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# Characteristics and determinants of ambient fungal spores in Hualien, Taiwan

Hsiao-Man Ho[a], Carol Y. Rao[b], Hsiao-Hsien Hsu[c], Yueh-Hsiu Chiu[c], Chi-Ming Liu[d,e], H. Jasmine Chao[c,*]

[a]*Department of Mathematics and Science Education, National Taipei Teachers College, No. 134 Sec. 2 Hoping E. Rd., Taipei, 106 Taiwan*

[b]*Department of Community Medicine, West Virginia University, Box 9190, Morgantown, WV 26506, USA*

[c]*Graduate Institute of Public Health, Taipei Medical University, No. 250 Wu-Hsing Street, Taipei 110, Taiwan*

[d]*Department of Medicine Research and Education, Cheng Hsin Rehabilitation Medical Center, No. 45 Cheng Hsin Street, Taipei 112, Taiwan*

[e]*Community Medicine Research Center and Institute of Public Health, National Yang-Ming University, No. 155 Sec. 2 Li-Nong St., Taipei 112, Taiwan*

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**Abstract**

Characteristics and determinants of ambient aeroallergens are of much concern in recent years because of the apparent health impacts of allergens. Yet relatively little is known about the complex behaviors of ambient aeroallergens. To address this issue, we monitored ambient fungal spores in Hualien, Taiwan from 1993–1996 to examine the compositions and temporal variations of fungi, and to evaluate possible determinants. We used a Burkard seven-day volumetric spore trap to collect daily fungal spores. Air pollutants, meteorological factors, and Asian dust events were included in the statistical analyses to predict fungal levels. We found that the most dominant fungal categories were ascospores, followed by *Cladosporium* and *Aspergillus/Penicillium*. The majority of the fungal categories had significant diurnal and seasonal variations. Total fungi, *Cladosporium*, *Ganoderma*, *Arthrinium/Papularia*, *Cercospora*, *Periconia*, *Alternaria*, *Botrytis*, and $PM_{10}$ had significantly higher concentrations ($p<0.05$) during the period affected by Asian dust events. In multiple regression models, we found that temperature was consistently and positively associated with fungal concentrations. Other factors correlated with fungal concentrations included ozone, particulate matters with an aerodynamic diameter less than 10 μm ($PM_{10}$), relative humidity, rainfall, atmospheric pressure, total hydrocarbons, carbon monoxide, nitrogen dioxide, and sulfur dioxide. Most of the fungal categories had higher levels in 1994 than in 1995–96, probably due to urbanization of the study area. In this study, we demonstrated complicated interrelationships between fungi and air pollution/meteorological factors. In addition, long-range transport of air pollutants contributed significantly to local aeroallergen levels. Future studies should examine the health impacts of aeroallergens, as well as the synergistic/antagonistic effects of weather, and local and global-scale air pollutions.


*Keywords:* Aerobiology; Aeroallergens; Asian dust storms; Fungi

*Corresponding author. Tel.: +886 2 2736 1661x6526; fax: +886 2 2738 4831.
*E-mail address:* hchao@tmu.edu.tw (H.J. Chao).

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## 1. Introduction

With increasing evidence of adverse health effects of outdoor allergens (Dales et al., 2004; Delfino et al., 1997; Lierl and Hornung, 2003; Neas et al., 1996; Ross et al., 2002), characteristics and determinants of ambient aeroallergens have drawn much attention in recent years. Among various outdoor allergens, fungal spores are of major concern because of their abundant sources and ubiquitous presence in environments (Burge and Rogers, 2000). Exposure to outdoor fungal spores has been associated with allergic respiratory symptoms, decreased lung functions among allergic asthmatics, asthma exacerbation, and asthma-related death (Cakmak et al., 2002; Dales et al., 2000, 2003, 2004; Rosas et al., 1998; Rutherford et al., 2000; Targonski et al., 1995). Sensitivity to certain fungal spores also predicts the existence of asthma (Lehrer et al., 1994; O'Hollaren et al., 1991). However, relatively little is known about the complex behaviors of fungal spores in ambient environments.

Various studies have been conducted worldwide to document the composition and variation of ambient fungal spores and to examine the association between fungal spores and meteorological factors (Corden and Millington, 2001; Di Giorgio et al., 1996; Henriquez et al., 2001; Mitakakis et al., 1997; Nikkels et al., 1996; Oh et al., 1998; Satheesh and Rao, 1994). However, the relationships between ambient fungal spores and meteorological factors are explored thoroughly in rather few studies (Katial et al., 1997; Li and Kendrick, 1994; Troutt and Levetin, 2001). The associations between fungi and air pollutants also lack comprehensive evaluation. Because of the complicated interrelationships among climatic factors, air pollutants, and aeroallergens (Lin and Li, 2000), it will be difficult to evaluate individual health risk without clear understanding of their interactions in atmosphere.

Transoceanic dust storms are another emerging issue concerning regional ambient aeroallergen compositions and concentrations. Although dust storm has been a long-existing weather phenomenon in Asia and Africa, not until recently abundant and viable microorganisms were discovered in dust clouds (Griffin et al., 2003; Shinn et al., 2003; Taylor, 2002). Asian dust storms are common meteorological events in the deserts of Mongolia and China during late winter and early spring. Long-range transported coarse particles from dust storms (yellow sand) have been documented to deteriorate air qualities and visibilities in other East Asian countries, including Taiwan (Chun et al., 2001; Fang et al., 2002; Ma et al., 2001). Two studies found higher concentrations of several fungal taxa during Asian dust events (Wu et al., 2004; Yeo and Kim, 2002). More investigations are needed to evaluate biological components of Asian dust and the potential health impacts of these possibly foreign microorganisms.

Outdoor allergen exposure is of particular concern in Taiwan, where subtropical climate with high temperature and relative humidity year-round provides appropriate environmental conditions for microbial growth. However, long-term ambient fungal levels have been documented in very few studies in Taiwan and are mostly restricted to the Taipei area (Tseng and Chen, 1979; Wang and Chen, 1979). Han and Chuang (Han and Chuang, 1981) monitored fungal air spora in Hualien and several other cities in Taiwan in 1976 and found that Hualien had the highest fungal concentration among all study areas. To address the above issues, we conducted a longitudinal study to monitor ambient fungal spores for 3 years in Hualien, Taiwan. The major goals were to assess the composition and temporal variation of ambient fungi in Hualien City and to examine the effects of meteorological factors, air pollutants, and long-range transported events on aeroallergen levels.

## 2. Materials and methods

### 2.1. Fungal sample collection and analysis

Airborne fungal spores were monitored from April 1993 to March 1996 in Hualien, Taiwan. Hualien City, the capital of Hualien County, is in eastern Taiwan (121° 36′ 23″E, 23°58′ 20″N), facing the Pacific Ocean, with an area of 29.41 $km^2$ and a population of approximately 110,000 people. Although Hualien City has been gradually industrialized in recent years, it is still one of the least polluted areas in Taiwan and famous for its tourism. The average temperature in Hualien City is about 27.8 °C in summer and 20.5 °C in winter.

Air sampling was conducted on the 3rd-floor roof of the Hualien County Bureau of Health, located in downtown Hualien. We used a Burkard seven-day volumetric spore trap (Burkard Manufacturing Co. Limited, Rickmansworth Hertfordshire, England) to collect daily fungal spores with a flow rate of 10 l $min^{-1}$. The flow rate of the sampler was calibrated once a week. Briefly, Melinex® tape, coated with a thin layer of Gelvotal solution, was mounted on the drum inside the sampler. The tape was exposed to airflow through a 2 mm × 14 mm orifice, rotating with the drum at a speed of 2 mm $hr^{-1}$. The drum was changed weekly and the exposed tape was removed and cut in 48 mm segments (a 24 h interval) for identification. Samples were analyzed under microscope every fourth day using the 12 traverse method to obtain 2 hr average concentrations (spores $m^{-3}$) (Rogers and Muilenberg, 2001). The spore categories counted included *Cladosporium*, *Arthrinium/Papularia*, *Aspergillus/Penicillium*, *Ganoderma*, *Curvularia*, *Fusarium*, *Ulocladium/Stemphylium*, *Cercospora*, *Alternaria*, *Drechslera*, *Torula*, *Botrytis*, *Nigrospora*, *Pithomyces*, *Periconia*, ascospores, and other fungi. We classified unidentifiable spores and known spores not otherwise categorized as "other fungi."

### 2.2. Data of environmental parameters

Air pollution and meteorological data were provided by Taiwan Environmental Protection Administration (Taiwan EPA). The nearest EPA monitoring station is about 4 km southwest of the sampling location. Computerized hourly data is available starting from 1st January 1994. Environmental parameters subjected to statistical analysis included temperature, relative humidity (RH), rainfall, atmospheric pressure, sulfur dioxide ($SO_2$), carbon monoxide (CO), ozone ($O_3$), particulates with aerodynamic diameters less than 10 μm ($PM_{10}$), nitrogen monoxide (NO), nitrogen dioxide ($NO_2$), total hydrocarbons (THC), methane ($CH_4$), and non-methane hydrocarbons (NMHC). The sampling dates affected by China dust storms starting 1994 were also supplied by Taiwan EPA (Taiwan EPA Grant EPA-92-L105-02-207, http://cisk.atmos.pccu.edu.tw/duststorm/).

### 2.3. Statistical analysis

We used SAS statistical package (v.8.02, SAS Institute Inc., Cary, NC) to perform data analyses. Kruskal–Wallis test was utilized to examine diurnal and seasonal effects of ambient fungi. Mann–Whitney *U* test was used to evaluate the impact of Asian dust storms on environmental factors. We used multiple regression to examine the relationships between ambient fungal spores and environmental parameters. We developed regression models for total fungi and major fungal categories, including ascospores, *Cladosporium*, *Aspergillus/Penicillium*, *Ganoderma*, *Arthrinium/Papularia*, and other fungi. To account for the serial correlations of fungal measurements, autoregressive error models were used, selected automatically by stepwise autoregression in SAS (PROC AUTOREG procedure). To perform regression analysis, fungal concentrations and several environmental factors, including $PM_{10}$, $SO_2$, NO, and NMHC, were transformed using base-10 logarithm to approximate normality. In order to perform log transformation, we added 1 to the concentrations in the major fungal categories to avoid zero values.

## 3. Results

### 3.1. Characteristics of ambient fungal spores

Table 1 summarizes the distributions of ambient fungal spores during the study period using 2 h averages ($n = 3224$). Ascospores, *Cladosporium*, *Aspergillus/Penicillium*, *Ganoderma, Arthrinium/Papularia* and other fungi were the most dominant fungal categories in Hualien City, present in more than 60% of the samples.

Table 1
Descriptive statistics for airborne fungal concentrations (spore $m^{-3}$) from April 1993 to March 1996 in Hualien, Taiwan

| Fungal categories | Freq (%)[a] | Mean | Median | Std Dev | Min | Max |
|---|---|---|---|---|---|---|
| Total spores | 100.00 | 6548.34 | 3166.67 | 9337.61 | 13.33 | 85533.33 |
| Other fungi | 90.91 | 4625.26 | 1230.00 | 8388.21 | 0.00 | 83940.00 |
| Ascospores | 91.50 | 870.64 | 180.00 | 1871.52 | 0.00 | 22300.00 |
| *Cladosporium* | 96.22 | 556.54 | 220.00 | 1316.01 | 0.00 | 31086.67 |
| *Aspergillus/Penicillium* | 84.12 | 184.13 | 73.33 | 412.02 | 0.00 | 15553.33 |
| *Ganoderma* | 85.51 | 130.70 | 40.00 | 221.48 | 0.00 | 2000.00 |
| *Arthrinium/Papularia* | 60.67 | 58.31 | 6.67 | 167.93 | 0.00 | 2086.67 |
| *Cercospora* | 50.51 | 33.60 | 6.67 | 107.68 | 0.00 | 2066.67 |
| *Periconia* | 57.20 | 22.89 | 6.67 | 78.33 | 0.00 | 2246.67 |
| *Fusarium* | 41.07 | 17.00 | 0.00 | 52.13 | 0.00 | 1166.67 |
| *Curvularia* | 49.50 | 16.63 | 0.00 | 42.46 | 0.00 | 680.00 |
| *Drechslera* | 31.85 | 12.20 | 0.00 | 50.12 | 0.00 | 1086.67 |
| *Nigrospora* | 42.31 | 7.49 | 0.00 | 18.18 | 0.00 | 380.00 |
| *Torula* | 30.25 | 4.76 | 0.00 | 11.51 | 0.00 | 186.67 |
| *Ulocladium/Stemphylium* | 21.65 | 4.14 | 0.00 | 16.63 | 0.00 | 280.00 |
| *Alternaria* | 20.35 | 2.40 | 0.00 | 6.66 | 0.00 | 106.67 |
| *Pithomyces* | 11.10 | 1.15 | 0.00 | 4.43 | 0.00 | 86.67 |
| *Botrytis* | 4.46 | 0.76 | 0.00 | 5.33 | 0.00 | 200.00 |

[a]Frequency was the percentage of samples (total $n = 3224$) with presence of that specific fungal category.

Total fungi, other fungal spores, and ascospores had similar diurnal patterns, with highest levels in the early morning (4–6 am) and lowest in the afternoon (2–4 pm) (Fig. 1). The concentration of *Cladosporium* was highest during 8 to 10 am and lowest during 2 to 6 pm. Similar to ascospores, *Ganoderma*, *Arthrinium/Papularia*, and *Drechslera* had highest concentrations in early morning (4–6 am) and kept decreasing until late afternoon. The diurnal variations of *Aspergillus/Penicillium* and *Fusarium* were somewhat different, with highest concentrations around midnight. *Cercospora* had lower levels during daytime (10 am–4 pm). The fungal categories not plotted had rather zigzag diurnal variations, except for *Alternaria*, which had similar diurnal rhythm as *Cladosporium*. All the fungal categories identified had statistically significant diurnal (hourly) variations ($p<0.05$).

Fig. 2 shows the seasonal trends of ambient fungi in Hualien, Taiwan. All the plotted fungal categories had highest levels during summer, except *Periconia*. Categories not shown in Fig. 2 did not display apparent seasonal variation. To examine the significance of seasonal (monthly) variation for ambient fungi, daily average data were used ($n = 271$). All the fungal categories had statistically significant seasonal effects, except for *Aspergillus/Penicillium*, *Alternaria*, *Botrytis*, and ascospores. Seasonal trend for each fungal category was similar every year during the study period, but fungal concentrations decreased over the years. This might result from the vegetation changes in vicinity during the investigation time.

### 3.2. Associations between ambient fungal spores and Asian dust storm events

A total of 33 episodes of Asian dust storms affected Taiwan from January 1994 to March 1996. Among our fungal measurements, 20 days of data ($n = 235$) were affected by dust events. Fungal measurements of nearest days before and after the selected episode days were used as background (about 25 days of data, $n = 297$). Several episodes would often occur in a short time frame so that only one background day fell between two dust episodes.

Table 2 shows the distributions of fungi and selected environmental parameters during background and episode days. Total fungi, *Cladosporium*, *Ganoderma*, *Arthrinium/Papularia*, *Cercospora*, *Periconia*, *Alternaria*, *Botrytis*, and $PM_{10}$ had significantly higher concentrations ($p<0.05$) during episode days. Relative humidity and pressure were slightly lower during the dust storm periods. No other environmental parameters were significantly associated with Asian dust storms episodes.

### 3.3. Associations between ambient fungal spores and environmental parameters

We examined the relationships between environmental factors and ambient fungal concentrations using daily averages (every fourth day) from January 1994 to March 1996 ($n = 200$). Table 3 lists the descriptive statistics for major fungal categories and environmental parameters examined in the multiple regression analyses.

Multiple regression models for major fungal categories are summarized in Table 4. Among the meteorological factors, temperature was the most consistent predictor of fungal concentrations. Except for *Aspergillus/Penicillium* and other fungi, higher temperature was positively associated with higher fungal levels. Rainfall had a negative association with *Ganoderma* and other fungi. Relative humidity was positively correlated with ascospores and *Cladosporium*, and negatively associated with *Aspergillus/Penicillium*. Pressure only had a negative correlation with other fungi. Several air pollutants were also significant predictors of ambient fungi. Ozone was negatively correlated with total fungi, other fungi, and *Ganoderma*. $PM_{10}$ was positively associated with total fungi and *Cladosporium*. Other environmental factors, such as THC, CO, $NO_2$, and $SO_2$, also had statistically significant relationships with fungal concentrations. Year was another important predictor of fungal levels in Hualien City. Most of the fungal categories had higher levels in 1994 than in 1995–96, concordant with the temporal trends previously mentioned.

## 4. Discussion

The dynamics of aeroallergens in the atmosphere are complicated, attributable to climate, weather, local biological sources, anthropogenic pollutants, and natural events (e.g., dust storms). Long-term monitoring and appropriate statistical modeling are valuable methods to explore the interrelationships among environmental factors on ambient aeroallergens. This 3-year study examined the relationships between ambient fungal spores and air pollution/meteorological factors, using multiple regression analysis and accounting for serial correlations of the repeated measurements.

The most dominant genus identified in Hualien City was *Cladosporium*, similar to many other geographical areas in the world (Henriquez et al., 2001; Mitakakis et al., 1997; Nikkels et al., 1996; Satheesh and Rao, 1994). Ascospores, *Aspergillus/Penicillium,* and *Ganoderma* were also prevalent in Hualien City, recovered from more than 80% of our samples and with daily mean concentrations over 130 spores $m^{-3}$. Our results are similar to a previous study that used a gravity slide method, which found that the most prevalent fungal genera in Hualien was also *Cladosporium*, followed by *Fusarium*, *Nigrospora*, *Alternaria*, *Curvularia*, *Drechslera,* and *Ganoderma.* Ascospores and *Aspergillus/Penicillium* were not identified separately (Han and Chuang, 1981). The different rank order of the fungal

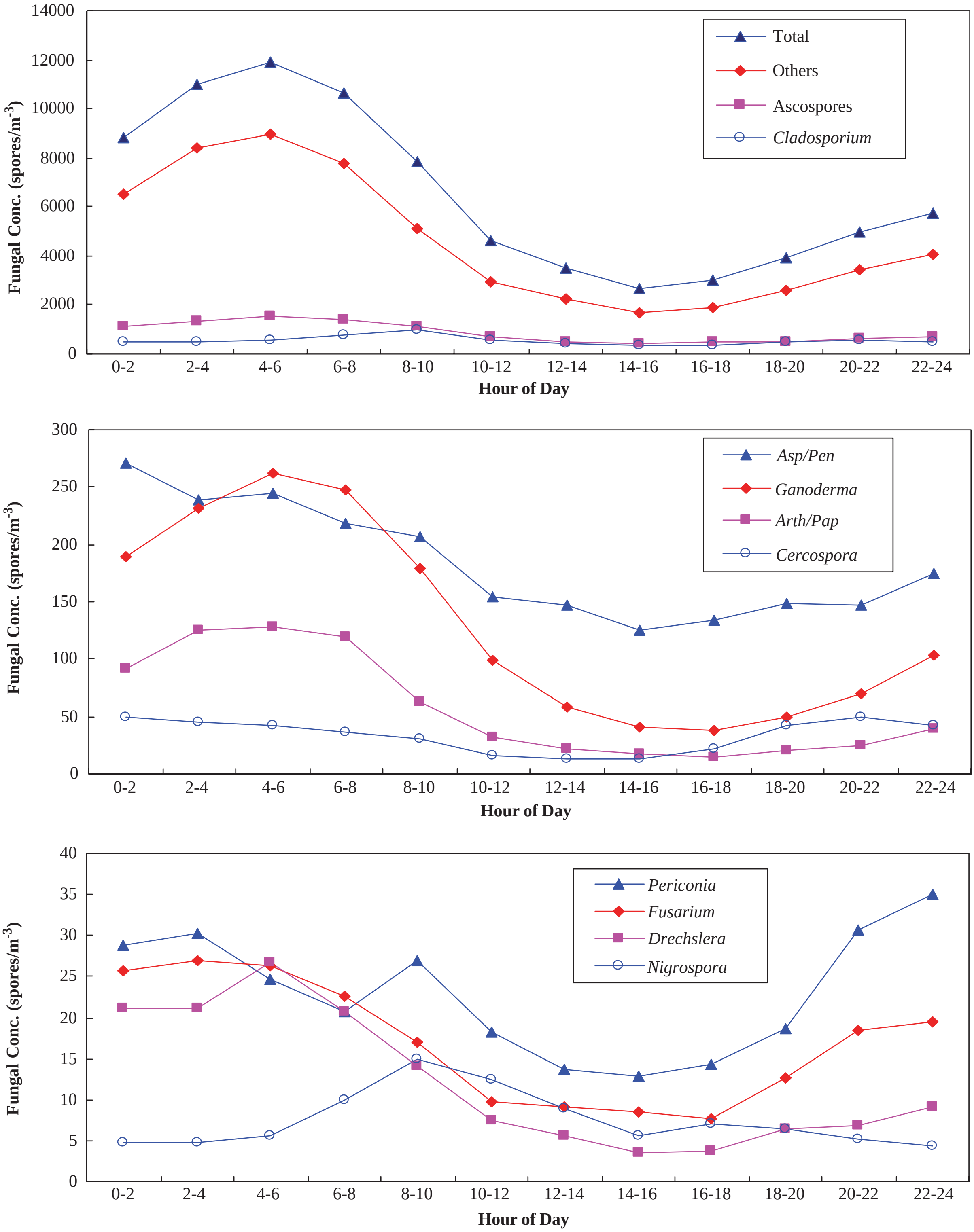


Fig. 1. Average diurnal fungal variations in Hualien, Taiwan.

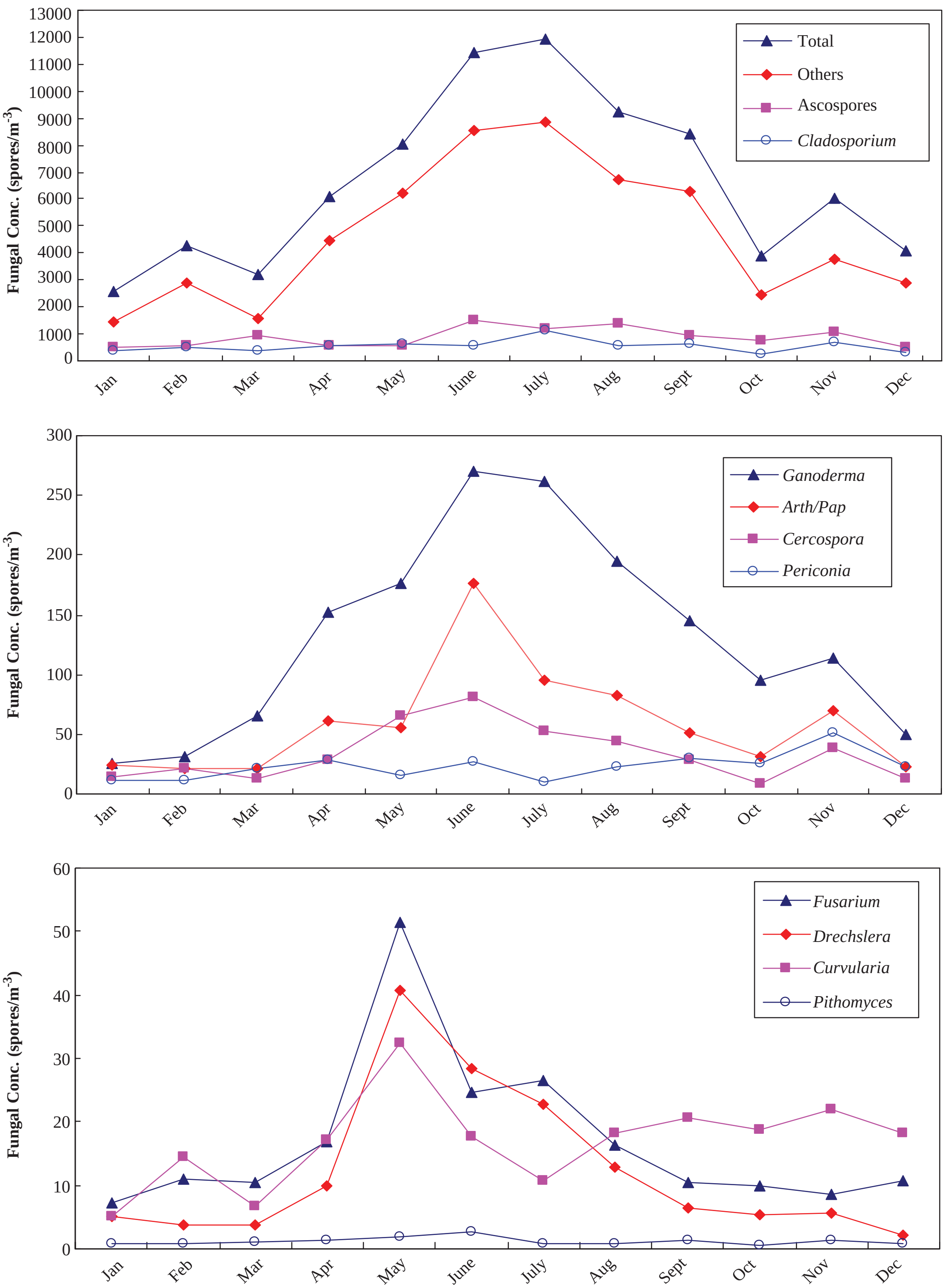


Fig. 2. Seasonal fungal variations in Hualien, Taiwan.

Table 2
Distributions of fungi and selected environmental factors during background and dust storm episode days in Hualien, Taiwan

| | Episode days ($n = 235$) | | | | Background days ($n = 297$) | | | | $p$ value[a] |
|---|---|---|---|---|---|---|---|---|---|
| | Freq (%)[b] | Mean | Med | SD | Freq (%) | Mean | Med. | SD | |
| Fungi (spores $m^{-3}$) | | | | | | | | | |
| Total | 100.0 | 6078.1 | 3566.7 | 7247.2 | 100.0 | 4839.1 | 2586.7 | 6026.5 | 0.0073 |
| Other | 92.3 | 4002.9 | 1573.3 | 6557.2 | 92.6 | 3418.6 | 1060.0 | 5614.4 | 0.3458 |
| Ascospores | 91.5 | 791.5 | 246.7 | 1514.7 | 95.6 | 624.0 | 166.7 | 1332.6 | 0.2412 |
| *Cladosporium* | 99.6 | 759.7 | 293.3 | 1319.5 | 97.3 | 474.0 | 240.0 | 686.4 | 0.0076 |
| *Aspergillus/Penicillium* | 79.2 | 205.5 | 80.0 | 311.7 | 92.6 | 133.3 | 66.7 | 189.6 | 0.3967 |
| *Ganoderma* | 91.0 | 104.9 | 46.7 | 133.3 | 84.2 | 67.6 | 26.7 | 103.0 | $<0.0001$ |
| *Arthrinium/Papularia* | 73.2 | 91.5 | 20.0 | 164.3 | 57.9 | 34.1 | 6.7 | 108.5 | $<0.0001$ |
| *Cercospora* | 58.7 | 40.6 | 6.7 | 78.0 | 53.5 | 18.7 | 6.7 | 32.4 | 0.0130 |
| *Curvularia* | 39.2 | 6.6 | 0.0 | 12.7 | 51.5 | 16.7 | 6.7 | 37.0 | 0.0005 |
| *Periconia* | 68.5 | 32.6 | 13.3 | 58.7 | 57.9 | 14.6 | 6.7 | 24.7 | $<0.0001$ |
| *Fusarium* | 40.9 | 8.4 | 0.0 | 14.7 | 42.8 | 13.9 | 0.0 | 28.2 | 0.3499 |
| *Drechslera* | 33.6 | 10.5 | 0.0 | 28.3 | 37.4 | 7.5 | 0.0 | 16.8 | 0.6102 |
| *Nigrospora* | 40.4 | 8.0 | 0.0 | 21.8 | 34.3 | 5.1 | 0.0 | 11.8 | 0.0854 |
| *Torula* | 34.5 | 5.2 | 0.0 | 11.0 | 32.7 | 5.0 | 0.0 | 11.9 | 0.6569 |
| *Ulocladium/Stemphylium* | 22.1 | 3.8 | 0.0 | 19.5 | 21.6 | 3.0 | 0.0 | 14.0 | 0.7873 |
| *Alternaria* | 31.5 | 4.5 | 0.0 | 9.8 | 17.2 | 1.7 | 0.0 | 4.8 | $<0.0001$ |
| *Pithomyces* | 8.1 | 0.7 | 0.0 | 2.5 | 12.1 | 1.2 | 0.0 | 4.1 | 0.1269 |
| *Botrytis* | 11.1 | 1.4 | 0.0 | 5.3 | 3.0 | 0.2 | 0.0 | 1.3 | 0.0002 |
| RH (%) | — | 75.2 | 74.8 | 8.0 | — | 76.8 | 76.7 | 7.7 | 0.0022 |
| Pressure (mmHg) | — | 764.1 | 764.0 | 3.5 | — | 764.8 | 765.6 | 3.9 | 0.0196 |
| $PM_{10}$ ($\mu g\,m^{-3}$) | — | 57.6 | 53.0 | 29.1 | — | 46.1 | 42.5 | 25.3 | $<0.0001$ |

[a]Mann–Whitney $U$ Test was used to examine the differences of fungal levels between episode and background days, except for Botrytis [for which Exact Test was used because of low recovery frequency ($<10$ times)].
[b]Frequency was the percentage of samples with presence of that specific fungal category.

Table 3
Descriptive statistics for major fungal categories and environmental factors from January 1994 to March 1996 in Hualien, Taiwan

| Variables | Mean | Median | SD | Min. | Max. |
|---|---|---|---|---|---|
| Fungi (spores $m^{-3}$) | | | | | |
| Total | 4844.41 | 3618.06 | 4428.70 | 46.67 | 22476.67 |
| Other | 2896.58 | 880.28 | 4204.83 | 0.00 | 19734.44 |
| Ascospores | 1109.54 | 336.94 | 1599.74 | 0.00 | 8254.44 |
| *Cladosporium* | 446.25 | 283.89 | 507.98 | 5.56 | 3678.89 |
| *Aspergillus/Penicillium* | 155.20 | 88.52 | 206.24 | 0.00 | 1351.11 |
| *Ganoderma* | 105.20 | 72.50 | 109.81 | 0.00 | 624.44 |
| *Arthrinium/Papularia* | 48.16 | 10.00 | 92.33 | 0.00 | 569.44 |
| Temperature (°C) | 22.86 | 23.00 | 4.54 | 13.32 | 30.23 |
| RH (%) | 77.40 | 78.05 | 8.39 | 57.77 | 97.46 |
| Pressure (mmHg) | 764.65 | 764.85 | 5.06 | 748.44 | 775.79 |
| Rainfall (mm) | 0.22 | 0.00 | 0.78 | 0.00 | 6.55 |
| $PM_{10}$ ($\mu g\,m^{-3}$) | 44.58 | 42.58 | 17.36 | 17.54 | 134.08 |
| $SO_2$ (ppb) | 1.11 | 0.98 | 0.72 | 0.13 | 6.67 |
| CO (ppm) | 0.79 | 0.76 | 0.22 | 0.17 | 1.55 |
| $O_3$ (ppb) | 16.79 | 16.31 | 7.48 | 2.03 | 37.95 |
| NO (ppb) | 7.62 | 6.60 | 4.54 | 1.12 | 32.61 |
| $NO_2$ (ppb) | 15.92 | 15.86 | 5.60 | 2.31 | 30.25 |
| THC (ppm) | 2.09 | 2.12 | 0.24 | 0.39 | 2.80 |
| $CH_4$ (ppm) | 1.85 | 1.86 | 0.21 | 0.16 | 3.44 |
| NMHC (ppm) | 0.26 | 0.23 | 0.17 | 0.04 | 1.82 |

Table 4
Multiple regression models for major fungal categories in Hualien, Taiwan

| | $\beta$ Coeff. | SE | $p$ value | $R^2$ |
|---|---|---|---|---|
| *Total Fungi* | | | | 0.44 |
| Intercept | 2.7224 | 0.4196 | <0.0001 | |
| Temperature | 0.0267 | 0.0094 | 0.0050 | |
| $Log_{10}$ ($PM_{10}$) | 0.4066 | 0.1980 | 0.0414 | |
| $O_3$ | −0.0110 | 0.0042 | 0.0094 | |
| THC | −0.3532 | 0.1483 | 0.0183 | |
| CO | 0.4117 | 0.1613 | 0.0115 | |
| Yr 1994 | 0.2608 | 0.0981 | 0.0085 | |
| Yr 1995–6 | 0 | — | — | |
| *Other Fungi* | | | | 0.77 |
| Intercept | 27.5590 | 9.7799 | 0.0054 | |
| Pressure | −0.0323 | 0.0128 | 0.0126 | |
| Rainfall | −0.1129 | 0.0469 | 0.0171 | |
| $O_3$ | −0.0143 | 0.0055 | 0.0096 | |
| $NO_2$ | 0.0261 | 0.0102 | 0.0114 | |
| THC | −0.4700 | 0.2095 | 0.0260 | |
| Yr 1994 | 1.2617 | 0.2838 | <0.0001 | |
| Yr 1995–6 | 0 | — | — | |
| *Ascospores* | | | | 0.64 |
| Intercept | −1.5503 | 0.6313 | 0.0150 | |
| Temperature | 0.0424 | 0.0154 | 0.0064 | |
| RH | 0.0401 | 0.0066 | <0.0001 | |
| $Log_{10}$ ($SO_2$) | 0.3448 | 0.1510 | 0.0235 | |
| *Cladosporium* | | | | 0.27 |
| Intercept | −0.9169 | 0.7408 | 0.2174 | |
| Temperature | 0.0215 | 0.0102 | 0.0363 | |
| RH | 0.0227 | 0.0055 | <0.0001 | |
| $Log_{10}$ ($PM_{10}$) | 0.5665 | 0.2212 | 0.0112 | |
| Yr 1994 | 0.4463 | 0.1206 | 0.0003 | |
| Yr 1995–6 | 0 | — | — | |
| *Aspergillus/Penicillium* | | | | 0.02 |
| Intercept | 2.6914 | 0.3958 | <0.0001 | |
| RH | −0.0106 | 0.0051 | 0.0385 | |
| *Ganoderma* | | | | 0.65 |
| Intercept | 0.0128 | 0.2278 | 0.9552 | |
| Temperature | 0.0821 | 0.0086 | <0.0001 | |
| Rainfall | −0.0886 | 0.0297 | 0.0032 | |
| $O_3$ | −0.0120 | 0.0037 | 0.0015 | |
| Yr 1994 | 0.2049 | 0.0891 | 0.0225 | |
| Yr 1995–6 | 0 | — | — | |
| *Arthrinium/Papularia* | | | | 0.51 |
| Intercept | −0.1573 | 0.2715 | 0.5631 | |
| Temperature | 0.0434 | 0.0115 | 0.0002 | |
| Yr 1994 | 0.6938 | 0.1346 | <0.0001 | |
| Yr 1995–6 | 0 | — | — | |

spore types might be a result of different sampling methods (the gravity slide method underestimates small spores) and local vegetation shift.

All the fungal categories had statistically significant diurnal variations in our study. Several fungal categories (i.e., total fungi, other fungal spores, ascospores, *Ganoderma*, *Arthrinium/Papularia*, and *Drechslera*) followed a Night Pattern as coined by Gregory (1973) with highest concentrations occurring between sunset and sunrise. The causes of this diurnal pattern are probably due in part to vertical mixing depth of air and radiation inversion at night. In addition, ascospores and basidiospores are released as humidity increases, and thus tend to be more concentrated at late night and before dawn (Burch and Levetin, 2002). Spore types such as *Cladosporium* and *Alternaria*, are usually found in higher concentrations during the warmest part of the day, dry weather conditions, with greatest wind speed and turbulence, usually referred as Middle-Day Pattern (Burch and Levetin, 2002; Gregory, 1973; Troutt and Levetin, 2001). In our study, the concentrations of *Cladosporium* and *Alternaria* were highest during 8 to 10 am.

Seasonal variations were similar every year over the study period. Most fungal categories peaked during warmer months, from May to July (Fig. 2). Although peak months varied slightly with differing local climates, similar findings were observed in studies conducted in other parts of the world (Bush and Portnoy, 2001; Di Giorgio et al., 1996; Oh et al., 1998). In spite of the consistent seasonal variations, the concentrations of total fungal spores and several major fungal categories decreased over the sampling years in Hualien City (Table 4). Sources of ambient fungi are usually considered to remain constant over time. However, major changes in the primary fungal source, plant materials, will alter the concentrations/compositions of fungal air spora (Burge, 2002). Urbanization of Hualien City and diminishing vegetation in vicinity might be some major reasons for decreasing ambient fungal levels during the study period.

To date, only very limited data are available for biological composition of Asian dust storms. In a study in Seosan, Korea, conducted during Asian dust and non-Asian dust periods in Spring 2000, researchers qualitatively found more diverse culturable fungi in samples taken during Asian dust events, including *Fusarium*, *Aspergillus*, *Penicillium,* and *Basipetospora*. Although the multi-stage impactor filter samples might result in low viability for many fungi, this study pointed out potential effects of Asian dust storms on fine biological particles (1.1–2.1 μm) (Yeo and Kim, 2002). Wu et al. (Wu et al., 2004) found basidiospores, *Aspergillus*/*Penicillium*, *Nigrospora*, *Arthrinium*, *Curvularia*, Rusts, *Stemphylium*, *Cercospora*, *Pithomyces,* and unidentified fungi had significantly higher concentrations during dust storm days than those of background days in Tainan City (southern Taiwan). Our study, which was conducted approximately 4 years earlier, found that total fungal spores, *Cladosporium*, *Ganoderma*, *Arthrinium*/*Papularia*, *Cercospora*, *Periconia*, *Alternaria*, *Botrytis*, as well as $PM_{10}$ had significantly higher concentrations during episode days. Moreover, in our study, *Periconia* and *Botrytis* had significantly higher levels during episode days; *Curvularia* and *Pithomyces* levels increased during background days. These outcomes were opposite to the findings in Tainan City. The discrepancies between the two studies are in part due to different geographical regions and weather conditions. In a study of ambient air pollutants monitored throughout Taiwan, the researcher found that Asian dust events contributed higher concentrations of particulate pollutants in northern and eastern Taiwan (Liu, 2004). Therefore, the long-range transported air pollutants might not influence Tainan City (southern Taiwan) as much as in Hualien City (eastern Taiwan). More studies are needed to clarify the associations between Asian dust events and ambient fungal composition/variation, preferably in areas with minimal influences of local pollutants.

Temperature and sampling year were the most consistent variables predicting fungal concentrations (Table 4). Temperature is one of the most important environmental factors that influence fungal survival and growth (Burge and Otten, 1999). Similar to the results of other studies (Di Giorgio et al., 1996; Mitakakis et al., 1997; Troutt and Levetin, 2001), we found positive correlations between temperature and most fungal categories, except for *Aspergillus*/*Penicillium* and other fungi. The negative association between sampling year and fungal concentrations was likely due to local vegetation change, as previously discussed.

Water is another essential environmental factor determining fungal survival and growth (Burge and Otten, 1999). Both relative humidity and rainfall are measures associated with water availability outdoors. The effect of rainfall on ambient fungal level is mixed. Rainfall may cause release of fungal spores by splash and "tap-and-puff" mechanisms. Ascospores and basidiospores require sufficient moisture for dispersal, either during rainfall or as humidity increases. On the other hand, rain removes ambient fungal spores by both rainout and washout effects, especially for dry-weather spores, such as *Cladosporium* and *Alternaria* (Burge and Rogers, 2000; Weber, 2003). Therefore, variable correlations have been observed in the literature. Studies in Melbourne, Australia and Denver, Colorado, USA, found that total fungal spores, *Cladosporium* and *Alternaria* were negatively correlated with rainfall and positively correlated with average temperature and relative humidity (Katial et al., 1997; Mitakakis et al., 1997). In Seoul, Korea, *Leptospheria* ascospores concentrations can abruptly increase during the monsoon season (Oh et al., 1998). Li and Kendrick (Li and Kendrick, 1994) found that, in Southern Ontario, Canada, the concentrations of *Leptosphaeria* and unidentified ascospores were higher during light rain, and basidiospores were most numerous during medium RH and rain. In our study, relative humidity was positively associated with ascospores and *Cladosporium* and negatively associated with *Aspergillus*/*Penicillium*. Rainfall was negatively associated with other fungi and

*Ganoderma,* a basidiospore. This negative relationship might implicate a long-term washout effect of rainfall in Hualien City.

*Penicillium* and *Aspergillus* are both common soil fungi decaying dead plant material outdoors and are produced in response to temperature and moisture conditions (Burge and Rogers, 2000). Nevertheless, relative humidity was the only measured environmental factor significantly associated with this fungal category. In addition, our statistical model could only account for 2% of the variation of *Aspergillus*/*Penicillium* concentrations, as opposed to 27 to 77% of the other fungal categories. Additional environmental factors, such as local agricultural activities, near-by biological sources, wind direction and speed, and sunlight, should be considered in future studies to fully understand the behavior of *Aspergillus*/*Penicillium*.

Fungal concentrations were associated with several air pollutants in our study, including $O_3$, $PM_{10}$, CO, $NO_2$, $SO_2$, and THC (Table 4). Ozone was negatively correlated with total fungi, other fungi, and *Ganoderma*. A negative association between total fungal concentrations and ambient $O_3$ was also observed in several other studies (Delfino et al., 1996; Lin and Li, 2000). Ozone is toxic to ambient microorganisms, especially after reacting with atmospheric olefins and forming so-called "open air factors" (Cox, 1995; Cox et al., 1973). On the other hand, it has been suggested that ozone might potentiate the health effect of ambient aeroallergens (Higgins et al., 2000). Therefore, the confounding effects of ozone and ambient fungi on respiratory symptoms should be examined in epidemiological studies, although the effects were not consistent in the past (Lewis et al., 2000; Rutherford et al., 2000).

In our study, $PM_{10}$ was positively associated with total fungi and *Cladosporium*. According to a research conducted in Queensland, Australia, fungal spores dominated the bioaerosol counts in the 2–10 μm range and fungal spores contributed approximately 5% to the total particulate mass (Glikson et al., 1995). Delfino et al. (Delfino et al., 1996) also found a positive correlation between total fungal spores and $PM_{2.5}$. Therefore, it is not surprising to observe associations between fungi and particulate matters. However, the relationships were variable in different studies (Glikson et al., 1995; Lin and Li, 2000; Wu et al., 2004). We also found fungal concentrations were associated with several other air pollutants, including CO, $NO_2$, $SO_2$, and THC. More studies are needed to understand the mechanisms of these associations.

## 5. Conclusion

This study provided essential information of long-term fungal compositions and variations in Hualien City, where air spora were more abundant than most of the areas in Taiwan. Significant diurnal rhythms were found for all fungal categories and coincided with the periodicities of Night Pattern and Middle-Day Pattern. Most fungal spores varied with season and usually peaked at warmer months. Associations between ambient fungi and environmental parameters were complex. Temperature was the most consistent factor positively associated with fungal levels. Several meteorological factors, such as rainfall and relative humidity, had mixed effects on fungal concentrations. Moreover, both local and long-range transported pollutants were likely to interact with aeroallergens, although most of the mechanisms remain to be studied. Because of significant temporal variation and the complicated interrelationships among aeroallergens and air pollution/meteorological factors, futures studies should examine the health impacts of aeroallergens using a longitudinal study design and examining the synergistic/antagonistic effects of these environmental parameters.

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