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ORIGINAL PAPER

Airborne fungi associated with ornamental plant propagation in greenhouses

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Abstract The objective was to determine potential exposure to airborne fungi in greenhouses and to characterize the temporal patterns of airborne fungi in relation to environmental conditions. We analyzed air samples collected in two greenhouses. Results showed that the top 5 fungi in greenhouse 1 were *Trichoderma*, hyphal fragments, *Aspergillus*/*Penicillium*-like, *Cladosporium*, and *Botrytis* in a descending order. Those in greenhouse 2 were *Aspergillus*/*Penicillium*-like, *Cladosporium*, *Botrytis*, yeast-like, and hyphal fragments. Maximum concentrations of *Trichoderma* and total spores in greenhouse 1 were 36,426 and 49,729 spores/m^3, respectively. Maximum concentrations of *Aspergillus*/*Penicillium* and total spores in greenhouse 2 were 46,961 and 71,037 spores/m^3, respectively. Airborne fungal populations fluctuated dramatically within 2 h during work hours, tenfold for *Aspergillus*/*Penicillium*, 66-fold for *Trichoderma*, and sevenfold for total spores. QPCR detected *Trichoderma harzianum* ranging from 7 to 3,500 conidia E/m^3. *Aspergillus*/*Penicillium* and *Botrytis* showed diurnal patterns, but not *Trichoderma*. *Aspergillus*/*Penicillium* and *Cladosporium* were positively correlated with temperature, relative humidity, dew point, heat index, and light and negatively with air movement and air pressure. *Botrytis* and *Trichoderma* were not correlated with the environmental factors. Greenhouse workers were potentially exposed up to 71,037 spores/m^3 of airborne fungi.

Keywords Bioaerosols · Greenhouses · Occupational exposure · Propagating · Fungal spores · *Trichoderma*

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1 Introduction

Certain fungi are allergens and some are human or plant pathogens and mycotoxin producers. Those fungi might pose a potential health risk to building occupants, if exposure occurred in a building (Miller 1992, 1993; Gravesen et al. 1994; Samson et al. 1994). Potential detrimental effects of fungi on human health are through inhalation, ingestion, or skin contact (Matossian 1989; Johanning et al. 1993; Miller 1993; Dill et al. 1997). Airborne fungi may cause allergenic diseases (Chapman 1999; Gravesen 1979; Horwitz and Bush 1997). About 20% of the human population consists of allergic individuals whose allergies can be triggered by inhalation or infestation of fungi (Kaplan et al. 1991). Airborne and phylloplane fungi are common in greenhouses, and at times the concentration of airborne fungi in

greenhouses can be high. Season averages of airborne fungi ranged from 1,158 to 1,502 CFU/m^3 in a tropical greenhouse in a botanic garden (Rodolfi et al. 2003).

Unfortunately, greenhouses as working environments have received very little attention from a public health aspect (Rodolfi et al. 2003). The nursery and greenhouse industry is the fastest growing segment of US agriculture, accounting for $26 billion in sales, 261,408 jobs and $18.1 billion in value added impacts in 2004 (Hall et al. 2005). Greenhouses are very unique workplaces and provide not only a great environment for plants to grow, but also permit the development of plant diseases, many of which are caused by fungi (Baker and Linderman 1979).

Plant diseases that occur in greenhouses can result in increased airborne fungal populations. Watering and high humidity also create conditions very conducive to other fungi, such as phylloplane fungi on leaf surfaces and saprotrophic fungi in soil and potting media. Phylloplane fungi are not only additional sources to airborne fungi, but also may be a potential exposure for greenhouse workers to fungi by direct skin contact. Greenhouse workers are an under-represented population in occupational health research due to the fact that most workers are seasonal, and it is difficult to conduct long-term research on occupational health in greenhouses.

Many studies on indoor and airborne fungi have been conducted in office buildings, schools, and residences (Li and Kendrick 1995a; Cooley et al. 1998; Jovanovic et al. 2001; Shelton et al. 2002). A few studies were conducted in buildings related to agricultural production, such as a mushroom farm, cotton mills, woolen mills, or a citrus warehouse (Crook and Olenchock 1995), but only one study has been conducted in greenhouses in Italy (Rodolfi et al. 2003). Unfortunately, research data for dose/exposure level and response relationships are insufficient to establish practical thresholds for making public health decisions (Li and Yang 2004). It is important to conduct research in the greenhouse industry to better understand workers' occupational exposure to airborne fungi and their health significance to greenhouse workers.

The objectives of this study were to determine potential exposure to airborne fungi in greenhouses during annual ornamental plant propagation, to characterize the temporal patterns of airborne fungi and their relationships with exposure to airborne fungi, and to study phylloplane fungi of greenhouse crops and their relationships with airborne fungi.

2 Materials and methods

The study was conducted in two greenhouses (designated as greenhouse 1 and greenhouse 2) in Connecticut from January to April, 2006. Both greenhouses specialize in the production of ornamental plants. In spring, the most common production crops were begonia, Easter lily, fuchsia, hedera, impatiens, ivy geranium, chrysanthemum, New Guinea impatiens, rosemary, regal geranium, royal geranium, and zonal geranium. The propagating plant materials used in the two greenhouses were primarily imported from South American countries and shipped in by air.

Greenhouse 1 (GH1) is a large production facility with a modern design. It was built with aluminum structures and a hoop top covered with a layer of polyacetylene film. Each house is equipped with a retractable white curtain to adjust sunlight intensity for propagating plants. A ventilation fan is installed at each end in addition to an exhaust fan on the ceiling. In GH1, all propagating plants in flats were placed directly on concrete floors with a drainage system. The plants were watered by a moving overhead watering system over the whole span of the house, and the system is programmed and controlled by a computer.

Greenhouse 2 (GH2) has a more traditional wooden structure with a glass ceiling and sides and gravel floors. A ventilation fan was installed in the walls at each end of the house. Plants were placed on shelves 80 cm in height with a metal mesh surface and wooden frames. The plants were watered manually.

2.1 Air samples for airborne fungal spores

Two Allergenco, MK-3 samplers (Environmental Monitoring Systems, Inc., Mt. Pleasant, SC) were used to take air samples. One sampler was posited in a propagating house in the greenhouses. In GH1, the sampler was put on a cart, while it was placed on a shelf in GH2. At both locations, the sampler was placed at ca. 80 cm height. A second sampler was placed at a height of 1 m off the ground outdoors, 10 m away from the propagating houses. The

sampling started at 12:15 in the first sampling day and finished at 12:14 in the following day. Samples were taken at 2-h intervals for 24 h each month. All of the samples were taken at 15 l/min for 10 min. A single longitudinal traverse method was used to read all 12 samples on a slide. The slides used to collect samples were 75 mm × 25 mm slides coated with a mixture of 90% petrolatum (Fisher, Pittsburgh, PA) and 10% paraffin wax (high melting point 54°C) (Li and Kendrick 1995b). Lacto-fuchsin mounting medium and 22 × 30 cover glasses were used to mount the samples. All 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 1,000× magnification.

2.2 Air samples of airborne fungal spores for QPCR analysis

Samples for QPCR analysis were collected with 37-mm 3pc cassettes with 0.8-μm polycarbonate filters (Zefon International, St. Petersburg, FL). Cassette samples connected to ems Megalite pumps (Environmental Monitoring Systems, Charleston, SC) were collected at 20 l/min for 60 min from 11:00 to 12:00. Samples from inside the propagating house and outside were collected simultaneously during each sampling period at the locations 0.5 m away from Allergenco, MK-3 samplers. *Trichoderma harzianum* was analyzed with QPCR methods based on the preliminary results of predominant fungi and availability of QPCR primers and probes. Air sample filters were vortexed in 5 ml of 0.05% Tween solution (in QH_2O) for 10 min to remove spores from the filter. The resultant suspensions were centrifuged for 5 min @ 35,000 rpm. The pellet and 200–300 μl of supernatant were resuspended, and the resuspensions were transferred to microcentrifuge tubes and centrifuged for 2 min. The pellet was used for DNA extraction. The extraction of DNA and other procedures for the study followed the procedure described by (Haugland et al. 1999). *Geotrichum candidum* was used as an internal reference for monitoring DNA recovery, PCR inhibition, and distinguishing true target negatives from PCR inhibition. The primers and probe of the analysis are specific for detecting *Trichoderma harzianum*, and their sequences are forward primer TharzF1: 5′-TTGCCTCGGCGGGAT, reverse primer TharzR1: 5′-ATTTTCGAAACGCCTACGAGA, and probe TharzP1: 5′-CTGCCCCGGGTGCGTCG (USEPA 2008).

Environmental data in the propagating areas were collected with a Davis Vantage Pro Weather Station (Davis Instruments Corp. Hayward, CA).

Data were analyzed using NCSS (NCSS, 329 North 1000 East, Kaysville, Utah 84037) for descriptive statistics, one-way ANOVA, and Spearman correlation analysis for multiple variables. Data analyses were focused on potential exposure to airborne fungi, temporal patterns of airborne fungi, the relationships of airborne fungi with crop growth conditions, and environmental parameters.

3 Results

3.1 Air samples for airborne fungal spores

The composition and concentrations of airborne fungi in the two greenhouses differed. Overall average concentrations of the predominant airborne fungi in GH1 were *Trichoderma* 3,539 conidia/m^3 (38.3%), hyphal fragments 2,055 pieces/m^3 (22.2%), *Aspergillus*/*Penicillium*-like 1,421 conidia/m^3 (15.4%), *Cladosporium* 727 conidia/m^3 (7.9%), *Botrytis* 479 conidia/m^3 (5.2%), and yeast-like 327 cells/m^3 (3.5%) in descending order (Table 1). The total concentration of airborne fungi in GH1 was 9,233 spores/m^3. Twenty-four taxa/groups of airborne fungi were present in GH1 (Table 1).

Overall average concentrations of the predominant airborne fungi in GH2 were *Aspergillus*/*Penicillium*-like 1,748.4 conidia/m^3 (34.6%), *Cladosporium* 1,268.5 conidia/m^3 (25.1%), *Botrytis* 601.4 conidia/m^3 (11.9%), yeast-like 397.4 cells/m^3 (7.9%), hyphal fragments 382.5 pieces/m^3 (7.6%), and ascospores 315.6 ascospores/m^3 (6.2%) in descending order (Table 1). The total concentration of airborne fungi in GH2 was 5,053 spores/m^3. Twenty-seven taxa/groups of airborne fungi were present in GH2 (Table 1).

Overall average concentrations of the top three outdoor airborne fungi at GH1 were ascospores 219.9 spores/m^3 (25.8%), basidiospores 219.9 spores/m^3 (25.8%), and *Trichoderma* 158.7 conidia/m^3 (18.6%). The total concentration of outdoor airborne fungi at GH1 was 853.9 spores/m^3. Eighteen fungal taxa were present (Table 1). Overall average concentrations of

Table 1 Airborne fungi recovered by Allergenco MK-3 samplers in the greenhouses (unit: spores/m^3)

Fungal taxa	GH1[a]	%	GH1 outdoors	%	GH2[b]	%	GH2 outdoors	%
Acremonium	55.2	0.6			80.0	1.6		
Alternaria	1.9	0.0			4.3	0.1	1.7	0.4
Ascospores	121.5	1.3	219.9	25.8	315.6	6.2	6.2	1.4
Asp/Pen-like[c]	1,421.0	15.4	43.8	5.1	1,748.4	34.6	50.3	11.5
Aureobasidium					5.0	0.1		
Basidiospores	175.5	1.9	219.9	25.8	101.1	2.0	108.3	24.8
Botrytis	479.3	5.2	0.8	0.1	601.4	11.9	1.9	0.4
Burgoa	1.2	0.0						
Cercospora					22.9	0.5		
Cladosporium	727.3	7.9	33.1	3.9	1,268.5	25.1	49.6	11.4
Coprinus	5.6	0.1	0.0	0.0	1.2	0.0		
Curvularia	1.9	0.0	0.8	0.1	12.4	0.2	0.6	0.1
Diplococcium			1.7	0.2				
Drechslera/Bipolaris							0.6	0.1
Epicoccum					0.6	0.0	0.6	0.1
Exserohilum							0.6	0.1
Fusarium	0.6	0.0	0.8	0.1	1.9	0.0		
Ganoderma					0.6	0.0		
Hyalodendron	3.1	0.0						
Hyphal fragments	2,055.3	22.3	55.4	6.5	382.5	7.6	114.1	26.1
Leptosphaeria	4.3	0.0			2.5	0.0		
Lycoperdon							5.8	1.3
Monodictys-like							20.1	4.6
Myxomycetes	186.6	2.0					28.5	6.5
Humicola	1.2	0.0						
Oidium	1.2	0.0			0.6	0.0		
Periconia	2.5	0.0	5.8	0.7	0.6	0.0	1.3	0.3
Pestalopsis							1.3	0.3
Pithomyces			3.3	0.4	1.2	0.0	0.6	0.1
Polythrincium							0.6	0.1
Rusts	9.3	0.1	2.5	0.3	1.9	0.0		
Scopulariopsis					8.1	0.2		
Smuts			2.5	0.3	1.2	0.0		
Spegazzinia							0.6	0.1
Stemphylium							1.3	0.3
Torula herbarum			0.8	0.1			4.7	1.1
Trichoderma	3,539.0	38.3	158.7	18.6	42.2	0.8		
Trichotecium					0.6	0.0		
Ulocladium							1.3	0.3
Unidentified fungi	60.1	0.7	22.3	2.6	36.6	0.7	5.0	1.1
Venturia	0.6	0.0			13.6	0.3		
Wallemia	51.5	0.6					0.8	0.2
Xylariaceae							0.6	0.1

Table 1 continued

Fungal taxa	GH1[a]	%	GH1 outdoors	%	GH2[b]	%	GH2 outdoors	%
Yeast-like	327.4	3.5	81.8	9.6	397.4	7.9	28.9	6.6
Total spores	9,233.0	100.0	853.9	100.0	5,053.0	100.0	436.4	100.0

[a] GH1, greenhouse 1

[b] GH2, greenhouse 2

[c] Asp/Pen-like = *Aspergillus*/*Penicillium*-like

the top three outdoor airborne fungi at GH2 were hyphal fragments 114.1 pieces/m^3 (26.1%), basidiospores 108.3 spores/m^3 (24.8%), and *Aspergillus*/*Penicillium*-like 50.3 conidia/m^3 (11.5%). The total concentration of outdoor airborne fungi at GH2 was 436.4 spores/m^3. Twenty-five fungal taxa were present (Table 1). The average concentrations of total airborne fungi in both greenhouses were >10 times the ones outdoors Table 1). The average concentration of total airborne fungi in GH1 was higher than the one in GH2.

3.2 Temporal patterns of airborne fungi

Aspergillus/*Penicillium* concentrations in air demonstrated a diurnal pattern with a daytime peak in the greenhouses (Fig. 1). However, its population elevated more than tenfold within 2 h from 3,482 conidia/m^3 at

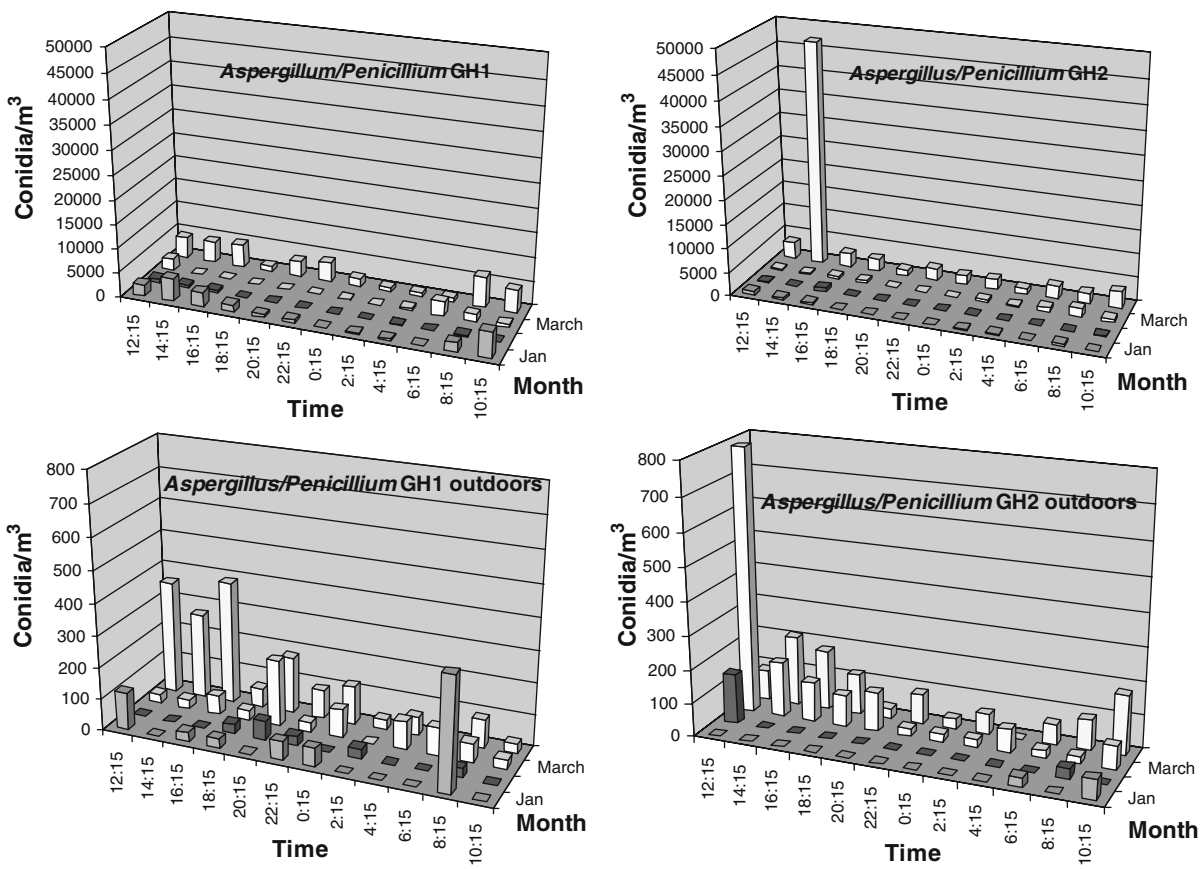

Fig. 1 Temporal patterns of *Aspergillus*/*Penicillium* in the greenhouses. *Upper left* greenhouse 1, *Upper right* greenhouse 2, *Lower left* greenhouse 1 outdoors, *Lower right* greenhouse 2 outdoors

12:15 to 46,961 conidia/m^3 at 14:15 in April in GH2. Outdoor populations had a similar diurnal pattern at much lower levels (Fig. 1). Overall population levels in the greenhouses were comparable at 1,421 and 1,748 conidia/m^3, respectively.

Botrytis showed a daytime-peak diurnal pattern in GH1 and an afternoon-peak pattern in GH2. In GH2, the population fluctuated drastically within a few hours. It was absent in outdoor air most times from January to March and present in April in the greenhouses (Fig. 2).

The populations of airborne *Trichoderma* conidia were at different levels in the greenhouses, 3,539 and 42 conidia/m^3 in GH1 and GH2, respectively, with no well-defined diurnal pattern. However, airborne concentrations exhibited several elevated spikes. One spike reached 36,426 conidia/m^3 at 8:15 PM on April 26 in GH1, where *T. harzianum* was applied routinely for plant disease management. The difference between the peak and the lowest population within 24 h was 200 times in GH1 on April 26. *Trichoderma* populations fluctuated 66-fold within 2 h from 23,719 to 357 conidia/m^3. *Trichoderma* populations were very low in GH2 and outdoors. Outdoor *Trichoderma* at GH1 was absent at most times (Fig. 3).

Hyphal fragments showed a double-peak diurnal pattern in the greenhouses. Variation in concentration in air was higher within 24 h than among months, and concentrations were much higher in GH1 than in GH2. Outdoor populations at GH1 demonstrated a similar pattern, but were quite different at GH2 (Fig. 4).

Total fungal spores in the air in GH1 showed a diurnal pattern with three peaks, one in the morning, one in the afternoon, and one in the evening. The peaks during morning and evening corresponded to the spikes that occurred during those periods of time in April. The highest population (49,729 spores/m^3) occurred at 20:15 in GH1. Total fungal spores in the

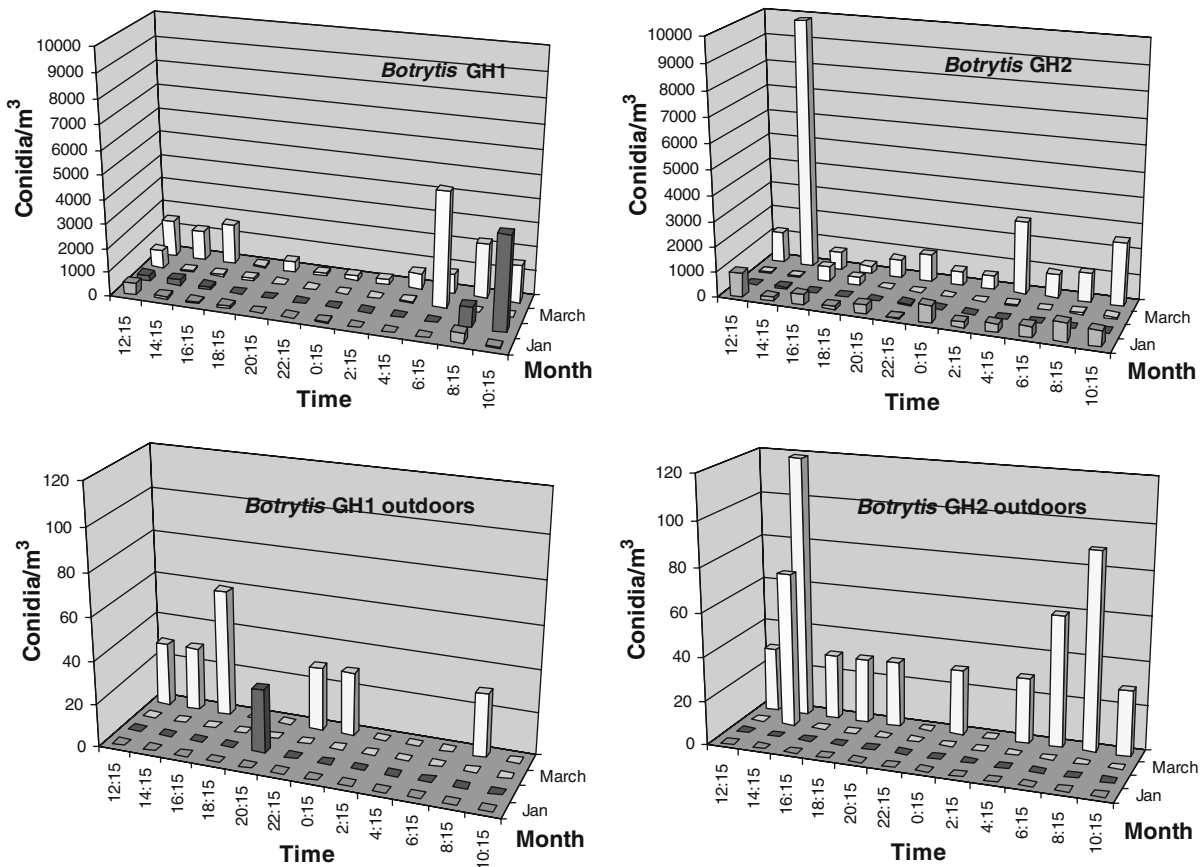

Fig. 2 Temporal patterns of *Botrytis* in the greenhouses. *Upper left* greenhouse 1, *Upper right* greenhouse 2, *Lower left* greenhouse 1 outdoors, *Lower right* greenhouse 2 outdoors

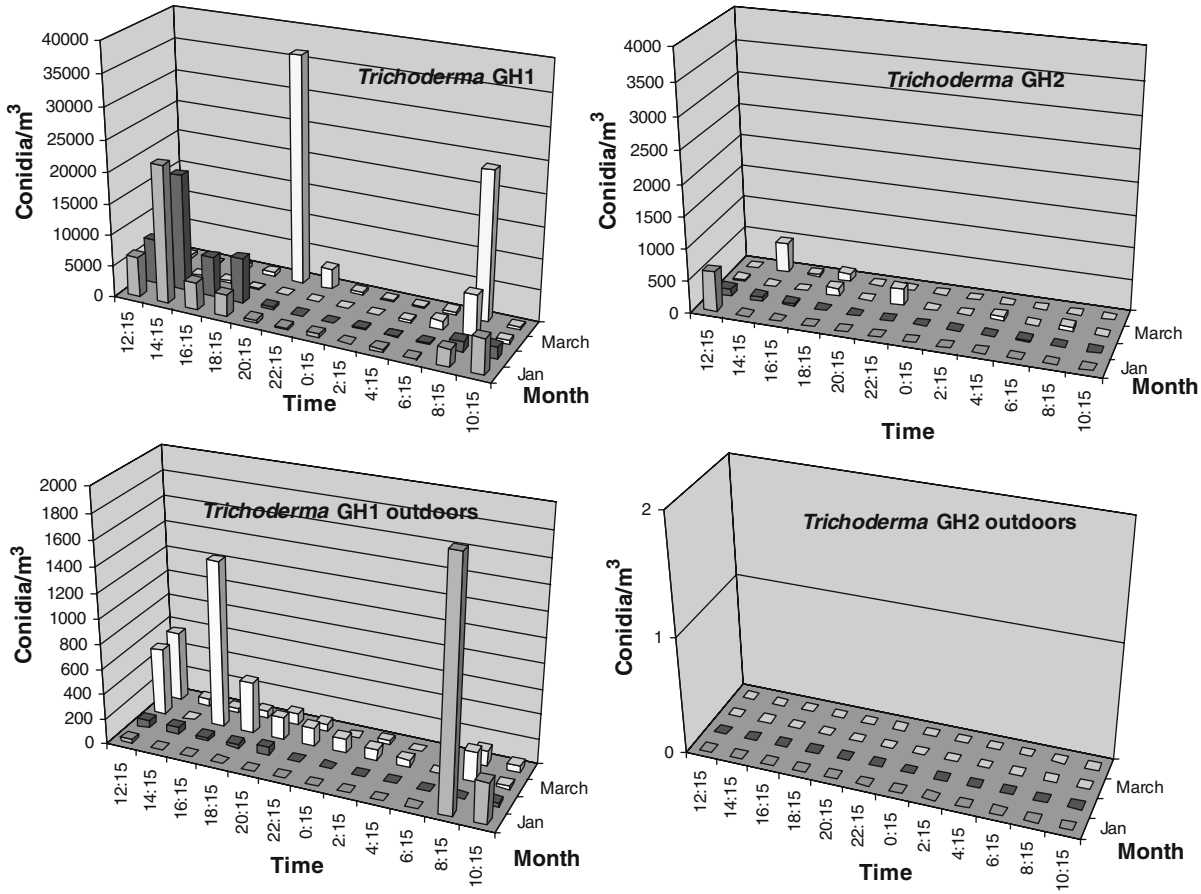

Fig. 3 Temporal patterns of *Trichoderma* in the greenhouses. *Upper left* greenhouse 1, *Upper right* greenhouse 2, *Lower left* greenhouse 1 outdoors, *Lower right* greenhouse 2 outdoors

air in GH2 showed a diurnal pattern with an afternoon peak at 14:15. The peak of the diurnal pattern occurred at the same time of the spike (71,037 spores/m^3) for total fungal spores observed in April (Fig. 5). Outdoor total fungal spores showed a daytime diurnal pattern with a peak in early afternoon at both greenhouses (Fig. 5).

3.3 QPCR analysis for airborne *Trichoderma harzianum*

QPCR analysis on the filter cassette samples showed that in GH1 airborne conidia of *Trichoderma harzianum* were detected at average concentrations of 3,500, 1,100, 7, and 96 conidia E/m^3 (conidia E = conidia equivalence) in the propagating room between 11:00 and 12:00 from January to April, respectively, while concentrations detected outdoors were 15, 1, 0, and 53 conidia E/m^3 in January to April, respectively (Fig. 6). In GH2, airborne conidia of *T. harzianum* were not detected with QPCR in most months in the propagating room and outdoors, except for April when 1 conidium E/m^3 was present in the propagating room (Fig. 6).

3.4 Correlation of airborne fungi and environmental factors

Among the predominant airborne fungi detected, several fungi were significantly correlated with environmental factors (Table 2). *Aspergillus*/*Penicillium* and *Cladosporium* were positively correlated with temperature (T), humidity (H), dew point, heat index, and solar radiation and negatively correlated with air pressure. *Aspergillus*/*Penicillium* was negatively correlated with wind speed also. Yeast-like

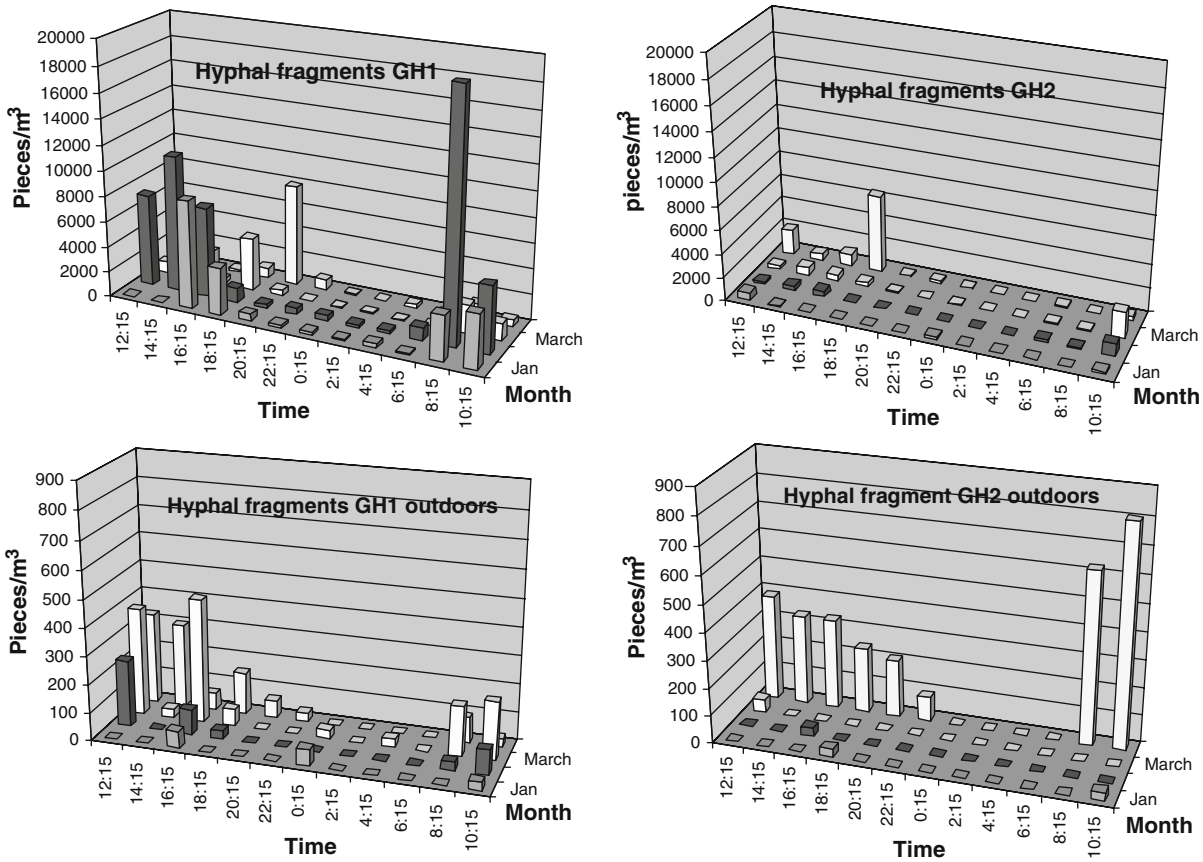

Fig. 4 Temporal patterns of hyphal fragments in the greenhouses. *Upper left* greenhouse 1, *Upper right* greenhouse 2, *Lower left* greenhouse 1 outdoors, *Lower right* greenhouse 2 outdoors

cells were positively correlated with relative humidity. *Botrytis*, *Trichoderma*, and hyphal fragment did not show any correlation with the environmental factors. *Aspergillus*/*Penicillium* and *Trichoderma* showed significant, strong and positive correlations with hyphal fragments (Table 2).

4 Discussion

This study documented rather high levels of airborne fungi in the two greenhouses. The most abundant 5 fungi in GH1 were *Trichoderma*, hyphal fragments, *Aspergillus*/*Penicillium*-like, *Cladosporium*, and *Botrytis* in a descending order. Those in GH2 were *Aspergillus*/*Penicillium*-like, *Cladosporium*, *Botrytis*, yeast-like, and hyphal fragments. Maximum concentrations of *Trichoderma* and total spores in GH1 were 36,426 conidia/m^3, and 49,729 spores/m^3, respectively. Maximum concentrations of *Aspergillus*/*Penicillium* and total spores in GH2 were 46,961 conidia/m^3, and 71,037 spores/m^3, respectively.

Trichoderma species were introduced into the greenhouses as biocontrol agents, and the other fungi may be associated with plant diseases or as saprobes on growing media. Using *Trichoderma* species to control plant diseases can be traced back to the 1920s (Harman 2006). *Trichoderma harzianum* has been applied as a biocontrol agent for managing various plant diseases in fields and greenhouses. It was applied as a potting mix routinely in GH1, while it was applied only once in the fall in GH2 applied to both soil and foliage. The intentional application of *T. harzianum* is largely responsible for the high airborne conidial population of *T. harzianum* in GH1.

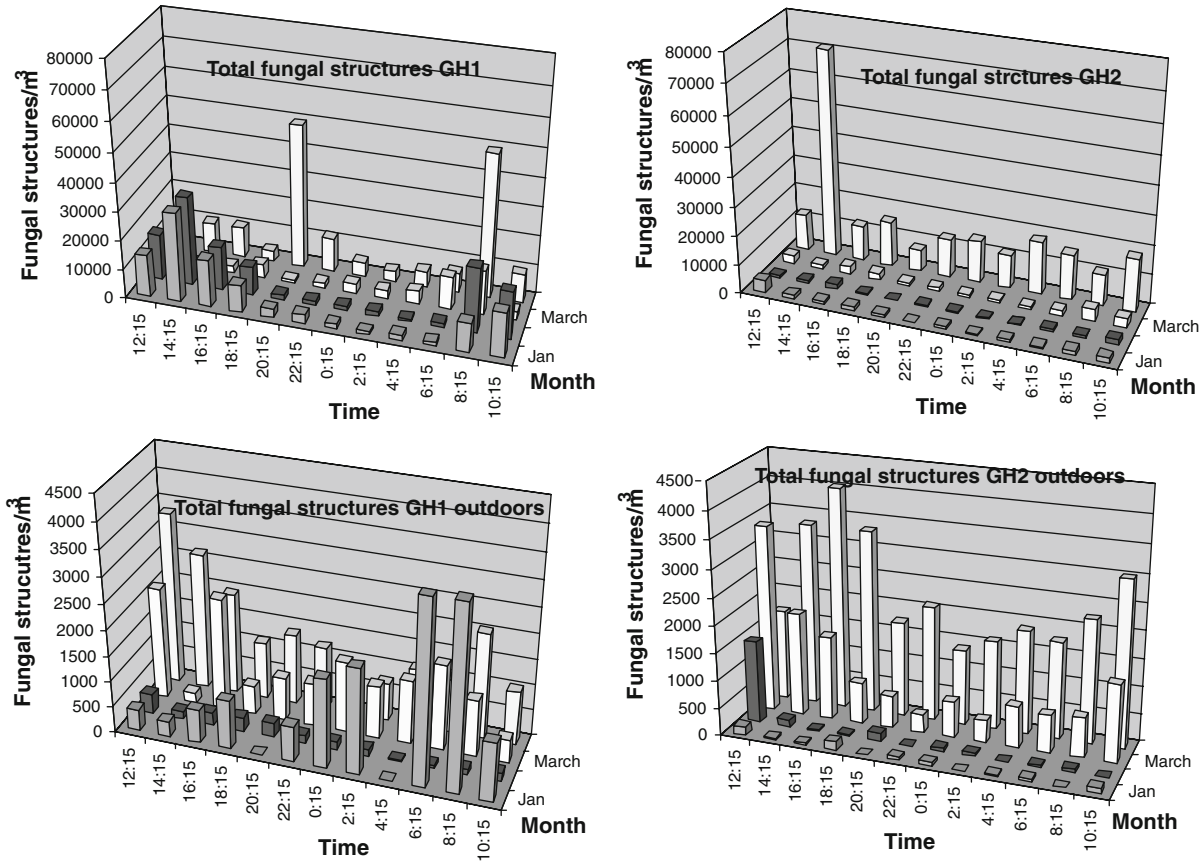

Fig. 5 Temporal patterns of total spores in the greenhouses. *Upper left* greenhouse 1, *Upper right* greenhouse 2, *Lower left* greenhouse 1 outdoors, *Lower right* greenhouse 2 outdoors

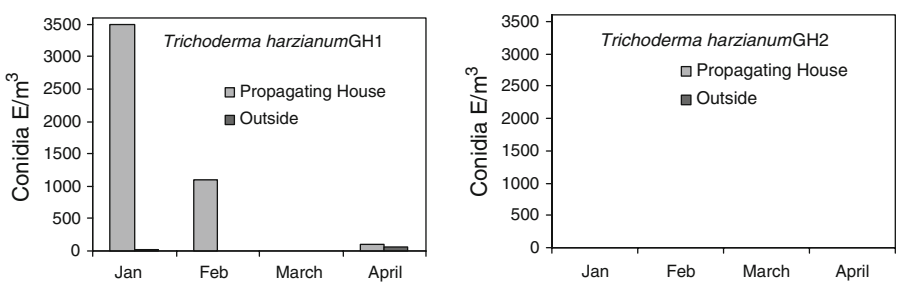

Fig. 6 Airborne conidia of *Trichoderma harzianum* collected by 37-mm 3pc cassettes with 0.8-μm polycarbonate filters in the greenhouses and outdoors and detected by QPCR. *Left* greenhouse 1, *Right* greenhouse 2

The spore trap method determined that the populations of *Trichoderma* in the air were 6,428, 7,232, 2530, 565 spores/m^3 from 12:15 to 12:25 from January to April, while the corresponding populations of *Trichoderma harzianum* enumerated by the QPCR method were 3,500, 1100, 7, and 96 conidia E/m^3, respectively, from 11:00 to 12:00. The workers took a lunch break from 12:00 to 12:30. During workers' break, the results from spore trap were expected to be lower than the ones from QPCR due to settlement of airborne conidia, not the contrary. The results from the two methods suggested that the QPCR method was not as efficient as expected. These results suggested that an unknown inhibitory factor might

Table 2 Spearman correlations of predominant airborne fungi and environmental factors

Fungi	*T*	Humidity	Dew point	Air movement	Heat index	Air pressure radiation fragment	Solar radiation	Hyphal fragment
Aspergillus/Penicillium								
r	0.31	0.31	0.31	−0.26	0.31	−0.40	0.35	0.32
p	0.00*	0.00*	0.00*	0.02*	0.00*	0.00*	0.00*	0.01*
Botrytis								
r	0.06	0.18	0.10	−0.08	0.06	−0.16	0.23	0.03
p	0.66	0.15	0.43	0.52	0.62	0.20	0.06	0.82
Cladosporium								
r	0.35	0.22	0.35	−0.17	0.35	−0.23	0.33	0.02
p	0.00*	0.05*	0.00*	0.14	0.00*	0.04*	0.00*	0.86
Trichoderma								
r	−0.04	0.11	−0.03	−0.03	−0.02	−0.26	0.02	0.49
p	0.78	0.43	0.81	0.85	0.91	0.06	0.89	0.00*
Hyphal fragments								
r	0.17	0.09	0.09	−0.14	0.14	−0.39	0.39	
p	0.12	0.43	0.44	0.21	0.23	0.00*	0.00*	
Yeast-like								
r	0.20	0.41	0.41	0.00	0.33	−0.01	−0.03	
p	0.14	0.00*	0.00*	1.00	0.01*	0.96	0.85	

* *p* is significant

have interfered with the enzyme reactions of QPCR. Gypsum powder (calcium sulfate dihydrate) was routinely added into the potting medium along with *Trichoderma* biological in GH1 production to adjust the pH value of the potting medium. Gypsum dust originating from dry walls had been observed to be an inhibitory factor interfering with QPCR analysis in the past (Chin Yang, pers. comm.). However, further study is necessary to determine whether gypsum powder in the air is the inhibitory factor in greenhouses. An improved DNA extraction method may help solve the problem.

Aspergillus/Penicillium-like is one of the predominant fungi in both greenhouses. Members of *Aspergillus* and *Penicillium* can be either saprotrophic or pathogenic to plants (Domsch et al. 1993). They are common on plant debris or organic matter. *Penicillium olsonii* is saprotrophic and characteristic due to its long conidiophores (Pitt 2001). It was found to colonize infected tissues of begonia and New Guinea impatiens (unpublished data), and its saprotrophic nature suggests that it is a secondary pathogen on foliage in the greenhouses. Rodolfi et al. (2003) noted that *Penicillium olsonii* is one of predominant airborne fungal species in greenhouses of a Botanical Garden. In GH1, potted wheat is used to raise a parasitic wasp for aphid control. The un-germinated wheat seeds were colonized by *Penicillium* sp. on the potting medium in the pots, which may partially explain why *Aspergillus/Penicillium* is the third predominant airborne fungal group in GH1. *Aspergillus/Penicillium* was found be one of the predominant fungi indoors and outdoors (Li and Kendrick 1995b; Ren et al. 1999).

Botrytis cinerea Pers. is a ubiquitous fungal pathogen causing gray mold, one of the most common diseases worldwide on a very broad range of hosts including ornamentals, vegetables, and fruit crops (Fokkema 1996). It was reported to cause serious losses in >200 crop species worldwide (Williamson 2007). Greenhouse crops are among the major hosts of *B. cinerea* (Friedrich et al. 2005; Pitchy et al. 2007). In both greenhouses, visible colonies of *B. cinerea* with fully developed conidiophores and conidia were found on begonia, zonal geranium, fuchsia, and New Guinea impatiens. *Botrytis cinerea* colonies on these

crops were the likely sources for the airborne *Botrytis* in the greenhouses.

Botrytis in the present study followed a daytime diurnal pattern with a peak in the morning in GH1 and an afternoon peak in GH2. In strawberry fields, Blanco et al. (2006) found that conidial populations of *B. cinerea* followed a daytime diurnal pattern also. However, the population peaked between 9:00 and 11:00 during the 2001–2002 season, while it peaked between 12:00 and 14:00 during the second season. These differences suggested that the peak of the diurnal pattern was not determined by any intrinsic factor for conidial release, but rather by environmental factors or production activities. Production activities, such as watering, ventilating, transplanting, and chemical spraying will be able to release conidia of *B. cinerea* into air. These production activities may be responsible for several sudden increased populations of this pathogen in the air.

Cladosporium are the most predominant airborne fungi outdoors in most areas in the world due to their saprotrophic and phytopathogenic nature (Domsch et al. 1993). *Cladosporium cladosporioides* was the most dominant airborne fungus in the greenhouses in an Italian Botanic Garden (Rodolfi et al. 2003). In the present study, *Cladosporium* was overtaken by *Trichoderma* and *Aspergillus*/*Penicillium* as the most dominant due to the application of *T. harzianum* as a biofungicide, and the presence of pathogenic and saprotrophic species of *Penicillium* on the crops, plant debris, and on the wheat seeds. Sooty mold and powdery mildew were present on rosemary in GH2. Microscopic examination showed that sooty mold is caused by *C. oxysporum* and *C. cladosporioides*. It explained why the average concentration of *Cladosporium* in GH2 was much higher than that in GH1. However, rosemary was not a major crop in GH2 and the presence of *C. oxysporum* and *C. cladosporioides* on a limited number of plants failed to make *Cladosporium* the most dominant airborne fungus in GH2.

Epiphytic yeasts are a common component of mycota on plant surfaces (di Menna 1959; Buck et al. 1998) and the predominant colonizers on phylloplane (Fokkema and Schippers 1986). These yeasts are nutrient competitors and observed to be natural antagonists against fungal plant diseases (Buck 2002; Nix-Stohr et al. 2008). Despite the slimy colonies they develop, yeast cells were found in the air (Rantio-Lehtimaki 1988). The airborne yeast-like cells found in the present study in greenhouses were most likely originated from the phylloplane of the ornamental crops.

A vast majority of hyphal fragments recovered from the greenhouses were dark brown and originated from dematiaceous fungi. Some of the potting work was conducted in the propagating area in GH1, while 100% of the potting work was done in a separate potting room in GH2. Potting may be partially responsible for the much higher population of hyphal fragments in GH1. *Botrytis cinerea* and *Cladosporium* spp. are dematiaceous fungi, but their populations in the air were not correlated with airborne pigmented hyphal fragments. Thus, the possibility of pigmented hyphal fragments in the air originating from these two fungi is slim.

Both *Aspergillus*/*Penicillium* and *Trichoderma* were strongly correlated with airborne hyphal fragments. The hyphae or mycelia of *Aspergillus*/*Penicillium* and *T. harzianum* are colorless and most airborne hyphal fragments were dark brown. Thus, hyphal fragments in the air were not largely originated from *Aspergillus*/*Penicillium* and *Trichoderma harzianum*. Rather, it is possible that certain production activities, such as potting, which promote the release of conidia of *T. harzianum* and *Aspergillus*/*Penicillium* into the air, also facilitate the release of pigmented hyphal fragments. Hyphal fragments released into the air from peat moss are likely responsible for airborne hyphal fragments. Examination of peat moss under a compound microscope revealed that peat moss contains a significant amount of pigmented hyphal fragments similar to those recovered from air samples. Potting, watering, ventilating, chemical application, and transplanting may also be associated with the release of *Trichoderma harzianum* and also pigmented hyphal fragments into the air. However, further study is necessary to determine the production activities which elevate the populations of *T. harzianum* and pigmented hyphal fragments.

The diurnal pattern of total fungal spores corresponded with *T. harzianum*, not surprising as *Trichoderma harzianum* was one of the predominant fungi in GH1. The highest population occurred at 20:15 in GH1. The diurnal pattern of total fungal spores in GH2 corresponded with *Botrytis cinerea*. The spike in total fungal spores observed in GH2 at 14:15 in April corresponded well with the spike of *B. cinerea* that occurred at the same time.

Spore trap results indicated that airborne fungal populations of *Trichoderma*, *Botrytis*, and total spores spiked in several incidences during off-work hours. Only watering, ventilation, and heating were in operation during these off-work sampling times. The ventilation, heating, and watering were not set to an arbitrary schedule; rather, they were controlled by the status of humidity, temperature, and leaf wetness in the greenhouses, therefore further study is necessary to determine the possibility that the operation of ventilation fans and/or automatic irrigation, etc. was related to such spikes. This hypothesis needs to be verified in future research.

Concentrations of *Aspergillus*/*Penicillium* and *Cladosporium* were positively correlated with solar radiation. Leach (1975) indicated that light triggered spore release of several fungi. It is possible that solar radiation assisted conidial release of *Aspergillus*/*Penicillium* and *Cladosporium*. *Aspergillus*/*Penicillium* was negatively correlated with wind speed in the greenhouses. Wind/air movement is the most unpredictable factor in the release and dispersal of fungal spores (Li and Yang 2004). Dry spore release of hyphomycetous fungi is frequently influenced by wind speed (Lyon et al. 1984). Sutton et al. (1978) noticed that *Botrytis squamosa* conidia were released at very low wind speed. The relationship between release and dispersal of fungi and air movement in greenhouses is not well studied. Air movement in greenhouses is primarily generated by ventilation fans. The speed of air movement in the greenhouses might exceed the optimum and lead to a negative correlation between airborne conidia of *Aspergillus*/*Penicillium* and wind speed.

Trichoderma and hyphal fragments did not show a diurnal pattern and any correlation with the environmental factors. *Trichoderma harzianum* was applied as a biocontrol agent, and the development, release, and dispersal of its conidia were not influenced solely by the environmental conditions in the greenhouse. The environmental conditions might only influence the re-suspension of these conidia. Most airborne hyphal fragments appeared aged and not viable. They resembled those present in peat moss, the major component of the potting media.

Blanco et al. (2006) found that populations of airborne conidia of *B. cinerea* were positively correlated with solar radiation and mean temperature, and negatively correlated with rainfall and relative humidity in field studies. However, in greenhouses, airborne *B. cinerea* were not correlated with any environmental factors measured. The greenhouse is an artificial environment in which close to optimal growth condition was provided for crop production. These environmental factors in greenhouses did not vary as significantly as those in the field, and may be the reason for the differences between the two studies.

The differences between high and low airborne conidial populations of *Trichoderma* were 132 times within 24 h. Within 2 h, the airborne conidial population levels changed 66-fold. The airborne population of *Cladosporium* changed from 60 to 4,643 conidia/m^3 within 2 h and the difference was 77-fold. Such a huge change in airborne fungal populations within 2 h poses a big challenge to properly assess potential exposure to airborne fungi in greenhouses and raises a legitimate question whether a 10-min sample at an arbitrary time is appropriate and sufficient to assess occupational exposure to airborne fungi in greenhouses. With such a huge variation in airborne conidia within 2 h, the possibility for a snap-shot air sampling to miss the highest populations is rather high. Without determining the major factors which are responsible for the sudden elevation of population of airborne fungi, any air sample with a 2–10 min sampling time for measuring occupational exposure to airborne fungi is just a wild guess. Greenhouse workers' activities such as potting, watering, heating, and ventilation are factors that need to be studied in detail in the future to determine whether these factors have effects on the drastic fluctuation of airborne fungal populations, and the potential exposure of greenhouse workers to these airborne fungi.

Monsó et al. (2002) found that 1/4 of workers in flower and/or ornamental production greenhouses were sensitized to workplace flowers or molds and developed occupational asthma, a disease that is suffered by 8% of the workers producing these crops in Europe. The fungi associated with occupational asthma in greenhouses were *Aspergillus* spp. *Alternaria* spp., *Penicillium* spp., and *Cladosporium herbarum* (Monsó et al. 2002). *Aspergillus*, *Penicillium*, and *Cladosporium* spp. were the predominant fungi in the greenhouses in the present study. *Alternaria* spp. are pathogens of a number of crops in greenhouses (Howard et al. 1994), but not a common disease on the ornamental crops produced in

the greenhouses under study. It explains why the low concentrations of *Alternaria* were observed in both greenhouses in the present study. The potential occupational exposure to total airborne fungi in the present study are quite high, with the highest reaching 49,729 spores/m^3 in GH1 and 71,037 spores/m^3 in GH2. Future research on occupational exposure to airborne fungi in greenhouses and greenhouse workers' occupational health is warranted.

No doubt biocontrol agents are environmentally sound alternatives to fungicides. As these biocontrol agents are living organisms, their potential effects on human health, such as triggering allergy should be fully evaluated so that their adverse health effects, if any, could be avoided or contained. This is the reason why the ethics of biofungicide use was discussed and examined (Ricard and Ricard 1997). Westwood et al. (2005) found that occupational exposure to the biocontrol agent, *Beauveria bassiana* for insect control triggered allergies. The health effects of occupational exposure to airborne conidia of *Trichoderma* spp. have not been studied extensively. However, several species of *Trichoderma* were found to cause allergenic sinusitis or hypersensitivity pneumonitis (Tang et al. 2003; Enríquez-Matas et al. 2007). It will be important to conduct research on the occupational exposure to *Trichoderma harzianum* and to determine any adverse effects on human health.

The concentrations of airborne fungi in the propagation areas of the greenhouses were >10 times the ones outdoors in January to April. It is necessary to study the relationships of airborne fungi inside and outside the greenhouses in the future.

Since the present study was conducted in one production season, which is relatively short, it will be necessary to conduct a multiple year study in the future so that the results can be compared.

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