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Respiratory Illness in Workers of an Indoor Shiitake Mushroom Farm

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Shiitake mushrooms *(Lentinus edodes)*, the second most important mushroom in world commerce, are usually grown on logs outdoors. Because of the relatively short harvesting period and weather dependency of shiitake strains that are cultivated outdoors, there is a trend toward indoor cultivation of strains that can permit year-round production. An evaluation of an indoor shiitake farm was conducted following reports that workers of the facility were experiencing symptoms of respiratory illness. During private interviews that included the administration of a questionnaire, 11 workers reported seven or more symptoms which they had experienced either during work or shortly after leaving work; six of these workers reported 15 or more symptoms. Predominant symptoms were dry cough, nasal discharge, sneezing, chest tightness, productive cough, and dyspnea. Based upon the results of the acute symptoms questionnaire and preliminary bioaerosol sampling, the management of the shiitake farm discontinued operations until implementation of an interim respiratory protection program. After operations resumed, additional bioaerosol screening was conducted with two-stage impactors and all-glass impingers for culturable mesophilic and thermophilic bacteria and fungal spores. Air concentrations of culturable fungal spores measured with the impactor exceeded 8.4×10^3 colony forming units/m^3. The highest airborne fungal spore concentration measured with the all-glass impingers was 2.4×10^6 spores/m^3. Sampling results showed extensive airborne contamination with a variety of *Penicillium* species, which produce respirable-size spores that may function as potent allergens. While the risk of respiratory illness from the inhalation of shiitake spores has been suggested previously, this is a report of an additional respiratory health risk associated with exposures to airborne allergenic molds in shiitake farming. Lenhart, S.W.; Cole, E.C.: Respiratory Illness in Workers of an Indoor Shiitake Mushroom Farm. Appl. Occup. Environ. Hyg. 8(2):112-119; 1993.

Introduction

Commercial mushroom production in the United States is dominated by the conventional button mushroom *(Agaricus bisporus)* with an annual production for 1989-1990 (July 1-June 30) of 715 million pounds worth $645 million.[1] The second most important mushroom produced in the United States and in world commerce is the shiitake *(Lentinus edodes)*. Annual sales of shiitake mushrooms in the United States for 1989-1990 were 3.75 million pounds with a value of $16.6 million.[1]

The methods used for commercial cultivation of shiitake mushrooms differ significantly from the production process of button mushrooms, which has been described elsewhere.[2-5] Shiitake mushrooms are not cultivated in compost or grown in the dark, as are button mushrooms. (Light is required during the fruiting of shiitake for proper mushroom shape and color.[6]) Rather, shiitake are grown on logs usually outdoors, but more recently indoors as well. Also, while button mushrooms are harvested before spore formation has occurred, shiitake are mature and sporulating at harvest.

An evaluation of a newly constructed indoor shiitake mushroom farm was conducted in 1989, after a county health director received reports that workers of the facility were experiencing symptoms of respiratory illness. A strain of shiitake is grown at this facility which permits year-round indoor production primarily for the fresh market.

The cultivation process of shiitake at the facility studied began with inoculation of mushroom mycelia (spawn) into recently felled red and white oak logs from 1.2 to 1.5 m in length and from 100 to 130 mm in diameter. This process is commonly referred to as "planting." Prior to planting, each log was sprayed with a 0.1 percent solution of copper sulfate pentahydrate and water to control contamination by endogenous fungi. Next, several 12-mm diameter holes were drilled to a depth of 25 to 50 mm around the circumference

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of each log. Each hole was then filled and compacted with a mixture of spawn and sawdust by a person using a hand-held pneumatic tool. (Planters are shown inoculating logs in Figure 1.) Planting is a seasonal activity which occurs only between fall and spring. Therefore, the majority of workers at this facility were local residents hired temporarily to work in the planting area. Depending upon the availability of logs, 500 to 1000 logs were inoculated during a work shift; approximately 50,000 logs were inoculated each planting season.

After hot wax was applied with a brush to seal each plug, inoculated logs were moved from the planting area to an area designated as the culture force room. Logs remained in the culture force room for 10 to 12 months where the temperature and relative humidity were controlled to permit optimum growth of the spawn across and into the wood. Once a group of logs was completely colonized, the logs were then ready for mushroom production, which involves a four-step fruiting cycle consisting of induction, pinning, fruiting, and resting.

The induction step began by manually moving colonized logs on carts from the culture force room to a cool shower room. During induction, environmental stresses (i.e., a sudden drop in temperature, increased moisture, and depleted nutrients) bring about a change in the mycelium, which shifts from its vegetative phase to the production of fruiting bodies.[6] After induction, logs were moved to a germination room for pinning. Hyphae aggregate to form knots at the wood/bark interface; at this earliest stage of their growth, mushrooms are called pins or primordia. Mushrooms are from 85 to 95 percent water and require a considerable water reserve in the substrate to mature. The water shower of the induction step thoroughly saturates the logs and provides the large water reservoir needed for mushroom development.[6]

After the pinning step, the logs were moved to the growth room for harvesting of a flush (i.e., all the mushrooms on a group of logs subjected to the same induction conditions). This fruiting step begins after the primordia have emerged and a cap and stem are discernible. (Shiitake are shown in Figure 2 prior to being harvested.) After a harvesting period of approximately 1 week, logs were returned to a culture control room for a period of 1 to 2 months. The four-step fruiting cycle was repeated at the conclusion of each resting step. During the resting step, the mycelium recovers from fruiting and replenishes depleted nutrients in preparation for further fruiting. The environment in a culture control room is warm and humid, with a temperature of 25°C and a relative humidity of 90 percent. In addition to moving carts of logs among the various rooms and assisting with harvesting activities, employees also spend part of their work shift in the culture control room using paint scrapers to remove extensive, visible fungal contamination from the bark of logs during the resting step of the fruiting cycle.

FIGURE 1. Planters inoculating drilled oak logs with a mixture of shiitake spawn and sawdust.

Methods

Two site visits were conducted at the indoor shiitake farm soon after the request for evaluation was received. During the first visit, a questionnaire was administered privately to each of the 14 hourly employees of the facility involved in shiitake cultivation, and preliminary bioaerosol sampling was conducted. Additional bioaerosol sampling was conducted at various locations of the facility during the second visit. The questionnaire was administered with the informed consent of each worker and consisted of a demographics section (name, address, birth date, race, sex, and marital status), a tobacco smoking history, and the 23 acute symptoms listed in Table I. Workers were asked to respond affirmatively to those symptoms they had experienced either during or shortly after leaving work, and that they believed were associated with their job. They were also asked whether they ever had hay fever or any type of allergies.

A short-term air sample was collected in the center of the culture control room for airborne spores. The sampling train consisted of an open-face, three-piece 37-mm cassette containing a 5-μm polyvinylchloride filter and cellulose support pad connected by flexible tubing to a personal sampling pump operated at a flow rate of 1.5 L/min. The sampling duration was 55 minutes. At the conclusion of sampling, the cassette was delivered to the laboratory for analysis. The filter was removed from its cassette, placed in 5 ml of 0.9 percent sterile normal saline, and vortexed vigorously with glass beads for 5 minutes. A Neubauer haemocytometer[7] was used to quantitate spores per volume of saline and per cubic meter of sampled air.

A 2-cm × 2-cm section of material cut from the coarse filter of one of the four overhead air-conditioning units in the mushroom growth room was placed in 10 ml of 0.9 percent sterile normal saline and vortexed vigorously for 5 minutes. Quantitation of spore content was achieved with a

FIGURE 2. Growth room of an indoor shiitake mushroom farm.

Neubauer haemocytometer, and spores were sized using a Porton graticule[8,9] at a magnification of 430 ×.

Andersen two-stage microbial impactors[10-12] and all-glass impingers (AGI-30)[13-15] were used during sampling for culturable bioaerosols conducted during the second site visit at the mushroom farm. Bioaerosol monitoring was conducted with Andersen samplers at five locations of the facility. Three 10-minute air samples were collected at each of three locations in Building 1 where shiitake cultivation occurs on oak logs: 1) in the hallway near the culture control rooms during log scraping, 2) in culture control room #1 during log scraping, and 3) in culture force room #1. Three 10-minute air samples were also collected at a fourth sampling location just outside Building 1. Two 15-minute air samples were collected at the fifth sampling location in a jar breaking room of Building 2 where shiitake cultivation occurs on artificial growth media.

Andersen samplers were operated at a flow rate of 28.3 L/min. Plates containing 20 ml of trypticase soy agar (TSA) were used in each sampler to isolate and quantitate airborne bacteria, and were incubated at 25°, 37°, and 55°C to isolate mesophilic and thermophilic microorganisms. Plates containing 20 ml of MacConkey agar (MAC) were used for the selective isolation and quantitation of gram-negative bacteria and were incubated at 37°C. Plates containing 20 ml of Sabouraud dextrose agar (SDA) were used to isolate and quantitate fungi (molds and yeasts) and were incubated at 25°C. Airborne microbial concentrations were calculated using positive-hole corrections.[16]

Bioaerosol sampling with AGI-30 was conducted at four locations of the facility. In Building 1, a 15-minute air sample was collected in a hallway near the culture control rooms during log scraping, and a 40-minute air sample was collected in culture control room #1 during log scraping. In Building 2, a 15-minute air sample was collected at two locations during jar breaking and during mushroom harvesting.

Each AGI-30 contained 30 ml of sterile 0.9 percent sodium chloride solution and was operated at a flow rate of 12.5 L/min. Each collection fluid was thoroughly mixed and inoculated into a Neubauer haemocytometer to quantitate spores for subsequent calculation of airborne concentrations. Collection fluids were then centrifuged for 12 minutes at 3500 rpm with sediment reconstituted in 3.0 ml of the supernatant. TSA and SDA plates were inoculated with 0.1 ml aliquots and incubated at 25° and 37°C to identify predominant molds.

Results

Questionnaires were analyzed from 14 workers. Nine women and five men had worked at the mushroom farm for periods ranging from 1 to 32 weeks (mean period: 13 weeks). Their ages ranged from 21 to 48 years (mean: 32 years), and seven workers were current smokers. One of the exsmokers

TABLE I. Acute Symptoms Reported by 14 Workers of an Indoor Shiitake Mushroom Farm

Acute Symptom	Number of Workers Reporting Symptom	Nonsmokers and Exsmokers Reporting Symptom (n = 7)	Smokers Reporting Symptom (n = 7)
Dry cough	11	5	6
Nasal discharge	11	6	5
Sneezing	11	6	5
Chest tightness or heaviness	10	5	5
Cough with fluid production	9	4	5
Dyspnea	9	5	4
Malaise	8	4	4
Nasal congestion	8	4	4
Rash	8	5	3
Chills	7	3	4
Headache	7	4	3
Nausea	7	5	2
Nose irritation	7	3	4
Sinus congestion	7	4	3
Throat irritation	7	4	3
Wheezing	6	3	3
Eye irritation	5	4	1
Fever	5	3	2
Joint pain	5	2	3
Upper airway irritation	5	0	5
Chest pain	4	2	2
Muscle weakness	3	2	1
Muscle pain	3	2	1

reported a history of both hay fever and allergies; two nonsmokers and one smoker reported a history of allergies alone. Seven workers reported that since beginning work at the facility they had each received treatment by their own private physician and had taken antibiotics for respiratory problems, but that their symptoms had not subsided.

The total number of workers, the number of nonsmokers and exsmokers, and the number of smokers who answered affirmatively to each of the 23 acute symptoms on the questionnaire are presented in Table I. Eleven of the 14 workers interviewed reported seven or more work-related symptoms that they had experienced either during work or shortly after leaving work. Six of these 11 workers reported 15 or more symptoms. The most prevalent symptoms reported were dry cough, nasal discharge, and sneezing (11 of 14), followed by chest tightness (10 of 14), and cough with fluid production and dyspnea (9 of 14).

Because the total number of workers interviewed is small, application of statistical methods for investigating differences between the reporting of symptoms by nonsmokers and exsmokers and by smokers would be of questionable value. However, it can be suggested from the data that for the vast majority of the acute symptoms there was essentially no difference between the number of workers from each category who reported a particular symptom. Possible exceptions are nausea, eye irritation, and upper airway irritation.

A concentration of 3.3×10^6 spores/m^3 was determined from the area air sample collected by filter cassette in the center of the culture control room. An estimated 5×10^7 spores/cm^2 were contained on the small section of filter cut from one of the four air-conditioning units in the mushroom growth room. The spores from this filter had an ellipsoid shape with a width of approximately 3.5 μm and a length of approximately 7 μm. Their shape, dimensions, and overall appearance were consistent with those of shiitake spores.[17]

Air concentrations from impactor samples for mesophilic bacteria (incubated at 25°C) measured inside Building 1 were $> 8.3 \times 10^3$ colony forming units (CFU)/m^3 in the hallway during log scraping, 5.8×10^3 CFU/m^3 in culture control room #1 during log scraping, and $> 4.3 \times 10^3$ CFU/m^3 in culture force room #1. The air concentration measured outside Building 1 was 268 CFU/m^3.

Air concentrations of mesophilic bacteria (incubated at 37°C) measured inside Building 1 were 4.1×10^3 CFU/m^3 in the hallway during log scraping, 2×10^3 CFU/m^3 in culture control room #1 during log scraping, and 410 CFU/m^3 in culture force room #1. An air concentration of 1×10^3 CFU/m^3 was measured in the jar breaking room of Building 2. The air concentration measured outside Building 1 was 2×10^3 CFU/m^3. The predominant microorganisms identified were *Bacillus* and *Micrococcus* species.

Air concentrations of thermophilic bacteria were 4 CFU/m^3 outside Building 1, 4 CFU/m^3 in the hallway during log scraping, and 8 CFU/m^3 in culture force room #1. Air concentrations of gram-negative bacteria were 14 CFU/m^3 outside Building 1, 42 CFU/m^3 in the hallway during log

TABLE II. Results of Sampling for Airborne Mold Spores Using Andersen Two-Stage Microbial Impactors[A]

Location (Activity)	Temperature (°C)/ Relative Humidity (%)	Concentration (CFU/m³) Nonrespirable (Stage 1)	Respirable[B] (Stage 2)	Predominant Mold
Building 1				
Hallway (log scraping)	23/63	$>4.2 \times 10^3$	$>4.2 \times 10^3$	*Penicillium* sp.
Culture control (log scraping)	25/89	$>4.2 \times 10^3$	$>4.2 \times 10^3$	*Penicillium* sp.
Culture force (none)	22/86	2.3×10^3	$>4.2 \times 10^3$	*Penicillium* sp.
Outside (none)	19/54	1.6×10^3	0.6×10^3	*Cladosporium* sp.
Building 2				
Small room (jar breaking)	22/62	$>4.2 \times 10^3$	$>4.2 \times 10^3$	*Penicillium* sp.

Note: Each Petri dish contained 20 ml of Sabouraud dextrose agar and was incubated at 25°C under fluorescent light.
[A]One air sample collected for mold at each location.
[B]Particles collected have an aerodynamic mass median diameter of 7 μm or less.

scraping, 67 CFU/m³ in culture control room #1 during log scraping, and 4 CFU/m³ in culture force room #1.

The results of bioaerosol sampling for culturable airborne fungal spores collected with Andersen two-stage microbial impactors and for airborne fungal spores collected with AGI-30 are presented in Tables II and III, respectively. The air concentration of culturable mold spores collected outside the facility was 2.2×10^3 CFU/m³ and consisted predominantly of *Cladosporium* species. In contrast, the sampling plates from the four locations inside the facility had fungal growth at all 200 impaction points of each stage with the exception of stage one of the sample collected in culture force room #1, and consisted predominantly of *Penicillium* species. The total air concentration of culturable airborne fungal spores at each of the four inside sampling locations exceeded 8.4×10^3 CFU/m³. The highest air concentration of fungal spores measured with the AGI-30 was 2.4×10^6 spores/m³ in culture control room #1, as determined by haemocytometer count and *Penicillium* spore morphology. The three other samples collected with the AGI-30 consisted predominantly of *Penicillium* species, as quantitated and identified upon growth culture.

Discussion

The area dedicated for shiitake cultivation on natural wood undercover and indoors more than doubled in the United States between 1988–1989 and 1989–1990 from 244,000 sq ft to 541,000 sq ft; the area of these logs reported in 1987–1988 was only 27,000 sq ft.[1] In contrast, the area dedicated for shiitake cultivation on natural wood outdoors declined between 1988–1989 and 1989–1990 from 561,000 sq ft to 485,000 sq ft.[1] The increase in indoor cultivation of shiitake suggests an important trend that could result in additional workers being employed in this industry. Therefore, it is important that the health risks associated with the indoor cultivation of shiitake mushrooms be understood.

The wide spectrum of symptoms reported by several workers of this shiitake farm suggests the existence of work-related allergic rhinitis[18] (nasal discharge and congestion, and sneezing), bronchitis[19–21] (productive cough and dyspnea), asthma[21–23] (cough, chest tightness, dyspnea, and wheezing), and, to a lesser extent, hypersensitivity pneumonitis[20,21,24,25] (dry cough, dyspnea, malaise, chills, headache, nausea, fever, and joint and muscle pain) and organic dust toxic syndrome[21,26–28] (dry cough, chills, fever, and joint pain). Unfortunately, because medical evaluations of the workers were not conducted, definitive diagnoses of their conditions and the extent of their illnesses could not be made.

The consistently warm temperatures and high relative humidities needed throughout an indoor shiitake farm to promote mycelia and mushroom growth are also ideal for the growth of molds. Initial antifungal treatment of the oak logs was apparently not effective for long time periods.

TABLE III. Results of Sampling for Airborne Mold Spores Using AGI-30[A,B]

Location (Activity)	Temperature (°C)/ Relative Humidity (%)	Concentration[C] (Spores/m³)	Predominant Mold
Building 1			
Hallway (log scraping)	23/63	4.2×10^3	*Penicillium* sp.
Culture control (log scraping)	25/89	2.4×10^6	*Penicillium* sp.
Building 2			
Growth room (harvesting)	22/62	1.4×10^4	*Penicillium* sp.
Small room (jar breaking)	22/62	1.2×10^4	*Penicillium* sp.

Note: 0.1 ml aliquots of mixed collection fluid were inoculated onto Sabouraud dextrose agar and incubated at 25°C.
[A]One 15-minute air sample collected at each location, except for a 40-minute sample collected at the culture control location.
[B]Collection fluid: 30 ml sterile saline 0.9 percent.
[C]Determined upon growth culture, except for the culture control sample, which was determined by haemocytometer count.

Bioaerosol sampling at various locations of the indoor shiitake farm resulted in the reporting of high concentrations of culturable airborne mold spores consisting predominantly of *Penicillium* species. *Penicillium* species are among the most common aeroallergenic fungi.[29] Their spores are of respirable size (3 to 4 μm) and they have been implicated as the cause of respiratory illness in both domestic[30] and occupational environments.[31-33] Hypersensitivity pneumonitis and asthma are the most frequently reported respiratory disorders caused by exposure to *Penicillium* spores.[31-34]

Shiitake mushrooms are grown typically on red and white oak logs. Although work-related wood dust exposures have been associated with the development of rhinitis, bronchitis, asthma, hypersensitivity pneumonitis, and organic dust toxic syndrome,[28,35,36] only one case of asthma related to oak dust exposure was found to be reported in the literature.[37] Therefore, exposure to oak dust alone would seem to be an unlikely cause for the widespread illness among the workers of the facility studied. Exposure to mold-contaminated wood has been reported to cause respiratory illness, and cutting oak logs contaminated with *Penicillium* species was suspected to cause a case of hypersensitivity pneumonitis in a tree cutter employed by a municipality.[38]

The potential also exists for workers on shiitake farms to develop respiratory illness associated with inhalation exposures to mushroom spores.[39-42] The millions of shiitake spores collected by the filter of an air-conditioning unit in the mushroom growth room is evidence that the spores become airborne. Shiitake release tremendous numbers of spores into the air because, unlike button mushrooms, they are harvested at maturity during active spore release. Therefore, shiitake spores are released during cap growth prior to attaining their marketable size.[42] As with *Penicillium* spores, hypersensitivity pneumonitis and asthma are the respiratory disorders reported to be most frequently associated with exposure to shiitake spores.[39-42]

Respirators are often used to protect workers from inhalation of airborne contaminants in environments where engineering controls are not feasible or remain to be developed. However, conflicting reports have been published concerning the effectiveness of respirators as a means of protecting both sensitized and healthy individuals against overexposure to organic dusts.[43-45] A tree cutter who developed hypersensitivity pneumonitis as a result of his exposure to *Penicillium* species reportedly had no further episodes of symptoms while cutting trees after being advised to use a "filtering face mask." [38] Another individual received similar results while wearing a powered air-purifying respirator when handling moldy fuel chips.[46] A third individual with hypersensitivity pneumonitis induced by *Penicillium* species remained symptomatic despite wearing a face mask.[47]

Although nonapproved disposable respirators have been recommended to be worn when working with oyster mushrooms,[48] the symptoms of workers harvesting shiitake mushrooms persisted despite the wearing of face masks.[42] Caution should be used when respirators are assigned to shiitake mushroom workers until the results of environmental and in-mask sampling and medical evidence clearly demonstrate that the use of any respirator does indeed provide protection against the inhalation of mold and mushroom spores.

Conclusions and Recommendations

Upon reviewing the results of the acute symptoms questionnaire and learning that allergenic mold and mushroom spores were available for inhalation in high concentrations, the management of the indoor shiitake farm made a decision to close the facility until an interim respiratory protection program could be implemented. Investigators of the study advised that at a minimum National Institute for Occupational Safety and Health/Mine Safety and Health Administration-approved half-mask air-purifying respirators with high efficiency cartridges be selected for routine use to reduce exposures. While engineering controls should be installed that will eliminate the need for the routine use of respiratory protection, effective and practical engineering controls for an indoor shiitake farm remain to be developed.

In addition to respiratory protection, it was also recommended that uniforms or disposable garments that remain at the facility at the conclusion of the work day be provided to each worker of the shiitake farm so that personal work clothing contaminated with moldy wood or shiitake spores would not be worn home. This measure was intended to protect the workers' family members from exposures to allergenic mold and mushroom spores.

A medical surveillance program should be made available to employees exposed to bioaerosols of moldy wood and/or shiitake spores. Preplacement, periodic, and termination (of exposure) medical evaluations should include: 1) a medical and occupational history addressing past and present respiratory illnesses, symptoms, and exposures, and 2) tests of pulmonary function. (Pertinent medical records, including medical surveillance results from previous employment, should be reviewed as part of the preplacement evaluation.) The evaluations should be repeated at 6-month intervals, or more often if indicated by previous finding or employee reports of respiratory symptoms.

Pulmonary function tests should be administered by trained technicians and equipment and test procedures should conform to guidelines recommended by the American Thoracic Society.[49] The medical surveillance program should be under the supervision of a physician with knowledge of occupational respiratory disease. Any worker detected by the surveillance program as having findings suggestive of occupational respiratory disease should be further evaluated by a physician with expertise in this field.

Generally, someone with an occupational respiratory disease is advised to avoid further exposure to any substance that contributes to the condition.

The number of bioaerosol samples collected during this study was small and, therefore, the study should be considered only as a screening survey. Additional bioaerosol monitoring should be conducted to effectively characterize the environment and health risks associated with the indoor cultivation of shiitake mushrooms on logs and on artificial growth media. Such additional air sampling should involve the collection of multiple samples of varying durations. In addition, personal air monitoring should be conducted to estimate exposures to shiitake spores by workers in the growth room. Also, because the seasonal activities associated with planting were completed the day after the acute symptoms questionnaires were administered, air sampling was not done in the planting room of Building 1. Therefore, air sampling should be conducted to estimate the exposures to both bioaerosols and wood dust that occur in this work area of the shiitake farm as well.

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Disclaimer

Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

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