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ORIGINAL ARTICLE

Seasonal variation of *Ganoderma* spore concentrations in urban and suburban districts of the city of Szczecin, Poland

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Abstract

According to recent studies, *Ganoderma* may be the third genus, after *Alternaria* and *Cladosporium*, the spores of which cause symptoms of allergy, and concentration is related to meteorological factors. The aerobiology of *Ganoderma* spores in Szczecin in urban and suburban districts was examined using Lanzoni Volumetric Spore Traps in 2008–2010. *Ganoderma* spores were present in the atmosphere on more than 90% of the days from June through September with peak concentrations in June, July and September. The number of days with spores was lower in the suburban district, while the total number of spores collected was higher there than in the urban district. Correlation and multiple regression analyses revealed weak relationships between *Ganoderma* and meteorological conditions, while testing the significance of differences between the districts showed that urban development did not have a clear impact on the values of meteorological parameters. A significantly higher abundance of spores in the suburbs of Szczecin seemed to be conditioned by the closeness of potential area sources. This study indicates that a single measuring site in the city centre insufficiently reflected the dynamics and level of *Ganoderma* spore concentration in peripheral districts.

Key words

Ganoderma spores, seasonal variation, urban area, suburban area

INTRODUCTION

Fungal spores constitute a significant fraction of airborne bioparticles, and it is usual to find concentrations above 1,000 spores·m^{-3}, which is several times more numerous than other airborne particles such as pollen grains.

Basidiomycota is the most morphologically complex fungal division, numbering approximately 25,000 species [1]. The spores of this Basidiomycota division are borne on basidia and can be divided into three classes: the Uredinomycetes (Rusts), Ustomycetes (Smuts) and Basidiomycetes (jelly fungi, rusts, smusts, bracket fungi, mushrooms and puffballs). Common basidiospores include *Ganoderma*, *Agrocybe*, *Coprinus* and *Agaricus* and those from crop pathogens such as the Rusts and Smuts [2].

The *Ganoderma* genus, involves wood-rotting basidiomycetes with laccate and non-laccate pilei. Historically, laccate taxa were referred to as the *G. lucidum* complex, and non-laccate species as the *G. applanatum* complex. These species complexes correspond to subgenera *Ganoderma* and *Elfvingia*, respectively, which are mainly distinguished by the pileus nature. The taxonomy of *Ganoderma* species has been based on classical descriptive criteria. As a result, the concept of a species in the genus is not well established nor universally accepted; most species are poorly circumscribed and their geographic range remains unknown [3].

Studies from various parts of the world have clearly implicated *Ganoderma* spores as aeroallergens [4]. Gregory and Hirst [5] first suggested that basidiospores might be associated with respiratory allergy. In some reports, 10–48% of human sensitization in skin prick tests has been attributed to *Ganoderma* spores [6, 7]. Tarlo et at. [8], during their studies on human sensitization to *Ganoderma* antigen, summarized that the demonstration of high counts of spores in the air, *in vivo* and *in vitro* are evidence of sensitization to *Ganoderma* antigen, and together with the apparent unique antigenic character of the *Ganoderma vis-a-vis* other clinical allergens, indicates that it should receive further consideration as a summer aeroallergen. Knowledge of the relationships between spore production and different environmental growth conditions, such as meteorological factors, can be used to improve diagnosis and treatment of respiratory allergic diseases [9].

The purpose of this study was to analyse the concentration of *Ganoderma* spores in the air of two districts of the city of Szczecin, Poland, differing in type of development and environment, and to determine whether different weather conditions affected spore concentrations in both locations.

MATERIALS AND METHOD

Daily spore concentrations were sampled between 2008–2010 using two Hirst-type volumetric spore traps (VPPS Lanzoni 2000). The flow rate was adjusted to 10 l/min and checked weekly. Spores were collected on Melinex tape coated with a thin film of silicone. Sampler drums were changed weekly, cut into strips 48 mm segments representing the previous 7 days, mounted on microscope slides in glycerol jelly containing basic fuchsine (0.1%), and protected with a cover glass. The daily mean number of fungal spores was measured by use

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of an optical microscope at 400 × along two full lengthwise traverses. Data were expressed as daily average spore per cubic meter of air. The spore data were analysed to determine the start, end and duration of the spore season using the 90% method. The start of a season was defined as the date when 5% of the seasonal cumulative spore count was trapped, and the end of a season as the date when 95% of the seasonal cumulative spore count was reached.

In the urban area of Szczecin (Śródmieście District, Felczaka Street, ST 1 in Fig. 1), the sampler was located on the roof of the building of the Faculty of Biology (height: 21 meters above ground level) The Śródmieście District is located in the city centre and characterized by dense, post-German buildings. Townhouses are located close to each other along the main and side streets. Along the streets and in the immediate area grow ornamental and non-ornamental trees, shrubs and herbaceous species.

In the suburban area of Szczecin (Gumieńce Disctrict, Krakowska Street, ST 2 in Fig. 1), the sampler was located on the roof of the campus building (height: 15 meters above ground level). The Gumieńce District is a peripheral district of the city, characterized by loose, low buildings with many backyard gardens. The area around the sampler had greenhouses, meadows and forests (Fig. 1).

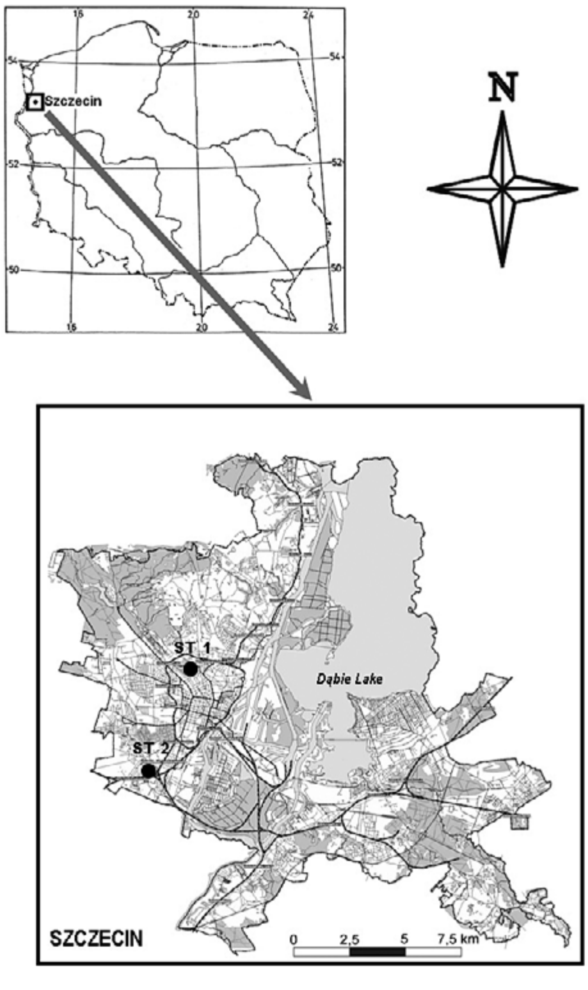

Figure 1. Locality of the monitoring site at the Faculty of Biology, Szczecin University (urban area, ST 1), and on the roof of a campus building (suburban area, ST 2)

In both locations, the meteorological data covering three years of the study were provided by automatic weather stations (Vaisala MAWS101, Finland). The meteorological stations were located in the immediate neighbourhood of the Lanzoni trap.

The final data set subjected to statistical analyses included the following variables: concentration of *Ganoderma* spores, air temperature, relative humidity, air pressure, wind velocity and precipitation. Due to some deviations from normality (assessed with the Kolmogorov-Smirnov, Lilliefors and chi-square tests), observed mainly in the case of spores, wind velocity and precipitation, the significance of differences in spore content and meteorological conditions between districts was assessed with the U Mann-Whitney test, while statistically significant differences between years at a given site were revealed using the Kruskal-Wallis and medians tests. The Spearman's rank correlation coefficients were applied in order to determine and assess the following:

1) relationships between spore concentration and meteorological conditions at a given site;
2) whether the dynamics of changes in spore level and meteorological parameters at the two districts studied were similar;
3) the actual strength of dependence of *Ganoderma* spore concentration on meteorological parameters with multiple regression applied to log(x+1) transformed data.

All the analyses were performed in STATISTICA 10 [10].

RESULTS

Spore seasons in the two districts studied started at the end of May or the beginning of June and ended in September (Tab. 1, Fig. 2). The number of days with spores was generally lower in the suburban district than in the urban, with the shortest duration of spore season in 2010 (86 days). On the other hand, the total number of spores collected was higher in Gumieńce than in Śródmieście, and at both sites it decreased year by year. The maximum numbers of spores per 24h were generally similar in both districts – in 2008 and 2010 they were observed in June and July, in 2009 in September, and also a downward trend was observed in the course of time. The average daily spore concentrations recorded in the urban district decreased from year to year (in 2010 it was significantly lower than in previous years), while in the suburbs mean concentrations for spore seasons were stable. Statistically significant differences were also observed between districts – each year and in the whole period studied the average daily concentrations of *Ganoderma* spores in the suburbs were higher than in the urban district.

Meteorological conditions in the Śródmieście district were similar in consecutive years, except for wind velocity (Tab. 2). It turned out that wind speed was significantly higher in 2008 comparing to 2009 and 2010. A similar downward trend was observed in the suburbs of Szczecin where wind velocity in 2010 was significantly lower than in the previous years. In turn, relative humidity in 2009 and 2010 was higher than in 2008. Comparison of the two districts studied in this investigation showed that wind velocity in the suburbs was higher in consecutive years, as well as in the whole study period, opposite to air pressure. Additionally, relative humidity differed significantly between sites in 2009 and 2010 and, as a result, for the three years altogether. Air in the

Table 1. Results of aerobiological study of *Ganoderma* spores in examined districts of Szczecin during spore seasons 2008-2010. Spore concentration expressed as average value ± standard deviation (median)

Year	Śródmieście: Season	Śródmieście: *Ganoderma* spore concentration [spores × m⁻³]		Gumieńce: Season	Gumieńce: *Ganoderma* spore concentration [spores × m⁻³]
2008	ss 1: 3 VI – 21 IX ss 2: 112 tn: 14609 max 1: 438 max 2: 27 VI	130.2 ± 77.1 **A** (123.0)	*	ss 1: 30 V – 12 IX ss 2: 107 tn: 16217 max 1: 444 max 2: 27 VI	155.5 ± 87.1 (166)
2009	ss 1: 1 VI – 23 IX ss 2: 111 tn: 12678 max 1: 330 max 2: 15 IX	114.2 ± 70.8 **B** (111.0)	*	ss 1: 9 VI – 18 IX ss 2: 103 tn: 14993 max 1: 333 max 2: 7 – 8 IX	145.7 ± 78.3 (139.0)
2010	ss 1: 30 V – 13 IX ss 2: 107 tn: 8512 max 1: 244 max 2: 3, 9, 17, 20 VII	79.6 ± 57.1 **A,B** (77.0)	*	ss 1: 12 VI – 5 IX ss 2: 86 tn: 11402 max 1: 334 max 2: 3 VII	132.6 ± 66.6 (113.0)
Total		108.3 ± 71.9 (99.0)	*		145.3 ± 78.6 (138.0)

ss 1 – spore season established by the 90% method
ss 2 – number of days in spore season
tn – total number of *Ganoderma* spores collected in spore season established by the 90% method
max 1 – maximum number of spores per 24 h
max 2 – date of maximum spore concentration
* statistically significant differences between districts (U Mann-Whitney test, $p<0.05$)
A-B statistically significant differences between years (Kruskal-Wallis and median tests, $p<0.05$)

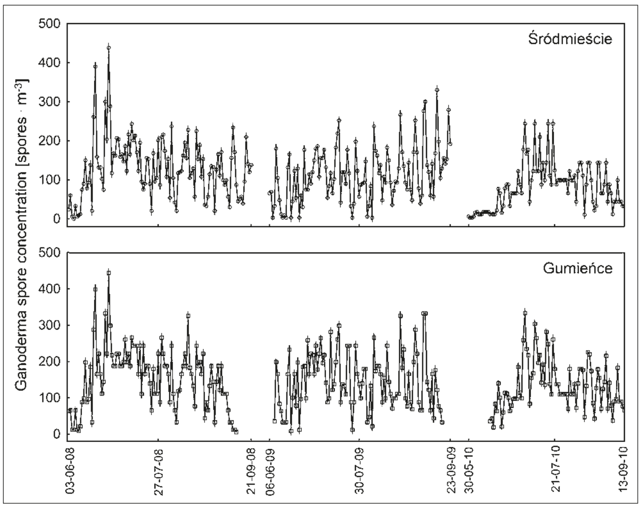

Figure 2. Concentration of *Ganoderma* spores in examined districts of Szczecin during spore seasons 2008–2010

suburbs was more humid than in the urban district although the average precipitation and seasonal sum of precipitation were similar.

The dynamics of changes in meteorological conditions and *Ganoderma* spore concentrations were similar for Śródmieście and Gumieńce. The only parameter which correlated moderately between those two sites was wind velocity (Tab. 3). The highest Spearman's rank correlation coefficients were obtained in both districts between the level of *Ganoderma* spores and air temperature (positive), as well as air humidity (negative). The strength of relationships

Table 2. Meteorological parameters recorded in examined districts of Szczecin during spore seasons 2008-2010. Data expressed as average value ± standard deviation (median)

Meteorological variable	District: Śródmieście	District: Gumieńce
2008		
Air temperature [°C]	16.7 ± 3.5 (16.8)	16.3 ± 3.3 (16.7)
Relative humidity [%]	66.2 ± 13.1 (68.0)	67.7 ± 12.6 (70.0) **C,D**
* **Air pressure** [hPa]	1008 ± 6 (1009)	990 ± 7 (1000)
* **Wind velocity** [m/s]	1.0 ± 0.8 (0.9) **A,B**	2.2 ± 0.8 (2.3) **E**
Precipitation [mm]	1.5 ± 3.2 (0.0)	3.4 ± 16.2 (0.2)
Precipitation, seasonal sum [mm]	162	378
Predominant wind direction	W, NW	SW
2009		
Air temperature [°C]	16.8 ± 3.1 (16.5)	17.3 ± 3.8 (17.1)
* **Relative humidity** [%]	70.1 ± 11.4 (69.5)	80.0 ± 11.8 (80.5) **C**
* **Air pressure** [hPa]	1009 ± 7 (1010)	1001 ± 6 (1001)
* **Wind velocity** [m/s]	0.7 ± 0.5 (0.6) **A**	2.7 ± 1.4 (2.5) **F**
Precipitation [mm]	2.8 ± 8.2 (0.0)	2.2 ± 5.3 (0.0)
Precipitation seasonal sum [mm]	305	246
Predominant wind direction	W, NW	SW
2010		
Air temperature [°C]	17.7 ± 4.4 (17.3)	16.9 ± 4.3 (16.6)
* **Relative humidity** [%]	71.3 ± 15.9 (70.0)	81.5 ± 14.2 (84.0) **D**
* **Air pressure** [hPa]	1009 ± 5 (1009)	1000 ± 5 (1000)
* **Wind velocity** [m/s]	0.6 ± 0.5 (0.4) **B**	1.8 ± 0.9 (1.8) **E,F**
Precipitation [mm]	2.6 ± 5.9 (0.0)	2.1 ± 5.6 (0.0)
Precipitation seasonal sum [mm]	276	156
Predominant wind direction	W, NW	SW
Total		
Air temperature [°C]	17.0 ± 3.7 (16.8)	16.8 ± 3.8 (16.7)
* **Relative humidity** [%]	69.1 ± 13.7 (69.0)	76.3 ± 14.3 (77.5)
* **Air pressure** [hPa]	1009 ± 6 (1009)	1000 ± 6 (1000)
* **Wind velocity** [m/s]	0.8 ± 0.6 (0.6)	2.2 ± 1.1 (2.2)
Precipitation [mm]	743	780
Predominant wind direction	W, NW	SW

* statistically significant differences between districts (U Mann-Whitney test, $p<0.05$)
A-F statistically significant differences between years (Kruskal-Wallis and median tests, $p<0.05$)

Table 3. Spearman's rank correlation coefficients for *Ganoderma* spore concentration and meteorological parameters between examined districts of Szczecin during spore seasons 2008–2010. All coefficients are statistically significant with $p<0.05$

Variable	2008	2009	2010	Total
Ganoderma spores	0.83	0.79	0.92	0.84
Air temperature	0.97	0.91	0.99	0.95
Relative humidity	0.98	0.93	0.98	0.91
Air pressure	0.98	0.93	0.99	0.97
Wind velocity	0.52	0.51	0.50	0.53
Precipitation	0.78	0.64	0.60	0.68

Table 4. Spearman's rank correlation coefficients between *Ganoderma* spore concentration and meteorological parameters in examined districts of Szczecin during spore seasons 2008–2010

District		Total	2008	2009	2010
Śród-mieście	*Ganoderma* &Air temperature	**0.27***	0.13	**0.23***	**0.61***
	Ganoderma & Relative humidity	**-0.16***	0.08	-0.14	**-0.40***
	Ganoderma & Air pressure	-0.02	**-0.19***	0.01	**0.21***
	Ganoderma & Wind velocity	**0.13***	0.15	-0.03	-0.01
	Ganoderma & Precipitation	0.05	**0.27***	-0.04	-0.14
Gu-mieńce	*Ganoderma* &Air temperature	**0.40***	0.11	**0.47***	**0.69***
	Ganoderma & Relative humidity	**-0.21***	0.01	-0.15	**-0.45***
	Ganoderma & Air pressure	**-0.12***	**-0.22***	**-0.20***	0.20
	Ganoderma & Wind velocity	-0.09	0.01	-0.15	-0.33
	Ganoderma & Precipitation	**0.18***	**0.28***	0.09	0.01

* statistically significant Spearman's rank correlation coefficients (p<0.05)

with the remaining meteorological factors was either low or insignificant and changed from one year to another (Tab. 4).

In order to verify the actual dependence of *Ganoderma* concentration on meteorological parameters, mutliple regression models were applied (Tab. 5). Only in 2010 the models explained the moderate part of the variability in spore concentration (R^2 equal to 0.39 in Śródmieście and 0.49 in Gumieńce). The model for the urban district included air temperature and air pressure while in the suburbs it was only air temperature that played a significant role.

DISCUSSION

The number of airborne fungal spores of a locality varied according to the weather, geographical location and presence of the local spore sources. Data collected in Szczecin clearly indicate the significance of *Ganoderma* basidiospores as bioaerosols [11]. The percentage part of *Ganoderma* spores in the all analysed spore types in the air of Szczecin was 10%. Seasonally, spore levels of *Ganoderma* peaked towards the summer and autumn. *Ganoderma* spores were present in the atmosphere on 90% of the days from May-June to November of 2008–2010. These results are similar to results from previous studies in other parts of the world [12, 13]. The highest spore concentration in late summer (January and February) was also reported by Cutten et al. [14] in Auckland, New Zealand, and by Singh et al. [6] in Delhi, India. In Poland, the highest concentrations occurred in July and August [15, 16].

Wind velocity in Śródmieście was significantly higher in 2008 than in the next two years, but the concentrations of *Ganoderma* spores decreased in the course of the years studied, and with no clear relationship between spore content and wind speed. Obviously, meteorological conditions did not contribute to the changes in *Ganoderma* concentrations between years.

Wind velocity in Gumieńce was significantly lower in 2010 than in previous years, but no significant changes were observed in *Ganoderma* spore concentrations between the three spore seasons studied and, moreover, the content of spores did not correlate significantly with wind speed.

Table 5. Multiple regression analysis of relationships between *Ganoderma* spore concentration and meteorological parameters in examined districts of Szczecin during spore seasons 2008–2010

Year	Explanatory variables	District							
		Śródmieście				Gumieńce			
		Beta	t	p	Model performance	Beta	t	p	Model performance
2008	Air temperature	0.18	1.44	0.15	F(5,105) = 1.71 p = 0.14 R^2 = 0.04	0.16	1.26	0.21	F(5,96) = 1.16 p = 0.33 R^2 = 0.01
	Relative humidity	0.15	1.13	0.26		0.04	0.34	0.74	
	Air pressure	-0.11	-0.95	0.34		-0.21	-1.91	0.06	
	Wind velocity	0.13	1.32	0.19		-0.00	-0.04	0.97	
	Precipitation	0.07	0.64	0.52		0.04	0.34	0.73	
2009	Air temperature	0.17	1.46	0.15	F(5,104) = 1.63 p = 0.16 R^2 = 0.03	**0.35**	**3.47**	**0.00**	**F(5,96) = 5.34** **p = 0.00** **R^2 = 0.19**
	Relative humidity	-0.11	-0.88	0.38		-0.08	-0.83	0.41	
	Air pressure	0.07	0.64	0.52		-0.15	-1.58	0.12	
	Wind velocity	0.00	0.03	0.98		-0.11	-1.17	0.24	
	Precipitation	0.21	1.77	0.08		0.05	0.48	0.63	
2010	Air temperature	**0.64**	**5.91**	**0.00**	**F(5,101) = 14.48** **p = 0.00** **R^2 = 0.39**	**0.65**	**5.54**	**0.00**	**F(5,96) = 20.87** **p = 0.00** **R^2 = 0.48**
	Relative humidity	0.05	0.37	0.71		-0.04	-0.30	0.76	
	Air pressure	**0.21**	**2.26**	**0.03**		0.10	1.07	0.29	
	Wind velocity	0.09	1.04	0.30		-0.04	-0.48	0.63	
	Precipitation	0.04	0.40	0.69		0.03	0.49	0.72	
Total	Air temperature	**0.23**	**3.38**	**0.00**	**F(5,322) = 6.14** **p = 0.00** **R^2 = 0.09**	**0.34**	**5.61**	**0.00**	**F(5,267) = 11.25** **p = 0.00** **R^2 = 0.17**
	Relative humidity	-0.07	-0.91	0.36		-0.08	-1.28	0.20	
	Air pressure	0.02	0.35	0.73		**-0.16**	**-2.74**	**0.01**	
	Wind velocity	**0.14**	**2.33**	**0.02**		-0.05	-0.95	0.34	
	Precipitation	0.11	1.68	0.09		0.07	1.17	0.24	

Another factor that differentiated the years studied was relative humidity – it was significantly lower in 2008 compared to the remaining years, but the level of *Ganoderma* spores in the air correlated negatively with air humidity only in 2010.

Calderon et al. [17] found that large concentrations of basidiospores were often associated with daily mean wind speed of 2–3 $m{\cdot}s^{-1}$. The reports of Lopez and Salvaggio [18] and Hasnain [19] also confirmed that wind velocities >5 $m\ s^{-1}$ were associated with decreased concentrations of basidiospores. The reason for the decrease could be the diluting effect of high wind speeds on concentrations of airborne particles. In addition, increasing wind speed would also increase water loss and limit spore production.

A similar negative correlation (determined using Spearman's rank correlation coefficient) between relative humidity and *Ganoderma* spore concentration was found in Portugal [12] and in Taiwan [20] using the multiple regression model. McCracken [21] reports that humidity levels of about 70% are associated with increased concentrations of basidiospores.

Air temperature and relative humidity correlated considerably with the concentration of *Ganoderma* spores, but those relationships were unstable (even insignificant in 2008).

Actual relationships between *Ganoderma* spore concentrations and meteorological parameters revealed by multiple regression models showed that the only considerable dependences were observed in 2010 (primarily between spore content and air temperature), while the models for previous years were weak. According to Burge and Otten [22], temperature is one of the most important environmental factors that influences fungal survival and growth. A positive, significant relationship between *Ganoderma* spores and air temperature was noted in Tulsa, USA [11] and in two cities of Portugal [12]. Similar studies were carried out in Saudi Arabia and significant correlation 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 [4]. Calderon et al. [16] noted a negative correlation with temperature and basidiospores concentrations and suggested that spore production or fungal growth decreased at higher temperatures (about >26 °C).

It can be concluded that differences in buildings and type of vegetation do not significantly affect the length of the season, or the amount and date of the maximum concentrations of spores. Differences were seen only in the total number of spores in the season, and in the suburb was about 10% higher than those in the city centre. The presented study shows that the type of building did not radically change the values of meteorological parameters in the studied districts, and the relationships between the concentration of *Ganoderma* spores and meteorological conditions were weak. Thus, the higher abundance of spores in the suburbs of Szczecin seemed to be conditioned by the closeness of potential area sources of spore (among other things – forest). Such results indicate that a single measuring site located in the city centre is insufficient since it does not reflect the dynamics and level of *Ganoderma* spore concentration in peripheral districts.

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