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Characterization of *Coprinus* spores in the NW of the Iberian Peninsula. Identification and count in aerobiological samples

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Abstract – A morphological and biometric study of the genus *Coprinus* spores present in the NW of the Iberian Peninsula produced four different groups. In addition, a year's count of the spores of this genus in the atmosphere of the city of Santiago de Compostela produced a total of 29,324 spores, most of them recorded in March, May and November. The statistical correlation with meteorological parameters is positive for humidity and rainfall and negative for temperature. The specific identification of spores was carried out using aerobiological samples with records above the yearly average value. The results obtained revealed that on those days most of the spores belong to *Coprinus micaceus* (Bulliard: Fr.) Fr. (80%), whereas *Coprinus disseminatus* (Pers.: Fr.) *Gray* spores represent 7%, and *Coprinus comatus* (Müller: Fr.) Gray, *Coprinus cinereus* (Schaeff.: Fr.) Gray and *Coprinus atramentarius* (Bulliard: Fr.) Fr. spores the remaining 13%.

***Coprinus* / Spores / NW Iberian Peninsula / Biometric / Meteorology / Aerobiology / Correlation**

Résumé – Une étude morphologique et biométrique des spores du genre *Coprinus* dans le nord-ouest de la Péninsule Ibérique a conduit à la délimitation de quatre groupes différents. Par ailleurs, le décompte des spores de ce genre, captées dans l'atmosphère de la ville de Santiago de Compostela atteint un total de 29 324 spores, la plupart d'entre-elles ont été enregistrées durant les mois de mars, mai et novembre. La corrélation statistique

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avec les paramètres météorologiques est positive pour l'humidité et la pluie, et négative pour la température.
L'identification spécifique des spores a été réalisée sur les échantillonnages aérobiologiques dont les relevés étaient au-dessus de la valeur moyenne annuelle. Les résultats obtenus révèlent que la plupart des spores appartiennent au *Coprinus micaceus* (Bulliard: Fr.) Fr. (80 %), alors que les spores de *Coprinus disseminatus* (Pers.: Fr.) Gray ne représentent que 7 %, et le reste (13 %) correspondant aux *Coprinus comatus* (Müller: Fr.) Gray, au *Coprinus cinereus* (Schaeff.: Fr.) Gray et au *Coprinus atramentarius* (Bulliard: Fr.) Fr.

***Coprinus* / Spores / Nord-ouest de la péninsule ibérique / Biométrie / Météorologie / Aérobiologie / Corrélation**

INTRODUCTION

The *Coprinus* genus belongs to the Basidiomycota division, Holobasidiomycetes class, Agaricales order and Coprinaceae family, and is one of the most widely distributed fungi in the Iberian Peninsula. The most remarkable morphological feature of the genus is that its carpophore becomes deliquescent when mature. However, some species are good to eat if they are picked early.

Consumption of these fungi has some restrictions, since, with the exception of *Coprinus comatus,* the other species can cause gastrointestinal disorders when eaten in combination with alcohol. This combination may induce the so-called coprinus syndrome or antabus reaction, with cardiac and respiratory arrest in the most severe cases (Piqueras, 1981). Some authors also advise against the ingestion of *Coprinus comatus*, since it accumulates lead, a potentially toxic heavy metal in very small doses (Alonso *et al*., 2004). Some records of invasive mycosis involving this fungus can be found in medical literature. Thus, Speller & Mac Ives (1971) reported the isolation of *Coprinus cinereus* in the aortic valve and myocardial tissue of a patient with acute endocarditis, and more recently invasive lung mycosis by *Coprinus* sp. has been reported in a patient with leukaemia (Nenoff *et al.*, 1996).

As far as the allergogenic effects of basidiospores in general are concerned, there are records of both perennial rhinitis and bronchial asthma induced by sensitivity to *Agaricus bisporus* spores (Dieguez *et al.,* 1996) and rhinoconjunctivitis and bronchial asthma by sensitivity to *Boletus edulis* spores (Nieto *et al.*, 1996). Some studies also indicate that these spores may aggravate eczemas in patients with atopic dermatitis (Fischer *et al.,* 1999). The wide occurrence in the atmosphere of these spores and the characterization of their allergens (Davis *et al*., 1988) have revealed a correlation of basidiospores with asthma epidemics of considerable proportions in New Orleans, England and Australia. It has also been demonstrated that 20-30% of patients with respiratory allergic symptoms show positive results in the corresponding skin tests (Lehrer & Homer, 1990; Lehrer *et al.*, 1994), and specifically for species of the *Coprinus* genus (Herxheimer *et al.*, 1969; López *et al.,* 1976; Hasnain *et al*., 1985; Sprenger *et al.*, 1988; Weissman *et al.,* 1987).

Despite the high occurrence of *Coprinus* basidiospores in the air, aerobiological studies do not usually make a count of them but concentrate on other genera such as *Cladosporium* and *Alternaria* (Infante *et al*., 1999a, b). In the NW of the Iberian Peninsula only two specific studies provide data on *Coprinus*, one in an urban environment and the other in a rural environment. They both confirm the high occurrence of *Coprinus* spores (Díaz, 1999; Aira *et al*., 2003).

Given the interest of basidiospores for the aetiology of allergic diseases and the high occurrence of *Coprinus* in the atmosphere of the NW of the Iberian Peninsula, the aim of this research is to see whether it is possible to distinguish between the spores of the different genus species by means of an optical microscope and whether that distinction may be applied to the usual counting of aerobiological samples or whether, by contrast, these spores should still be considered as belonging to one single type. The different allergic reaction induced by each species makes it advisable to specifically distinguish between the spores in the aerobiological samples, since this would help in the diagnosis and treatment of the symptoms involved.

For this purpose, we carried out a daily counting of *Coprinus* basidiospores in a city located in the study area for a whole year. The research revealed not only the seasonal and hourly dynamics of these spores, but also the impact of the main meteorological parameters on them. Finally, a morphobiometric study was also implemented.

MATERIAL AND METHODS

The study deals with the morphology of the genus *Coprinus* spores present in the NW of the Iberian Peninsula, within the geographical limits of Galicia, and with the frequency of these spores in the atmosphere (Fig. 1).

From the material collected in the LOU–Fungi mycotheque, 15 species belonging to this genus were identified. Of these, the most abundant species are *C. atramentarius* (Bulliard: Fr.) Fr., *C. cinereus* (Schaeff.: Fr.) Gray., *C. comatus* (Müller: Fr.) Gray, C. *disseminatus* (Pers.: Fr.) Gray, *C. micaceus* (Bulliard: Fr.) Fr. and *C. picaceus* (Bull.: Fr.), whereas *C. auricomus* Patouillardii, *C. hercules* Uljé & Bas, *C. impatiens* (Fr.) Quel, *C. kuehneri* Uljé & Bas, *C. leiocephalus* Orton, *C. patouillardii* (Quél) Gray, *C. radians* (Desm.: Fr.) Fr., *C. sterqulinus* (Fr.) Fr. and *C. urticicola* (Berk. & Br.) Buller occur only rarely and sporadically.

With the spores of these species, a morphological and biometric study was carried out. For this purpose, we measured the following parameters in a hundred samples: P (polar axis length), E (equatorial diameter), p (germinal pore width), mucron presence or absence and colour. Moreover, in the atmosphere of the city of Santiago de Compostela *Coprinus* spores were collected and counted for a year using a Hirst-type volumetric spore trap (Lanzoni VPPS 2000) and following the methodology suggested by the Red Española de Aerobiología (Galán *et al.*, 2007).

Average – either daily or hourly – concentrations are expressed as number of spores/m^3 in the air, whereas the annual or monthly total records are absolute values. In order to study the intraday behaviour we used the

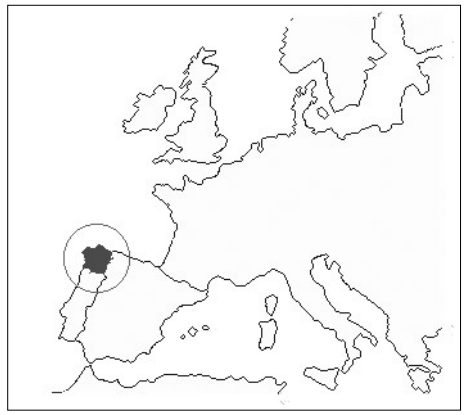

Fig. 1. Location of the study area.

guidelines of Galán *et al.* (1991) and to find out the correlation between the concentration of spores and the meteorological variables we applied Spearman's test with the aid of the *Statistica* programme with three confidence intervals (99%, 95%, 90%). The Centro Meteorológico Territorial de Galicia (which comes under the Instituto Nacional de Meteorología of the Ministerio de Medio Ambiente) and the Observatorio Astronómico "Ramón María Aller" (which comes under the Universidad de Santiago de Compostela) provided the meteorological data.

RESULTS

Biometric study. A cluster analysis was made with the measurements and the morphological features of the genus *Coprinus* spores. The results are represented in the following dendrogram (Fig. 2).

Our statistical analysis led to the distinction of four groups. The first one (Group 1) is monospecific and only includes *Coprinus impatiens,* distinctly recognizable for its spores of a yellowish colour, a P/E ratio of 1.54 µ and a tiny germinal pore (0.7 µ).

Group 2 includes *Coprinus kehneri* (9.0 × 5.6 µ), *Coprinus urticicola* (6.5 × 4.7 µ), *Coprinus comatus* (12.6 × 7.9), *Coprinus disseminatus* (6.8 × 4.7 µ) and *Coprinus cinereus* (11.8 × 8.3 µ). The P/E ratio ranges between 1.30 to 1.61 µ, and the size of the germinal pore ranges from 2.2 to 3.8 µ. Group 3 includes *Coprinus patouillardii* (5.9 × 5.1 µ), *Coprinus radians* (8.1 × 5.6 µ), *Coprinus*

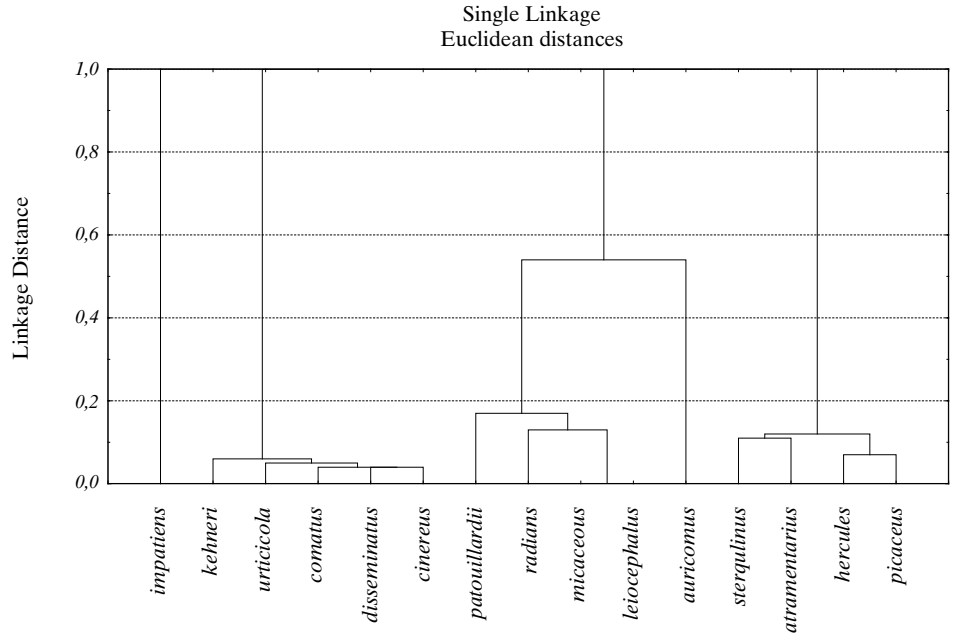

Fig. 2. Dendrogram obtained in the cluster analysis by means with the *Coprinus* spores main data.

micaceus (6.7 × 5.0 µ), *Coprinus leiocephalus* (9,3 × 6.9 µ) and *Coprinus auricomus* (12.6 × 6.4 µ). The P/E variation range was 1.16-1.97 µ and the size of the germinal pore varied between 1.4 and 4.3 µ.

Finally, Group 4 includes *Coprinus sterqulinus* (13.5 × 9.2 µ), *Coprinus atramentarius* (10.7 × 6.3 µ), *Coprinus hercules* (11.6 × 10.1 µ) and *Coprinus picaceus* (16.8 × 13.6 µ). The P/E ratio ranged from 1.15 to 1.70 µ and the size of the germinal pore ranged between 1.4 to 4.7 µ.

Aerobiological study. The samples collected from 1st January to 31st December 1998 in the city of Santiago de Compostela included 29,324 spores of the *Coprinus* genus. Most of these spores are present in the atmosphere in March, May and November, with over 5,000 spores every month (Fig. 3).

The annual average value of mean daily concentrations was 80 spores/m^3. With the exception of April and the months between June and September, all other months exceeded that average value. The highest daily value was recorded on 16th March, with 2,033 spores/m^3, and the daily values were also very high at the end of January and November (Table 1).

As far as the hourly distribution is concerned, the *Coprinus* spores are particularly abundant in the small hours and the early hours of the morning, with a peak at 4 a.m. From that moment the concentration progressively decreases to reach the lowest value between midday and 3 p.m. From then on, the concentration increases again, though more gently, up to midnight (Fig. 4).

The correlation between average daily concentrations and meteorological parameters was positive for humidity (with a significance level of 99%) and rainfall (95%), and negative for the variables of temperature (highest, average and lowest values) and hours of sunshine. As far as the wind direction and wind run are concerned, the NE-S and the S-SW direction have a positive impact on the concentration of spores, whereas the N-NE direction correlates negatively. The correlation is lower and less significant as far as wind run is concerned. These results are usually coincidental if the correlation is made either with the values of the meteorological variables of one particular day or with the values recorded 1 or 2 days before (Table 2).

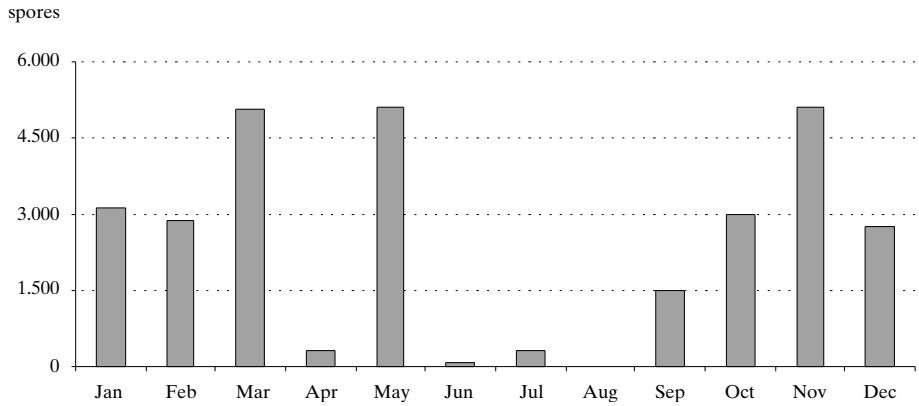

Fig. 3. Monthly distribution of *Coprinus* spores.

Table 1. Monthly average values and highest daily values

	Monthly average value spores/m^3	*Highest daily value spores/m^3*	*Date*
January	100	1,432	31/01
February	103	1,746	1/02
March	164	2,033	16/03
April	11	60	28/04
May	165	1,379	4/05
June	3	14	14/06
July	10	154	3/07
August	1	2	17/08, other
September	50	381	14/09
October	97	596	14/10
November	170	1,716	20/11
December	89	841	24/12

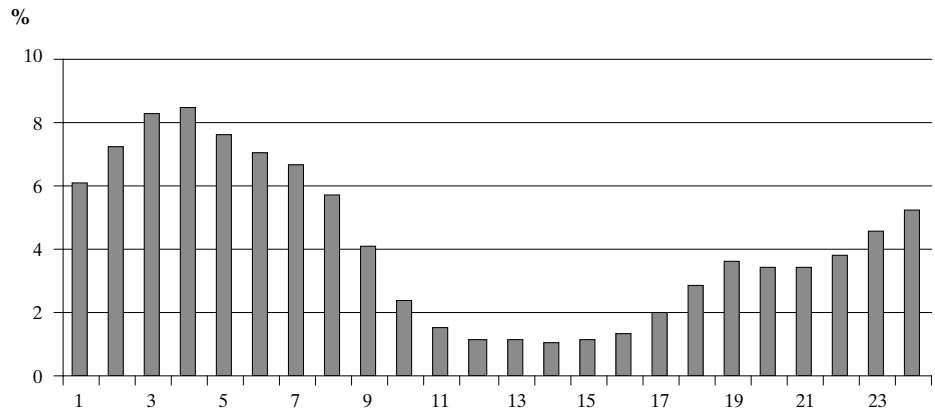

Fig. 4. Intradaily values of the atmospheric spore concentration.

With the aim of applying the results of the statistical study carried out with the biometric data of the 15 *Coprinus* species present in the study area, of all the aerobiological samples taken in the city of Santiago we selected those corresponding to days with an average daily concentration above the annual average value (80 spores/m^3). As a result, we obtained a total of 40 samples (Fig. 5). The highest average daily concentrations were recorded on 16th March (2,033 spores/m^3), 1st February (1,746 spores/m^3) and 20th November (1,716 spores/m^3).

In the samples analysed no *Coprinus impatiens* spores (Group 1) are found. Of all the species included in Group 2, the *Coprinus disseminatus* spores represent 7%

Table 2. Correlation between spore concentrations of *Coprinus* and meteorological variables

	R (Spearman)		*R (Spearman)*
RAINFALL	Ns	**CALM WIND**	0.134**
one day before	0.161**	one day before	Ns
two days before	0.175***	two days before	0.120**
HUMIDITY	0.204***	**N-NE WIND**	– 0.108**
one day before	0.264***	one day before	– 0.179***
two days before	0.283***	two days before	– 0.213***
MAX. TEMP.	– 0.416***	**NE-S WIND**	0.184***
one day before	– 0.453***	one day before	0.156**
two days before	– 0.451***	two days before	0.172***
MIN. TEMP.	– 0.414***	**S-SW WIND**	Ns
one day before	– 0.362***	one day before	0.137**
two days before	– 0.366***	two days before	0.182***
AVER. TEMP.	– 0.448***	**SW-N WIND**	Ns
one day before	– 0.446***	one day before	Ns
two days before	– 0.449***	two days before	Ns
SUN HOURS	– 0.286***	**SPEED WIND**	– 0.202***
one day before	– 0.394***	one day before	– 0.118**
two days before	– 0.378***	two days before	– 0.105**

Confidence intervals (90% *; 95% **; 99.9%***).

of all the identified species, and in Group 3 the *Coprinus micaceus* spores are the most abundant, reaching 80% of the total. The remaining 13% included in "Others" correspond to *Coprinus comatus*, *Coprinus cinereus* and *Coprinus atramentarius* spores.

While the *Coprinus micaceus* spores tend to be concentrated from the end of January to early May with a secondary peak in November, the *Coprinus disseminatus* spores tend to be concentrated in March and towards the end of the year. Finally, the species of the "Others" group have been recorded in the atmosphere mostly between May and November (Fig. 6).

The highest daily record of *Coprinus micaceus* spores was 1,913 spores/m^3 (16th March), with very high values on 31st January (1,426 spores/m^3) and 20th November (1,324 spores/m^3). Meanwhile, the highest record of *Coprinus disseminatus* spores was only 144 spores/m^3 (20th November), with similar levels on 15th and 16th March (115 and 120 spores/m^3).

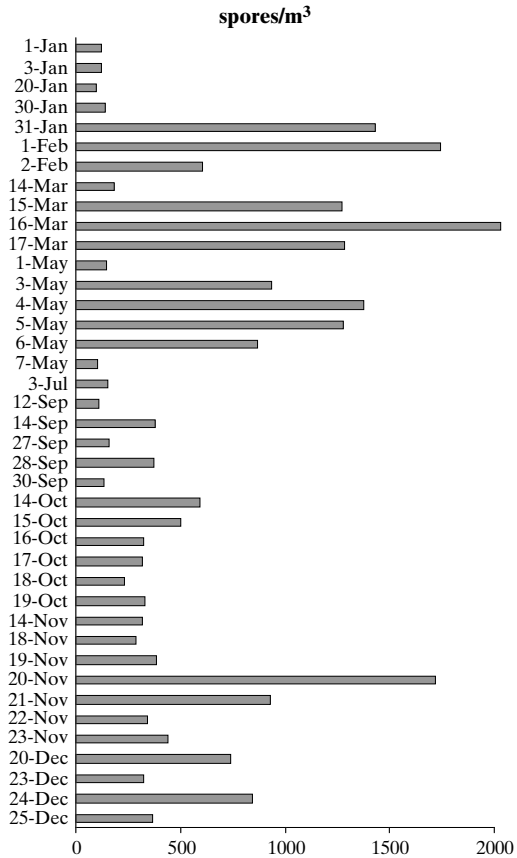

Fig. 5. Average daily concentrations for the selected days.

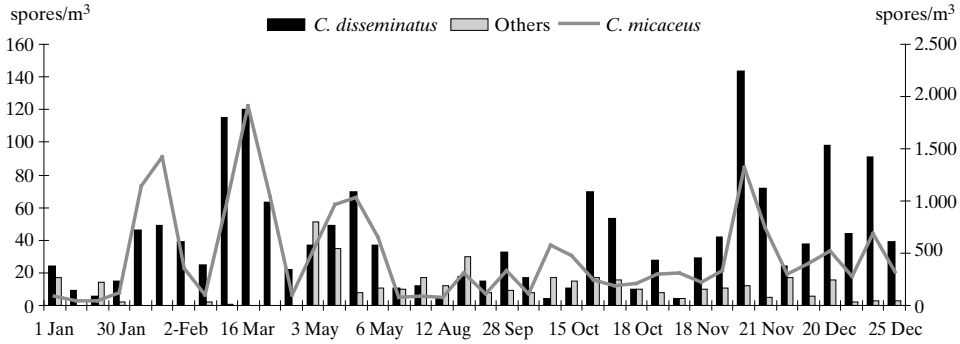

Fig. 6. Representation of the spores of *C. disseminatus*, *C. micaceus* and other *Coprinus.*

DISCUSSION

According to their morphological features, the *Coprinus* basidiospores of the species recorded in the NW of the Iberian Peninsula may be arranged in 4 different groups easily recognizable in the aerobiological samples. The most abundant species in the atmosphere of Santiago is *Coprinus micaceus and Coprinus disseminatus*, noticeably more frequent than the rest. Meanwhile, the spores of *Coprinus comatus*, *Coprinus cinereus* and *Coprinus atramentarius*, taken as a whole, show considerably lower records.

This kind of biometric studies can be applied to other geographical areas having the same species as the NW of the Peninsula. Quite understandably, any other study should take into account the corresponding biodiversity and incorporate the relevant local species.

As far as the seasonal behaviour of *Coprinus* in the reference city (Santiago) is concerned, the highest concentrations are recorded in autumn and winter, although the records of March and May are also high. During these months the spores of *Coprinus micaceus* and *Coprinus disseminatus* are almost the only ones present in the atmosphere.

A number of authors have also recorded high concentration levels during this same period in other sites in the Iberian Peninsula (Fernández *et al.*, 1993; Herrero *et al.*, 1995; Gonzalo *et al.*, 1997), in the Canary Islands (La-Serna & Domínguez, 1996) and in Italy (Palmas & Cosentino, 1989). The highest average daily concentrations are over 1,000 spores/m^3 in most of the bibliography.

The intraday distribution of the *Coprinus* basidiospores, which in our study clearly follows a night pattern, coincides with the research of other authors. Gonzalo *et al.* (1997), for example, recorded the highest values at 11 p.m. and 5 a.m. This distribution pattern is completely different to the one observed in other genera widely researched in environmental mycology, such as *Cladosporium* and *Alternaria*, whose spores tend to occur in the atmosphere during the evening, a fact which correlates positively with the temperatures (Dopazo *et al*., 2003).

As far as *Coprinus* is concerned, the results of the statistical correlation between spore concentration and meteorological variables clearly reveal that temperature (highest and average records) is the meteorological factor which

most significantly reduces its presence in the atmosphere. This fact would explain not only its scant representation during the hottest months of the year, but also its dominance during the early hours, since temperatures are then lower than in the daytime. Other authors have also reported a significant and negative correlation with temperature. They point out that basidiospores are rarely found at temperatures over 20°C, whereas the highest values are recorded in periods with average temperatures around 10-15°C (Gonzalo *et al.,* 1997).

As far as the impact of rainfall and humidity is concerned, in our study the correlation is positive for both parameters. The significance increases when data are referred to preceding days, a pattern also recorded by Airaudi & Filipello (1996). This fact could reflect the deletereal impact of these factors on the carpophore, which would facilitate the release of basidiospores into the atmosphere.

Annex. References of LOU–Fungi, *C. atramentarius* (1499), *C. auricomus* (5973), *C. cinereus* (4572), *C. comatus* (5654), *C. disseminatus* (6202), *C. hercules* (1522), *C. impatiens* (1501), *C. kuehneri* (6280), *C. leiocephalus* (1492), *C. micaceus* (7525), *C. patouillardii* (8750), *C. picaceus* (4809), *C. radians* (4168), *C. sterqulinus* (1497) and *C. urticicola* (7273).

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