Aerobiologia **16:** 75–81, 2000.

Multi-year study of *Ganoderma* aerobiology

Rebekah L. Craig & Estelle Levetin*
Faculty of Biological Science, University of Tulsa, Oklahoma, USA
*(*author for correspondence, e-mail: estelle-levetin@utulsa.edu)*

(Received 27 October 1999; accepted in final form 8 November 1999)

Key words: aerobiology, airborne spores, basidiospores, *Ganoderma*, Oklahoma

Abstract

Ganoderma basidiospores are dominant members of the airspora in many regions of the world and are considered important airborne allergens. The aerobiology of *Ganoderma* spores in the Tulsa, Oklahoma area was examined using Burkard Volumetric Spore Traps from 1987–1996. *Ganoderma* spores were present in the atmosphere on more than 95% of the days from June through October with peak concentrations generally occurring from late August to mid-October. The data showed marked interannual variation, with seasonal totals in 1994 and 1995 significantly higher than other years. Stepwise backward multiple regression showed that cumulative season total was significantly related to June temperature and May through August precipitation ($R^2 = 0.97$, $p < 0.01$).

Abbreviations: CST – cumulative season total

1. Introduction

Basidiomycetes are a complex group of organisms within the Kingdom Fungi consisting of approximately 25,000 species. Almost all of the known edible and poisonous mushrooms are contained within this subdivision, along with all the bracket fungi and other large fleshy fungi. At least nine species of *Ganoderma* thrive in North America. Typically, these polypores are found growing on dead or living hardwood and conifers. *Ganoderma applanatum*, *G. lucidium*, and *G. curtisii* can all be found in Oklahoma. The spores are easily recognizable with a smooth transparent outer wall and golden brown inner wall. Interwall connections and a prominent germ pore with truncated apex are also distinctive features (Levetin, 1989).

Studies from various parts of the world have clearly implicated *Ganoderma* spores as aeroallergens (Tarlo et al., 1979; Hasnain et al., 1984). Evidence from New Zealand; Ontario, Canada; and Delhi, India have reported atmospheric concentrations of *Ganoderma* ranging from 6% to 34% of the air spora (Tarlo et al., 1979; Hasnain et al., 1984, Cutten et al., 1988; Singh et al., 1995). In various reports, 10–48% human sensitization in skin prick tests has been attributed to *Ganoderma* spores (Tarlo et al., 1979; Butcher et al., 1987; Hasnain et al., 1993; Singh et al., 1995).

It is well known that weather conditions influence the day-to-day variability as well as seasonal levels of atmospheric spore concentrations. For instance, variations in precipitation and temperature can influence *Ganoderma* spore release (McCracken, 1987). During routine aerobiological analysis of Burkard slides, it appeared that *Ganoderma* spores were more abundant during certain years; however, these spores had not been itemized for the whole period. The present study was undertaken to specifically determine the daily concentration of airborne *Ganoderma* spores from 1987 to 1996 and to identify the meteorological factors that contributed to the yearly fluctuations.

2. Materials and methods

Since December 1986, the atmosphere in Tulsa has been monitored with Burkard Volumetric Spore Traps. The concentration of *Ganoderma* spores in the Tulsa atmosphere was analyzed from June 1 to October 31, from 1987 through 1996, using data from three different sites. One sampling station (Site A) was located

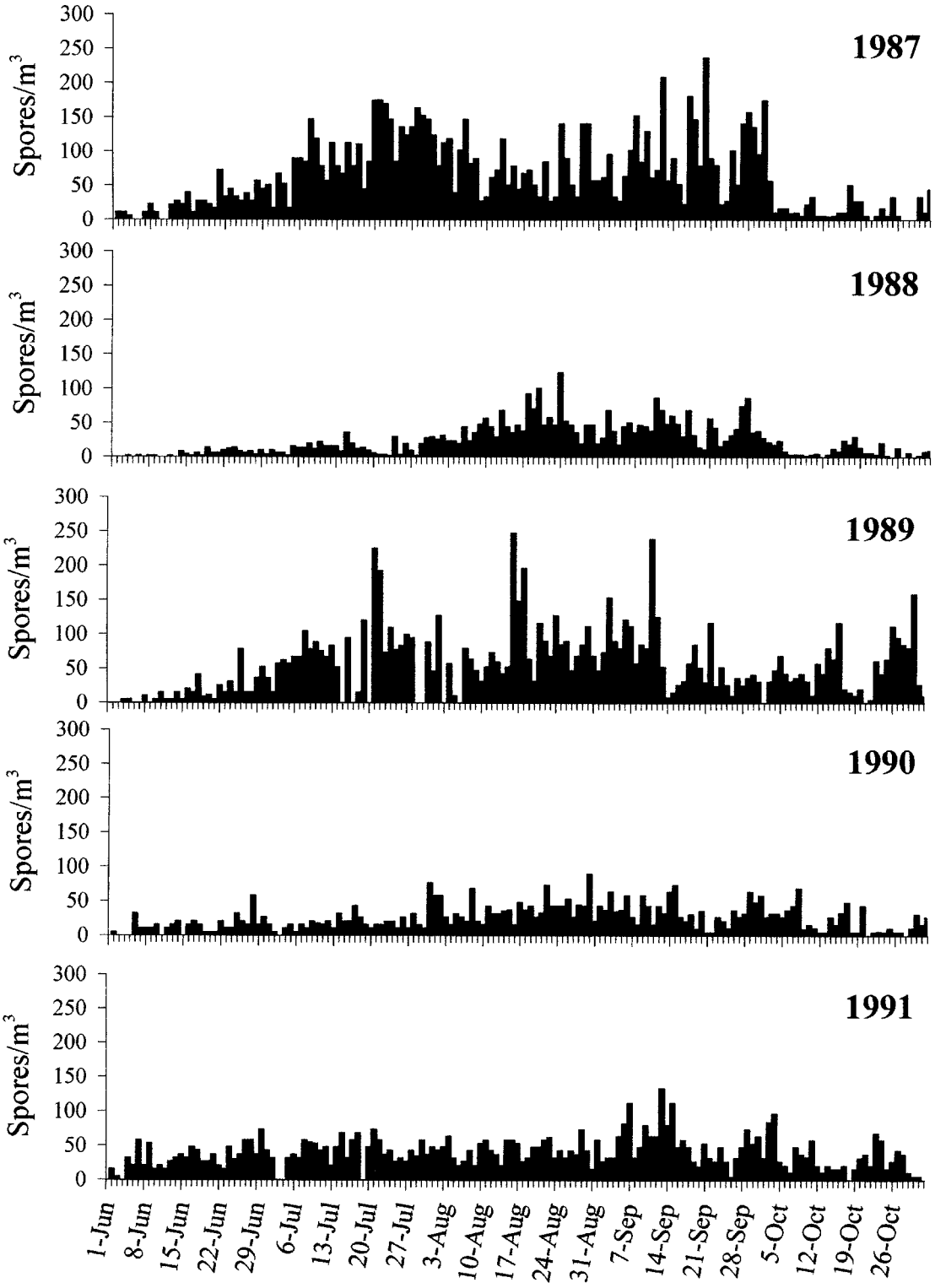
1987
1988
1989
1990
1991
Spores/m3
300
250
200
150
100
50
0
1-Jun
8-Jun
15-Jun
22-Jun
29-Jun
6-Jul
13-Jul
20-Jul
27-Jul
3-Aug
10-Aug
17-Aug
24-Aug
31-Aug
7-Sep
14-Sep
21-Sep
28-Sep
5-Oct
12-Oct
19-Oct
26-Oct

Figure 1. ►

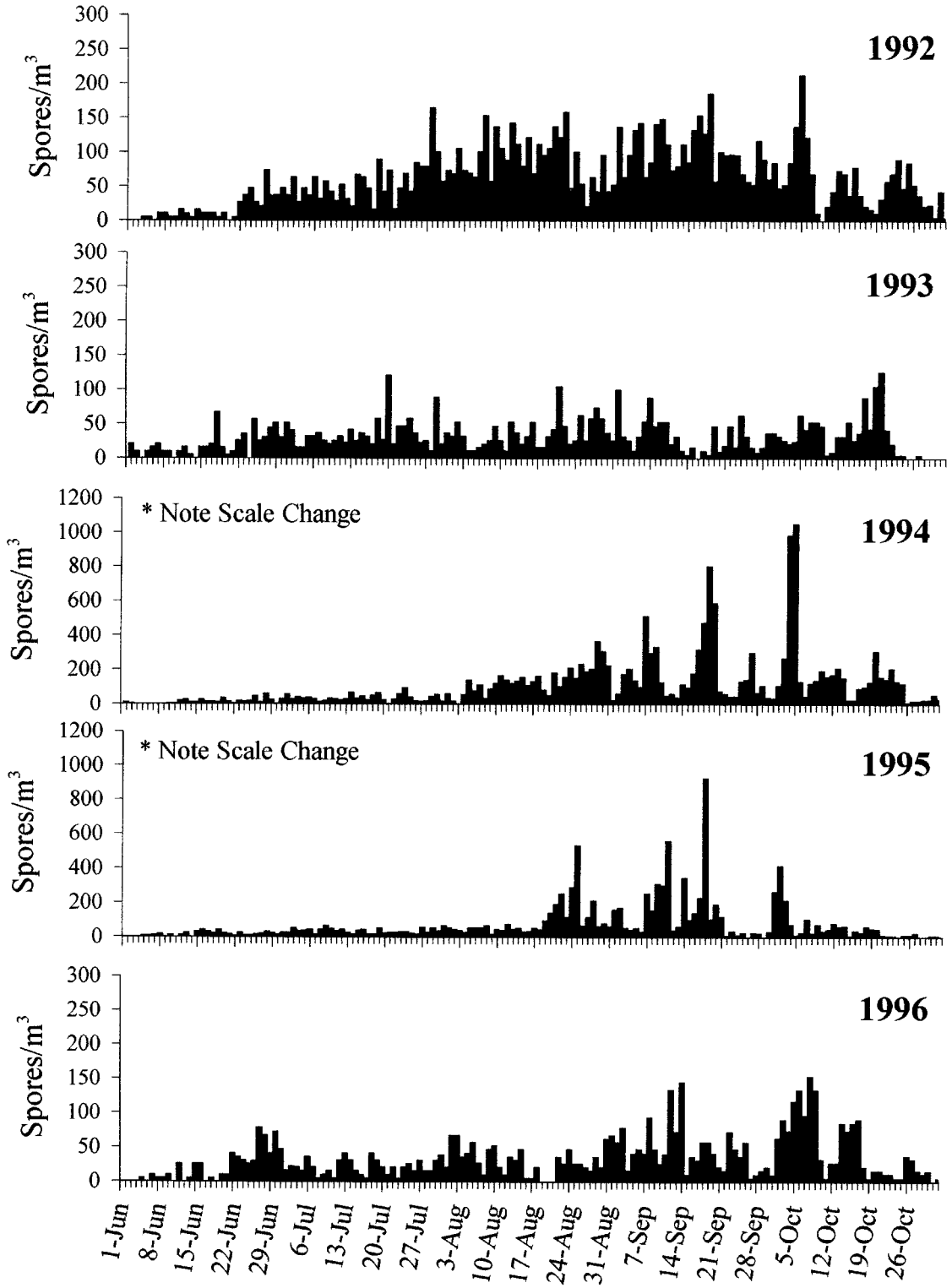

Figure 1. Yearly profiles of average daily concentration of *Ganoderma* spores in the Tulsa atmosphere from 1987–1996. Note different scales for 1994 and 1995. In 1994, data from Site B was used from July 5 through August 31.

on the roof of a flat-topped building at The University of Tulsa approximately 12 meters above ground. A second sampling station (Site B) was located 1.5 meters above ground in an older residential neighborhood 6.5 kilometers southwest of the University. The third sampler (Site C) was located approximately 40 kilometers southwest of Tulsa in a cattle pasture. The Burkard traps were set for seven-day sampling onto Melenex tape coated with a thin film of Lubriseal. The tapes were changed weekly and cut into one-day segments that were mounted on microscope slides. Slides were stained with glycerin-jelly containing basic fuchsin and examined microscopically. Slides from Site A and Site B were analyzed at 1000× magnification using the single traverse method (one longitudinal traverse parallel to the direction of movement); slides from Site C were analyzed at 1000× magnification using the 12 traverse method (Sterling et al., 1999).

Microscope counts were converted into atmospheric concentrations and expressed as spores per cubic meter of air. Previous studies had shown that few *Ganoderma* spores occurred in the atmosphere before June 1 or after Oct 31 (Levetin, 1990). Therefore the average daily concentration of spores from June 1 to October 31 was used to generate the cumulative season total (CST), the mean spore concentration for the season, and the peak concentration during the season.

Meterological data (average temperature, relative humidity, precipitation, heating degree days, and cooling degree days) were obtained from the NOAA (National Oceanic and Atmospheric Administration). The data were collected at the National Weather Service Station approximately 8 km northeast of the sampling station at the university. Yearly spore concentrations were correlated with the following meterological parameters: monthly precipitation for January through October, mean monthly temperature for January through October, mean monthly relative humidity for January through October, total yearly heating degree days, and total yearly cooling degree days. Simple regression, backward stepwise multiple regression, and t-tests were performed on the spore data and meteorological data using Statistica 5.0 software.

3. Results

The onset and duration of the *Ganoderma* season were fairly consistent; *Ganoderma* spores were present in the Tulsa atmosphere on 95% of the days from June 1 through October 31 at Site A (Figure 1). Peak concentrations generally occurred from late August to mid-October. The average cumulative season total was 7,998.

Table 1. *Ganoderma* spore season statistics for Tulsa 1987–1996.

Year	Cumulative Season Total	Mean Daily Concentration (spores/m^3)	Peak Concentration (spores/m^3)	Date of Peak
1987	9873	65	237	Sep 19
1988	3679	24	124	Aug 23
1989	8625	56	248	Aug 15
1990	3935	26	90	Aug 29
1991	6131	40	133	Sep 12
1992	9848	64	213	Oct 4
1993	4781	31	126	Oct 19
1994	17220	113	1055	Oct 4
1995	10800	71	926	Sep 17
1996	5093	33	154	Oct 7

Examination of yearly *Ganoderma* spore statistics showed that several spore parameters varied considerably from year to year (Table 1). None of the spore parameters studied (CST, Mean, and Peak) showed statistically significant variation over time during the years that were analyzed. However, a two-way ANOVA showed there were significant differences ($p < 0.05$) between the monthly means and also between seasonal means. The CST for 1994 was unusually high, 17,220, exceeding all other yearly totals by at least 6,200 (Figure 2). During this year, the highest daily peak was recorded, 1055 spores/m^3, along with the highest seasonal mean, 113 spores/m^3. The CST and peak concentration for 1995 were also high, exceeding all other years except 1994 by 927 and 678 spores/m^3, respectively. The mean for 1994 and 1995 (92 spores/m^3) was significantly greater than the mean for the other 8 years (43 spores/m^3, $T_{152} = -5.90$, $p < 0.01$). The 1988 season had the lowest CST, 3,679, and the lowest mean, 24 spores/m^3. The 1990 season had the lowest daily peak, 90 spores/m^3 and a seasonal mean of 26 spores/m^3 (Figure 3).

The diurnal periodicity from ground level sampling at Site C in 1996 revealed peak concentrations at 04:00 hours (Figure 4). This peak constituted 12.7% of the total daily concentration. Lowest levels were observed at 16:00 hours.

Multiple regression analysis was performed to determine which meteorological variables were the

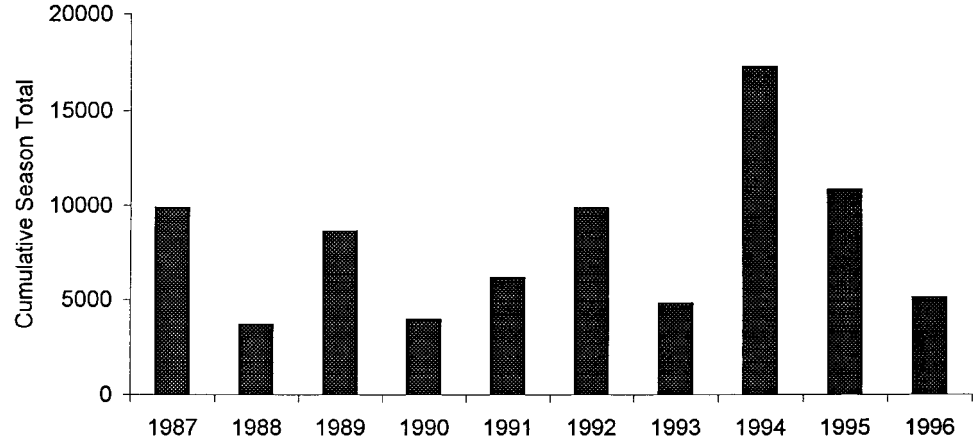

Figure 2. Cumulative season total of airborne *Ganoderma* spores in Tulsa.

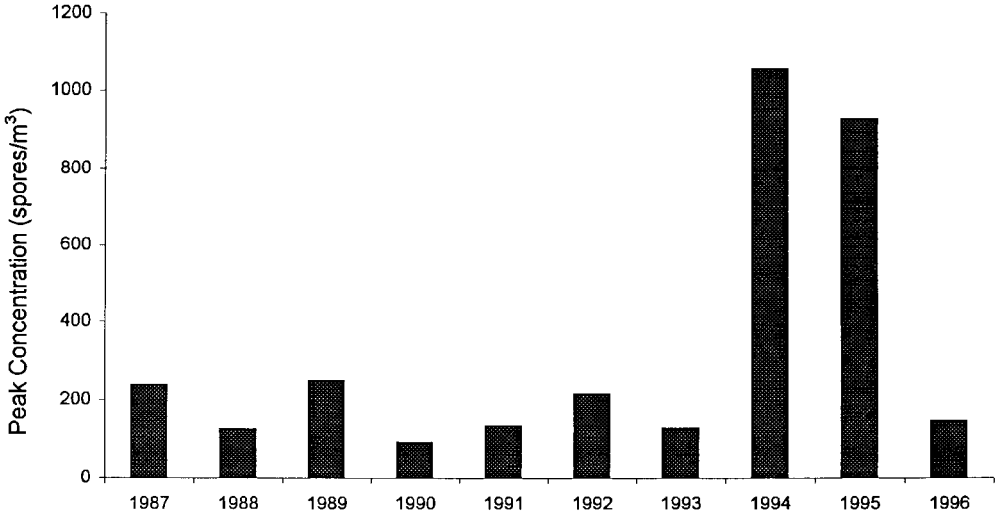

Figure 3. Peak airborne *Ganoderma* concentration per year.

most important predictors for CST. Backward stepwise multiple regression analysis showed that June temperature and May through August precipitation were significant factors for predicting CST (Table 2). For this 10-year period, these factors explained 97% of the variation.

4. Discussion

Data collected in this study clearly indicate the significance of *Ganoderma* basidiospores as bioaerosols. *Ganoderma* spores were present in the atmosphere on 95% of the days from June to October of 1987–1996. These results are similar to results from previous studies in other parts of the world. Singh reported that maximum *Ganoderma* spore release occurred during the late summer and autumn, the rainy season in Delhi (Singh et al., 1995). Cutten also reported that highest spore output was observed in January and February, which is late summer in Auckland (Cutten et al., 1988). Despite the consistent occurrence of spores throughout the 10 years studied, the seasonal levels of *Ganoderma* spores were highly variable.

The CST for 1994 exceeded all other yearly totals by at least 6,200. Due to a roofing project at Site A, spore concentrations from Site B were used for July 5 through August 31 of 1994. In a previous study, concentrations of aeroallergens at Site B were found to be greater than at Site A. In 1987, the peak concentration of *Ganoderma* basidiospores at Site A was close to 250 spores/m^3; however, the peak at Site B was

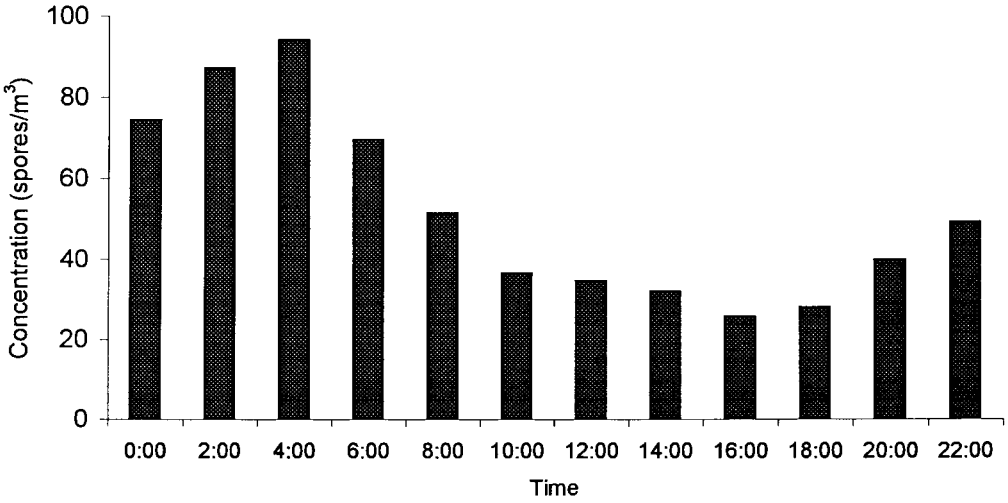

Figure 4. Diurnal periodicity of *Ganoderma* basidiospores.

Table 2. Multiple regression model showing the influence of meteorological factors on cumulative season total of airborne *Ganoderma* basidiospores ($R^2 = 0.97$, $p < 0.01$).

Meteorological Variable	Beta Value	t-test
June Temperature	0.938	6.107*
May Precipitation	0.256	3.783*
June Precipitation	1.161	7.885*
July Precipitation	0.721	9.907*
Aug. Precipitation	0.378	4.378*

*$p < 0.05$.

over 950 spores/m^3. Concentrations of basidiospores at Site B may have been influenced by nearby sources (Levetin, 1990). The unusually high CST for 1994 may have been influenced by increased average daily concentrations during the weeks that data was collected at Site B. However, the data from Site B used in this study was collected well before the 1994 season peak, which was recorded at Site A on October 4. This peak, 1055 spores/m^3, exceeds all other years, except 1995, by 807 spores/m^3.

Weather conditions in the months prior to the season peak may be important for determining the cumulative season total. Data indicate that June temperature and May through August precipitation influence the magnitude of *Ganoderma* spore levels during the season extending from June 1 to October 31. It is likely that the relationship with precipitation and temperature reflects fungal requirements for optimum growth within the substrate.

The concentrations reported in this 10 year study were daily averages; however, hourly concentrations also differ throughout the day. *Ganoderma* spore release follows a diurnal rhythm with peak concentration at approximately 04:00 hours and lowest levels at 16:00 hours. A similar early morning maximum was observed by Haard and Kramer (1970) at 06:00 hours. Mean temperature and relative humidity during daily peak concentrations were about 22 °C and 79%, respectively. These results are consistent with those reported by McCracken who found that periods of maximum daily spore release were related to mean temperature of 17 °C and mean relative humidity of 77% (McCracken, 1987).

In conclusion, this study showed that (i) in northeastern Oklahoma the season for airborne *Ganoderma* spores lasts from June through October; (ii) there has been a significant difference among years in cumulative season total; (iii) June temperature and May through August precipitation were important predictors for cumulative season total. Additional years of data could provide a more accurate determination of any variation in spore concentration over time.

Acknowledgements

Support for this project was provided in part, by a NSF-EPSCoR grant (Project Number EPS9550478) which provided funds to enhance the Oklahoma Mesonet. The authors wish to thank Melinda Sterling for counting *Ganoderma* spores from Site C and

Dr. Christine Rogers for assistance with data management.

References

Butcher B.T., O'Neil C.E., Reed M.A., Altman L.C., Lopez M. and Lehrer S.B.: 1987, Basidiomycete allergy: measurement of spore specific IgE antibodies. *J. Allergy Clin. Immunol.* **80**, 803–809.

Cutten A.E.C., Hasnain S.M., Segedin B.P., Bai T.R. and McKay E.J.: 1988, The basidiomycete *Ganoderma* and asthma: collection, quantitation, and immunogenity of the spores. *N. Z. Med. J.* **101**, 361–363.

Haard R.T. and Kramer C.I.: 1970, Periodicity of spore discharge in the Hymenomycetes. *Mycologia* **62**, 1145–1169.

Hasnain S.M., Newhook F.J., Wilson D.J. and Corbin J.B.: 1984, First report of *Ganoderma* allergenicity in New Zealand. *N. Z. J. Sci.* **27**, 261–267.

Hasnain S.M., Wilson J.D. and Newhook F.J.: 1985, Allergy to basidiospores: immunologic studies. *N. Z. Med. J.* **98**, 393–396.

Levetin E.: 1990, Studies on airborne basidiospores. *Aerobiologia* **6**, 177–180.

Levetin E.: 1989, Basidiospore identification. *Ann. Allergy* **62**, 306–310.

McCracken F.I.: 1987, Factors affecting the spore release of *Ganoderma applanatus. J. MI. Acad. Sci.* **32**, 55–60.

Singh A.B., Gupta S.K., Pereira B.M.J. and Prakash D.: 1995, Sensitization to *Ganoderma lucidum* in patients with respiratory allergy in India. *Clin. Exp. Allergy* **25**, 440–447.

Sterling M, Rogers C. and Levetin E.: 1999, An evaluation of two methods used for microscopic analysis of airborne fungal spore concentrations from the Burkard Spore Trap. *Aerobiologia* **15**, 9–18.

Tarlo S.M., Bell B., Srinivasan J., Dolovich J. and Hargreave F.E.: 1979, Human sensitization to *Ganoderma* antigen. *J. Allergy Clin. Immunol.* **64**, 43–49.