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International Biodeterioration & Biodegradation 42 (1998) 9–16

INTERNATIONAL BIODETERIORATION & BIODEGRADATION

Production of mycotoxins on artificially inoculated building materials

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Accepted 29 January 1998

Abstract

Isolates of *Stachybotrys chartarum*, *Aspergillus versicolor* and *Trichoderma* spp. from water damaged Danish buildings were grown on old and new gypsum boards, wood pieces, chipboard, gypsum board with wallpaper and acoustic ceiling tiles. Production of sterigmatocystin and 5-methoxysterigmatocystin by each of 5 isolates of *A. versicolor* growing on pine wood, wallpaper, gypsum board and chipboard, were detected using HPLC with diode array detection and TLC with $AlCl_3$ staining. After derivatization to the heptafluorobutyrlated ester and using gas chromatography ion trap mass spectrometry, negative ion chemical ionisation, for detection, trichothecenes of the verrucarol type were found in 4 of 5 isolates of *S. chartarum* growing on old and new gypsum boards. None of 8 *Trichoderma* isolates produced the trichothecenes T-2 toxin, HT-2 toxin, diacetoxyscirpenol, fusarenon-X, deoxynivalenol, nivalenol or trichothecenes of the verrucarol or trichodermol type on any of the above mentioned materials.

Keywords: Mycotoxins; Building materials; Artificially innoculated

1. Introduction

Stachybotrys chartarum (synonym: *S. atra*), *Penicillium chrysogenum*, *Aspergillus versicolor*, *Trichoderma* spp. and *Chaetomium* spp. are some of the major fungal contaminants on water damaged building materials in Denmark (Gravesen et al., 1997). At present *S. chartarum*, *A. versicolor* and *Trichoderma* spp. are suspected of contributing to mucous membrane symptoms such as itching eyes, stuffy nose, irritation of skin, headache and fatigue among occupants of affected Danish buildings.

S. chartarum is a tertiary colonist (Grant et al., 1989) capable of growing at high water activities $a_w > 0.90$–0.95, and is frequently seen on water damaged gypsum boards, wallpaper and glass-fibre wallpaper (Grant et al., 1989; Gravesen et al., 1997; Nikulin et al., 1994). *S. chartarum* is known to produce six toxic macrocyclic and four non-macrocyclic trichothecenes (Table 1). Satratoxin H (Fig. 1) and satratoxin G are the trichothecenes most often produced by *S. chartarum* on water damaged building materials (Nikulin et al., 1994; Johanning et al., 1996; Croft et al., 1986; Sorensen et al., 1987), but trichoverrol A and B, verrucarin B and J were detected in a natural sample from a house in Chicago (Croft et al., 1986).

Trichoderma is also a tertiary colonist only capable of growth when $a_w > 0.9$, and is commonly found on wooden products (Gravesen et al., 1994). The most common species found in Danish water damaged buildings are *Trichoderma harzianum*, *T. longibrachiatum*, *T. viride* and *T. atroviride* (unpublished results from the Department of Biotechnology).

Some *Trichoderma* species are capable of producing trichothecenes (Fig. 1 and Table 1), e.g. trichodermol, harzianum A and trichodermin produced by *T. harzianum* and trichodermin produced by *T. longibrachiatum*. All these trichothecenes can be hydrolysed to the parent alcohol trichodermol (Tamm and Tori, 1984; Godfredsen and Vangedal, 1965; Corley et al., 1993). There are also reports of production of T-2 toxin (Smoragiewicz et al., 1993) and diacetoxyscirpenol (DAS) (Cvetnic and Pepeljnjak, 1997).

A. versicolor is a primary colonist capable of growth down to $a_w > 0.65$–0.70 (Grant et al., 1989), making this fungus one of the most frequently isolated fungi from building materials (Gravesen et al., 1994). *A. versicolor* consistently produces the carcinogenic mycotoxin sterigmatocystin on most laboratory media (Frisvad, 1989). On wallpaper paste agar 2 of 4 isolates produced sterig-

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S0964-8305/98/$19.00
PII: S0964-8305(98)00038-9

Table 1
Trichothecenes produced by S. chartarum, T. longibrachiatum, T. harzianum and T. viride

Genus	Species	Toxin	Hydrolysis product	
Stachybotrys	*chartarum*	Roridin E	Verrucarol	Eppley and Bailey, 1973
Stachybotrys	*chartarum*	Satratoxin H	Verrucarol	Eppley et al., 1977
Stachybotrys	*chartarum*	Verrucarin J	Verrucarol	Eppley et al., 1977
Stachybotrys	*chartarum*	Satratoxin F	Verrucarol	Eppley et al., 1980
Stachybotrys	*chartarum*	Satratoxin G	Verrucarol	Eppley et al., 1980
Stachybotrys	*chartarum*	Trichoverrol A and B	Verrucarol	Jarvis et al., 1986
Stachybotrys	*chartarum*	Verrucarin B	Verrucarol	Croft et al., 1986
Stachybotrys	*chartarum*	Trichoverrin A and B	Verrucarol	Croft et al., 1986
Stachybotrys	*cylindrospora*	Trichodermol	Trichodermol	Ayer and Miao, 1993
Stachybotrys	*cylindrospora*	Trichodermin	Trichodermol	Ayer and Miao, 1993
Trichoderma	*viride*	Trichodermin	Trichodermol	Godtfredsen and Vangedal, 1965
Trichoderma	*harzianum*	Harzianum A	Trichodermol	Corley et al., 1994
Trichoderma	*harzianum*	Trichodermin	Trichodermol	Ichineo and Kurate, 1983
Trichoderma	*Longibrachiatum*	Trichodermin	Trichodermol	Ichineo and Kurate, 1983

matocystin (Larsen and Frisvad, 1999). Production of cyclopiazonic acid (Ohmomo et al., 1973) has not been verified and is probably due to misidentification with other *Aspergillus* species (Frisvad, 1989).

For analysis of trichothecenes, gas chromatography (GC) of various derivatives has usually been used whereas high performance liquid chromatography (HPLC) has been less successful due to poor excitation values, except for some macrocyclic trichothecenes (Krishnamurthy and Sarver, 1986; Krishnamurthy and Sarver, 1987; Frisvad and Thrane, 1993). Sterigmatocystin is usually detected by TLC with $AlCl_3$ staining (AOAC official method 973.38), but HPLC of the native and derivatised toxin using single UV, diode array or fluorescence detection has also been used (Frisvad and Thrane, 1987; Abramsom and Thorstein, 1989; Lepom, 1986; Neely and Emerson, 1990).

The purpose of this paper is to verify the production of mycotoxins from *A. versicolor*, *S. chartarum*, *T. harzianum*, *T. longibrachiatum* and *T. atroviride* grown on artificially inoculated building materials.

2. Materials and methods

2.1. Fungal identification.

S. chartarum (5 isolates), *Trichoderma* (8 isolates) and *A. versicolor* (5 isolates) are held at the IBT Culture

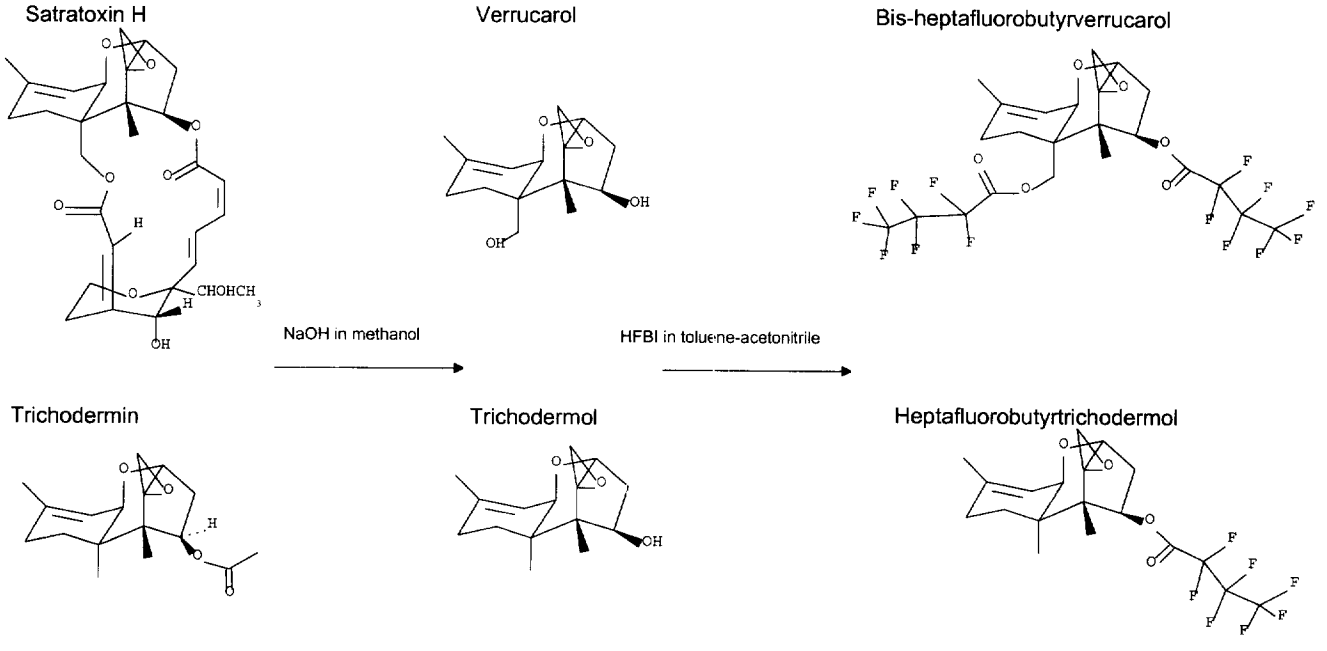

Fig. 1. Hydrolysis and heptafluorobuturylation of trichothecenes.

Collection, Department of Biotechnology, Technical University of Denmark. All fungi were identified by cultivation as described by Samson et al. (1995).

2.2. Cultivation on building materials

New gypsum boards and chipboard sheets 9 mm (bought 2 weeks before the inoculation) were cut into discs 120 mm in diameter. Acoustic ceiling and old gypsum boards (approx. 25 years) were cut into $9 \times 90 \times 90$ mm pieces and pine wood into $8 \times 60 \times 60$ mm pieces. The materials were placed in 140 mm Petri dishes, one in each except for the pine wood, where 3 pieces were placed in each Petri dish before sterilisation with 20 kGy γ-radiation. Autoclaved double distilled water was added and the materials were inoculated with 1 mL spore suspension containing 10^5 spores/mL.

In Petri dishes (140 mm) discs of new gypsum boards 9 mm thick $\times$ 120 mm in diameter were coated with a thin layer of wallpaper paste and 0.5 mL spore suspension containing 10^5 spores/mL was added, wallpaper was applied to the pasted surface of the gypsum disc and 0.5 mL spore suspension containing 10^5 spores/mL was added. Finally autoclaved double distilled water was added. The Petri dishes were placed in a ventilated room at 25°C, and inspected once a week. Autoclaved double distilled water was added as the materials dried out (7–14 days) to maintain a high water activity (a_w).

3. Extraction

Extraction of the infested building materials was performed in one of two ways:

- After 50 days of cultivation, materials were extracted by soaking in 400 mL methanol overnight followed by 400 mL dichloromethane overnight. The combined phases were evaporated to dryness on a rotary evaporator *in vacuo* at 40°C, and taken up in 2×5 mL methanol.
- After 25 or 100 days of cultivation the fungal biomass was scraped off the materials and extracted overnight in 50 mL methanol and evaporated to dryness on a rotary evaporator *in vacuo* a 40°C, and taken up in 2×5 mL methanol.

All samples were stored at −80°C until analyses were performed.

Spiking experiments involving addition of T-2 toxin, roridin A, trichodermol, sterigmatocystin and dihydrosterigmatocystin from dichloromethane solutions before and after addition of the extraction solvents were performed for estimation of recovery percentage.

3.1. Trichothecene analysis

Part of the extract (2 mL of 10 mL) was cleaned up on C_{18} SPE modules using a water-methanol system according to Nielsen et al. (1998).

3.2. Silica gel clean-up.

Part of the extract (2 mL of 10 mL) was evaporated *in vacuo* at 40 C at 5 mbar in a Chris Rotational Vacuum Concentrator (RVC), and taken up in 300 μL dichloromethane, loaded onto a Sep-Pak® VAC 6 cc Silicagel (Waters, Wat036910 1 g) module and eluted with 14 mL dichloromethane using the modified method of Rosen et al. (1986). The eluate was evaporated to dryness in the RVC.

4. Derivatization procedure for GC-MS analysis

For analysis for verrucarol and trichodermol trichothecenes, samples were hydrolysed in 0.2 M NaOH in methanol and derivatized with heptafluorobuturylimidazol according to Nielsen et al. (1998) (Fig. 1).

Extracts from *Trichoderma* were also derivatized without the hydrolysis step, thereby analysing for T-2 toxin, HT-2 toxin, DAS, fusarenon-X (F-X), deoxynivalenol (DON) and nivalenol (NIV).

5. GC-MS analysis

A Finnigan GCQ® integrated gas chromatograph-mass spectrometer (GC-MS), ion-trap with external ionisation, was used for the analysis. Injection was splitless on a 0.18 mm, 0.18 μm, 10 m polydimethyl siloxane column (DB1 121-1021) fitted with a 2 m 0.20 mm deactivated fused silica retention gap, using the temperature program described by Nielsen et al. (1998). The MS was operated in the negative chemical ionization (NCI) mode using methane as reagent gas at 1 bar.

5.1. Sterigmatocystin analysis

5.1.1. HPLC Analysis. Extracts were filtered through a 0.45 μm filter (Millipore, HVL PO4700) and analysed on a HP 1090 Series 2 HPLC equipped with a diode array detector scanning 200–600 nm in 4 nm steps, 6 mm flow-cell. A sample of 10 μl was injected on a HP Hypersil BDS 3 μm C_{18} column with the water-acetonitrile system described by Smedsgaard (1997).

5.1.2. TLC. Extracts were analysed by TLC, spraying with $AlCl_3$ solution according to Lund (1995), using sterigmatocystin as external standard.

6. Results and discussion

6.1. Evaluation of growth

Growth of *Trichoderma* could be detected after 3–4 days on the chipboard discs and was profuse. Collectively

Trichoderma spp. were capable of growing on all the materials except for the new gypsum boards (Table 2). Growth of *S. chartarum* IBT 7711 could be recognized after 5 days on the new gypsum board. Growth of the other *S. chartarum* isolates was not as rapid, although the gypsum boards were black after 14 days. All isolates of *A. versicolor* were able to grow on all materials except acoustic ceiling tiles.

6.2. Mycotoxin analysis

Extraction of the mycotoxins was best performed by scraping the fungal mycelium off the material into methanol instead of soaking the material in the solvent, thereby avoiding large quantities of interfering compounds from the building materials.

It was found that the mycotoxin standards of sterigmatocystin, dihydrosterigmatocystin, roridin A, trichodermin and T-2 toxin added to the building materials from dichloromethane were bound to the materials in a way which prevented extraction with methanol or dichoromethane (results not shown). This indicates that it may be impossible to clean extracellular mycotoxins from building materials, making dust from the material a potential hazard.

NCI scan detection of the heptafluorobuturylated trichothecenes was superior (Fig. 2) to positive electron impact (EI^+), EI^+ MS/MS and NCI MS/MS. No trichothecenes of the trichodermol type, verrucarol type, DAS, DON, F-X, T-2, HT-2 or NIV could be detected from any of the 8 *Trichoderma* isolates.

Trichothecene production of the verrucarol type, was detected in four of five *S. chartarum* isolates (Fig. 3). This supports findings of Nielsen et al. (1998), who detected these mycotoxins in both of two buildings examined. The

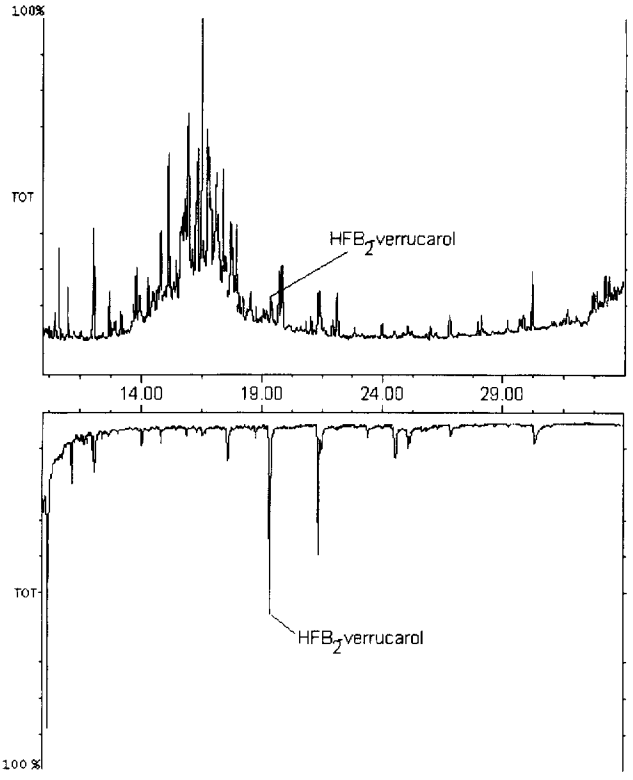

Fig. 2. Total-ion chromatograms of the same mixture analysed by GC-MS EI^+ scan (upper) and NCI scan (lower).

Table 2
Growth of inoculated fungi on building materials after 2 weeks

Species	IBT #	New gypsum	Old gypsum	Chipboard	Acoustic ceiling tiles	Pine wood	Wall paper
A. versicolor	14940	+ +	÷	+ + +	÷	+ +	+ + +
A. versicolor	15903	+ +	+	+ + +	÷	+ +	+ + +
A. versicolor	15942	+ +	+	+ + +	÷	÷	+ + +
A. versicolor	16000	+ +	+ +	+ + +	÷	÷	+ + +
A. versicolor	18238	+	÷	+ + +	÷	÷	+ + +
S. chartarum	7711	+ + +	+ + +	÷	÷	÷	Not tested
S. chartarum	9262	+ + +	+	÷	÷	÷	Not tested
S. chartarum	9263	+ +	+ + +	÷	÷	÷	Not tested
S. chartarum	14915	+ +	+	÷	÷	÷	Not tested
S. chartarum	14916	+	÷	÷	÷	÷	Not tested
T. atroviride	9127	÷	÷	+ + +	÷	÷	Not tested
T. atroviride	9133	÷	÷	+ + +	÷	÷	Not tested
T. atroviride	9144	÷	÷	+ + +	÷	÷	Not tested
T. harzianum	9142	÷	+ + +	+ +	+	÷	Not tested
T. harzianum	9143	÷	÷	+ +	÷	+ +	Not tested
T. longibrachiatum	9128	÷	+ + +	+ + +	+	+ +	Not tested
T. longibrachiatum	9132	÷	+	+ + +	+	+ +	Not tested
T. viride	9131	÷	÷	+ + +	÷	+ +	Not tested

+ + + material was covered by mycelia within 2 weeks, + + within 4 weeks, + only a fraction of the material was covered after 4 weeks. ÷ no growth.

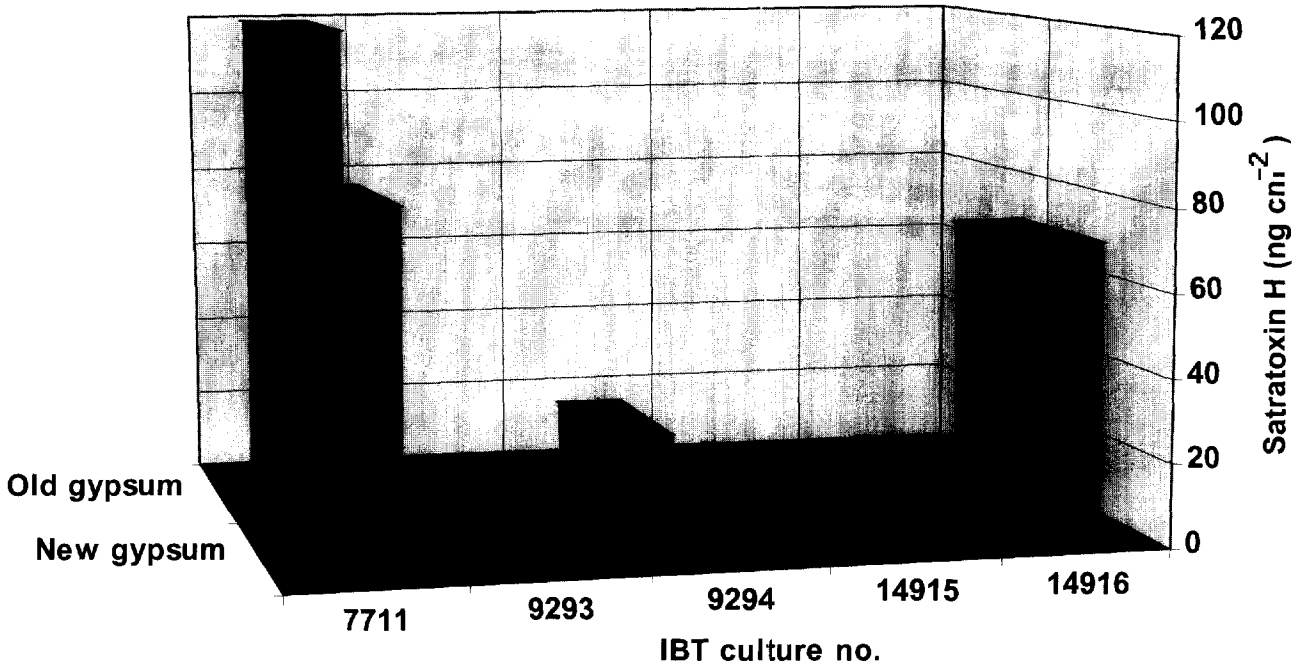

Fig. 3. Production of trichothecene of the verrucarol type (calculated as satratoxin H) from of *S. chartarum* isolates on gypsum boards.

verrucarol had probably originated mostly from satratoxin H and G, as these are the primary trichothecene metabolites from *S. chartarum* growing on building materials (Nikulin et al., 1994; Croft et al., 1986; Johanning et al., 1996). The quantities detected (20–140 ng cm^{-2}) are different from but of the same order as that reported by Johanning et al. (1996). They detected 1 μg satratoxin H in 60 mg *S. chartarum* biomass scraped from a 30 cm^2 infested building material giving 33 ng cm^{-2}. This is lower than reported by Nikulin et al. (1994), who detected 2–3.5 μg cm^{-2} satratoxins from a highly toxic strain of *S. chartarum*.

A. versicolor produced large quantities of sterigmatocystin and 5-methoxysterigmatocystin (Fig. 4) on the building materials (Figs 5 and 6). As the quantities of *A. versicolor* biomass scraped from the materials were under 0.1 g, the total contents of sterigmatocystins may have been more than 1% of the biomass.

Since sterigmatocystin has been classified by the International Agency for Research on Cancer (IARC) as a 2A human carcinogen (IARC, 1993), these findings point to a potential carcinogen risk in buildings infested by *A. versicolor*, especially for workers making structural changes or cleaning up contaminated materials.

7. Conclusions

The findings of trichothecenes from 4 out of 5 *S. chartarum* isolates indicates that most Danish isolates produce toxins when growing on building materials. *Trichoderma* spp. were not found to produce trichothecene mycotoxins on building materials.

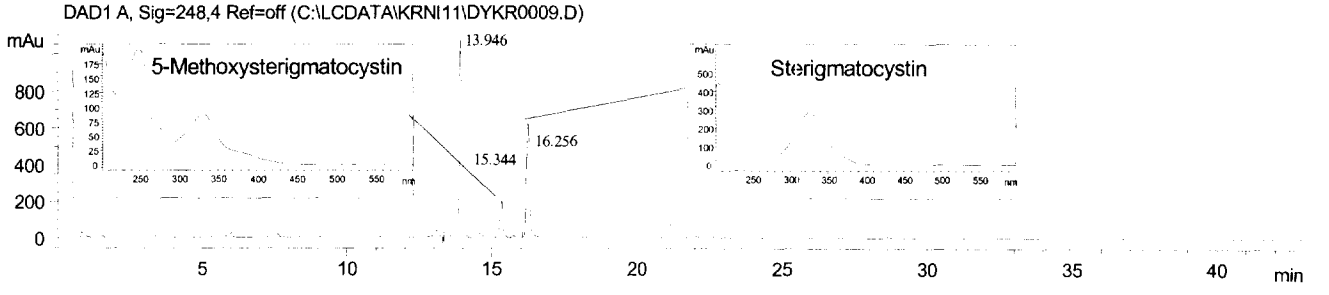

Fig. 4. HPLC chromatogram of extract of *A. versicolor* IBT 16000 grown on wallpaper.

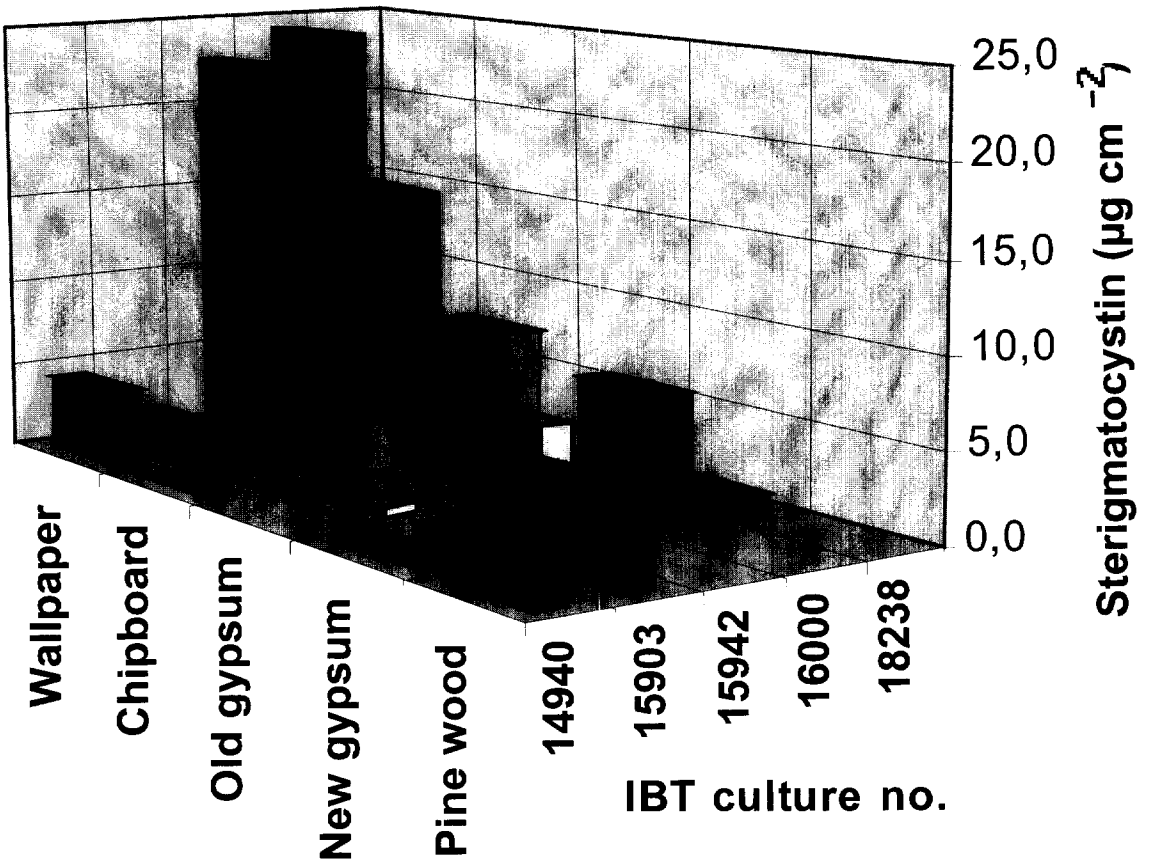

Fig. 5. Production of sterigmatocystin by *A. versicolor* on building materials.

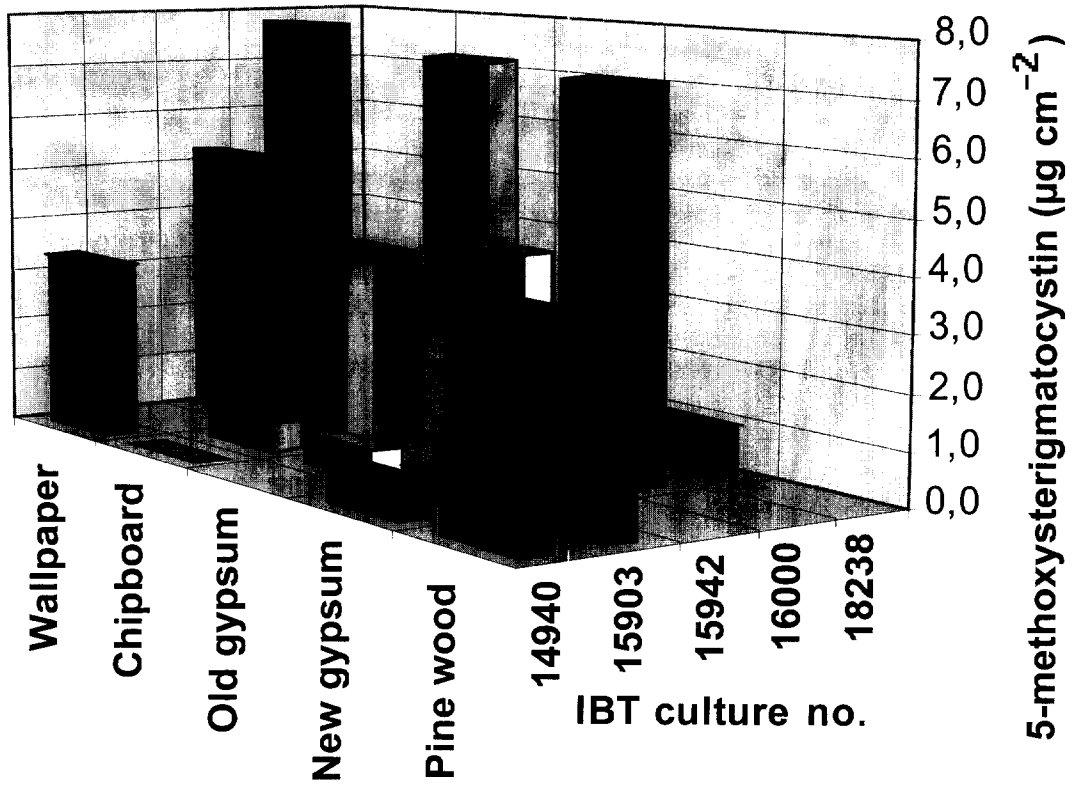

Fig. 6. Production of 5-methoxysterigmatocystin by *A. versicolor* on building materials.

The findings of high quantities of the carcinogenic mycotoxin sterigmatocystin, especially on wallpaper, demonstrate a potential health hazard in water damaged buildings, since *A. versicolor* is almost always present on water damaged building materials. The finding of sterigmatocystin production of *A. versicolor* on building materials has not been reported before.

Acknowledgements

This work was supported by a grant from the Danish Building Ministry # B4-1255-13 as a part of the 3 year research project *Micro-organisms in water damaged buildings*. Our thanks are expressed to Lisbeth Larsen, ALK Laboratories for supplying isolates of *S. chartarum* from water damaged buildings. We gratefully acknowledge English language assistance by Margaret Flannigan.

References

Abramson, D., Thorsteinson, T., 1989. Determination of sterigmatocystin in barley by acetylation and liquid chromatography. Journal of AOAC. 72, 342–344.

AOAC, 1996 AOAC Official method 973.38, Sterigmatocystin in barley and wheat. Official Methods of analysis of AOAC International. 16th Edition AOAC International, Gaithersburg.

Ayer, W.A., Miao, S., 1993. Secondary metabolites of the aspen fungus *Stachybotrys cylindrospora*. Canadian Journal of Chemistry, 71. 487-493.

Corley, D.G., Miller-Widerman, M., Durley, R.C., 1994. Isolation and structure of harzianum A: A new trichothecene from *Trichoderma harzianum*. Journal of Natural Products. 57, 422–425.

Croft, W.A., Jarvis, B.B., Yatawara, C.S., 1986. Airborne outbreak of trichothecene toxicosis. Atmospheric Environment. 20, 549–552.

Cvetnic, Z., Pepeljnjak, S., 1997. Distribution and mycotoxin-producing ability of some fungal isolates from the air. Atmospheric Environment. 31, 491–495.

Eppley, R.M., Bailey, W.J., 1973. 12, 13-Epoxy-Δ-trichothecenes as probable mycotoxins responsible for stachybotryotoxicosis. Science. 181. 758–760.

Eppley, R.M., Mazzola, E.P., 1977. Structure of satratoxin H, a metabolite of *Stachybotrys atra*. Journal of Organic Chemistry. 42, 240 243.

Eppley, R.M., Mazzola, E.P., Stack, M.E., Dreifuss, P.A., 1980. Structure of satratoxin F and satratoxin G, metabolites of *Stachybotrys atra*. Journal of Organic Chemistry. 45, 2522–2523.

Frisvad, J.C., Thrane, U., 1987. Standardised High-Preformance Liquid Chromatography of 182 mycotoxins and other fungal metabolites based on alkylphenone retention indices and UV-VIS spectra (diode array detection). Journal of Chromatography. 404, 195-214.

Frisvad, J.C., 1989. The connection between the Penicillia and Aspergilli and mycotoxins with special emphasis on misidentified isolates. Archives of Environmental and Contamination Toxicology. 18, 452–467.

Frisvad, J.C., Thrane, U., 1993. Liquid column chromatography of mycotoxins. In: V. Betina (ed), Chromatography of Mycotoxins: Techniques and applications. Journal of Chromatography Library. 54, 253–372.

Godtfredsen, W.O., Vangedal, S., 1965. Trichodermin, a new sesquiterpene antibiotic. Acta Chemica Scandinavia. 19, 1088 1102.

Grant, C., Hunter, C.A., Flannigan, B., Bravery, A.F., 1989. The moisture requirements of moulds isolated from domestic dwellings. International Biodeterioration. 25, 259–284.

Gravesen, S., Frisvad, J.C., Samson, R.A., 1994. Microfungi. Munksgaard, Copenhagen.

Gravesen, S., Nielsen, P.A., Nielsen, K.F., 1997. SBI report 282: Microfungi in water damaged buildings (in Danish). Danish Building Research Institute, Hørsholm.

IARC, 1993. IARC Monographs on the evaluation of carcinogenic risks to humans. V.56. Some naturally Occurring Substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins International Agency for Research on Cancer, Lyon.

Ichinoe, M., Kurata, H., 1983. Trichothecene-producing Fungi. In: Y. Ueno (ed.), Trichothecenes - Chemical, Biological and Toxicological Aspects, pp. 73–82. Elsevier-North-Holland Publishing Co., Amsterdam.

Jarvis, B.B., Yin-Won, L., Cömezoglu, S.N., Yatawara, C.S., 1986. Trichothecenes produced by *Stachybotrys atra* from eastern Europe. Applied and Environmental Microbiology. 51, 915–918.

Johanning, E., Biagini, R., Hull, D., Mory, P., Jarvis, B.B., Landsbergis, P. 1996. Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. International Archives of Occupational and Environmental Health. 68, 207–218.

Krishnamurthy, T., Sarver, E.W., Greene, S.L., Jarvis, B.B., 1987. Mass spectral investigations on trichothecene mycotoxins. II. Detection and quantitation of macrocyclic trichothecenes by gas chromatography/negative ion chemical ionisation mass spectrometry. Journal of AOAC. 70, 132–140.

Krishnamurthy, T., Sarver, E.W., 1986. Mass spectral investigation on trichothecene mycotoxins. III. Synthesis, characterization and application of pentafluoropropionyl and trifluoroacetyl esters of simple trichothecenes. Journal of Chromatography. 355, 253–264.

Larsen, T.O., Frisvad, J.C., 1994. Production of volatiles and presence of mycotoxins in conidia of common indoor Penicillia and Aspergillii. In: R.A. Samson, B. Flannigan, M.E. Flannigan, A.P. Verhoeff, O.C.G. Adan, E.S. Hoekstra (Eds), Health Implications of Fungi in Indoor Air Environment, pp. 251–279. Elsevier North-Holland Publishing Co., Amsterdam.

Lepom, P., 1986. Determination of sterigmatocystin in feed by high-preformance liquid chromatography with column switching. Journal of Chromatography. 354, 518 523.

Lund, F., 1995. Diagnostic characterisation of *Penicillium palitans*, *P. commune*, *P. solitum*. Letters in Applied Microbiology. 21. 60–64.

Neely, F.L., Emerson, C.S., 1990. Determination of sterigmatocystin in fermentation broths by reversed-phase high-performance liquid chromatography using post-column fluorescence enhancement. Journal of Chromatography. 523, 305 311.

Nielsen, K.F., Hansen, M.Ø, Larsen, T.O., Thrane, U., 1998. Production of trichothecene mycotoxins on water damaged gypsum boards in Danish buildings. International Biodeterioration & Biodegradation (in press).

Nikulin, M., Pasanen, A.-L., Berg, S., Hinitikka, E.-L., 1994. *Stachybotrys atra* growth and toxin production in some building materials and fodder under different relative humidities. Applied and Environmental Microbiology. 60, 3421–3424.

Ohmomo, S., Sugita, M., Matazo, A., 1973. Isolation of cyclopiazonic acid, cyclopiazonic acid imine and bissecodehydrocyclopiazonic acid from the cultures of *Aspergillus versicolor* (Vuil) Tiraboschi. Journal of the Agricultural and Chemical Society of Japan. 47, 57–63.

Rosen, J.D., Rosen, R.T., Hartman, T.G., 1986. Capillary gas chroma-

tography-mass spectrometry of several macrocyclic trichothecenes. Journal of Chromatography. 355, 241–251.

Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O., (Eds) 1995. Introduction to Food-borne Fungi, 5th edition Centraalbureau voor Schimmelcultures, Baarn.

Smedsgaard, J., 1997. Micro-scale extraction procedure for standardized screening of fungal metabolite production in cultures. Journal of Chromatography A. 760, 264–270.

Smoragiewicz, W., Cossette, B., Boutard, A., Krzystyniak, K., 1993. Trichothecene mycotoxins in the dust ventilation system in office buildings. International Archives of Occupational and Environmental Health. 65, 113–117.

Sorenson, W.G., Frazer, D.G., Jarvis, B.B., Simpson, J., Robinson, V.A., 1987. Trichothecene mycotoxins in aerosolized conidia of *Stachybotrys atra*. Applied and Environmental Microbiology. 53, 1370–1375.

Tamm, C., Tori, M., 1984. Trichothecenes. In V. Betina (Ed.), Mycotoxins - Production, Isolation, Separation and Purification, pp. 131–182. Elsevier, Amsterdam.