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Myrothecium: A new indoor contaminant?

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Abstract

In the natural environment *Myrothecium* species occur as soil or leaf surface saprobes or as weak plant pathogens. In addition, some species of *Myrothecium* are known to produce trichothecene mycotoxins. During a previous aerobiological investigation at two Las Vegas elementary schools, *Myrothecium* conidia were found to be the second most abundant spore type identified indoors from Burkard personal spore trap samples. The present study was undertaken to re-examine the schools to locate the source of *Myrothecium* spores and to examine the ability of *Myrothecium* to grow on indoor substrates. There were no obvious signs of contamination in the classrooms; however, *Myrothecium* spores occurred on about 30% of the Burkard samples. Two colonies of *Myrothecium* were identified from subcultures of the Andersen samples, and three colonies were identified from carpet dust samples. Culture studies showed that a strain of *Myrothecium cinctum* was able to grow on various culture media as well as on various indoor substrates including paper, cardboard, wallpaper, ceiling tiles, dry wall, carpets and cotton rug. Although there was no attempt to estimate any human health risks, these investigations are believed to be the first to document abundant *Myrothecium* spores from indoor air samples. © 1997 Elsevier Science Ireland Ltd.

Keywords: Myrothecium; Fungal spores; Mycotoxins; Indoor air

1. Introduction

In the natural environment *Myrothecium* species occur as soil or leaf surface saprobes or as weak plant pathogens causing leaf spot disease on various types of vegetation (Ellis and Ellis, 1985; Watanabe, 1994). It is considered a cosmopolitan genus on dead plant material, especially grasses. Studies have shown that *Myrothecium* spores have a biotin requirement for spore germination but not for mycelial growth (Griffin, 1994). Members of the genus *Myrothecium* are known to breakdown both starch and cellulose, and the cellulases of these fungi are important in the degradation of cotton textiles (Mueller and Loeffler, 1976).

The genus *Myrothecium* has gained notoriety in recent years because *M. verrucaria* and *M. roridum* are well known producers of mycotoxins (Bean et al., 1992; Bergman et al., 1992; Ueno, 1983; Moss, 1996). As a result, numerous studies have investigated the toxicity of the verrucarins and roridins, two types of macrocyclic trichothecene mycotoxins, produced by these fungi (Ueno, 1983; Moss, 1996). The macrocyclic trichothecenes are considered the most toxic trichothecenes. Studies have shown that these toxins are capable of causing a variety of adverse health effects including inhibition of protein synthesis, immune suppression, and impairment of alveolar macrophage function (Yang and Johanning, 1997). The macrocyclic trichothecenes also include satratoxins which are produced by another cellulolytic fungus, *Stachybotrys atra*. It should be noted that the spores as well as the mycelium of many toxigenic fungi contain mycotoxins. In fact, several studies have found airborne *Stachybotrys* conidia with trichothecene mycotoxins (Flannigan and Miller, 1994; Yang and Johanning, 1997).

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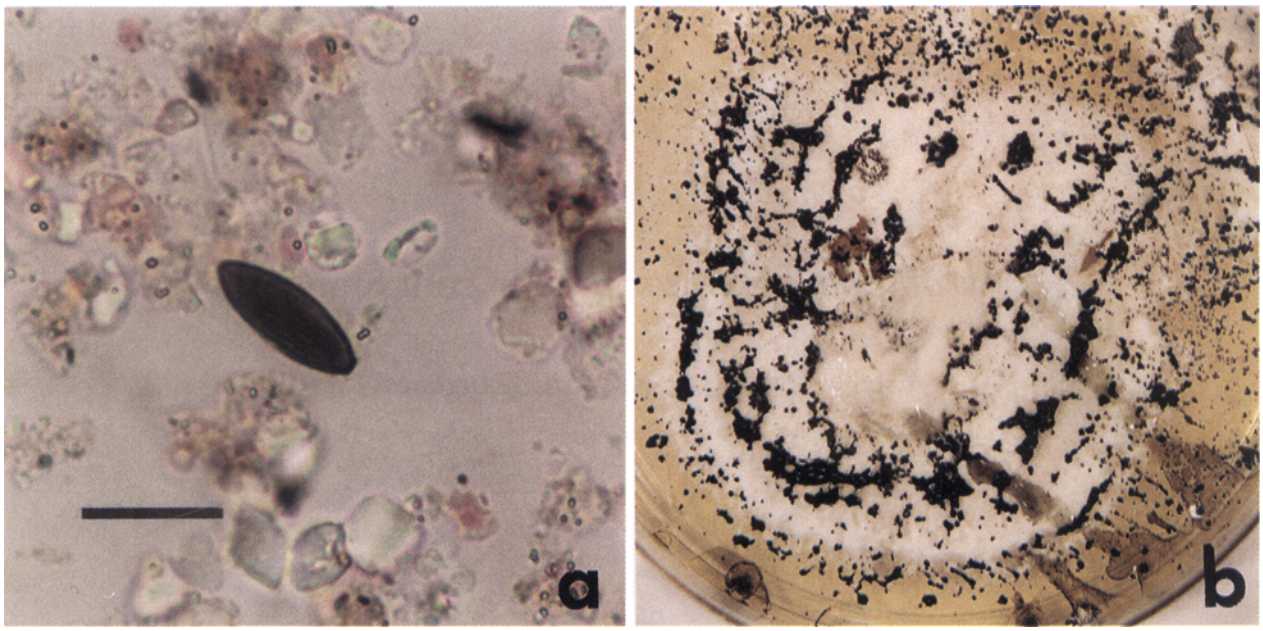

Fig. 1. *Myrothecium cinctum*: (a) conidium from a 1994 air sample at an elementary school in Las Vegas, Nevada (bar = 10 μm); (b) colony appearance growing on PDA showing abundant sporodochia.

Airborne *Myrothecium* conidia were identified during an aerobiological investigation at two elementary schools in Las Vegas, Nevada during the summer of 1994 (Levetin, 1995, Levetin et al., 1996). The study was part of the EPA School Evaluation Project and was designed to test modifications that had been made to the ventilation systems in the school. Data from that study showed that smut spores were the most abundant spore type identified indoors from Burkard Personal Spore Trap samples, and *Myrothecium* spores were the second most abundant spore type. Out of a total of 216 indoor Burkard samples (108 from each school), *Myrothecium* spores appeared on 125 slides (57.8% of the samples) and represented 16.8% of the total spore recovery. Although concentrations ranged up to 708 spores/m^3, the collective mean indoor concentration of *Myrothecium* spores at these two schools was 112 spores/m^3 (Levetin, 1995).

Many of the spores were identified as the species *Myrothecium cinctum* based on the distinct longitudinally striated appearance of the conidia (Ellis and Ellis, 1985). Spores were fusiform, approximately 4 × 12 μm, with longitudinal striations and a broad attachment (Fig. 1a). Other spores lacked striations and may belong to other species of *Myrothecium*. In contrast to the indoor occurrence, only four outdoor Burkard samples (out of 108) had *Myrothecium* spores. The indoor prevalence of these spores suggested an indoor source of *Myrothecium* may have been present at both schools. During the initial investigation at these schools, an equal number of Andersen samples for viable fungi were collected along with the Burkard samples. Surprisingly, only a single colony of *Myrothecium* was identified on the Andersen samples (Buttner et al., 1994).

The present study was undertaken to determine if *Myrothecium* could occur as an indoor contaminant. There were two goals: (1) to re-examine both schools in an effort to locate the sources of the *Myrothecium* spores, and (2) to examine the ability of *Myrothecium cinctum* to grow on common building materials and other indoor substrates.

2. Materials and methods

2.1. Sampling protocols

Air samples for both viable fungi and total spores were collected indoors in two classrooms as well as outdoors at each elementary school. Efforts were made

Table 1
Mean concentrations of airborne fungi at two Las Vegas elementary schools

Location	Mean concentration of viable fungi* (cfu/m^3)	Mean concentration of total spores** (spores/m^3)
School A		
Indoors	298	1383
Outdoors	232	2069
School B		
Indoors	142	1516
Outdoors	174	1038

* At each school 20 Andersen samples were collected indoors and eight outdoors.
** At each school 16 Burkard samples were collected indoors and seven outdoors.

Table 2
Fungal taxa identified during total spore analysis at school A

Taxa	Concentrations in spores/m^3			
	Room 17 mean*	Room 18 mean*	Indoor mean	Outdoor mean
Cladosporium	677	200	439	650
Basidiospores	217	238	228	30
Smut spores	316	131	224	773
Rust spores	119	21	70	8
Pencillium/Aspergillus	19	70	45	58
Myrothecium	34	49	42	—
Drechslera	63	7	35	8
Alternaria	40	22	31	56
Curvularia	—	7	4	—
Epicoccum	—	7	4	—
Stachybotrys	—	14	7	—
Ascospores	—	—	—	24
Spore fragments	79	38	59	—
Hyphal fragments	90	42	66	40
Other	138	179	159	365

* Mean of eight Burkard samples collected within each room.

Table 3
Fungal taxa identified during analysis of viable fungi at school A

Taxa	Concentrations in cfu/m^3			
	Room 17 mean*	Room 18 mean*	Indoor mean	Outdoor mean
Cladosporium	223	72	148	99
Penicillium	88	10	49	16
Yeast	45	33	39	58
Non-sporulating	54	22	38	32
Alternaria	9	10	10	12
Phoma	12	4	8	—
Pithomyces	1	4	3	—
Curvularia	4	—	2	—
Drechslera	4	—	2	3
Myrothecium	1	1	1	—
Aspergillus	—	1	<1	9
Chaetomium	—	—	—	2
Other	—	—	—	2

* Mean of ten Andersen samples collected within each room.

to duplicate the sampling locations as closely as possible to the previous study. However, one classroom in school B was unavailable for sampling, and it was necessary to use the next room. In addition, school A had been completely remodeled in the 16 months since the initial investigation. Four sets of air samples were collected at each school; three sets were collected during one sampling day, with an additional set of samples collected on a second day. Each set consisted of duplicate samples from each classroom and outdoors.

For viable fungi, samples were collected using paired Andersen single stage (N6) samplers containing malt extract agar and potato dextrose agar plates. Following sample collection, petri dishes were sealed with Parafilm for transport to the laboratory where they were incubated at room temperature for 5–7 days. Colonies were counted and identified microscopically. Positive hole corrections were applied to all the samples, and the concentration of viable fungi expressed as colony forming units/cubic meter (cfu/m^3) of air sampled. Because *Myrothecium* cultures normally do not produce spores for 1–3 weeks, all non-sporulating white or whitish colonies were subcultured onto malt extract agar and re-examined daily for several weeks.

Paired Burkard personal spore traps were used in the collection of total spore samples. Slides were precoated with a thin film of Lubriseal, and samples were collected for 5 or 10 min. Burkard slides were made permanent with glycerin jelly and analyzed microscopi-

Table 4
Fungal taxa identified during analysis of total spores at school B

Taxa	Concentration in spores/m^3			
	Room 52 mean*	Room 54 mean*	Indoor mean	Outdoor mean
Smut spores	225	496	361	212
Cladosporium	252	158	205	111
Basidiospores	176	197	187	116
Rust Spores	93	67	80	22
Drechslera	81	48	65	20
Ascospores	79	22	51	31
Myrothecium	46	44	45	19
Curvularia	63	—	32	46
Alternaria	42	14	28	56
Aureobasidium	21	14	18	11
Penicillium/Aspergillus	16	—	8	130
Arthrinum	—	8	4	-
Nigrospora	—	—	—	9
Spore fragments	121	82	102	9
Hyphal fragments	165	77	121	40
Other	290	132	211	169

* Mean of eight Burkard samples collected within each room.

Table 5
Fungal taxa identified during analysis of viable fungi at school B

Taxa	Concentrations in cfu/m^3			
	Room 52 mean*	Room 54 mean*	Indoor mean	Outdoor mean
Yeast	150	30	90	29
Non-sporulating	84	32	58	62
Cladosporium	61	15	38	37
Penicillium	21	17	19	15
Alternaria	16	4	10	20
Phoma	6	4	5	3
Aureobasidium	5	2	4	2
Drechslera	5	—	3	3
Pithomyces	3	5	4	—
Rhizopus	2	1	2	—
Arthrinum	—	—	—	2
Aspergillus	—	1	<1	2
Curvularia	—	—	—	2
Other	—	—	—	2

* Mean of ten Andersen samples collected within each room.

cally at 1000 × magnification. Spores were counted and identified. Airborne concentrations were calculated and expressed as spores/m^3 of air sampled.

In addition to air samples, dust (vacuum) samples, bulk samples, and tape samples were also collected from both schools. Dust samples were collected onto sterile filters held in a dust trap attached to the hose of a Hoover heavy duty vacuum cleaner. A 1 m^2 area of carpet was vacuumed for 5 min, the filter was removed and transferred to a plastic bag and sealed. The dust trap was cleaned with 70% ethanol solution after each sample collection and all samples were stored separately. The dust samples were sieved (45 μm pore size) to remove large particles, and the fine dust was weighed and dilution plated (with duplicates) onto malt extract agar containing streptomycin (500 μg/ml). Colonies were counted and identified microscopically. Counts were expressed as cfu/g of dust. All white or whitish non-sporulating colonies were subcultured as described above.

Bulk samples were collected from the ventilation system. This included insulation samples as well as drain pan residues. Materials were weighed and incubated on a rotator in 10 ml of sterile distilled water for 30 min. The resulting suspension was dilution plated (with duplicates) and treated as above. The results were expressed as cfu/g of material. Tape samples were examined microscopically.

Table 6
Mean concentrations of viable fungi isolated from dust samples at two Las Vegas Elementary schools

Sample	School	Site	Total fungi (cfu/g)	Fungal taxa (cfu/g)
V-1	A	Room 18	5.9×10^4	1.2×10^4 *Cladosporium* 3.2×10^4 Non-sporulating 9.0×10^3 Yeast 3.5×10^3 *Alternaria* 1.5×10^3 *Drechslera* 1.0×10^3 *Fusarium* 5.0×10^2 *Epicoccum* 5.0×10^2 *Pithomyces*
V-2	A	Room 18	2.0×10^6	8.6×10^5 Yeast 8.2×10^5 Non-sporulating 1.8×10^5 *Cladosporium* 2.0×10^4 *Drechslera* 2.0×10^4 *Penicillium* 4.0×10^4 *Alternaria* 4.0×10^4 *Fusarium* 4.0×10^4 *Myrothecium*
V-3	A	Room 17	5.7×10^4	2.7×10^4 Non-sporulating 8.0×10^3 *Cladosporium* 7.5×10^3 Yeast 6.0×10^3 *Alternaria* 3.0×10^3 *Penicillium* 2.0×10^3 *Drechslera* 1.5×10^3 *Fusarium* 5.0×10^2 *Aspergillus* 5.0×10^2 *Epicoccum* 5.0×10^2 *Myrothecium* 5.0×10^2 *Rhizopus*
V-4	A	Room 17	1.8×10^5	1.1×10^5 Non-sporulating 2.6×10^4 Yeast 1.9×10^4 *Penicillium* 1.5×10^4 *Cladosporium* 5.0×10^3 *Myrothecium* 3.0×10^3 *Fusarium* 1.0×10^3 *Nigrospora*
V-5	B	Room 54	8.6×10^4	5.9×10^4 Non-sporulating 1.9×10^4 Yeast 2.0×10^3 *Cladosporium* 2.0×10^3 *Fusarium* 1.5×10^3 *Alternaria* 1.5×10^3 *Penicillium* 5.0×10^2 *Aspergillus* 5.0×10^2 *Rhizopus*
V-6	B	Room 52	2.0×10^5	1.8×10^5 Yeast 1.4×10^4 Non-sporulating 2.5×10^3 *Drechslera* 2.5×10^3 *Penicillium* 5.0×10^2 *Cladosporium*
V-7	B	Room 52	5.6×10^4	2.9×10^4 Non-sporulating 2.1×10^4 Yeast 1.0×10^3 *Alternaria* 1.0×10^3 *Cladosporium* 1.0×10^3 *Fusarium* 1.0×10^3 *Penicillium* 1.0×10^3 Other 5.0×10^2 *Aspergillus* 5.0×10^2 *Pestalotiopsis* 5.0×10^2 *Rhizopus*
V-8	B	Room 54	4.7×10^4	2.0×10^4 Non-sporulating 1.8×10^4 *Yeast* 3.0×10^3 *Alternaria* 2.5×10^3 *Drechslera* 1.5×10^3 *Penicillium* 1.0×10^3 *Cladosporium* 5.0×10^2 *Epicoccum* 5.0×10^2 *Fusarium* 5.0×10^2 *Pithomyces*

2.2. Culture studies

Myrothecium cinctum (ATCC # 22270) was subcultured onto a variety of common laboratory media used for fungal growth including malt extract agar (MEA), potato dextrose agar (PDA), corn meal agar (CMA), Czapek's solution agar (CSA) and dichloran–glycerol agar (DG-18). Cultures were incubated at room temperature and examined daily for fungal growth and formation of conidia.

Portions of various building materials and other indoor substrates were placed in glass petri dishes, wrapped in aluminum foil, and sterilized by autoclaving for 15 min. Materials included used fiberglass insulation, dry wall, ceiling tile, prepasted wallpaper, cotton fabric, cotton throw rug, synthetic pile carpet, writing paper and cardboard. Aliquots of sterile distilled water, ranging from 3 to 25 ml, were added to each petri dish to thoroughly moisten each substrate. Each material was uniformly inoculated with a cylinder (4 mm diameter) cut from the perimeter of an actively growing culture of *Myrothecium cinctum*. Cultures were incubated at room temperature and examined periodically for growth and conidia formation.

3. Results

The two Las Vegas elementary schools were revisited, and air samples collected on 3 days in November 1995. A total of 102 air samples was collected consisting of 56 Andersen samples for viable fungi (40 indoors and 16 outdoors) and 46 Burkard samples for total spores (32 indoors and 14 outdoors). In addition eight dust samples, seven bulk samples, and 16 tape samples were also gathered.

The concentrations of airborne viable fungi and total spores were relatively low both indoors and outdoors (Table 1). There were no obvious signs of indoor contamination within either building. At school A, *Cladosporium* was the most abundant taxon identified indoors from both Burkard and Andersen samples. On the Burkard samples from school B, smut spores were the most frequent spore type identified, while yeast

Table 7
Growth and spore formation of *Myrothecium cinctum* on various culture media

Medium	Days to reach maximum colony size	Days until sporodochia visible
MEA	28	7
CSA	20	10
CMA	20	7
PDA	25	7
DG-18	—*	—**

* No mycelial growth.
** No sporodochia on DG-18.

colonies were the most abundant fungal type found on the Andersen samples. The taxa identified from all the air samples are summarized in Tables 2–5.

Myrothecium spores occurred on approximately 30% of the Burkard samples. The mean indoor concentration of *Myrothecium* spores was 45 spore/m^3 at one school and 42 spores/m^3 at the second school (Tables 2 and 4). The maximum concentration of *Myrothecium* conidia was 260 spores/m^3 in Room 52 of school B. Overall, *Myrothecium* conidia accounted for 2.9% of the total indoor spore recovery at each school. In contrast, *Myrothecium* spores were identified on only one outdoor sample.

No *Myrothecium* colonies were identified directly on the Andersen samples. Approximately 40 non-sporulating white or whitish colonies were subcultured from the Andersen plates and two colonies of *Myrothecium* were identified from these subcultures (Table 3). Both were from school A, but they were from separate classrooms.

All cultures from the carpet dust samples showed extensive fungal growth with a variety of different fungi (Table 6). No *Myrothecium* colonies were directly identified from these samples, but all white or whitish non-sporulating colonies were subcultured. Colonies of *Myrothecium* were identified on subcultures obtained from three of eight dust samples. Again, these were from both classrooms in school A (Table 6).

Cultures resulting from dilution plating of bulk samples also exhibited abundant fungal colonies. None of the colonies or subcultures from these developed into *Myrothecium*, and no evidence of *Myrothecium* spores was seen on the tape samples. Therefore, data from these analyses are not reported here.

Laboratory culture studies showed that the strain of *Myrothecium cinctum* was able to grow on various culture media, although spores generally did not develop for 1–2 weeks following inoculation. In culture, the fungus generally showed a floccose white mycelium that gradually developed black sporodochia (cushion-shaped aggregations of conidiophores) containing dense clusters of slimy conidia (Fig. 1b). The conidia start out pale yellow or green and only develop deep green pigmentation as the spores mature. In some older cultures the conidia appear brown. The fungus was able to grow readily on MEA, PDA, CMA, CSA (Table 7). On DG-18 a very small yeast-like colony appeared around the initial inoculum. There was no further development of the colony, and the only conidia were those that developed on the inoculum cylinder.

Building material studies showed that *Myrothecium cinctum* was able to grow and reproduce on various indoor substrates including paper, cardboard, the pre-pasted back of the wallpaper, ceiling tiles, dry wall, jute carpet backing, and the cotton throw rug (Table 8). In several of these it was difficult to detect the mycelium within the substrate and a qualitative scale was used to describe the mycelial development. Sporodochia occurred on all substrates but were most abundant on the cotton throw rug and the back side of the wallpaper.

Table 8
Growth and spore formation of *Myrothecium cinctum* on various building materials and indoor substrates

Substrate	Extent of mycelial development	Extent of spore formation
Insulation	+	++
Dry wall	++++	++
Ceiling tile	+++	++
Wallpaper	++++	++++
Cotton fabric	+++	+++
Throw rug	+++	++++
Pile carpet	++	+
Writing paper	+	++
Cardboard	+++	++

+, scant; ++, limited; +++, moderate; ++++, abundant.

4. Discussion

As in the previous investigation *Myrothecium* was identified from air samples at both elementary schools. *Myrothecium* spores occurred on approximately 30% of the Burkard samples, but the concentrations were generally lower than in the 1994 investigation (Levetin, 1995; Levetin et al., 1996). Two *Myrothecium* colonies were identified from subcultures of the 40 indoor Andersen plates representing a 2.5% occurrence. In the previous investigation only one *Myrothecium* colony was identified from 216 indoor Andersen samples at these schools for a 0.46% occurrence (Buttner et al., 1994); however, there had been no attempts to subculture any non-sporulating colonies.

In both investigations there was a considerable difference between the recovery of *Myrothecium* on the Burkard samples for total spores and Andersen samples for viable fungi. Even though *Myrothecium* is capable of growing on both MEA and PDA plates which were used in the Andersen samplers, few viable colonies were

recovered from air samples. There are several possible explanations for these results. The biotin requirement for germination (Griffin, 1994) may prevent the establishment of colonies on certain culture media. In addition, it is possible that *Myrothecium* spores may lose viability quickly. While the spores of some species remain viable for months or years, the spores of other fungi, such as *Botrytis cinera* and *Stachybotrys atra*, rapidly decline in viability (Flannigan and Miller, 1994). However, it is also possible that the slimy *Myrothecium* spores are not well adapted for airborne dispersal in indoor air, and only spores from desiccated, senescent colonies become airborne in dry indoor air.

In the natural environment spores produced in a slimy polysaccharide layer are typically dispersed by rain splash (Fitt et al., 1989; Huber and Gillespie, 1992). The slime inhibits their direct removal by wind; however, it also protects the spores from desiccation during dry weather. The first raindrops dissolve the mucilage and leave a spore suspension available for splash dispersal by additional raindrops. While very large droplets carrying spores are unaffected by wind, small droplets may evaporate and allow spores to be dispersed through the air as a true aerosol. Rain splash generally confines dispersal to periods when the wet conditions are also favorable for germination on new substrates. In the indoor environment such splash dispersal would not normally be possible, and dissemination of spores may only occur as colonies age and dry up. Although the dried slime layer would no longer inhibit dispersal, the drying may be accompanied by a loss of spore viability. It should be noted that there is no evidence that loss of viability would reduce the potency of any mycotoxins within the spore (Flannigan and Miller, 1994).

The initial investigation at both elementary schools was conducted during the summer of 1994. Since both schools had year-round programs, classes were in session and air conditioning was running. The present study was conducted 16 months later during the late fall. No air conditioning was in use; in fact, all the classrooms were heated. In addition, the classrooms in school A had been completely remodeled since the initial study. Although these variables clearly altered the environment, evidence of *Myrothecium* was still present in both schools. As in the previous investigation, the outdoor airborne concentrations of *Myrothecium* conidia were substantially lower than indoors suggesting a possible indoor source.

Although no visible evidence of contamination was found, *Myrothecium* was identified in the vacuum dust collected at school A as well as in the air samples. Carpeting can serve as a reservoir for settled spores from the indoor environment, but it can also serve as an amplification site for fungal contamination. The viable *Myrothecium* propagules from the carpet dust may have originated from settled airborne spores or from contamination in the carpeting itself. The laboratory studies have shown that *M. cinctum* is able to grow on carpet backing as well as many other indoor substrates.

The culture studies showed that colonies of *M. cinctum* were able to develop on a variety of culture media as well as the building materials. Only DG-18 could not support the growth of the fungus. Since DG-18 is a xerophilic medium (Hocking and Pitt, 1980), it suggests that *M. cinctum* has a high water activity requirement.

Although there was no attempt to establish any human health risks due to the presence of this fungus, the investigations at these schools in both 1994 and 1995 are believed to be the first to document abundant *Myrothecium* spores from indoor air samples. It is worth noting that since the initial investigation, *Myrothecium* conidia have also been identified on Burkard samples from schools in Santa Fe, New Mexico and Kansas City, Kansas as well as from a home in Tulsa, Oklahoma (Levetin, unpublished observations). Because of the ability of *Myrothecium* to grow on indoor materials and because some species of *Myrothecium* are capable of producing mycotoxins which are similar to *Stachybotrys* toxins, the presence of this genus as an indoor air contaminant must be regarded as a potential health threat.

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