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Trichothecene Mycotoxins in Aerosolized Conidia of *Stachybotrys atra*

W. G. SORENSON,[1,2]* DAVID G. FRAZER,[1,3] BRUCE B. JARVIS,[4] JANET SIMPSON,[1] AND VICTOR A. ROBINSON[1]

Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health, Morgantown, West Virginia 26505[1]; Departments of Microbiology[2] and Physiology,[3] West Virginia University, Morgantown, West Virginia 26506; and Department of Chemistry and Biochemistry, The University of Maryland, College Park, Maryland 20782[4]

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Stachybotrys atra **is the etiologic agent of stachybotryotoxicosis, and this fungus and its trichothecene mycotoxins were recently implicated in an outbreak of unexplained illness in homes. *S. atra* was grown on sterile rice, autoclaved, dried, and then aerosolized by acoustic vibration. The distribution of particles (mass and number) was monitored on an aerodynamic particle sizer interfaced with a computer. Dust was collected on preweighed glass-fiber filters and extracted with 90% aqueous methanol. Extracts were tested for the ability to inhibit protein synthesis in rat alveolar macrophages, the ability to inhibit the proliferation of mouse thymocytes, and the presence of specific trichothecene mycotoxins. Virtually all of the particles were <15 μm in aerodynamic diameter, and the mass median diameter was 5 μm. Thus, most of the particles were respirable. Microscopic analysis of the generated dust revealed that ca. 85% of the dust particles were conidia of *S. atra*, another 6% were hyphal fragments, and the remainder of the particles were unidentifiable. Thus, >90% of the particles were of fungal origin. The extracts strongly inhibited protein synthesis and thymocyte proliferation. Purified satratoxin H was also highly toxic in the same systems. Each of the individual filters contained satratoxin H (average, 9.5 ng/mg of dust). Satratoxin G and trichoverrols A and B were found in lesser amounts in some, but not all, of the filters. The limit of analysis is ca. 50 ng. These results establish that the conidia of *S. atra* contain trichothecene mycotoxins. In view of the potent toxicity of the trichothecenes, the inhalation of aerosols containing high concentrations of these conidia could be a potential hazard to health.**

Stachybotrys atra Corda is a cellulolytic saprobe which is one of several deuteromycete fungi known to produce trichothecene mycotoxins. These naturally occurring toxic sesquiterpene metabolites are potent inhibitors of protein synthesis in eucaryotic organisms. *S. atra* is known to produce several macrocyclic trichothecenes, including verrucarin, roridin E, and satratoxins F, G, and H (8). Stachybotryotoxicosis is a severe toxic syndrome of large domestic animals in eastern Europe, which has been produced experimentally in laboratory animals, including dogs, rabbits, guinea pigs, mice, and chicks (10), and was induced in human volunteers by Russian investigators (6). The typical form of the disease (5) occurs in animals with continuous exposure to low levels of the toxin. The disease is characterized by leukopenia, severe thrombocytopenia, hemorrhage, and arrhythmic heartbeat and often leads to death (5). Russian investigators also reported that the disease occurs frequently in human populations in areas where the disease is endemic in horses. The disease was associated with contaminated straw and dust aerosols heavily laden with conidia in cottonseed oil processing plants, grain elevators, textile mills, and other grain processing operations, including breweries (17). Since the macrocyclic trichothecenes as a group are highly toxic and produce biological effects in experimental animals similar to those observed in stachybotryotoxicosis, they are regarded to be the chemical agents responsible for stachybotryotoxicosis (4).

Kozak et al. (11), in a review of currently available methods for home mold surveys, reported a small child with a case of asthma associated with extensive contamination of jute-backed carpeting by *Stachybotrys* sp. The carpet had sustained water damage. In their attempts to demonstrate viable *Stachybotrys* sp., the authors reported that no viable organisms of this genus were detected by Andersen sampling even though *Stachybotrys* sp. conidia are readily dispersed in air. Further studies in another home with the same problem revealed *Stachybotrys* sp. conidia by rotorod air sampling. *Stachybotrys* sp. colonies were isolated from the carpet samples.

Recently Croft et al. (3) reported an outbreak of unexplained illness, occurring over a 5-year period, which appeared to be related to the extensive occurrence of *S. atra* contamination of home ductwork and ceiling fiberboard. The fiberboard and air samples collected from the home contained substances which elicited characteristic trichothecene toxicity when injected into mice. The toxic extracts were subsequently shown by these investigators to contain macrocyclic trichothecenes. This study raises the possibility that *S. atra* is the etiologic agent via its toxic metabolites and that such illness may be more common than is currently realized. The rationale for this statement is that, although *S. atra* is not commonly encountered in surveys of indoor airborne fungi because it does not compete well with *Aspergillus* and *Penicillium* species, which tend to predominate in such samples, it grows readily on ceiling tiles and other similar materials indoors when atomospheric conditions, especially moisture, permit. With the increasing tendency to permit higher humidity levels and a higher level of recirculated indoor air to control energy costs, there has been an increase in the number and frequency of outbreaks

* Corresponding author.

of unexplained illness in offices and residences (15). Although the etiology of these outbreaks has not been established, the possibility that fungi are involved is strongly suggested in certain of these cases.

The objective of this study was to determine whether conidia of *S. atra* contain macrocyclic trichothecenes.

MATERIALS AND METHODS

Source and culture of fungi. *S. atra* Budapest 1 and Debrecen 1132, obtained from B. Harrach, Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary, were used in these studies. These isolates had been cultured from incidents of stachybotryotoxicosis in eastern Europe. Strain Budapest 1 was grown in Morgantown in a 1-liter Roux bottle on 100 g of moist, sterile Japan Red Rose Rice (Japan Food Corp., San Francisco, Calif.). The rice culture was incubated at 22 to 25°C for 2 weeks and then transferred to 8°C for 2 weeks. After incubation, the rice culture was sterilized by autoclaving and allowed to dry at room temperature. Strain Debrecen 1132 was grown in College Park in a 1-liter Erlenmeyer flask on 200 g of moist, autoclaved Uncle Ben's Converted Rice (Uncle Ben's, Inc., Houston, Tex.). The culture was incubated at 22 to 26°C for 2 weeks and then incubated at 10°C for 2 weeks.

Aerosol generation and analysis. Respirable-size dust particles were generated from 10-g portions of *S. atra* culture in a miniature version of the Pitt-3 cotton dust generator. The minigenerator has a 3.5-in. (ca. 8.9-cm) diameter and 10-in. (ca. 25-cm) length of Plexiglas tube, with vibration provided by rubber dam material over a 2.5-in. (ca. 6.3-cm) speaker, powered at 132 Hz and 3 V. The dust was carried from the generator via an airflow of 5 liters/min.

A TSI aerodynamic particle sizer (model 3300; TSI, Inc., St. Paul, Minn.) was used to record mass and number distributions throughout the run. The aerodynamic particle sizer was interfaced with an Apple II$^+$ computer with all data stored on floppy disks for future analysis. A 20-s sample time was used.

Preweighed glass-fiber filters (37 mm) were used to collect dust for gravimetric analysis and mycotoxin assay. The dust was pulled through the filters with two portable DuPont pumps, each pulling 2.5 liters/min. Filters were housed in three-piece plastic cassettes with the top removed during sampling for open-faced collection. A numbered back-up pad was placed in back of the filter for support. Cellulose sealing bands were placed around the filter immediately after preweighing. After dust collection on the filters, they were weighed again, with the change in weight and the gravimetric concentration recorded.

Microscopic study of dust removed from filters was undertaken to assess the degree of purity of the conidial preparations. Small portions of dust were mixed in mounting medium, and a series of 10 microscopic fields (45×) was studied from each of a set of 10 slides. The number of particles in each of the following categories was recorded: (i) conidia, (ii) hyphal fragments, and (iii) other (particles not assignable to either of the other categories).

Isolation of trichothecenes from rice cultures of *S. atra*. Trichothecenes were isolated from rice cultures by preparative thin-layer chromatography on a Chromatotron (Harrison Research, Palo Alto, Calif.) and semipreparative high-performance liquid chromatography. Details of the procedures were described previously (9). The chemical structures of the metabolites isolated were determined by nuclear magnetic resonance spectroscopy (9). The isolated metabolites were used as authentic standards for high-performance liquid chromatography analysis, and purified satratoxin H (SH) was used as a positive control in biological activity studies.

Extraction of spore aerosol samples. Extraction of the mycotoxins was done essentially as described by Jarvis et al. (9), but the ferric gel was omitted. In essence, filters containing conidia were extracted with 90% methanol under agitation in a sonicator, the crude extracts were defatted with hexane, and the extract was concentrated in vacuo to remove methanol. Toxins were removed from the aqueous solution by partition into dichloromethane. The dichloromethane extracts were dried over sodium sulfate, concentrated in vacuo, transferred to vials, and concentrated to dryness under a stream of nitrogen. Dried extracts were stored at −20°C until needed for experiments to determine biological activity.

Trichothecene analysis in *S. atra* conidia. Replicate filters containing conidia from the same aerosols were used to determine the presence of specific trichothecenes. Samples of conidia (weight, 7 to 33 mg per sample) in separate flasks were sonicated for 30 min in 2 ml of 90% aqueous methanol (H_2O/MeOH ratio, 1:9, vol/vol). This procedure was repeated twice more with fresh portions of aqueous methanol. The extracts for each sample were combined (6 ml per sample) and concentrated to near dryness under nitrogen gas. Each aqueous sample was placed on a bed on C-18 bonded phase silica (500 mg, Baker Spe column; J. T. Baker Chemical Co., Phillipsburg, N.J.). The column was washed with 3 ml of 10% methanol in water (discarded) and then 3 ml of 75% methanol in water. The 75% aqueous methanol solution was concentrated to dryness, and the residue was dissolved in 20 ml of dichloromethane and injected into a high-performance liquid chromotograph (9). Peaks corresponding in retention time to SH and satratoxin G and trichoverrols A and B were analyzed with a diode-array detector (LKB model 2140; LKB Products, Inc., Washington, D.C.) which recorded UV spectral data for each eluate peak. The UV spectra of the peaks were identical to the spectra of authentic standards.

AM isolation and culture. Alveolar macrophages (AM) were harvested from male viral-antibody-free Sprague-Dawley rats by tracheal lavage by the method of Myrvik et al. (16). Rats were anesthetized by intraperitoneal injection with sodium pentobarbital and exsanguinated by cutting the abdominal aorta. The lungs from each rat were lavaged with a total of 60 ml of prewarmed calcium- and magnesium-free Hanks balanced salt solution. The cells from several animals were pooled, centrifuged at 500 × *g* for 10 min, and washed with phosphate-buffered saline. Supplements added to the medium included 2% heat-inactivated fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and heparin (20 U/ml). Medium and supplements were obtained from GIBCO Laboratories, Grand Island, N.Y. A Coulter Counter (Coulter Instrument Co., Hialeah, Fla.) was employed to determine the cell concentration. Cell purity, as determined from the cell volume distribution, was approximately 95%. Van Scott et al. (M. R. Van Scott, T. D. Sommerville, R. C. Lantz, P. R. Miles, and V. Castranova, *in* K. Van Dyke and V. Castranova, ed., *Cellular Chemiluminescence*, vol. III, in press) have shown that such a distribution count includes all subpopulations of AM obtained by pulmonary lavage.

AM suspensions were cultured at a cell concentration of 2.5×10^5 cells per cm^2 in Linbro tissue culture plates

(Linbro, Hamden, Colo.). AM monolayers in medium 199 (2% heat-inactivated fetal bovine serum, heparin, penicillin, and streptomycin) were incubated for 2 h at 37°C in 5% CO_2 to allow for adherence of AM, rinsed with phosphate-buffered saline to remove nonadherent cells, and incubated with medium 199 (10% heat-inactivated fetal bovine serum, penicillin, and streptomycin) until needed for experiments. The viability of the cultured AM was routinely greater than 95%, as determined by trypan blue exclusion (18).

Protein-synthesis inhibition assay. For protein-synthesis studies, AM monolayers were incubated in Eagle minimum essential medium with Earle salt solution and L-glutamine but without L-leucine (KC Biologicals, Lenexa, Kans.). The AM monolayers were incubated with sample extract or SH dissolved in dimethyl sulfoxide (DMSO) and 1 μCi of [^{3}H]leucine per ml (110 Ci/mmol; New England Nuclear Corp., Boston, Mass.) at 37°C in 5% CO_2 for 2 h. After incubation, the culture plates were chilled on ice to stop metabolism, the culture medium was removed, and the cells were washed three times with chilled phosphate-buffered saline. Monolayers were solubilized for 30 min with 0.5 ml of 0.1 M KOH, and proteins were acid precipitated with 2.0 ml of 10% trichloroacetic acid and 50 μl of 1% bovine serum albumin. The acid-precipitable material was collected on glass-fiber filters, washed with cold trichloroacetic acid, placed in scintillation vials with 10 ml of Aquasol-2 (New England Nuclear Corp.), and counted by liquid scintillation. Median effective dose values were determined by probit analysis (14).

Thymocyte proliferation inhibition assay. Female CD-1 mice (6 to 8 weeks old) (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were sacrificed by cervical dislocation, and the thymuses were removed aseptically. Thymuses were minced, forced through sterile stainless-steel mesh, and collected in RPMI 1640 medium. Nucleated cells were counted with a Coulter Counter, and the cell concentration was adjusted to 10^7 cells per ml in RPMI 1640 containing 10% fetal bovine serum, 100 U of penicillin G per ml, 100 μg of streptomycin sulfate per ml, and 20 μM 2-mercaptoethanol. Spore extracts were dissolved in DMSO and diluted in saline. Diluted extracts were then added to RPMI 1640. Negative control wells contained an equal concentration of DMSO, and the final concentrations of DMSO, thymocytes, and 2-mercaptoethanol were 0.25%, 10^6 per well, and 20 μM, respectively. Thymocytes were cultured in 96-well tissue culture plates (Costar, Cambridge, Mass.) at 37°C in a humidified incubator with 5% CO_2 and stimulated with crude interleukin-1 (IL-1) prepared from cultures of rat AM activated with *Escherichia coli* O111:B4 lipopolysaccharide as described by Lachman et al. (12). IL-1 (50 μl per well) was added to half the wells to study the effect of the conidial extracts on the IL-1-induced proliferation. Cultures were incubated for 72 h, and [^{3}H]thymidine was added for the last 7 h of incubation. Cells were harvested and washed with a PHD cell harvestor (Cambridge Technology, Inc., Cambridge, Mass.) and counted by liquid scintillation. The stimulation index is the quotient of the counts per minute of IL-1-treated cultures divided by the counts per minute of otherwise identical cultures lacking IL-1.

RESULTS AND DISCUSSION

The purpose of these studies was to determine whether the conidia of *S. atra* contain trichothecene mycotoxins and not to describe characteristics of typical aerosols as they might occur in the workplace. Therefore, the conditions of aerosol generation were selected to reduce the number of larger particles and, therefore, to enrich the small-particle component of the aerosol (gentle acoustic vibration, a relatively low airstream flow rate, and the location of the exit high in the generator so that heavier particles would settle out of the airstream before exit). Two parameters of the aerosols were monitored: (i) the number of particles versus particle size and (ii) the cumulative mass versus particle size. Figure 1 demonstrates that (i) virtually 100% of the aerosol particles from both cultures were <15 μm in aerodynamic diameter, (ii) the diameter at peak number concentration for both cultures was ≤5 μm, (iii) the mass median aerodynamic diameter of particles from both cultures was ≤5 μm, and (iv) the particle distributions from both cultures were similar. Thus the particles entrained in the airstream from the aerosol generator were respirable, and the vast majority of these particles could be expected to penetrate deeply into the lung (2).

A total of 7,163 particles in 100 microscopic fields were classified into the following categories: conidia (6,083 [84.9%]), hyphal fragments (469 [6.5%]), and other (611 [8.5%]) (Table 1). Fungal elements, i.e., conidia and respirable-size hyphal fragments, made up >90% of the total respirable airborne fragments. The data also demonstrate that the different microscopic fields were similar, i.e., the percentage of conidia in each field ranged from 79.3 to 88.6% (84.7 ± 2.9%), and the combined percentage of conidia and respirable hyphal fragments ranged from 88.4 to 94.5% (91.3 ± 2.3%). Thus, the extracts represent primarily respirable fungal particles of *S. atra*. Total separation of conidia from hyphal fragments and other particles was not possible, but assuming unit density for all particles, it is likely that the samples were ca. 85% conidia by weight because the other particles were of comparable size (1 to 15 μm). Microscopic analysis of dust from rice culture of Budapest 1 was not done because of limited material, but the data obtained from aerodynamic particle sizer studies suggest that the aerosol characteristics of the two dusts were similar.

Extracts from the spore preparations caused a dose-dependent inhibition of protein synthesis in rat AM within 1 h (Table 2). The median 50% effective dose (ED_{50}) after 2-h incubation was 0.05 μg/ml of extract residue for Debrecen 1132 culture (95% confidence interval, 0.04 to 0.07). Similar studies with SH yielded an ED_{50} of 7.6 nM (95% confidence interval, 6.2 to 9.3). It was not possible to determine the ED_{50} for the Budapest 1 extract because of the occurrence of values greater than 100% of control. This was a consistent finding in several experiments. Probit analysis requires values between 0 and 100%. The two samples were grown and extracted in different laboratories, and there were slight differences in the way this sample was handled (e.g., drying and autoclaving time) which could result in some chemical change. Alternatively, the different strains might produce different metabolites, especially if grown on different brands of rice. The results observed represent the combined effect of constituents in the extract. If strain Budapest 1 produces a metabolite(s) which stimulates protein synthesis, in addition to the toxic components of the extract, dilution of the extract could result in enhanced protein synthesis as the SH is diluted. In earlier studies of the effects of other mycotoxins on protein synthesis and phagocytosis in rat AM, doses of T-2 toxin and patulin affected protein synthesis and phagocytosis to an almost equal extent (7, 20).

SH and the spore extracts strongly inhibited the ability of mouse thymocytes to respond to crude preparations of rat IL-1 (Table 3). Untreated control cells had a stimulation

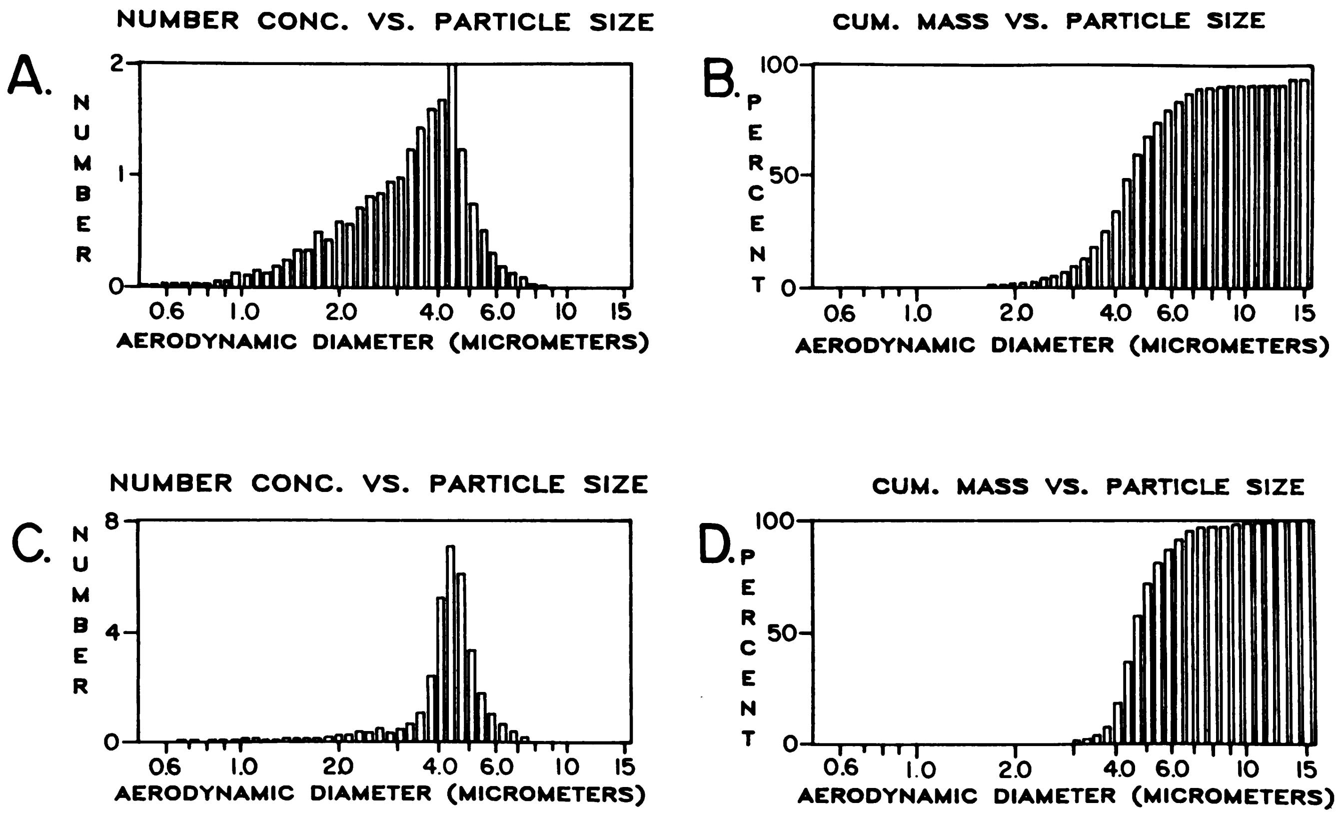

FIG. 1. Computer-generated plots of number and mass distributions of aerosol particles from the rice cultures during aerosol generation runs. The aerodynamic particle sizer was interfaced with an Apple II$^+$ computer with all data stored on floppy disks. Number distributions for strain Budapest 1 (A) and Debrecen 1132 (C) and mass distributions for strain Budapest 1 (B) and Debrecen 1132 (D) are shown.

index of ca. 40, whereas cells treated with either SH or spore extracts showed a dose-dependent decline in stimulation index. The ED_{50}s for SH, Budapest 1 extract, and Debrecen 1132 extract were 0.8 nM, 0.14 μg/ml, and 0.06 μg/ml, respectively. The 95% confidence intervals for SH, Budapest 1 extract, and Debrecen 1132 extract were 0.62 to 1.00 nM, 0.12 to 0.17 μg/ml, and 0.05 to 0.17 μg/ml, respectively.

Analysis of individual filters for specific trichothecenes showed that each of the filters studied contained one or more of these compounds (Table 4). Because of the small amount of sample on each filter, it is possible that the compounds were present in other samples at concentrations below the

TABLE 1. Microscopic analysis of aerosol particles from rice cultured with *S. atra* Debrecen 1132

Slide no.	Conidia (*A*)	Hyphal fragments (*B*)	Other	Total[a]	% Conidia	% *A* + *B*[b]
1	751	44	53	848	88.6	93.8
2	537	36	62	635	84.6	90.2
3	591	49	62	702	84.2	91.2
4	461	41	66	568	81.2	88.4
5	736	43	68	847	86.9	92.0
6	734	53	74	861	85.3	91.4
7	434	30	53	517	83.9	89.7
8	655	50	41	746	87.8	94.5
9	613	68	92	773	79.3	88.1
10	571	55	40	666	85.7	94.0

[a] Total particles in 10 microscopic fields.
[b] Conidia and hyphal fragments combined.

TABLE 2. Inhibition of protein synthesis in rat AM by SH and *S. atra* conidial extracts

Sample[a]	Concn	cpm[b]	% of control
SH	0.1 μM	34.9 ± 4.1	11.1
	0.03	48.9 ± 4.6	15.6
	0.01	108.0 ± 23.6	34.5
	0.003	206.8 ± 48.2	66.0
	0.001	302.4 ± 52.9	96.5
	0 (control)	313.4 ± 81.3	
B	5.2 μg/ml	35.6 ± 3.1	5.2
	1.56	63.4 ± 9.2	9.3
	0.52	246.5 ± 85.4	36.2
	0.156	867.7 ± 191.5	127.4
	0.052	710.6 ± 169.8	104.4
	0 (control)	680.9 ± 144.6	
D	1.38 μg/ml	39.9 ± 4.9	5.9
	0.46	58.2 ± 13.8	8.5
	0.138	178.9 ± 55.8	26.3
	0.046	488.0 ± 76.1	71.7
	0.014	540.1 ± 114.0	79.3
	0.005	547.7 ± 90.0	80.4
	0.0014	632.8 ± 150.7	92.7
	0 (control)	680.9 ± 144.6	

[a] B, Extract of conidia obtained from Japan Red Rose Rice inoculated with strain Budapest 1; D, extract of conidia obtained from Uncle Ben's Converted Rice inoculated with strain Debrecen 1132.
[b] Average of four replicate cultures ± standard deviation.

TABLE 3. Inhibition of mouse thymocyte proliferation[a] by SH and *S. atra* conidial extracts

Sample concn[b]	cpm[c]		% of control	SI[d]
	With IL-1	Without IL-1		
SH				
0.025 μM	1,344.6 ± 225.6	1,049.1 ± 773.7	2.1	1.3
0.0075	1,928.0 ± 314.2	1,832.8 ± 1,531.3	3.1	1.1
0.0025	16,508.6 ± 2,778.9	1,788.4 ± 974.9	26.1	9.2
0.00075	44,174.6 ± 8,131.8	1,974.8 ± 1,479.3	69.9	22.4
0.00025	40,131.7 ± 6,975.9	1,493.2 ± 1,056.6	63.5	26.9
B				
0.65 μg/ml	3,597.1 ± 997.3	1,592.9 ± 1,470.3	5.7	2.3
0.325	20,321.6 ± 3,203.1	3,102.8 ± 2,403.0	32.2	6.5
0.163	35,380.4 ± 2,725.4	1,468.5 ± 1,008.3	56.0	24.1
0.081	44,112.3 ± 4,915.8	1,683.6 ± 1,013.5	69.8	26.2
0.041	48,061.9 ± 5,737.6	1,542.7 ± 719.5	76.1	31.2
0.020	57,192.6 ± 11,895.8	1,694.6 ± 1,294.2	90.5	33.7
D				
0.58 μg/ml	1,837.5 ± 415.8	1,219.9 ± 379.3	2.9	1.5
0.29	1,710.1 ± 906.3	1,301.0 ± 531.6	2.7	1.3
0.145	6,131.5 ± 628.5	966.6 ± 493.7	9.7	6.2
0.073	25,947.0 ± 4,650.7	1,374.4 ± 465.5	41.1	18.9
0.036	49,276.0 ± 5,840.4	1,478.4 ± 938.4	78.0	33.3
0.018	59,241.5 ± 12,333.8	1,432.7 ± 472.3	93.0	41.3
Untreated control	63,176.3 ± 9,126.8	1,638.6 ± 802.4		38.6

[a] Stimulated with crude IL-1 prepared in rat AM cultures treated with *Escherichia coli* lipopolysaccharide.
[b] B, Extract of conidia obtained from Japan Red Rose Rice inoculated with strain Budapest 1; D, extract of conidia obtained from Uncle Ben's Converted Rice inoculated with strain Debrecen 1132.
[c] Average of four replicate cultures ± standard deviation.
[d] Stimulation index.

limit of analysis. Since SH was detected in all samples, we can compare the samples with respect to the concentration of this toxin. SH ranged from 6.8 to 12.7 ng/mg of dust and was fairly constant in concentration from sample to sample. All of the filters except code no. 014231 were from rice culture Delbrecen 1132. The concentration of SH in this filter was comparable to the amount of SH in the other filters.

Stachybotrys spp. are much less frequently noted in surveys of airborne fungi in homes and offices than such familiar genera as *Cladosporium*, *Penicillium*, *Alternaria*, and *Aspergillus*, and this fact might suggest to some that they are of minor importance. Kozak et al. (11) reported a severe case of asthma in a small child. A water-damaged carpet in his home was extensively contaminated by a *Stachybotrys* sp., the patient gave a positive prick test to antigens prepared from a *Stachybotrys* sp. isolated from the carpet, and dramatic improvement in his asthma occurred after removal of the damaged carpet and pads and careful cleaning of the contaminated areas. In this instance, a *Stachybotrys* sp. was not detected in the viable mold count, yet was isolated from the carpet, and appeared to be the agent responsible for his illness. Since this fungus does not compete well with certain other fungi commonly found on the culture media used in mold-count surveys, it is likely that it is frequently an underestimated component of indoor air. On the other hand, whether *S. atra* acts by eliciting adverse immune responses or by direct toxicity or by a combination, the presence of viable cells in the air is not essential for its effect. Croft et al. (3) reported a similar heavy infestation of *S. atra* in a household in suburban Chicago. The occupants of this home suffered a variety of maladies over a period of 5 years which disappeared after the house had been thoroughly cleaned of the *S. atra*-contaminated material (ductwork, insulation, and ceiling material). Extracts of the contaminated household materials were toxic to experimental animals, and several highly toxic trichothecene mycotoxins were isolated from these extracts. Andrassy et al. (1) reported an outbreak of illness in farm workers caused by *S. atra* in heavily contaminated hay. The patients unanimously complained of dyspnea, airway obstruction, sore throat, bloody nose or nasal secretions, conjunctivitis, and inflammation of the skin on the face and body.

TABLE 4. Trichothecene content in *S. atra* conidia

Code no.[a]	Toxin amt[b] (ng)		
	SH	SG	TV
014231 (14.65)	100 (6.8)	ND[c]	ND
014219 (17.69)	225 (12.7)	100 (5.7)	80 (4.5)
014225 (6.94)	50 (7.2)	ND	ND
014241 (11.65)	120 (10.3)	80 (6.9)	ND
014243 (16.02)	180 (11.2)	100 (6.2)	50 (3.1)
000004 (14.81)	110 (7.4)	ND	50 (3.4)
000013 (32.74)	350 (10.7)	225 (6.9)	100 (3.1)

[a] Individual filter code no. Number in parentheses is the total weight (in milligrams) of dust collected.
[b] Amount of toxin found. Values in parentheses are the concentrations in nanograms of toxin per milligram of dust. Toxin abbreviations: SG, satratoxin G; TV, trichoverrols A and B.
[c] ND, Not detected. The limit of analysis is ca. 50 ng.

The trichothecene mycotoxins are acutely toxic to a variety of mammalian species (23), strongly inhibit protein, DNA, and RNA synthesis in eucaryotic cells (7, 24), and are immunotoxic in rats and mice (13, 19, 21, 22). Additionally, the symptoms of stachybotryotoxicosis in humans suggest immunotoxic effects (5). The finding of trichothecene mycotoxins in aerosolized respirable conidia of *S. atra* demonstrates the possibility for pulmonary exposure of workers to these highly toxic substances and may help explain the findings of previous workers of human stachybotryotoxicosis and other illness caused by exposure to moldy hay, contaminated ductwork, and carpets heavily contaminated by *S. atra*.

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