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Myxomycete (slime mold) spores: unrecognized aeroallergens?

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ABSTRACT

Background: Myxomycete spores are present in the outdoor air but have not been studied for allergenicity. **Objective:** To determine whether patients with seasonal allergic rhinitis (SAR) symptoms are sensitized to myxomycete spores.

Methods: Myxomycete specimens were collected in the field. Nine species of myxomycetes were collected and identified: *Arcyria cinerea*, *Ceratiomyxa fruticulosa*, *Fuligo septica*, *Hemitrichia clavata*, *Lycogala epidendrum*, *Metatrichia vesparium*, *Stemonitis nigrescens*, *Tubifera ferruginosa*, and *Trichea favoginea*. Allergen extracts were made for each species. Protein content of each extract was measured by bicinchoninic acid assay. Protein electrophoresis was performed. Subjects with a history of SAR symptoms were enrolled, and allergy skin prick testing was performed with each extract.

Results: Protein content of the extracts ranged from 1.05 to 5.8 mg/mL. Protein bands were seen at 10 to 250 kD. Allergy prick testing was performed in 69 subjects; 42% of subjects had positive prick test results for at least 1 myxomycete extract, with 9% to 22% reacting to each extract. Five of the 12 subjects who tested negative for all allergens on the standard aeroallergen panel had positive prick test results for myxomycetes.

Conclusion: Forty-two percent of subjects with SAR were sensitized to myxomycete spores. A significant subset of subjects who had SAR symptoms and otherwise negative skin test results showed sensitization to myxomycetes. These spores are present in the outdoor air during the summer and autumn and might be significant aeroallergens.

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Introduction

The commonly recognized outdoor aeroallergens include tree, grass, and weed pollens and fungal spores. Patients with spring and autumn exacerbations of their allergic rhinitis symptoms are typically allergic to pollens, and patients with symptoms during wet weather and in the late autumn usually have fungal spore allergies. However, it is not unusual for a patient to present with symptoms typical of seasonal allergic rhinitis (SAR), exacerbated by outdoor exposures and improving with allergy medications, yet with no positive allergy skin test results. In these cases, it seems likely that the patient is reacting to an unidentified outdoor aeroallergen. Myxomycetes are a potential source of allergenic spores in the outdoor air.

Myxomycetes, popularly known as *slime molds*, are eukaryotes that have a complex life cycle, with phases typical of animal and fungal organisms. In the plasmodial (slime) phase, the organism moves slowly along its substrate, ingesting bacteria. When the supply of nutrients or water becomes low, the plasmodium morphs into a fungus-like fruiting body and makes spores similar to those of fungi (Fig 1).¹ The fruiting bodies of myxomycetes vary in size from microscopic to quite large, and the number of spores produced per fruiting body varies correspondingly. At one extreme is the

well-known “dog vomit slime mold” (*Fuligo septica*), which appears on mulch beds and decaying logs in the summer, with the plasmodial phase resembling its namesake. The fruiting bodies of *F septica* can reach up to 12 inches or larger in diameter and are packed with millions of spores. Many other species are smaller but very numerous.

The air sampling data for the author's area is performed using a rotorod impaction sampler, which undersamples small spores; nevertheless, many myxomycete spores are identified, especially during the summer and autumn (Fig 2). A longitudinal birth cohort study found myxomycete spores in 59% of the indoor and 80% of the outdoor air samples.² Thus, myxomycete spores are potential aeroallergens that have not yet been investigated, except for a study of basidiomycete spore allergies that included intradermal skin testing with *F septica*,³ and an occupational asthma case report related to *Dictyostelium discoideum*.⁴

The purpose of this study was to determine whether patients with SAR symptoms are sensitized to myxomycetes, and whether myxomycete allergy might account for some cases of SAR with negative aeroallergen skin test results.

Methods

Myxomycete Collection and Identification

Myxomycete fruiting bodies were located in local woods, parks, and landscaping mulch beds and collected. They were identified

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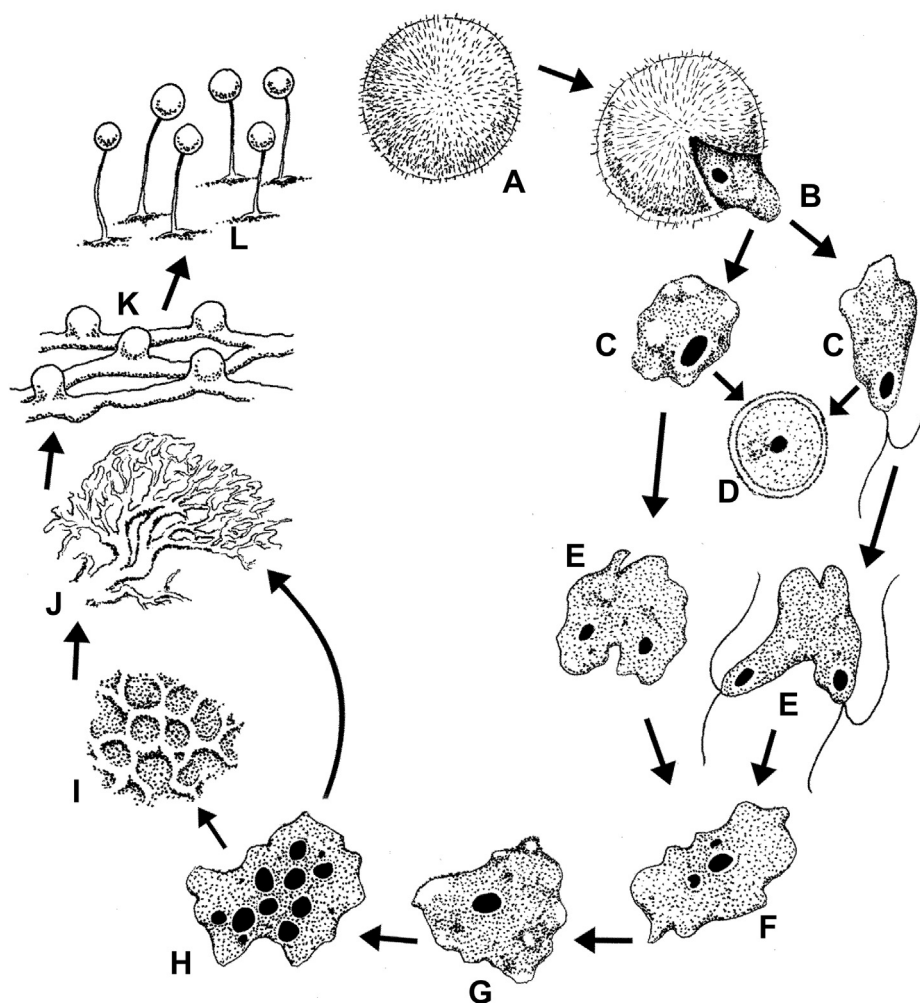


Figure 1. Generalized life cycle of the myxomycetes. (A, B) A protoplast emerges from the spore. (C) The protoplast can take the form of an amoeba or a flagellated cell during the first trophic stage. (D) Under dry conditions or in the absence of food, amoebae form a microcyst, or resting stage. (E, F) Compatible amoeboflagellates fuse to form a zygote (G). (H) The nucleus of the zygote divides by mitosis and each subsequent nucleus also divides without being followed by cytokinesis, thus producing a single large cell (J), the plasmodium, that represents the second trophic stage. Under adverse conditions, the plasmodium can form the second type of resting stage found in myxomycetes, the sclerotium (I). (K, L) Fruiting bodies are formed from the plasmodium. During formation of the fruiting bodies, spores are produced. Adapted from Stephenson¹ with permission of the author.

using a field guide by Stephenson and Stempen⁵ and by the Eumycetoan Project Web page (<http://slimemold.uark.edu/martin.htm>) using macroscopic and microscopic features. A sample of each species was sent to Steven L. Stephenson, PhD, at the University of Arkansas, to confirm the identity. The spores were collected from species with a contained spore mass (*Lycogala epidendrum*, *F. septica*) by dissecting the spore mass from the fruiting body. Capillitial elements were present in the spore mass. In other species (*Arcyria cinerea*, *Ceratiomyxa fruticulosa*, *Hemitrichia clavata*, *Metatrichia vesparium*, *Stemonitis nigrescens*, *Trichia favoginea*, *Tubifera ferruginosa*; eFigs 1–18), the spores were inseparable from the tiny sporangia, which were collected whole. Thus, extracts for these species included spores, capillitia, peridia, and stalks. The material was stored under desiccation until a sufficient quantity had been collected for extraction.

Extraction

The extraction method described by Liengswangwong et al⁶ and Horner et al⁷ for basidiospore extracts was attempted, but failed. This method involves extracting the spores in ammonium bicarbonate solution, centrifuging and filtering to remove the spores, lyophilizing the extract, and resuspending the lyophilate in

phosphate buffered saline (PBS) and glycerin. Most myxomycete lyophilates contained insoluble crystals and could not be successfully dissolved into the PBS. Therefore, the following extraction procedure, which is similar to that used for the production of commercial fungal extracts, was followed:

1. For acetone defatting, 1,500 mg of each specimen was mixed with acetone and shaken gently for 15 minutes. The mixture was centrifuged at 4,000 rpm for 30 minutes, and then the acetone supernatant was poured off. The myxomycete material was dried in a glass beaker under a hood.
2. The dried myxomycete material was suspended in a solution of 50% glycerin in PBS (pH 7) at 1:10 wt/vol (ie, 1 g of material per 10-mL solution). The mixture was intermittently shaken to keep the material homogeneously suspended in the buffer solution. The purity of the sample was assessed by placing a drop of the extract on a microscope slide and counting all elements present up to 100 particles at 400× magnification. Acceptable purity was defined as less than or equal to 1% foreign spores and less than 3% soil or bark debris.
3. The material was kept in PBS and glycerin for 48 hours at room temperature, and then the suspension was centrifuged at 7,000g

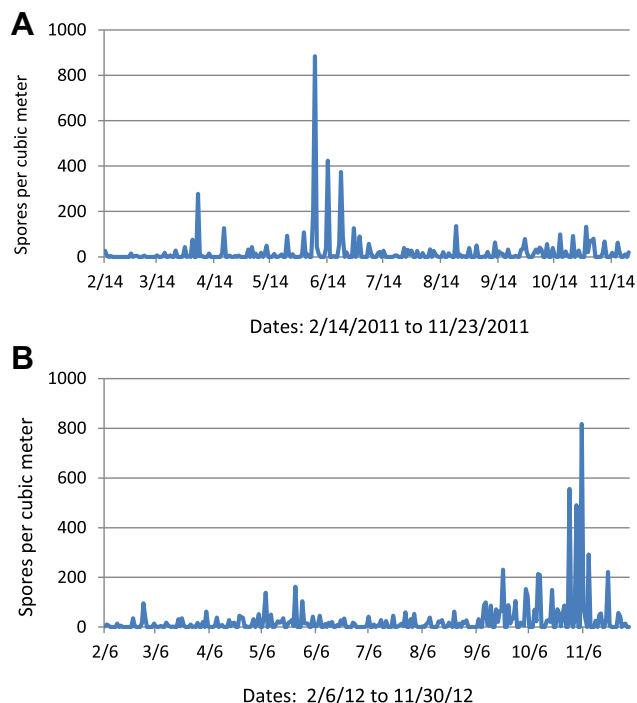


Figure 2. Myxomycete spore counts for (A) 2011 (range 0–884 spores/m³) and (B) 2012 (range 0–817 spores/m³). Counts were obtained by the Hamilton County Department of Environmental Services using a rotorod sampler.

for 30 minutes. The supernatant was passed through a 0.2- μ m Nalgene (Nalge Nunc International Corp, Rochester, New York) filter into a sterile vial. Each vial was labeled with an expiration date of 1 year from the date it was made. This expiration date was arbitrarily determined based on the shelf life of commercially available fungal extracts; there did not seem to be a decrease in the incidence of positive skin test results over the course of the 12-month period. Samples of each extract were sent to the infectious disease laboratory for sterility testing. Protein content of each extract was determined by the bicinchoninic acid assay. Electrophoresis and silver staining were performed on each extract to determine the weights of the proteins.

Allergy Prick Testing

The institutional review board approved the protocol. All subjects signed an informed consent; for children, the parents signed the parental permission form and the child signed an assent form.

Allergy prick testing was performed using the GREER Pick system (GREER, Lenoir, North Carolina), used as a prick test device. The skin was pricked at a 45° angle, catching the skin with an upward motion. All study prick testing was performed by the principal investigator for consistency of technique. The principal investigator tested the consistency of her results with this system by performing 10 prick tests with the histamine control in the same subject; the wheal sizes ranged from 5 to 8 mm (coefficient of variation, 0.21).

Prick testing was performed when the subject had not taken diphenhydramine, hydroxyzine, ranitidine, antihistamine eye drops, or antihistamine nasal sprays for 3 days or cetirizine, loratadine, or fexofenadine for 5 days. Subjects with asthma were enrolled when their asthma was clinically well controlled, with normal pulmonary examination and spirometry. Each of the myxomycete extracts was applied along with a positive (histamine base 1 mg/mL; Histatriol, ALK, Port Washington, New York) and negative (diluent) control. The

prick tests were read after 15 minutes, and the test result was considered positive if the myxomycete extract produced a wheal diameter at least 3 mm larger than the negative control reaction. In cases in which the wheal had an elongated shape, the mean wheal diameter was recorded (average of short and long axes).

Negative controls

Prick testing with the extracts was performed first in 10 volunteers who had no history of allergic rhinitis or asthma symptoms to ensure that there were no nonspecific irritant reactions to the extracts.

Safety testing of positive controls

The extracts were tested next in 10 adult volunteers who had a history of SAR. These subjects were tested with a 1:10 dilution of the extract; because there were no unduly large skin test reactions to the diluted extract, they were retested with the full-strength extract.

Patients with allergic rhinitis

After the safety testing was completed, subjects at least 6 years old were enrolled through the allergy clinic if they presented with a history of SAR symptoms; exacerbations during the spring, summer, and/or autumn; and improvement of symptoms with antihistamine treatment. The study procedures included prick testing with myxomycete extracts and the collection of information regarding the subjects' symptom patterns, medication responses, and home environment (Table 1). Most subjects also were having routine aeroallergen testing performed at the same time as part of their evaluation for SAR, and the response to the routine aeroallergen tests was recorded in the study database. Routine aeroallergen testing included the following allergens: fungal spores (1:10 wt/vol): *Epicoccum nigrum*, *Alternaria tenuis*, *Hormodendrum cladosporioides*, *Helminthosporium interseminatum*, *Penicillium notatum*, *Aspergillus niger*, *Aspergillus fumigatus*, *Mucor racemosus*, *Rhizopus nigricans*, *Fusarium vasinfectum*, *Pullularia pullulans*; tree pollens (1:20 wt/vol): box elder-maple mix, oak mix, pecan, sycamore (American), willow (black), ash (white), beech (American), birch mix, cottonwood (common), elm (American), walnut (black), mulberry mix, eastern red cedar, pine mix; grass pollens (standardized; 100,000 BAU/mL): Kentucky blue, orchard, redtop, ryegrass, sweet vernal, timothy; weed pollens (1:20 wt/vol): ragweed, giant; ragweed, common short; cocklebur, kochia, lamb's quarter, burweed, poverty weed, marsh elder, careless weed, rough redroot pigweed; indoor aeroallergens: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus* (standardized; 30,000 AU/mL), cat hair (acetone precipitated, 10,000 BAU/mL), dog hair (acetone precipitated; 1:100 wt/vol), feather mix (chicken, goose, duck), cockroach (1:10 wt/vol), German and American mix. The red cedar and pine mix extracts were obtained from GREER; all other extracts were obtained from Jubilant Hollister-Stier Laboratories LLC (Spokane, Washington).

Data analysis

Correlations were sought between positive skin test results for myxomycetes and other variables using the Fischer exact test (for

Table 1

Characteristics of subjects with seasonal allergic rhinitis (n = 69)

Age (y), range (mean)	6–50 (16.7)
Male/female, n	34/35
History of asthma, n (%)	35 (51)
Home environment, n (%)	
Suburban	54 (78)
Urban	8 (11)
Rural	7 (10)
History of indoor mold exposure, n (%)	8 (11)

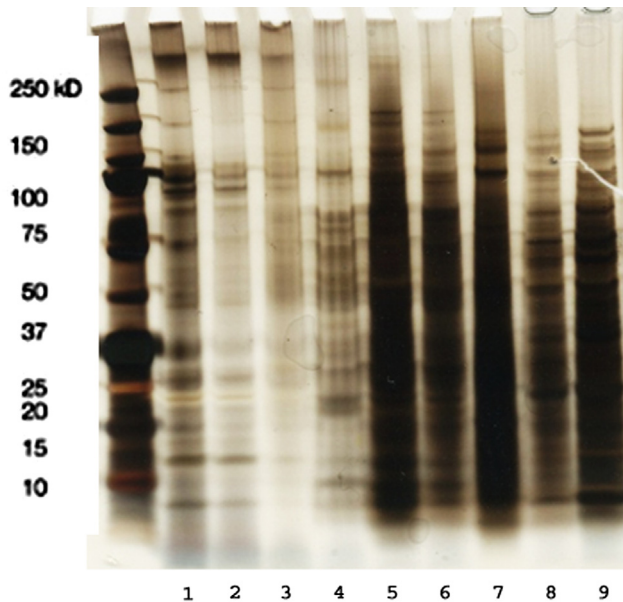


Figure 3. Electrophoresis with silver staining of myxomycete extracts. 1, *Arcyria cinerea*, 5.8 mg/mL of protein content by bicinchoninic acid assay. 2, *Ceratiomyxa fruticulosa*, 1.05 mg/mL. 3, *Fuligo septica*, 2.41 mg/mL. 4, *Hemitrichia clavata*, 2.70 mg/mL. 5, *Lycogala epidendrum*, 5.27 mg/mL. 6, *Metatrichia vesparium*, 2.90 mg/mL. 7, *Stemonitis nigrescens*, 5.60 mg/mL. 8, *Tubifera ferruginosa*, 5.00 mg/mL. 9, *Trichea favoginea*, 3.17 mg/mL.

variables with small numbers in some cells) or the Pearson goodness-of-fit χ^2 test. These tests were performed using SISA software (STSC, Irvine, California).

Results

Extracts

All extracts showed acceptable purity. Sterility testing showed that all extracts were sterile. The protein concentrations ranged from 1.05 to 5.8 mg/mL by bicinchoninic acid assay. For comparison, using the same assay, the protein concentration of the 11 Hollister-Stier fungal extracts used in the allergy clinic was found to range from 5 to 22 mg/mL (mean, 14.3 mg/mL).

Protein electrophoresis with silver staining displayed bands at 10 to 250 kD (Fig 3).

Subjects

In addition to the 10 nonallergic controls and 10 adult volunteers with SAR who underwent safety testing with the diluted and

then full-strength extracts, 59 subjects were enrolled through the allergy clinic and underwent allergy prick testing with the full-strength extracts. Thus, results of full-strength extract testing were available for 69 subjects with SAR (Table 1).

Results of Skin Testing

The positive (histamine) control prick test yielded positive results in all subjects, with wheal diameters ranging from 3 to 12 mm (mean wheal diameter, 6.9 mm). The negative (diluent) control prick test produced no wheal in any subject. None of the nonallergic control subjects had positive skin test reactions to the extracts. Of the 10 allergic control subjects, none had an unusually large wheal and flare reaction to the 1:10 dilution or full-strength myxomycete extracts. There were no systemic reactions to prick testing.

Twenty-nine of the 69 subjects with allergic rhinitis (42%) had a positive skin prick test reaction to at least 1 myxomycete extract (Table 2). Twenty-two of these 29 subjects had positive reactions to more than 1 species of myxomycete (range, 2 to 7 species positive). Of the 69 subjects who were tested with the myxomycete extracts, 59 also were tested with a panel of commercially available aeroallergens. Twelve of these 59 subjects, all of whom had SAR symptoms responding to antihistamines, tested negative for all pollens, molds, dust mite, cockroach, cat, and dog; 5 of these 12 subjects had positive skin test results for myxomycetes.

There was no correlation of positive skin test results to myxomycete extracts with positive prick test results to commercial fungal extracts or with positive skin test reactions to pollens or other aeroallergens. There was no correlation with diagnosis of asthma. There was a correlation with urban residence, although the number of urban subjects was small (Table 3).

Discussion

The myxomycete species studied in this project were selected based on availability. These were the species for which the investigator was able to collect spores in sufficient quantities to make allergen extracts. Thus, these are the larger species (fruiting body size from 3 mm to several centimeters, mostly ≤ 1 cm in diameter) that are prevalent in the Cincinnati area. There are many other very tiny or microscopic species prevalent in the environment; it is unknown whether these are also a source of allergenic airborne spores, although 1 study has described a case of occupational rhinoconjunctivitis and asthma related to *D discoideum*, a tiny cellular slime mold (fruiting bodies 1 mm tall) in a laboratory worker.⁴

The specific identification of myxomycete spores in an air sample is difficult, because the spores of many species appear similar; however, myxomycete spores have a characteristic appearance that allows them to be identified as a class. The data presented in Figure 2 represent the best estimate of myxomycete

Table 2
Results of skin prick testing with myxomycete extracts

Myxomycete species	Protein content of extract (mg/mL)	Positive prick test results, n (%)	Prick test wheal diameter (mm), mean (range) ^a
<i>Arcyria cinerea</i>	5.8	9 (13)	4.4 (3-7)
<i>Ceratiomyxa fruticulosa</i>	1.05	12 (17)	4.8 (3-10)
<i>Fuligo septica</i>	2.41	10 (14)	4.1 (3-6)
<i>Hemitrichia clavata</i>	2.7	6 (9)	4.8 (3-8)
<i>Lycogala epidendrum</i>	5.27	9 (13)	4.3 (3-5)
<i>Metatrichia vesparium</i>	2.9	8 (12)	4.6 (3-10)
<i>Stemonitis nigrescens</i>	5.6	6 (9)	3.5 (3-4)
<i>Trichea favoginea</i>	5	15 (22)	3.8 (3-6)
<i>Tubifera ferruginosa</i>	3.17	8 (12)	4.1 (3-5)
Total subjects with ≥ 1 positive myxomycete prick test result		29/69 (42)	
Subjects testing negative for standard aeroallergens, positive for ≥ 1 myxomycete		5/12 (42)	

^aThe mean histamine wheal diameter was 6.9 mm.

Table 3
Correlations of positive myxomycete skin tests with other variables

Variable	P value
Urban home	.008 ^a
Rural home	.69 ^a
Suburban home	.14 ^b
Indoor mold in home	.85 ^a
Asthma	.53 ^b
Positive skin test results for other molds	.2 ^b
Positive skin test results for other aeroallergens	.44 ^a

^aFischer exact test.

^bPearson goodness-of-fit χ^2 test.

concentrations by the Hamilton County Department of Environmental Services; however, air sampling is performed using a rotorod impactor, which is known to undersample smaller spores. Thus, the actual concentration of myxomycete spores is probably higher than presented. The sampler is located on an urban rooftop. Because most species of myxomycetes grow on decaying wood, concentrations would be expected to be higher in suburban and rural environments. Therefore, it was surprising to find an association of myxomycete spore sensitization with living in an urban home; this might be a spurious finding because of the small number of subjects living in urban homes (only 8 of the 69 subjects).

This study found that a significant number of subjects who had SAR symptoms had positive skin test results for myxomycetes. Most of these subjects also had positive skin test results for other aeroallergens, but there was no correlation between skin test reactivity to commercial fungal extracts and reactivity to myxomycetes; thus, cross-reactivity between myxomycete allergens and fungal allergens is unlikely. Most subjects with positive skin test results for myxomycetes reacted to more than 1 species; thus, cross-reactivity between myxomycete species is possible.

Further study will be needed to determine whether exposure to environmental myxomycete spores actually causes allergic rhinitis symptoms. However, it is worth noting that of the 12 patients with SAR symptoms who had otherwise negative allergy prick test results,

5 showed sensitization to myxomycete spores. Thus, myxomycetes could account for some of the “unknown seasonal aeroallergens” that are postulated to cause the SAR symptoms in these patients.

In summary, myxomycete spores are prevalent in the outdoor environment from early spring until late autumn. Forty-two percent of subjects with SAR had positive skin test reactions to at least 1 species of myxomycete spores, and most of these subjects were sensitized to 2 or more species. For some subjects with SAR symptoms, myxomycete spore extracts were the only extracts yielding positive skin test results. Thus, myxomycete spores might be previously unrecognized outdoor aeroallergens.

Acknowledgments

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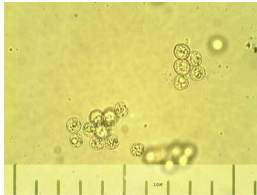
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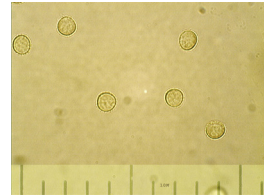
eFigure 1. *Arcyria cinerea*.



eFigure 7. *Hemitrichia clavata*.



eFigure 2. *Arcyria cinerea* spores 1000x.



eFigure 8. *Hemitrichia clavata* spores 1000x.



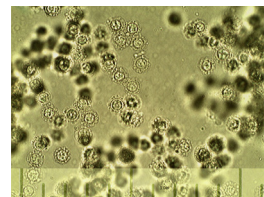
eFigure 3. *Ceratiomyxa fruticulosa*.



eFigure 9. *Lycogala epidendrum*.



eFigure 4. *Ceratiomyxa fruticulosa* spores 1000x.



eFigure 10. *Lycogala epidendrum* spores 1000x.



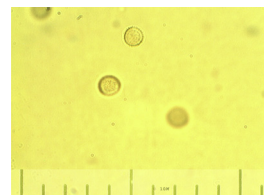
eFigure 5. *Fuligo septica*.



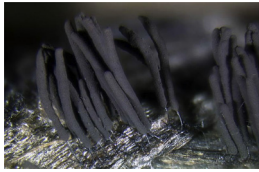
eFigure 11. *Metatrachia vesparium*.



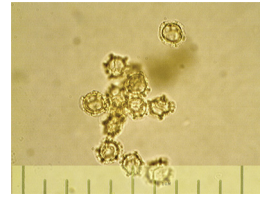
eFigure 6. *Fuligo septica* spores 1000x.



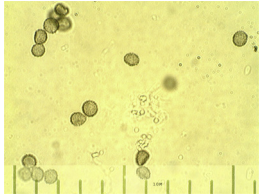
eFigure 12. *Metatrachia vesparium* spores 1000x.



eFigure 13. *Stemonitis nigrescens*.



eFigure 16. *Trichea favoginea* spores 1000x.



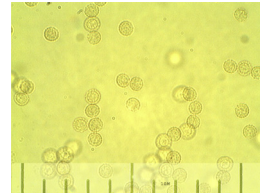
eFigure 14. *Stemonitis nigrescens* spores 1000x.



eFigure 17. *Tubifera ferruginosa*.



eFigure 15. *Trichea favoginea*.



eFigure 18. *Tubifera ferruginosa* spores 1000x.