

THE OCCURRENCE OF *Ganoderma* SPORES IN THE AIR AND ITS RELATIONSHIPS WITH METEOROLOGICAL FACTORS

Agnieszka Grinn-Gofroń

Department of Plant Taxonomy and Phytogeography, University of Szczecin,
Wąska 13, 71-415 Szczecin, Poland
e-mail: agofr@univ.szczecin.pl

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Abstract:

According to a recent study, *Ganoderma* may be the third genus, after *Alternaria* and *Cladosporium*, whose spores cause symptoms of allergy and whose levels are directly related to meteorological factors. There are only few articles from different parts of the world about the relationships between *Ganoderma* spore count and meteorological factors. The aim of the study was to review all available publications about airborne *Ganoderma* spores and to compare the results in a short useful form.

Key words: *Ganoderma*, airborne spores, meteorological factors, statistical correlation

INTRODUCTION

Airborne spores are the primary means of dispersal for fungi. Spores are single or multicellular units surrounded by a rigid (usually chitinous) cell wall. Each spore is capable of reproducing the entire organism (as opposed to pollen grains, which only carry half of the genetic complement of the plant). Fungi can have very complex life cycles, sometimes with up to five morphologically distinct spore types produced during a single cycle. This makes the classification of fungal sources based on airborne spores rather difficult.

Most commonly isolated and identified from air samples are spores of the two largest fungal groups: the Ascomycota and the Basidiomycota. The last group includes mushrooms, puffballs, and bracket fungi, the largest and most conspicuous fungi in the environment. Also included are the rusts and smuts (two groups of plant pathogens that lack fruiting bodies) as well as some yeasts. The characteristic sexual spore in the basidiomycetes (the common name for members of this division) is the basidiospore, which is externally

formed on a basidium. Each basidium typically bears four spores on peglike appendages. Karyogamy and meiosis occur within the basidium, and the four haploid products of meiosis give rise to the four basidiospores. Basidia line the gills of mushrooms and the pores of bracket fungi, with the resulting basidiospores exposed to the atmosphere throughout development. Except for the rusts and smuts, basidiospores are the primary means of dispersal for the Basidiomycetes. Basidiospores are always single-celled, and most have an asymmetrically placed attachment peg (the attachment point to the sterigmata of the basidium). Color, size, and shape of basidiospores vary within relatively narrow ranges. The colors range from nearly black to colourless, with yellow, gold and brown pigments prominent. Sizes range from about 2 to 25 μm , with many spores in the 5 to 10 μm range. Shapes are most often ovate, with occasional species producing elongated, angular, nodulose spores. Many basidiospores have a pore at the end opposite the attachment appendage.

The basidiospores of *Ganoderma*, commonly known as a bracket fungus or wood decay fungus, are an important and prevalent group of fungal air spora worldwide (Levetin, 1990, 1991; Hasnain, 1993; Halwagy, 1994; Lehrer et al. 1994; Li and Kendrick, 1995). At least six species of *Ganoderma* occur in Poland. Their polypores are found growing on dead or living hardwood and conifers. The spores have an orange inner wall and spines that penetrate a colourless outer wall, with a truncated end. This fungus is absolutely from outdoor sources. *Ganoderma* spores range in size from 6.5-13 x 5-9 micrometers.

Discharge of basidiospores, especially from bracket fungi, requires atmospheric moisture, although the mechanism is not completely understood. Basidiospores are frequently seen in the predawn hours when the humidity is high. However, Haard and Kramer

(1970) investigated spore discharge in 19 species of mushrooms and bracket fungi and found three basic patterns. In many species, a night-time maximum was evident, with spore release increasing in the late afternoon or early evening, peaking during the middle of the night, and then decreasing in the morning. Other species, especially some bracket fungi, showed a double peak of spore release in the early morning and evening hours, while many small mushrooms showed continuous spore discharge with a peak 24 to 48 h after the onset of spore discharge and then a gradual decrease as the basidiocarp aged.

Gregory and Hirst (1952) first suggested that basidiospores might be associated with respiratory allergy. Studies from various parts of the world have clearly implicated *Ganoderma* spores as aeroallergens (Tarlo et al. 1979; Hasnain et al. 2004), and they have been implicated in the elicitation of respiratory allergic diseases (Cutten et al. 1988; Sprenger et al. 1988).

Tarlo et al. (1979) found *Ganoderma* basidiospores to be the most prevalent fungal air spores in Ontario (Canada) and reported human sensitization to *Ganoderma* extracts. Hasnain et al. (1985), Cutten et al. (1988) and Hasnain (1993), in their studies from Auckland, New Zealand, reported that *Ganoderma* basidiospores constituted a major component of the air spora and were potential sources of allergens involved in asthma and allergic rhinitis. These authors also suggested that persistent exposure to *Ganoderma* basidiospores could stimulate a base level of allergic responsiveness in susceptible atopic patients. Positive skin and inhalation tests to *Ganoderma applanatum* have been reported by Herxheimer et al. (1969). Lehrer et al. (1986) and Sprenger et al. (1988) found in their studies that 32% of atopic populations in New Orleans, Louisiana, and 30% in Seattle, Washington, USA, were skin test positive to the basidiospores extracts including *Ganoderma*. Singh et al. (1994) reported sensitization to *Ganoderma lucidum* in the atopic population in India. Intradermal and skin prick tests performed using antigenic extract of *G. lucidum* revealed that 28.4%, and 17.4% of patients showed marked skin reactivity to the spores and whole body extracts, respectively. Nonetheless, the airborne basidiospores have been ignored in many studies, yet high concentrations of basidiospores exist in the atmosphere at various locations around the world (Gregory and Hirst, 1952; Salvaggio et al. 1971; Rubulis, 1984; Anderson, 1985; Hasnain et al. 1985; Misra, 1987; Levetin, 1990, 1991). In Sweden, basidiospores accounted for about 30% of the total air spora collected during a several-year study (Rubulis, 1984). In Arctic and subarctic areas, the air spora tends to be dominated by basidiospores and ascospores during the short spore-producing season (Lacey, 1981;

Anderson, 1985). Fall peaks in the concentration of airborne basidiospores have been reported in some of these studies, while both late spring and fall peaks occur in Tulsa, Oklahoma. Previously, studies have categorized basidiospores as coloured and colourless or identified one or two distinctive genera; recent work has identified airborne basidiospores from 18 genera (Levetin, 1991).

Presence in the air

Ganoderma spores very often occur together with those of *Cladosporium* and *Alternaria*. Seasonally, spore levels of *Ganoderma* peaked toward summer and autumn. Tarlo et al. (1979) found *Ganoderma* basidiospores to be the most prevalent fungal air spores in Canada, while Hasnain et al. (1985), Cutten et al. (1988), Hasnain (1993) in their studies from New Zealand reported that *Ganoderma* basidiospores constituted a major component of the air spora, probably reflecting the great forested area in this country, and the markedly higher concentrations occurred in summer and autumn, from December to April. Similar results were noted by Mitakakis and Quest (2001) in Melbourne (Australia) where *Ganoderma* spores accounted for approximately 2% of all identified taxa. The highest concentrations of *Ganoderma* spores which occurred from June to October were noted in Mexico (Calderon et al. 1995), Canada (Li and Kendrick, 1995) and India (Chakraborty et al. 2003). Oliveira et al. (2009), during three-year aerobiological monitoring in two areas of Portugal differing in the urbanization level, recorded higher airborne concentrations (including *Ganoderma* spores) in late summer and early autumn (September and October) and the lowest in the winter months (January and February). In Badajoz (Spain), the basidiospores, including inter alia *Ganoderma*, were present in the atmosphere mainly during autumn. The maximum concentrations were found in November, with the lowest values being found during summer (Gonzalo et al. 1997).

During a five-year study period in India, two very distinct periods of spore concentration were found, the first one in March and the second one in October. The highest number of *Ganoderma* spores occurred in October, probably because of high mean air temperature and relative humidity, low rainfall level and different wind speeds (0.8 – 4.2 km/h). In Kerala District, the presence of spores was not noted in January and February, whereas the highest concentration occurred in August. This was due to specific weather conditions (Chakraborty et al. 2003). During year-round air monitoring conducted in Santiago (Chile), the concentration of *Ganoderma* spores increased in November, while the highest values occurred in February and April (Ibanez Henriquez et al. 2001). In Saudi

Arabia (Jizan City) high concentrations were noted in December and January with air mean temperature between 15 and 20°C and relative humidity about 70% (Hasnain et al. 2004). In Poland the highest concentrations occurred in July (Grinn-Gofroń, 2008) and August (Kasprzyk et al. 2004; Stępałska and Wołek, 2005). In Rzeszów (Poland) *Ganoderma* was the taxon whose spore concentration peaks latest, i.e. in August and in the first days of September (Kasprzyk et al. 2004).

In their results of a 10-year study on *Ganoderma* aerobiology, Craig and Levetin (2000) reported, in addition to yearly fluctuations, that hourly concentrations also differ throughout the day. Its spore release follows a diurnal rhythm with a 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. Different results were obtained in Madras (India) by Vittal and Krishnamoorthi (1981). *Ganoderma* spores exhibited a peak concentration between midnight and dawn and were less common in the day time. It was noticed that there was a sudden increase in the numbers of spores from midnight onwards reaching a peak at 02:00 a.m. and then onwards there was a gradual decline till 18:00 hours after.

Lacey (1981) observed that concentrations of airborne basidiospores of all types showed a diurnal periodicity typical of this fungal spore type, with the maximum concentration at night, between 02:00 and 04:00 h, when dew is sufficient for spore liberation.

Correlations with meteorological factors

Minimum temperature

A strong, statistically significant correlation between *Ganoderma* spore concentration and minimum temperature was noted only in the western and southern parts of Poland (Grinn-Gofroń, 2008; Stępałska and Wołek, 2005). Both results can be considered reliable, because they were obtained by the same methodology and using the same statistical analysis – multiple regression.

Mean temperature

A positive, significant relationship between *Ganoderma* spores and average temperature was noted in Tulsa (Oklahoma, USA) (Craig and Levetin, 2000), and in two cities of Portugal (Porto and Amares) (Oliveira et al. 2009). Similar studies were carried out in Saudi Arabia and significant correlations for basidiospores were found only in Jizan, a small town close to the Red Sea. This town borders Yemen, which has a different flora and fauna with forested ranges, providing a possible source area for Jizan. High concentrations occurred in December and

January when temperatures are generally in the range 15-20°C.

Calderon et al. (1995) noted a negative correlation between temperature and basidiospore count and suggested that spore production or fungal growth decreased at higher temperatures (about >26°C).

Relative humidity

In Mexico City Calderon et al. (1995) observed the largest concentrations of basidiospores when relative humidity was 70 to 80%. In their study, the concentrations of airborne basidiospores were directly correlated with RH. It is well known that high humidity and rain favor the production and liberation of basidiospores. This compares with the reports of McCracken (1987) and Hasnain et al. (2004) that the level of humidity about 70% was associated with increased concentrations of *Ganoderma* spores.

Examining the correlation between spores and meteorological factors in two cities of Portugal, Oliveira et al. (2009) noted a similar positive correlation for *Ganoderma* spores. In Poland (Cracow) only Stępałska and Wołek (2005) observed a positive significant relationship between *Ganoderma* spore count and relative humidity. The results were obtained by the multiple regression method and occurred only in the pre-peak period.

Rainfall

Hasnain (1993) in Auckland, Craig and Levetin (2000) in Tulsa and Calderon et al. (1995) in Mexico noted a significant correlation with precipitation. *Ganoderma* is considered to be a moist-air spore type because its concentrations showed marked seasonal differences with the highest numbers during the wet season and water is an important factor involved in spore release. Lacey (1990) observed abundance of basidiospores during the rainy period in tropical countries. The rainy season accelerates the development of sporocarps and release of more spores because of high humidity and water availability. A significant negative correlation with precipitation was noted in Poland both in Szczecin and Cracow (Grinn-Gofroń, 2008; Stępałska and Wołek, 2005). In Szczecin the correlation was significant for two of the three seasons analyzed and in Cracow in the 1997 season. Both correlations were calculated using the same method (multiple regression).

Wind speed

Hasnain (1993) and Calderon et al. (1995) reported that large concentrations of basidiospores were often associated with daily average wind speeds of 2 to 3 m s⁻¹. This compares with the reports of Lopez and Salvaggio (1983) and Hasnain (1993), among others, that wind velocities >5 m s⁻¹

were correlated with decreased concentrations, perhaps because of the diluting effect of high wind speeds on concentrations of airborne particles. In addition, increasing wind speed would also increase water loss and may, in turn, suppress spore production.

Pressure

A positive significant, but not so strong, correlation with air pressure was noted only in Szczecin by Grinn-Gofroń (2008) during one season studied.

Statistical models

The only one statistical model for *Ganoderma* basidiospores in the air and its relationship to meteorological parameters using both the multivariate regression tree method (MRT) and classification artificial neural network (ANN) model were created in Poland (Grinn-Gofroń and Strzelczak, in press).

MRT analysis revealed that the presence or absence of *Ganoderma* spores depends mostly on dew point temperature, with the threshold value at the level of around 9°C. Similar results were obtained in neural network modelling. The sensitivity analysis of ANN model classification showed that the most important factors determining *Ganoderma* presence/absence were dew point temperature and humidity with the cut-off value for the first variable equal to about 11°C. Maximum and average wind speed and precipitation were less important, but still significant. No other author noted a correlation between dew point temperature and *Ganoderma* concentrations.

Based on the statistical models described above, the general conclusion is that very low performance of the regression models for the seasons suggests that there are some other variables influencing *Ganoderma* spore concentrations, more important than meteorological factors.

The presence of *Ganoderma* basidiospores within the air spora of the world and the high concentrations may be important for understanding allergic reactions. Aerobiological investigations should contribute to the understanding of allergic manifestations caused by basidiospores of *Ganoderma*.

CONCLUSIONS

- *Ganoderma* spores are noted in the air together with those of *Alternaria* and *Cladosporium* mostly during summer and early autumn.
- Minimum, mean temperature, and relative humidity were positively and statistically significantly correlated with *Ganoderma* spore concentration.
- An unclear situation occurred in the case of rainfall. Some authors noted a positive correlation, while others a negative one. In several cases, a statistically significant correlation did not occur.

- A positive significant correlation was reported also for average wind speed, but only in the range between 2 to 3 m s⁻¹.
- Dew point temperature was the most important factor in the statistical predictive models.
- Such discrepancies in the results of various studies suggest that, in addition to meteorological parameters, there are still other factors which strongly influence *Ganoderma* spore concentration in the air.

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Występowanie zarodników *Ganoderma* w powietrzu i korelacje z czynnikami pogodowymi

Streszczenie

Rodzaj *Ganoderma* jest trzecim po *Alternaria* i *Cladosporium* rodzajem grzybów wywołującym objawy alergii. Niestety nie opracowano dotąd wartości stężeń progowych dla tych zarodników, a liczba publikacji opisujących związek stężenia zarodników z czynnikami meteorologicznymi jest wciąż mała.

Najwyższe koncentracje zarodników tego rodzaju notowane są w okresie późnego lata, wczesnej jesieni i w porównaniu z innymi typami stanowią jedną z bardziej licznych grup. Statystycznie istotne, pozytywne korelacje były notowane dla dobowych średnich i minimalnych temperatur oraz dla wilgotności względnej a negatywne dla: wysokości opadów deszczu ale tylko w kilku przypadkach. Średnia prędkość wiatru w zakresie od 2 do 3 m/s również była pozytywnie skorelowana z liczbą zarodników w powietrzu. Wyższe i niższe prędkości wiatru nie wykazywały statystycznie istotnych korelacji.

Statystyczny model prognostyczny oparty na wieloregresyjnym drzewie neuronowym i sztucznych sieciach neuronowych za najważniejszy czynnik warunkujący wysokie stężenie zarodników *Ganoderma* w powietrzu uznał temperaturę punktu rosy.

Rozbieżności w wynikach mogą sugerować, że oprócz parametrów meteorologicznych istnieją inne czynniki, które w znaczący sposób wpływają na obecność zarodników *Ganoderma* w powietrzu.

