such materials. In contrast, the volatile metabolites produced by the fungi can diffuse through substrates such as insulation material and wallpaper (34). Some molds that are able to grow in buildings are known to produce highly toxic metabolites, such as the trichothecenes (7) produced by *Stachybotrys chartarum* (formerly *Stachybotrys atra*). Other microbially produced volatiles exhibit an unpleasant odor (5, 6, 9, 13, 16–20, 22, 27, 38), but even some of the nonpungent compounds must be considered possible health hazards. The levels of these substances might be low, but long-term exposure to combinations of several substances present at low concentrations might be harmful.

The hypothesis that microbial volatiles are one cause of sick building syndrome can be supported only if the identities of these compounds are known. Identification would also facilitate study of the health effects of the different metabolites on humans. It would also be important to study the connection between effects on humans and levels of microbial volatiles in affected buildings before using the method routinely. Studies on fungal proliferation in stored cereal have shown that fungal metabolites can be used as indicators of mold growth (2–5). Börjesson et al. (2–5) studied the growth of *Fusarium culmorum* and several *Penicillium* and *Aspergillus* species on cereal or cereal-based agar media. Differences in the production of volatile metabolites were reported to depend more on the fungal species than on the grain type. 3-Methylfuran was produced by the majority of the studied species and was suggested as a marker substance for mold growth in cereals. By analogy, commonly produced fungal metabolites might serve as possible indicators of mold growth in buildings.

Besides being dependent on fungal species, the production of metabolites is also influenced by media. Norrman (25, 26) studied the effects of carbon and nitrogen sources on the production of volatile compounds by *Dipodascus aggregatus*. Sprecher and Hanssen (33) concluded that terpene production by *Ceratocystis* species was highly influenced by the carbon source.

In this study, five fungal species, *Aspergillus versicolor*, *Penicillium commune*, *Cladosporium cladosporioides*, *Paecilomyces variotii*, and *Phialophora fastigiata*, all known to be found in affected buildings (29), were cultivated on two media, malt extract agar (MEA) and dichloran glycerol agar (DG18). These media have differing nutrient compositions and water contents. The objectives of the investigation were to study which volatile metabolites were produced, how the production of volatiles differed between species, and how it was affected by cultivation media. If some compounds were found to be com-

* Corresponding author. Mailing address: Analytical Chemistry Division, National Institute of Occupational Health, P.O. Box 7654, S-907 13 Umeå, Sweden. Phone: 46-90-16 50 88. Fax: 46-90-16 50 27. Electronic mail address: callnils@nioh.se.

† Present address: Environmental Toxicology Section, Research Institute of Toxicology, NL-3508 TD Utrecht, The Netherlands.

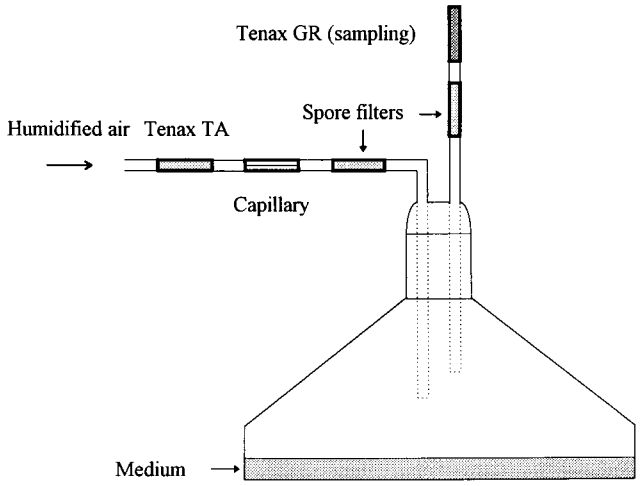


FIG. 1. Equipment used for cultivation and sampling.

monly produced by several species and on both media, they could be interesting as potential markers of mold growth in buildings.

MATERIALS AND METHODS

Fungal cultures. The following strains were used: *Aspergillus versicolor* (Vuill.) Tiraboschi, *Penicillium commune* Pitt, *Cladosporium cladosporioides* Ellis, *Paecilomyces variotii* Bain, and *Phialophora fastigiata* (Lagerb. & Melin) Conant. The strains were obtained from Pegasus Lab AB, Uppsala, Sweden.

Cultivation media. MEA was prepared by adding 20 g of malt extract (Difco Laboratories) and 15 g of agar (bacteriological agar no. 1; Oxoid) to 1,000 ml of demineralized water. After being stirred at 85°C for 1.5 h, the medium was poured into culture flasks and autoclaved at 125°C for 15 min. Each 2-liter flask contained 330 ml of medium. DG18 was prepared by adding 15.8 g of dichloran glycerol agar base (DG18; Oxoid) to 500 ml of demineralized water. The mixture was stirred at 85°C for 1 h, and 80 ml of glycerol (>99.5%; Prolabo) was added. The medium was poured into culture flasks and autoclaved as above.

Preparation of spore suspensions and inoculation. The fungi were cultivated on MEA and DG18 on petri dishes for 2 weeks. A 5-ml portion of sterile water with 0.05% Tween 80 (Kebo Lab AB) was added to the culture, the mixture was smoothly shaken, and the suspension was isolated. The spore concentration was determined by counting in a Bürker counting chamber (0.100 mm by 0.04/0.0025 mm^2) and adjusted with water to 1.0×10^3 spores per ml. To each culture flask was added 10 ml of the spore suspension. The flasks were gently rotated to create a homogeneous suspension layer on the agar surface, and the excess suspension (about 6 ml) was poured out. The medium blanks were treated as above but with sterile 0.05% Tween 80 instead of the spore suspension.

Cultivation and sampling equipment. The cultivation was performed in 2-liter culture flasks made of glass, with glass adapters for air inlet and outlet (Fig. 1). Air, cleaned through oil and particle filters (F50-004-A000 and F13-000-M300; Norgren Martonair AB), was humidified by being led through water-filled dispersion bottles and was then led via Tenax TA tubes (SKC) and sterile silanized glass wool (Chrompack) into the flasks at 30 ml/min. The pressure at the inlet of the cultivation system was maintained at 0.6×10^5 Pa with a pressure regulator (Norgren R38-200-RNCA), and the flow through the culture flasks was adjusted by using glass capillaries (50 mm by 0.15 mm [inner diameter]; Wernerglas, Stockholm, Sweden). The flow was measured with a flow meter (ROTA L0.025/1.9). At the outlet, the air passed through glass wool before being sampled on Tenax GR. The glass wool served as spore filters to avoid introducing unwanted microorganisms into the flasks and to prevent spores from the cultures from being trapped on the sampling tubes. Connections were made of Teflon tubing (HABIA, Stockholm, Sweden), and the connectors to the adapters were made of brass with rubber O-ring seals. For each medium and mold, two flasks were inoculated and one flask served as a medium blank.

Sampling of volatile metabolites. The volatile metabolites released by the cultures were sampled by connecting adsorbent tubes at the outlet of the culture flasks. The adsorbent tubes (Chrompack; length, 160 mm; outer diameter, 6 mm; inner diameter, 3 mm) were made of glass. They were all packed with 90 mg of Tenax GR (60-80 mesh; Buchem B.V.), with silanized glass wool (Chrompack) at both ends. The tubes were conditioned overnight at 300°C with a helium (AGA) flow of 30 to 40 ml/min before use. Samples were taken every third or fourth day from day 3 to day 12 or 15; additional samples were taken on later occasions (up to day 28) for some species and media. The sampling time varied between 5 and 140 min, depending on the rate of metabolite production. The flow was 30 ± 2 ml/min during all the cultivation and also during sampling. Three samples were taken from each flask on every sampling occasion. Two samples were run on a gas chromatograph with a flame ionization detector, and the third was used for mass-spectrometric identification.

Analysis and identification. The volatiles trapped on the adsorbent were thermally desorbed with a commercial thermal desorption injector (Chrompack 16400 purge and trap injector, modified for thermal desorption injection according to Chrompack modification manual M-16420-85-2).

The injection was run under optimized conditions (36). Desorption was performed at 220°C for 10 min. The desorption flow was 30 ml/min, and the pressure on the injector and column was 3×10^5 Pa. Helium was used as the carrier gas. The cold trap (Chrompack CP-TM-Sil-8CB, d_f (film thickness) of 5.0 μm; inner diameter, 0.5 mm) was first cooled and then maintained at −125°C during desorption, and the sample was injected onto the column by heating the trap to 130°C for 3 min. The gas-chromatographic measurements were carried out on an HP 5890 gas chromatograph with a fused-silica column (HP Ultra 2; 50 m by 0.2 mm [inner diameter], coated with cross-linked 5% phenylmethylsilicone, d_f 0.33 μm) and a flame ionization detector. An HP 3392A integrator was used as a recorder. The temperature of the detector and injection block was 250°C. The gas-chromatographic temperature program was 30°C for 5 min followed by a temperature rise of 10°C/min up to 220°C. This final temperature was maintained for 10 min.

The metabolites produced were quantified as equivalents of toluene. Toluene standards were dynamically generated as described by Sunesson et al. (35). Flame ionization detection was used for the quantification. Because of the low sensitivity of the detector toward sulfur-containing compounds, dimethyl disulfide was generated as the quantification standard for the sulfur-containing substances found (dimethyl sulfide and dimethyl disulfide).

For identification, a mass spectrometer (Finnigan INCOS 500) was used as the detector after the gas-chromatographic separation. The temperature of the ion source was 150°C. The emission current was 750 μA at an electron energy of 70 eV. The instrument scanned at *m/z* 12 to 15, 21 to 27, and 34 to 300 in 0.6 s during the first 15 min of the analysis and at *m/z* 36 to 400 in 0.6 s for the remaining 19 min. A reference standard library (NIST), containing 42,220 spectra, was used to aid the identification of the volatiles. When available, reference compounds were used to confirm the identities of the metabolites.

Reference compounds. The reference compounds used for identification were as follows. **(i) Hydrocarbons.** The hydrocarbons included *n*-heptane (PolyScience Corp., >99.5%), *n*-octane (Merck, >99.5%), 1-octene (Aldrich, 98%), toluene (Merck, >99.5%), 1,3,5-trimethylbenzene (PolyScience Corp., >99.5%), *m*-xylene (Aldrich, 98%), *o*-xylene (Aldrich, 97%), and *p*-xylene (Aldrich, 99%).

(ii) Alcohols. The alcohols included *o*-cresol (Merck, 99%), *m*-cresol (Kebo, >99%), *p*-cresol (BDH laboratory reagent, 98%), 2-ethylhexanol (Fluka, >97%), 2-methyl-1-butanol (Aldrich, 99%), 3-methyl-1-butanol (Baker, 98%), 3-methyl-3-buten-1-ol (Aldrich, 97%), 2-methyl-1-propanol (Sigma-Aldrich, 99.5%), 3-octanol (Aldrich, 99%), 1-octene-3-ol (Aldrich, 98%), and 2-propanol (Merck, >99.5%).

(iii) Aldehydes. The aldehydes included benzaldehyde (Kebo, >99%), decanal (Aldrich, 98%), furfural (Merck, >99.5%), 3-methylbutanal (Kebo, >98%), 2-methylpropanal (Kebo, >98%), nonanal (Aldrich, 95%), and octanal (Aldrich, 99%).

(iv) Ketones. The ketones included acetone (Merck, >99.5%), 2-butanone (Merck, >99%), cyclopentanone (Aldrich, 99%), 3-methyl-2-butanone (Tokyo Kasei Organic Chemicals, 99%), 3-methyl-2-pentanone (Aldrich, 99%), 3-octanone (Aldrich, 99%), and 3-pentanone (Aldrich, 97%).

(v) Ethers. The ethers included anisole (Riedel-De Haën, >95%), 2,5-dimethylfuran (synthesized as follows: 2,4-hexadione was dissolved in paraffin oil, in which one equivalent of zinc chloride [anhydrous] was suspended; the mixture was heated to 120°C, and the furan and water formed were distilled off; the 2,5-dimethylfuran layer was separated and dried over $MgSO_4$; the purity was >98% as determined by gas chromatography), 3-methylanisole (Aldrich, 99%), furan (Aldrich, 99%), 3-methoxyanisole (Tokyo Kasei Organic Chemicals, >99%), and 3-methylfuran (Tokyo Kasei Organic Chemicals, 99%).

(vi) Esters. The esters included ethyl acetate (Merck, p.a.), ethyl butanoate (Aldrich, 99%), ethyl propanoate (Aldrich, 99%), methyl acetate (Aldrich, 99%), methyl benzoate (Tokyo Kasei Organic Chemicals, >99%), 3-methylbutyl acetate (Kebo, purum), propyl acetate (Theodor Schuchardt, für Chromatographie), and 2-methylpropyl acetate (Aldrich, 99%).

(vii) Sulfur compounds. The sulfur compound used was dimethyl disulfide (Janssen, p.a.).

(viii) Terpenes and terpene derivatives. The terpenes included camphene (Aldrich, >90%), β-caryophyllene (Tokyo Kasei Organic Chemicals, >80%), α-curcumene [1-(1,5-dimethyl-4-hexenyl)-4-methylbenzene, synthesized by the method of Hall et al. (10), >95%], geosmin (synthesized by the method of Hansson et al. [11, 12], >98%), R-(+)-limonene (Fluka, 98%), S-(−)-limonene (Fluka, 97%), α-pinene (Aldrich, 98%), and β-pinene (Aldrich, 98%).

(ix) Others. Acetonitrile (Riedel-de Haën, 99.8%) was also used.

Toluene (Merck, >99.5%) was used as standard for quantification. The dimethyl disulfide used as quantification standard was the same as above.

TABLE 1. Production of volatiles from *Aspergillus versicolor*, *Penicillium commune*, *Cladosporium cladosporioides*, *Paecilomyces variotii*, and *Phialophora fastigata* on DG18 and MEA

No.	Compound	Production by[a]:					Purity/fit or reference compound[b]
		A. versicolor	*P. commune*	*C. cladosporioides*	*P. variotii*	*P. fastigiata*	
	Hydrocarbons						
1	1,3-Pentadiene	X					946/946
2	2-Methyl-1,3-pentadiene				M		965/987
3	Heptane		X				R
4	Octane				X, M		R
5	1-Octene	X−		X−			R
6	Xylene				X, M		R
7	Trimethylbenzene				X, M		R
8	2,3,5-Trimethyl-1,3-hexadiene	X					937/954
	Alcohols						
9	2-Propanol				M		R
10	2-Methyl-1-propanol	X−	X+		X++[c], M		R
11	2-Methyl-1-butanol	X	X+, M+		X, M		R
12	3-Methyl-1-butanol	X	X, M+		X++[c], M	X	R
13	3-Methyl-3-buten-1-ol	X−					R
14	1-Octen-3-ol	X				X	R
15	3-Octanol	X−					R
16	2-Ethylhexanol	M					R
17	Cresol		M				R
	Ketones						
18	Acetone		X++			X	R
19	2-Butanone		X		X	X	R
20	Cyclopentanone		X			X	R
21	3-Methyl-2-butanone	X					R
22	3-Pentanone			X			R
23	3-Methyl-2-pentanone	X−					R
24	3-Octanone	X				X−	R
25	4-Methyl-3-hexanone	X					813/826
26	5-Ethyl-4-methyl-3-heptanone	X					850/895
	Ethers						
27	Furan				X		R
28	3-Methylfuran	X−	X	X	X−, M−		R
29	2,5-Dimethylfuran		X		X−		R
30	2,4-Dimethylfuran				M		867/930
31	Anisole	X					R
32	3-Methylanisole		M+				R
33	3-Methoxyanisole	X+					R
	Esters						
34	1-Methylpropyl formate				X		872/893
35	2-Methylpropyl formate				X		726/795
36	Methyl acetate		M+		M−		R
37	Ethyl acetate		M++				R
38	Propyl acetate		M				R
39	2-Methylpropyl acetate		X, M+				R
40	3-Methylbutyl acetate		X, M+				R
41	Ethyl propanoate		M				R
42	Ethyl butanoate		M				R
43	Ethyl-2-methyl butanoate		M				817/824
44	Methyl-3-methyl butanoate					X−	875/877
45	Methyl benzoate					X	R
	Sulfur compounds						
46	Dimethyl sulfide		M++				960/970
47	Dimethyl disulfide	X	M			X−	R
48	Ethanthioic acid-*S*-(2-methyl)butylester		M+				745/953
	Terpenes and terpene derivatives						
49	α-Pinene		X				R
50	β-Pinene		X+				R
51	Camphene		X				R
52	Limonene		X+				R
53	Geosmin		X, M−				R
54	Caryophyllene					X+	R
55	Methyl-(1-methylethenyl)benzene		X				816/992
56	1,2,3,4-Tetramethyl-4-(1-methylethenyl)benzene				M		845/945
57	α-Curcumene (1-(1,5-dimethyl-4-hexenyl)-4-methylbenzene)		X		X		R
	Sesquiterpenes	X	X+	X	X++, M+	X+	

[a] X, DG18; M, MEA. The amounts of metabolites produced are indicated as toluene equivalents when determined by flame ionization detection: X++ and M++, metabolite production in amounts corresponding to >1,000 ng of toluene per h; X+ and M+, 100 to 1,000 ng/h; X and M, 5 to 100 ng/h; X− and M−, <5 ng/h.

[b] Substances identified with reference compounds are marked with R. For substances whose identities are indicated only by the mass spectrometric library, the reported purity and fit values are given.

[c] Production until day 8; no production at day 12.

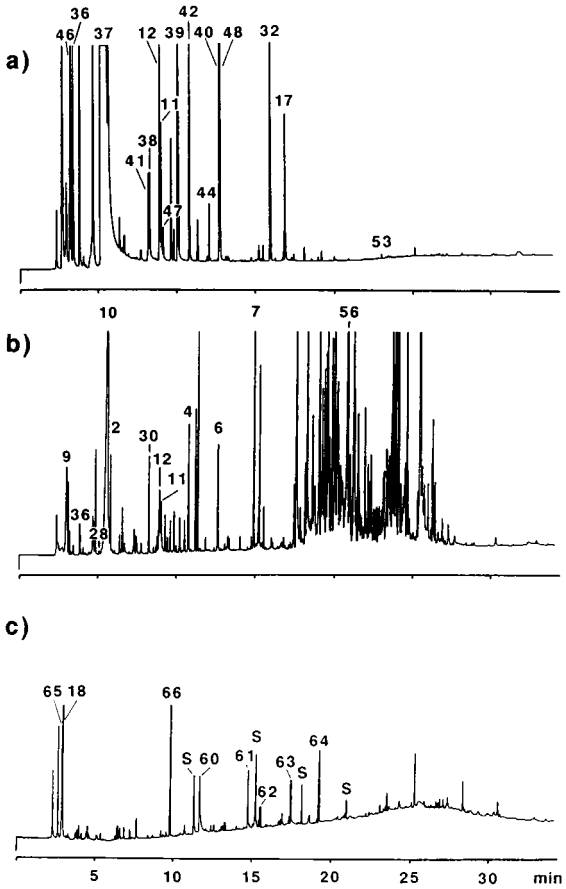


FIG. 2. Gas chromatograms of volatiles from *Penicillium commune* (after 16 days and 15 min of sampling) (a) and *Paecilomyces variotii* (after 9 days and 120 min of sampling) (b) on MEA. Also given is the MEA blank after 16 days and 15 min of sampling (c). The numbers identify the compounds as listed in Table 1 and 2. Compounds marked with S are silica-containing substances bleeding from the cold trap.

RESULTS

The volatiles produced by the fungi are summarized in Table 1. On MEA, both *Penicillium commune* and *Paecilomyces variotii* produced a complex pattern of metabolites (Fig. 2), while on DG18, all fungi produced several metabolites in different amounts (Fig. 3 and 4).

Penicillium commune was the only species that produced a series of esters on MEA, of which ethyl acetate was the dominant substance. Dimethyl sulfide, 2-methylpropyl acetate, and 3-methylanisole were the major products next to ethyl acetate. It is noteworthy that the strongly earth-smelling substance geosmin is produced by this species in small amounts. On DG18, the fungi produced both mono- and sesquiterpenes. Only some of the monoterpenes could be identified: α-pinene, β-pinene, camphene, and limonene. The only esters detected were 2-methylpropyl acetate and 3-methylbutyl acetate. Ethyl acetate, which was the dominant product of this species on MEA, was totally absent. Geosmin was also produced on this medium.

Paecilomyces variotii produced sesquiterpenes as main products on both media. The fungi also produced some aromatics like xylene and trimethylbenzene. 3-Methylfuran was found in small amounts on both media. On MEA, 2-methyl-1-butanol and 3-methyl-1-butanol were produced at the beginning of the cultivation period, but their levels declined rapidly. On DG18, 2-methyl-1-propanol and 3-methyl-1-butanol were produced in large amounts during the first week of growth, 2.5 and 5 μg/h, respectively, after 8 days, but at 12 days no production of those substances was detected.

Aspergillus versicolor showed very little production on MEA. The only metabolite that was produced in detectable amounts was 2-ethylhexanol. On DG18, the major constituents produced were 3-methoxyanisole, 1-octene-3-ol, and 3-octanone. Other alcohols and ketones were also detected. Dimethyl disulfide was produced in small amounts as the only sulfur-containing compound. Sesquiterpenes were detected as minor products.

Phialophora fastigiata did not produce anything in detectable amounts on MEA. On DG18, the fungi showed a dominating sesquiterpene region in the chromatograms. Only caryophyllene could be identified from this cluster. Methyl benzoate was identified as a considerable metabolite. Dimethyl disulfide was produced but only as a minor component.

Cladosporium cladosporioides was generally the least productive species. No metabolites were detected on MEA. On DG18, only three metabolites were identified: 1-octene, 3-methylfuran, and 3-pentanone. Sesquiterpenes were also detected in small amounts.

2-Methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol were the substances produced by most species and on both media. Only *Cladosporium cladosporioides* did not produce any of these alcohols. 3-Methylfuran is produced by four of the five species on DG18 and by *Paecilomyces variotii* on MEA as well, but only in small amounts. Dimethyl disulfide was identified from three species. Sesquiterpenes were produced by all species on at least one medium (preferably DG18).

Some of the substances identified in the media were consumed by the fungi during growth. This concerns mainly some aldehydes, like furfural and benzaldehyde, present in both media. Volatiles emitted from the media are presented in Table 2.

The time dependence of the production of metabolites is shown in Fig. 5 to 7. Values in parentheses are below the detection limit (0.4 ng/h). The volatiles shown in the figures were selected basically by three criteria: (i) a compound produced by more than one species; (ii) a major metabolite; or (iii) a compound with an unpleasant odor. The relative standard deviations of the amounts produced were generally below 20% ($n = 4$). Metabolites were usually detected after 3 to 6 days. Within the sampling period, most components were produced in rather constant amounts after the production had started or in slowly declining amounts after reaching a maximum. Some metabolites showed maximum amounts after 6 to 9 days of growth and thereafter exhibited a rapid decrease in production.

DISCUSSION

The production of volatiles proved to be dependent on both fungal species and media. A large number of metabolites could be identified, but many of them were produced by only one species and often on only one medium. A few metabolites could be considered commonly produced: 2-methyl-1-propanol, 2-methyl-1-butanol, and 3-methyl-1-butanol (which are often reported as fungal metabolites [2–4, 8, 22]), 3-methylfuran, and dimethyl disulfide were all produced by at least three species, and production occurred on both media. Being commonly produced, those substances would be of interest as indicators of mold growth in buildings, although it is possible that the production of volatiles under natural conditions in

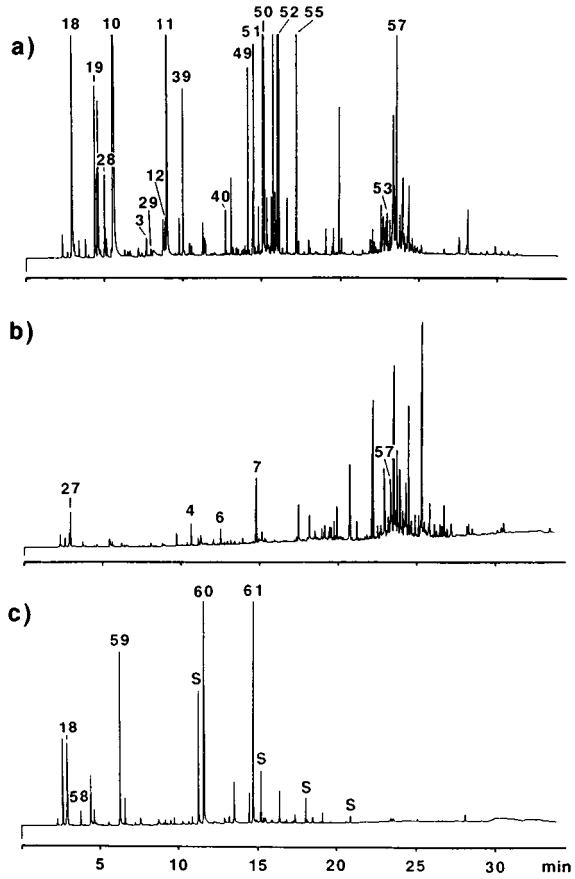


FIG. 3. Gas chromatograms of volatiles from *Penicillium commune* (after 12 days and 140 min of sampling) (a) and *Paecilomyces variotii* (after 12 days and 5 min of sampling) (b) on DG18. Also given is the DG18 blank after 12 days and 140 min of sampling (c). The numbers identify the compounds as listed in Table 1 and 2. Compounds marked with S are silica-containing substances bleeding from the cold trap.

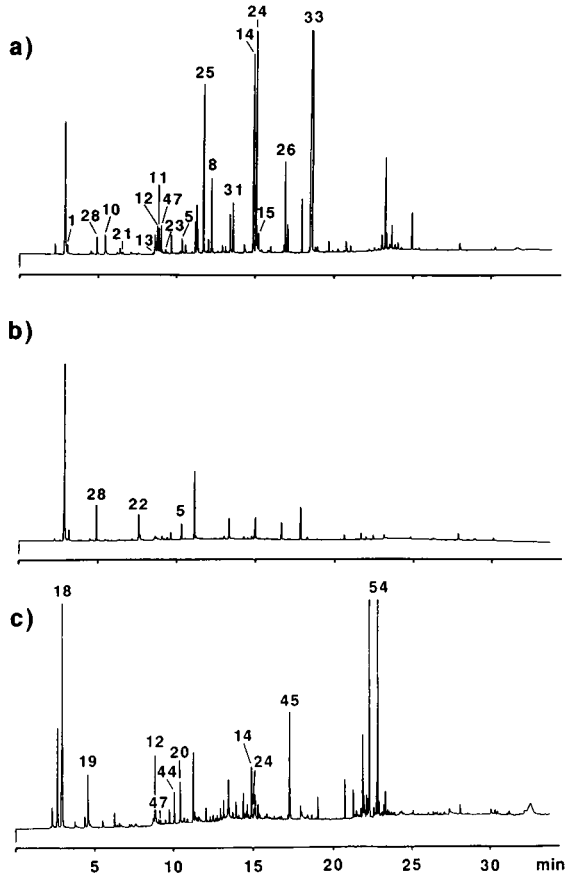


FIG. 4. Gas chromatograms of volatiles from *Aspergillus versicolor* (after 12 days and 140 min of sampling) (a), *Cladosporium cladosporioides* (after 12 days and 140 min of sampling) (b), and *Phialophora fastigiata* (after 12 days and 60 min of sampling) (c) on DG18. See Fig. 3c for the DG18 blank. The numbers identify the compounds as listed in Table 1.

buildings differs from the compounds produced on media such as the ones used in this study. 3-Methylfuran is also suggested by Börjesson et al. (4) as a marker substance for mold proliferation in stored cereals. Sesquiterpenes were produced by all fungi, but they would not be good as markers of fungal growth in indoor environments since terpenes could also originate from wood (14, 37) in the building material. Many of the volatile organic compounds produced by the fungi in this study could also have other sources explaining their presence in indoor air and would therefore not be useful as indicators of mold growth in buildings or could only be used with caution in combination with other indicator substances.

Some of the identified compounds have previously been reported as fungal metabolites. In addition to the substances presented above as the most frequently found in this study are included, for example 1-octen-3-ol, 3-octanol, 3-octanone, and geosmin (2–6, 9, 13, 16–20, 38). These compounds could also be interesting as markers of fungal growth in buildings. The production of the earthy-smelling compound geosmin from *Penicillium commune* was not previously known. Among the *Penicillium* species, geosmin has been previously reported as a metabolite from *P. expansum* (22), *P. citrinum* and *P. farinosum* (27), *P. vulpinum* and *P. aethopicum* (5), and *P. clavigerum* (20). Ethanol, which is a commonly produced microbial metabolite, was found in trace amounts (gas-chromatographic retention time 2.80 min) from some cultures (e.g., *Penicillium commune* on MEA). However, the low breakthrough volume of the adsorbent for such a low-boiling alcohol makes any quantification of ethanol impossible (35). In buildings, growth of microorganisms usually has been proceeding for some time before people experience problems. When choosing marker metabolites for fungal growth in buildings, it is therefore also important to consider the time dependence of the production. Metabolites produced in rather equal amounts during the period of growth would be preferable to metabolites produced, for example, only during the first days of growth.

Miller et al. (23) used 3-methyl-1-butanol, 2-hexanone, and 2-heptanone as markers for fungal growth in buildings in a study of 50 Canadian houses. They found an increased level of these compounds in problem buildings compared with reference houses. However, these higher concentrations correlated poorly with other measurements of fungal growth, like levels of CFU per cubic meter and ergosterol concentrations, as would be expected. Ström et al. (34) used 13 substances as indicators of growth of microfungi and bacteria in damp buildings. Among those substances were 3-methylfuran, 3-methyl-1-butanol, 1-octen-3-ol, 3-octanone, 3-octanol, and geosmin, which have also been suggested as possible markers in this study. Increased levels of the indicator substances were found in the problem buildings compared with reference buildings. Since the analysis was performed by mass spectrometry with selected

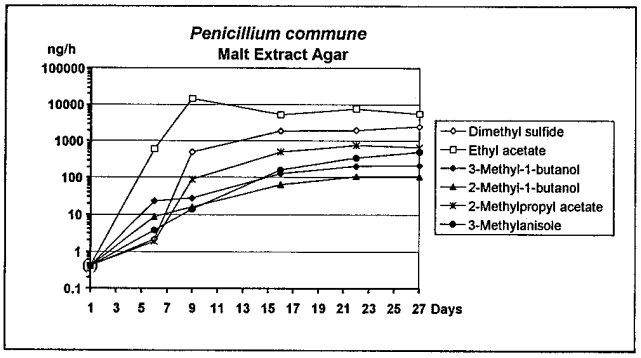
TABLE 2. Volatiles emitted from the cultivation media

No.[a]	Compound	Emission from:		Purity/fit or reference compound[b]
		MEA	DG18	
	Aldehydes			
58	2-Methylpropanal		X	R
59	3-Methylbutanal		X	R
60	Furfural	X	X	R
61	Benzaldehyde	X	X	R
62	Octanal	X		R
63	Nonanal	X		R
64	Decanal	X		R
	Others			
18	Acetone	X	X	R
65	Acetonitrile	X		R
66	Toluene	X		R

[a] Numbers are as in Table 1.
[b] The degree of identification (reference compound or mass spectrometric library only) is given as in Table 1.

ion monitoring, no additional information concerning other sampled substances is available. Both that investigation and the study by Miller et al. indicate that microbially produced volatile metabolites could be a useful tool in detecting problem buildings. However, in both studies the criteria for selection of buildings to investigate are not well specified.

The media chosen for cultivation in this study, MEA and DG18, are the two media principally recommended for cultivation of mold species found in indoor environments (29). They are both considered nutritionally rich media, but they differ in composition. Malt extract is a dried and powdered infusion of malt, while the dichloran glycerol agar base additionally consists of peptone, glucose, potassium dihydrogen phosphate, magnesium sulfate, and dichloran. A determining factor distinguishing between the media, however, is the water content. DG18 contains glycerol instead of some of the water, and this medium generally favors growth of xerophilic molds.



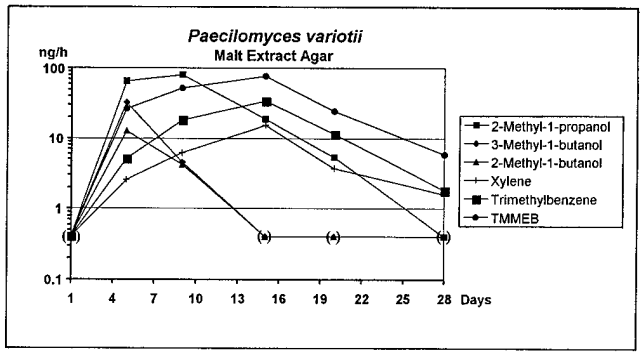
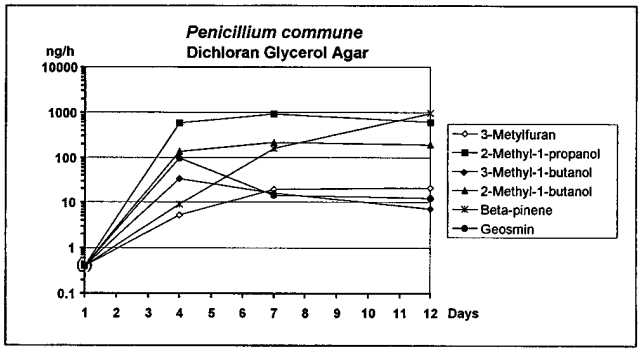


FIG. 5. Time dependence of metabolite production from *Penicillium commune* and *Paecilomyces variotii* on MEA. TMMEB, 1,2,3,4-tetramethyl-4-(1-methylethenyl)benzene.



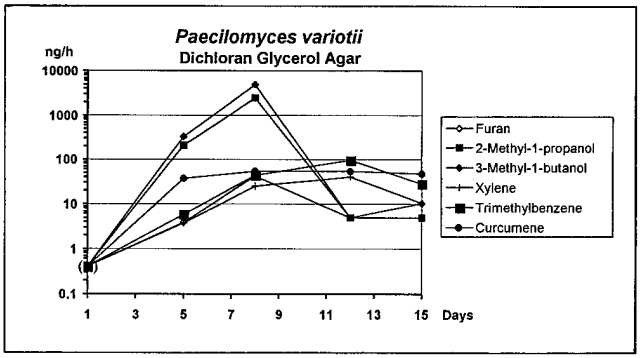


FIG. 6. Time dependence of metabolite production from *Penicillium commune* and *Paecilomyces variotii* on DG18.

In a parallel study, Tenax GR proved to have a catalytic effect on the breakdown of some terpeneoid structures during thermal desorption (35). Therefore, and because mass spectra of terpenes are very similar for different substances of the same formula, the majority of the terpeneoid compounds have not been possible to identify. It cannot be excluded that some of the identified monoterpenes, or the sesquiterpeneoid structures suggested, are not really metabolites produced by the fungi but breakdown products of other terpenes produced by the fungi. Caryophyllene, which is reported as a metabolite of *Phialophora fastigiata*, breaks down into a cluster of peaks during analysis, but since the cluster produced by the pure reference compound coincides with the cluster from the fungi in retention times and mass spectra, its identity is considered probable. The mass spectrum of geosmin is very characteristic, and the production of this strongly smelling compound from *Penicillium commune* is certain.

During the mass-spectrometric analysis, some scan intervals were omitted to avoid disturbances from air and water. This gives lower purity and fit values for some substances given in Table 1 than what would have been the case if the whole *m/z* interval had been scanned. This concerns, for example, ethyl-2-methyl butanoate and methyl-3-methyl butanoate, which both have purity and fit values below 900, although the spectra correspond very well to the library spectra within the scanned regions. Both compounds have reverse fit values over 970 (30). For the substances identified with reference compounds, both

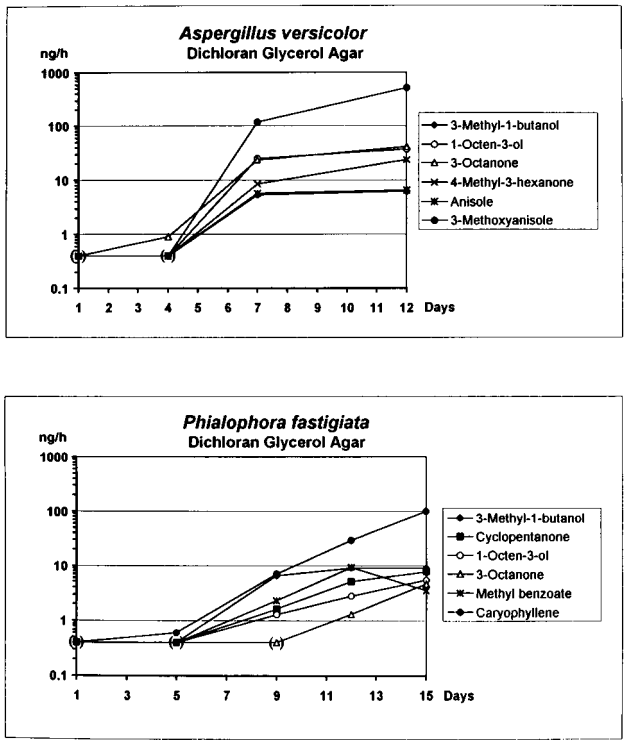


FIG. 7. Time dependence of metabolite production from *Aspergillus versicolor* and *Phialophora fastigiata* on DG18.

the spectra and the gas-chromatographic retention times agree. Reported in Table 1 are only substances that could be identified with reference compounds or substances whose spectra coincided well with the library spectra and that had gas-chromatographic retention times that were probable for the suggested compounds.

In conclusion, a large number of fungal metabolites were identified in this study. Some metabolites were produced by several species and on both media and would be of interest as indicators of mold growth in indoor environments.

ACKNOWLEDGMENTS

Generous funding provided by the Center for Environmental Research (CMF) for this project is gratefully acknowledged.

We thank Mårten Ellburg for synthesizing α-curcumene and Sven-Olof Westermark for valuable laboratory assistance in cultivation of the fungi.

REFERENCES

1. **Blomquist, G., and B. Andersson.** 1994. Measurements of microorganisms in non-industrial indoor environments in northern Sweden, p. 39–47. *In* R. A. Samson, B. Flannigan, M. E. Flannigan, A. P. Verhoeff, O. C. G. Adan, and E. S. Hoekstra (ed.), Health implication of fungi in indoor air environment. Elsevier/North-Holland Publishing Co., Amsterdam.
2. **Börjesson, T., U. Stöllman, P. Adamek, and A. Kaspersson.** 1989. Analysis of volatile compounds for detection of molds in stored cereals. Cereal Chem. **66:**300–304.
3. **Börjesson, T., U. Stöllman, and J. Schnürer.** 1990. Volatile metabolites and other indicators of *Penicillium aurantiogriseum* growth on different substrates. Appl. Environ. Microbiol. **56:**3705–3710.
4. **Börjesson, T., U. Stöllman, and J. Schnürer.** 1992. Volatile metabolites produced by six fungal species compared with other indicators of fungal growth on stored cereals. Appl. Environ. Microbiol. **58:**2599–2605.
5. **Börjesson, T., U. Stöllman, and J. Schnürer.** 1993. Off-odorous compounds produced by molds on oatmeal agar: identification and relations to other growth characteristics. J. Agric. Food Chem. **41:**2104–2111.
6. **Collins, R. P.** 1976. Terpenes and odoriferous materials from microorganisms. Lloydia **39:**20–24.
7. **Croft, W. A., B. B. Jarvis, and C. S. Yatawara.** 1986. Airborne outbreak of trichothecene toxicosis. Atmos. Environ. **20:**549–552.
8. **Flannigan, B., E. M. McCabe, and F. McGarry.** 1991. Allergenic and toxigenic micro-organisms in houses. J. Appl. Bacteriol. Symp. Suppl. **70:**61S–73S.
9. **Halim, A. F., J. A. Narciso, and R. P. Collins.** 1977. Odorous constituents of *Penicillium decumbens* (Thom). Egypt. J. Pharm. Sci. **18:**253–259.
10. **Hall, S. S., F. J. McEnroe, and H.-J. Shue.** 1975. A convenient synthesis of the sesquiterpene (±)-α-curcumene. VI. Application of alkylation-reduction to the total synthesis of terpenes. J. Org. Chem. **40:**3306–3307.
11. **Hansson, L., and R. Carlson.** 1990. Synthesis of (±)-geosmin. Part 2. A one-pot four-step conversion of 1,4a-dimethyl-1α,8aα-epoxyperhydronaphtalen-2-one into (±)-geosmin. Acta Chem. Scand. **44:**1042–1045.
12. **Hansson, L., R. Carlson, and A.-L. Sjöberg.** 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.
13. **Harris, N. D., C. Karahadian, and R. C. Lindsay.** 1986. Musty aroma compounds produced by selected molds and actinomycetes on agar and whole wheat bread. J. Food Prot. **49:**964–970.
14. **Imamura, H.** 1989. Contribution of extractives to wood properties, p. 843–860. *In* J. W. Rowe (ed), Natural products of woody plants II. Springer-Verlag KG, Berlin.
15. **Jantunen, M. J., A. Nevalainen, A.-L. Rytkönen, M. Pellikka, and P. Kalliokoski.** 1987. The effect of humidification on indoor air fungal spore counts in apartment buildings. Proceedings of the 4th International Conference on Indoor Air Quality and Climate, vol. 1, p. 643–647. Institute for Water, Soil and Air Hygiene, Berlin.
16. **Kaminski, E., S. Stawicki, and E. Wasowicz.** 1974. Volatile flavor compounds produced by molds of *Aspergillus*, *Penicillium*, and *Fungi Imperfecti.* Appl. Microbiol. **27:**1001–1004.
17. **Karahadian, C., D. B. Josephson, and R. C. Lindsay.** 1985. Contribution of *Penicillium* sp. to the flavours of Brie and Camembert cheese. J. Dairy Sci. **68:**1865–1877.
18. **Karahadian, C., D. B. Josephson, and R. C. Lindsay.** 1985. Volatile compounds from *Penicillium* sp. contributing musty-earthy notes to Brie and Camembert cheese. J. Agric. Food Chem. **33:**339–343.
19. **Kikuchi, T., K. Shigetoshi, H. Suehara, A. Nishi, and K. Tsubaki.** 1981. Odorous metabolites of a fungus, *Chaetomium globosum* Kinze ex Fr. Identification of geosmin, a musty-smelling compound. Chem. Pharm. Bull. **29:** 1782–1784.
20. **Larsen, T. O., and J. C. Frisvad.** 1994. A simple method for collection of volatile metabolites from fungi based on diffusive sampling from Petri dishes. J. Microbiol. Methods **19:**297–305.
21. **Marbury, M. C., and J. E. Woods, Jr.** 1991. Building-related illness, p. 306–322. *In* J. M. Samet and J. D. Spengler (ed.), Indoor air pollution: a health perspective. The John Hopkins University Press, Baltimore.
22. **Mattheis, J. P., and R. G. Roberts.** 1992. Identification of geosmin as a volatile metabolite of *Penicillium expansum*. Appl. Environ. Microbiol. **58:** 3170–3172.
23. **Miller, J. D., A. M. Laflamme, Y. Sobol, P. Lafontaine, and R. Greenhalgh.** 1988. Fungi and fungal products in some Canadian houses. Int. Biodeterior. **24:**103–120.
24. **Nevalainen, A., A.-L. Pasanen, M. Niininen, T. Reponen, P. Kalliokoski, and M. J. Jantunen.** 1991. The indoor air quality in Finnish homes with mold problems. Environ. Int. **17:**299–302.
25. **Norrman, J.** 1971. A gas chromatographic investigation of the influence of different carbon sources on the production of volatile compounds by *Dipodascus aggregatus*. Arch. Microbiol. **75:**145–162.
26. **Norrman, J.** 1971. The influence of different nitrogen sources on the production of volatile compounds by *Dipodascus aggregatus*. Arch. Microbiol. **80:**338–350.
27. **Pisarnitskii, A. F., and I. A. Egorov.** 1988. Low-molecular-weight metabolites produced by some *Penicillium* species. Prikl. Biokhim. Mikrobiol. **24:**760–764. (In Russian.)
28. **Rytkönen, A.-L., P. Pasanen, P. Kalliokoski, A. Nevalainen, and M. Jantunen.** 1988. The effect of air temperature and humidity on the growth of some fungi. Proceedings of Healthy Buildings '88, vol. 2, p. 345–350. Swedish Council for Building Research, Stockholm.
29. **Samson, R. A., B. Flannigan, M. E. Flannigan, A. P. Verhoeff, O. C. G. Adan, and E. S. Hoekstra (ed.).** 1994. Health implication of fungi in indoor air environment. Elsevier/North-Holland Biomedical Press, Amsterdam.
30. **Sokolow, S., J. Karnofsky, and P. Gustafson.** 1978. The Finnigan library search program. Finnigan application report no. 2. Finnigan Instruments, San Jose, Calif.
31. **Spengler, J. D.** 1991. Sources and concentrations of indoor air pollution, p. 33–67. *In* J. M. Samet and J. D. Spengler (ed.), Indoor air pollution: a health perspective. The John Hopkins University Press, Baltimore.
32. **Sprecher, E., and H.-P. Hanssen.** 1982. Influence of strain specificity and culture conditions on terpene production by fungi. Planta Med. **44:**41–43.
33. **Strachan, D. P., B. Flannigan, E. M. McCabe, and F. McGarry.** 1990.

Quantification of airborne molds in the homes of children with and without wheeze. Thorax **45:**382–387.
34. **Ström, G., J. West, B. Wessén, and U. Palmgren.** 1994. Quantitative analysis of microbial volatiles in damp Swedish houses, p. 291–305. *In* R. A. Samson, B. Flannigan, M. E. Flannigan, A. P. Verhoeff, O. C. G. Adan, and E. S. Hoekstra (ed.), Health implication of fungi in indoor air environment. Elsevier/North-Holland Biomedical Press, Amsterdam.
35. **Sunesson, A.-L., C.-A. Nilsson, and B. Andersson.** 1995. Evaluation of adsorbents for sampling and quantitative analysis of microbial volatiles using thermal desorption-gas chromatography. J. Chromatogr. **699:**203–214.
36. **Sunesson, A.-L., C.-A. Nilsson, B. Andersson, and R. Carlson.** 1992. Thermal desorption cold trap-injection in high-resolution gas chromatography: multivariate optimization of experimental conditions. J. Chromatogr. **623:**93–103.
37. **von Schantz, M., and R. Hiltunen.** 1983. Coniferae (Nadelbäume). Commentationes Physico-Mathematicae 57. The Finnish Society of Science and Letters, Helsinki. (In German.)
38. **Wasowicz, E., and E. Kaminski.** 1988. Volatile components of sound and musty wheat grains. Chem. Mikrobiol. Technol. Lebensm. **11:**161–168.