AIRBORNE BASIDIOSPORES OF BOLETES AND THEIR POTENTIAL TO INFILTRATE A RESIDENCE IN CENTRAL CONNECTICUT

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ABSTRACT
Bolete basidiospores at all locations showed diurnal patterns with night-time peaks. Bolete basidiospore concentrations inside a residence showed strong correlations with those on the outdoor deck. Dew and wind velocity correlated with both large and small basidiospores at all locations positively and negatively, respectively. RH was correlated with basidiospore concentrations outdoors, but not the small basidiospores on the deck. Rain was not correlated with bolete basidiospores. Airborne small basidiospores near the ground were correlated with the ones on the deck, but not so for large basidiospores. Spatio-temporal distributions of large basidiospores varied within a 5-m range. Human activity had a significant effect on large bolete basidiospores in the living room, but not on small ones. A significant number of bolete basidiospores entered the residence, and the concentration of small bolete basidiospores indoors reached 33301 spores/m3. However, overall the basidiospore concentration in the residence was 21 % of outdoor.

INDEX TERMS
basidiospore, boletes, diurnal pattern, meteorological factors, residence.

INTRODUCTION
The boletes, species of Boletaceae, are fleshy pore fungi and very common throughout the world (Bessette et al. 2000). Several hundred species are distributed in North America (Arora 1986). Bessette et al. (2000) described over 300 species in their book “North American Boletes.” These macrofungi are among the most fascinating wild mushrooms and highly prized by edible mushroom collectors (Arora 1986, Bessette et al. 1997, Bessette et al. 2000, Læssøe 1998). The majority of boletes are ecologically interesting and important due to the symbiotic association: ectomycorrhizae these mushrooms developed with woody plants (Arora 1986, Bessette et al. 2000). During the fruiting season, basidiospores released from boletes could be significant to local airborne fungal spore compositions and populations due to the relative large sizes of basidiomata.

Basidiospores in certain geographic areas in the world were a significant part of airborne fungi outdoors from summer to fall and were found to be dominant airborne fungal spores in Hamilton, Ontario and among top three dominant ones in Madras, India and Cagliari, Italy (Cosentino et al. 1990, Tarlo et al. 1979, Vital and Krishnamoorthi 1988). However, studies on airborne basidiospores have been scarce due to the difficulties of identification based on their morphological characteristics. Very few studies were specific on the taxonomic groups below basidiomycetes. Airborne Agrocybe, Chondrostereum purpureum Coprinus, Ganoderma, and Marasmius rotula were among the genera that were investigated (Craig and Levetin 2000, Dye 1974, Gilliam 1975, Henríquez et al. 2001, Li and Kendrick 1995a, Tarlo et al. 1979). Also, basidiospores are important airborne allergens in different geographic areas and may be particularly important in asthmatic patients (Helbling 1998, Lehrer et al. 1994, O'Neil et al. 1988, Sprenger et al. 1988). The infiltration of outdoor airborne bolete basidiospores into indoor environments have not been reported.

The objectives of this study were to determine the spatio-temporal characteristics of bolete basidiospores, to elucidate the potential of these basidiospores to infiltrate a nearby residence, and to correlate basidiospores concentrations with environmental factors.

RESEARCH METHODS
Air samples for basidiospores of boletes were taken from September 30 to October 12, 2004 from three locations including two locations outside and one inside of a residence in Avon, Connecticut, USA. The residence is a

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two-story colonial single family building with a walk-out basement. Air samplers were set up to take samples at 2-hour intervals, 12 samples per day. Three samplers, Allergenco, Mark-3 (Environmental Monitoring Systems, Inc., Mt. Pleasant, SC) were used to take air samples. The Allergenco sampler collects multiple, discrete fungal spore deposits on 75 × 25 mm slides. Twelve samples per day were taken on a slide. All the samples were taken at 15 L/min for 10 min. One sampler was positioned at ground level. It was placed on a metal pan to prevent water from soaking and damaging the sampler and to minimize the disturbance caused by outlet air to ground surface. Another one was placed on a deck with a height of 2.7 m off ground and 5.2 m away from the first sampler on the ground. The 2nd sampler was 2.4 m away from the residence. The third one was placed on an end table in the living room of the residence. Since the samplers are not water proof, both samplers outdoors were sheltered by a cover of 62.5 × 45 × 60 cm (Length × Width × Height) from rain damage. The slides used for sampling were replaced in the samplers at 6:30 pm daily. Once slides were removed from the samplers, all spore deposits were marked with an extra fine sharpie marker at the lower ends of the deposits to allow locating the samples easily for analysis. Resident activity and door/window status were recorded daily.

Samples were collected on 75 × 25 mm slides coated with a mixture of 90 % petrolatum (Fisher, Pittsburgh, PA) and 10 % paraffin wax (high melting point 54ºC) (Li and Kendrick, 1995a). Lacto-fuchsin mounting medium and 22 × 30 cover glasses were used to mount the samples. All the samples were analyzed under an Olympus BX 40 compound microscope equipped with phase contrast and DIC optics. The fungal spore identification was conducted under 400 × or 1000 × magnifications. Airborne bolete basidiospores were separated into two groups according to their non-overlapping sizes: a small spore group with length <12 μ and a large spore group with length >17 μ.

Weather data for Avon CT were provided by Rick Bunton. The weather data were collected with a Davis Vantage Pro Weather Station (Davis Instruments Corp. Hayward, CA). Data were analyzed with NCSS (NCSS, 329 North 1000 East, Kaysville, Utah 84037).

RESULTS

Overall average concentrations of large bolete basidiospores on the ground, on a deck and in a residence living room were 95, 74, 18 spores/m3, respectively, during the sampling period. The highest concentrations for these groups reached 1399, 804, and 179 spores/m3, respectively. The overall average of small basidiospore concentrations on the ground, on the deck and in the residence were 8723, 5875, 1887 spores/m3, respectively, and the highest concentrations for small basidiospore groups reached 68299, 57824, 33301 spores/m3, respectively. Both groups at all sites showed diurnal patterns with night-time peaks and the lowest concentrations during the day (Figures 1, 3). However, the peak period for large basidiospores was from 21:00 to 02:00 at night and minimum in the afternoon (Figure 1), while small basidiospores showed much earlier and longer peak periods from 18:00 to 02:00 and a minimum in the morning (Figure 3). Diurnal patterns of basidiospores of both groups indoors showed more similar patterns with the ones on the deck than the one near the ground (Figures 1, 3). There was a plume of large basidiospores (1399 spores/m3) that occurred at 6:00 am on October 1, 2004. Daily concentrations of basidiospores of both groups indoors and outdoors varied significantly from day to day at all locations (Figures 2, 4). Diurnal and daily variations of basidiospores of both groups indoors and outdoors were very high and reached > 10 fold for the comparison of maximum and minimum concentrations (Figures 1-4).

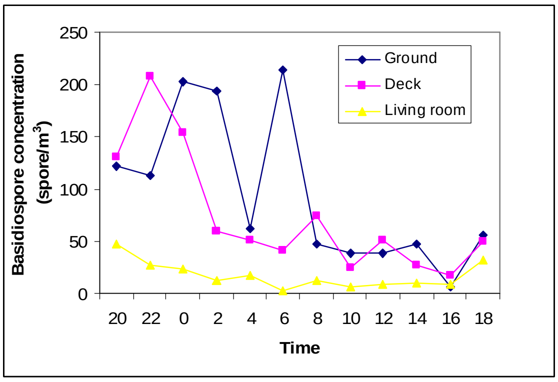

Figure 1. *Diurnal pattern of airborne large basidiospores of boletes indoors and outdoors in Avon,*

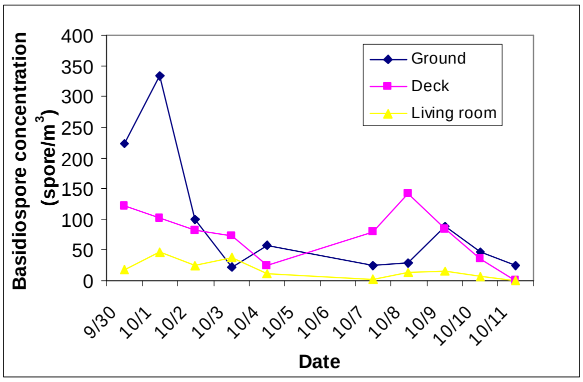

Figure 2. *Daily populations of airborne large basidiospores of boletes indoors and outdoors in Avon,*

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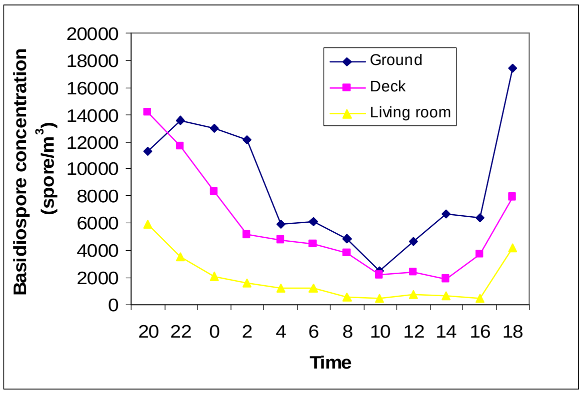

Figure 3. *Diurnal pattern of airborne small basidiospores of boletes indoors and outdoors in Avon, CT.*

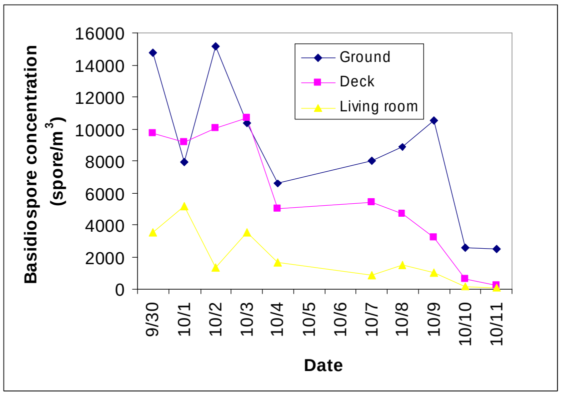

Figure 4. *Daily populations of airborne small basidiospores of boletes indoors and outdoors in Avon, CT.*

Pearson Correlation Analysis showed that large basidiospores near the ground level and on the deck correlated with the large basidiospores in the living room (Table 1). However, large basidiospores near the ground and on the deck did not show a significant correlation. Small basidiospores near the ground and on the deck correlated with small basidiospores in the living room. The correlation between small basidiospores on the deck and in the living room was very strong (r=0.76). There is a correlation between small basidiospores near ground and on the deck (Table 1).

Dew showed significant correlation with basidiospores of both groups in all locations (Table 1). Wind velocity was negatively correlated with basidiospores of both groups in all locations. Southwest wind showed stronger correlations with basidiospores of both groups on the deck and in the living room. RH was correlated with large basidiospores on the deck and the ground and small basidiospores on the deck, but not those on the ground. Rain was not correlated with basidospore concentrations of either group of boletes. Air pressure showed correlation with the basidiospores of both groups, but only on the deck. Temperature showed a correlation with only the small basidiospores on the deck. Keeping a window or door open is an important factor to allow basidiospores to infiltrate a residence. Open windows or doors were correlated with the basidiospores of both groups in the living room. Activity of the residents showed a correlation with large basidiospores in the living room, but not with small ones (Table 1).

Table 1. *Pearson Correlation Analysis of environmental factors and airborne basidiospores of boletes at 3 locations.*

Factor	LS[1]/ground		LS/deck		LS/LR[2]		SS[3]/ground		SS/deck		SS/LR	
	r	p[4]	r	p	r	p	r	p	r	p	r	p
Large spore/ground	1.00	0.00	0.08	0.37	0.19	0.04	0.19	0.04	0.20	0.02	0.25	0.01
Large spore/deck	0.08	0.37	1.00	0.00	0.29	0.00	0.03	0.74	0.53	0.00	0.23	0.01
Large spore/LR	0.19[5]	0.04	0.29	0.00	1.00	0.00	0.13	0.15	0.68	0.00	0.62	0.00
Small spore/ground	0.19	0.04	0.00	0.74	0.13	0.15	1.00	0.00	0.31	0.00	0.32	0.00
Small spore/deck	0.20	0.03	0.53	0.00	0.68	0.00	0.31	0.00	1.00	0.00	0.76	0.00
Small spore/LR	0.25	0.01	0.23	0.01	0.62	0.00	0.32	0.00	0.76	0.00	1.00	0.00
Open window/door	-0.10	0.26	-0.02	0.83	0.22	0.01	0.06	0.52	0.11	0.24	0.19	0.04
Resident activity	-0.16	0.07	-0.04	0.68	0.22	0.02	0.06	0.53	0.13	0.16	0.16	0.07
Barometric pressure	0.10	0.26	0.18	0.04	0.13	0.15	0.09	0.32	0.21	0.02	0.16	0.08
Dew	0.19	0.04	0.27	0.00	0.18	0.04	0.42	0.00	0.21	0.02	0.18	0.05
Heat D D	0.02	0.81	-0.07	0.46	-0.13	0.15	-0.32	0.00	-0.09	0.30	-0.13	0.16
Heat index	-0.02	0.86	0.06	0.53	0.11	0.21	0.31	0.00	0.07	0.43	0.12	0.20
Rain	-0.05	0.57	-0.06	0.48	-0.03	0.74	0.08	0.39	-0.07	0.46	-0.01	0.91
RH	0.28	0.00	0.28	0.00	0.09	0.30	0.15	0.11	0.18	0.05	0.08	0.37
Temperature-max	-0.04	0.67	0.04	0.65	0.11	0.22	0.29	0.00	0.07	0.47	0.11	0.22

Temperature-mean	-0.03	0.67	0.04	0.66	0.10	0.25	0.29	0.00	0.06	0.51	0.11	0.25
Temperature-min	-0.03	0.71	0.05	0.61	0.11	0.22	0.29	0.00	0.07	0.46	0.11	0.22
Wind velocity	-0.23	0.01	-0.37	0.00	-0.25	0.01	-0.27	0.00	-0.40	0.00	-0.25	0.01
Wind direction	-0.13	0.16	-0.44	0.00	-0.42	0.00	-0.16	0.08	-0.49	0.00	-0.43	0.00

[1] LS=large basidiospore. [2] LR=living room. [3] SS=small basidiospore. [4] Bonferroni Probability. [5]numbers in bold indicate correlation is statistically significant.

DISCUSSION

The concentration of airborne bolete basidiospores can reach very high levels, up to 8723 and 68299 spores/m^3 for large and small groups in central CT, respectively. It may have important effects on individuals with strong allergenic response to fungi. Basidiospore release and concentrations in air usually show well defined diurnal patterns with peak periods during late night to early morning (Levetin and Horner 2002). In the present study both groups showed diurnal patterns with night-time peaks. A number of basidiomycetes, such as *Coprinus* and *Ganoderma*, showed well defined diurnal patterns with a maximum period at night (Li and Kendrick 1995a, Craig and Levetin 2000). Haard and Kramer (1970) found *Crepidotus*, *Panaeolus*, and *Oudemansiella* showed a diurnal pattern with a maximum period during the midnight and a minimum during the daytime. However, the peak period for basidiospores of both bolete groups occurred before 02:00 AM, which was much earlier than the peak periods of *Ganoderma* and Coprinaceae (Li and Kendrick 1995a, Craig and Levetin 2000). The peak period of large bolete basidiospores occurred at a similar time to *Cortinarius*, *Lactarius*, *Collybia*, *Laccaria*, *Crepidotus*, *Panaeolus*, and *Oudemansiella* (Haard and Kramer 1970). Small basidiospores boletes showed much earlier and longer peak periods from 18:00 to 02:00 and a minimum in the morning, which is different from the diurnal patterns of most basidiomycetes studied, but the earlier peak period was similar to the results of Haard and Kramer (1970). However, the peak period in the study of Haard and Kramer (1970) was shorter than the one observed in the present study. Rain that occurred in the 1st and 3rd days of sampling may be, in part, responsible to the long peak period in the present observation. The differences in diurnal patterns may be due to differences in species of boletaceae.

Large bolete basidiospores showed the presence of spore plumes, which is a phenomenon of spore concentrations increasing dramatically over a very short period of time. Burch and Levetin (2002) observed the presence of spore plumes in their study. The occurrence of spore plumes may be significant to individuals who are allergenic to certain fungal spores during exposure to high spore concentrations (Burch and Levetin 2002) and to IAQ investigation.

Environmental factors are always very important in the release and dispersal of fungal spores, especially by basidiospores. Dew was a significant factor. Its importance may relate with the discharge mechanism of bolete basidiospore. Wind velocity was negatively correlated with the populations of bolete basidiopores. Hasinain (1993) observed a similar correlation between basidiospores of *Ganoderma* and wind velocity. However, Li and Kendrick (1995b) found there was no correlation between wind velocity and *Ganoderma* from May to October, and a negative correlation from November to October in southern Ontario. The differences between the studies may partially be due to the differences in fungal species and geographic areas. It is understandable that open window/door was a conducive factor for bolete basidiospores to infiltrate a residence. The activities of residents were found to correlate with large bolete basidiospores, but not with small bolete basidiopores. Resuspension of large bolete basidospores into air provoked by resident activity could be part of the reason.

CONCLUSION AND IMPLICATIONS

Spatial distribution of large bolete basidiospores was different within a 5-m range, but the difference for small bolete basidiospores was much less. Bolete basidisopores showed diurnal patterns with peak periods at night and minimal populations in daytime. Daily variation of basidiospores was significant. Dew, wind velocity, and RH were important factors, which were correlated with airborne basididospores of boletes. Fewer than 21 % of basidiospores infiltrated the residence. However bolete basidiospores indoors and outdoors could reach very high levels (>33000 spore/m^3 and >60000 spore/m^3 for indoor and outdoor bolete basidiospores, respectively) if windows or doors were kept open. Diurnal and daily variations of bolete basidiospores indoors and outdoors were very high and > 10 fold for the comparison of maximal and minimal basidiospore populations. Understanding spatio-temporal variations of bolete basidiospores will be crucial for using a proper strategy to take air samples to avoid non-representative results. Occurrence of high populations of airborne bolete basidiospores may have significant effects on allergic individuals. Such data and information are helpful for individuals and medical practitioners to better understand bolete-related allergy for proper diagnosis and treatment. It will be helpful for allergic individuals to stay indoors or close windows during peak times. Further studies are necessary to determine functional and causal relationships of airborne basidiospores of boletes outdoors with the ones indoors and with environmental factors.

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