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ORIGINAL PAPER

Comparison of *Alternaria* spore levels between two areas within the same city (Salamanca, Middle West Spain)

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Abstract The purpose of this study is to contribute to the knowledge about fungal spores in the atmosphere of the city of Salamanca (Middle West Spain), through the comparative study of *Alternaria* spore levels in two different sampling points within the same city. The study was done in terms of seasonal and hourly distribution and the possible influence of the main meteorological parameters on their atmospheric concentrations. The sampling was carried out from 17 February 2014 to 16 February 2016, both included, with two Hirst-type volumetric spore trap samplers, in two buildings in the city: one in a semi-urban environment, on the outskirts of the city, and the other in the city centre, 1.4 km apart. After the two years of sampling, the total annual values of *Alternaria* varied very little concerning the location of the samplers. The maximum values coincided in the two spore traps during 2014–2015 on the same day with similar amounts, whereas in 2015–2016 the difference was more noticeable both in date and amount. In the study of the seasonality of *Alternaria*'s atmospheric distribution, there were no differences in the length of the main spore season nor the number of days with health risk levels (concentrations above 100 spores/m^3). With regard to correlations, the analyses carried out between daily concentrations in both samplers obtained highly significant and positive results. The influence of meteorological parameters on spore levels, showed a positive effect of temperature and sunshine, as well as a negative one for humidity and rainfall.

Keywords *Alternaria* · Spore count · Trap comparison · Spain

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1 Introduction

Due to their ubiquitous nature, the Fungi kingdom can be found in most environments on Earth. These organisms are capable of creating a large number of spores that influence various aspects of human life, including food, agriculture and even human health (Caretta, 1992). Allergic diseases have increased in the last decades affecting the population more frequently, which generates greater interest in aerobiological studies with a particular focus on fungal spores present in the atmosphere (Erkara et al., 2009). These studies cover multiple aspects, from the interaction of spores with meteorological factors (Olsen et al., 2019a) to the creation of spore calendars (Ščevková & Kováč, 2019), the study of seasonal and intradiurnal variation (Khan et al., 2016) and the interaction between spores and their allergenicity (Grewling et al., 2019).

There are a large number of fungal spores related to allergic processes, of which the most commonly studied are those of the ascomycete genera *Alternaria* Nees, *Cladosporium* Link, *Aspergillus* P. Micheli and *Penicillium* Link (O´Gorman & Fuller, 2008). This is due to the large amount of these spore types in the air and the fact that more and more people are sensitized to them. That implies that they may be involved in diseases such as asthma and allergic rhinitis, due to the sensitization to fungal allergens. Also, the influence of toxic components present on them, called mycotoxins, have a direct impact on human health (Fernández Rodríguez et al., 2014; Stetzenbach & Krauter, 2016).

The role of *Alternaria* spores in allergic processes has been known for a long time. The works of Deamer and Graham (1947) pointed out the allergenic potential of this spore type, particularly in children. Research on the effect of these spores on allergic processes in both adults and children continues nowadays (Aydogdu & Asan, 2008; Lee et al., 2017). In addition, it is a very common spore as it is a fungus involved in the decomposition of organic matter (Erkara et al., 2008) and is associated with crops such as cereals, potatoes and tomatoes as a plant pathogen (Bardei et al., 2017; Bessadat et al., 2017; Escuredo et al., 2019) and can be isolated from the leaf surface of ornamental and native plants (Kumar & Chandel, 2018; Varpe, 2020). That is the main reason why the presence of *Alternaria* is linked to agricultural land, and the release of its spores into the atmosphere is closely related to harvesting processes (Sánchez Reyes et al., 2016). For this reason, this spore type is particularly frequent in the Mediterranean region (Marchesi, 2020; Maya Manzano et al., 2016) although in other areas of northern Europe it is also frequent and reaches high concentrations (Grinn Gofroń et al., 2016).

In Spain, the study of spores and specifically of *Alternaria* has been approached from the perspective of their relationship with meteorological parameters (Recio et al., 2012; Sabariego et al., 2000), and, more recently, their influence on human health is also being taken into account (Armentia et al., 2019). However, in the Spanish region of Castile and Leon, these studies are still scarce and most of the work carried out includes data from decades ago, such as the studies carried out in Leon (Fernández et al., 1998), Palencia (Herrero et al., 1996), Zamora (González Parrado et al., 2009) or in Salamanca itself (Pérez Gorjón et al., 2003). In Valladolid, located near the city of Salamanca, more recent studies have been carried out related to *Alternaria* and its dynamics in the atmosphere, as well as to its role and influence on human health (Armentia et al., 2019; Sánchez Reyes et al., 2009, 2016).

One type of study that is becoming increasingly abundant is the comparison between different areas about the number of airborne spores depending on the location of the different samplers, either between different areas of the same city (Patel et al., 2018), between environments in nearby cities (Oliveira et al., 2009a), or even between different cities that are far away from each other (Kasprzyk et al., 2015). However, such space-based studies of *Alternaria* are still scarce in Europe (Skjøth et al., 2016).

In a previous article, Fuentes et al. (2019) evaluated the airborne fungal spores in Salamanca's atmosphere, publishing its first fungal spore calendar, together with the analysis of seasonality and daily distribution of the most abundant spores. The present manuscript aims to provide more detailed information on *Alternaria* airborne spore by comparing the results obtained after analysing aerobiological samples for these spores from two different areas of the city, in terms of seasonal behaviour, intradiurnal patterns and correlations with the main meteorological parameters, as well as to compare *Alternaria* atmospheric levels in the city with the threshold levels established for the allergic population.

2 Materials and methods

Aerobiological sampling took place at two different points in the city using two Hirst-type spore trap samplers (Hirst, 1952). The first spore trap was located on the roof of a historical building in the city centre, at the height of 14 m. The second trap was located on the roof of the Faculty of Pharmacy of the University of Salamanca in a semi-urban environment at a height of 25 m. The distance separating both samplers is approximately 1.4 km (Fig. 1) and they were operating uninterruptedly from 17 February 2014 to 16 February 2016. The methodology to be followed for obtaining and processing the samples was that established by the Spanish Aerobiology Network (Galán et al., 2007); thus, the minimum requirements for aerobiological sampling as established by Galán et al. (2014) were met. This methodology consists of performing four longitudinal scans equidistant from each other along the length of the slide, at 400× magnification in an optical microscope. The data obtained were expressed as spores/m^3, using the term Annual Spore Integral (ASIn) to express the total annual quantities obtained or monthly spore integral as indicated in the methodology (Galán et al., 2017).

To understand the behaviour of *Alternaria* spores in the atmosphere, the Main Spore Season (MSS) was established, expressed according to the methodology developed by Galán et al. (2017). To this end, the season was set at 90%, with the beginning established when 5% of total annual spores were recorded and the end when 95% were captured (Nilsson & Persson, 1981). Once the MSS was established for the two samplers, the most important factors of this period were studied (start, end and length duration in days). Together with the MSS study, the seasonality of *Alternaria* spores was analysed in order to determine their prevalence in the atmosphere, as well as their peak day and the maximum values reached. For this purpose, graphs with the distribution between the months of maximum presence (May–November) were established, using the average of the previous five days to smooth out trends. Regarding the threshold values above which the presence of *Alternaria* spores can influence human health, the limit of 100 spores/m^3 proposed by Gravesen (1979) was established.

The study of the intradiurnal variation was carried out using the period covered by the MSS by calculating the percentage of *Alternaria* spores present in each hour of the day (Galán et al., 1991) and expressing the results in a column chart, one for each period of study (2014–2015 and 2015–2016). The graphical representation was carried out by showing the results in a bi-hourly manner, expressing the concentration in terms of two hours total percentage.

The comparison of *Alternaria* spore concentrations obtained in both spore traps was performed using Spearman's nonparametric test (since the data did not follow a normal distribution) of SPSS software (version 23), expressing the results at two significance levels (95% and 99%) for the comparison between spore traps and for the correlations with the meteorological parameters. Correlations were calculated according to the main spore season for each period

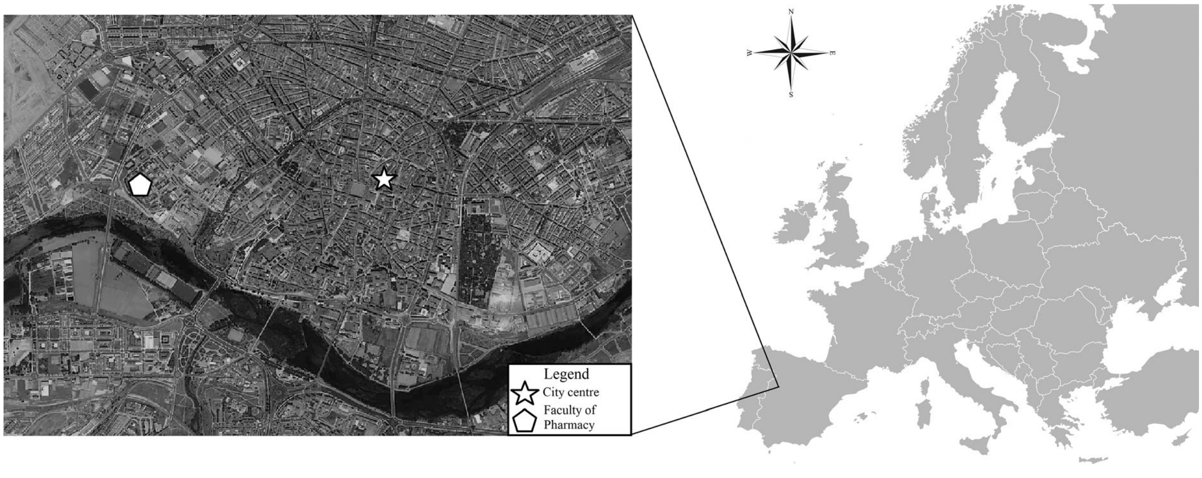

Fig. 1 Location of the two spore traps in the city of Salamanca

of study independently. Also, the two years were analysed jointly in order to find possible differences. Correlations were made, on the one hand, with the daily totals of the MSS for each period and, on the other hand, by separating the data by month, both for each period individually and for the two periods together.

Correlations with the main meteorological parameters were performed to determine the potential influence of meteorology on the concentrations recorded in the interior and the outskirts of the city. To this end, correlations were established between *Alternaria* spores and various meteorological factors during the MSS. On the one hand, each study period was analysed in both locations in order to observe the possible differences in the influence of the meteorological parameters on the number of spores in the atmosphere and, on the other hand, the two study years were analysed together. The meteorological parameters studied were temperature (maximum, minimum and average) (°C), rainfall (mm), relative humidity (%), wind speed (km/h), wind frequencies from the four quadrants (NE, SE, SW and NW) (%), calm frequency (%) and hours of sunshine (hours). The meteorological data were provided by the State Agency of Meteorology (AEMET), obtained from the airbase of Matacan, located 14 km from the centre of Salamanca.

3 Results

3.1 Comparison of meteorological values during the study period

The meteorological parameters that showed the major differences during the two study periods (2014–2015 and 2015–2016) were temperature and rainfall (Table 1). Average monthly temperatures did not differ much; they were slightly higher from March to August 2015–2016 than the previous period, and December and January presented values that doubled the temperature of the same months in 2014–2015. Concerning maximum temperatures, the pattern was quite similar, except in June and July 2015, where the temperature was 2.6 and 4.1 degrees higher, respectively, and December was 5 degrees higher than the previous year (8.6 °C in 2014 and 13.6 °C in 2015). The minimum temperatures collected were reasonably similar, except for September 2014–2015, which was 3.4 degrees warmer than 2015–2016, and January 2015, which was very different from January 2016 (− 3.8 °C in 2015 compared to 2.3 °C in 2016). On the other hand, rainfall showed considerable differences concerning its distribution throughout the year. During 2014–2015, the maximum rainfall was recorded in November, followed by February, while the rest of the year there were rather low values that were zero in August. During 2015–2016, rainfall was distributed in a completely different way; the maximums collected occurred in June and April, while during the rest of the year lower values or rainfall were recorded, although always above zero. In January 2016, the maximum values for the entire study period reached a total of 101.5 mm. The relative humidity showed hardly any difference between the values collected for each year, with almost identical values during the summer months.

Wind speed showed similar values both years; minimum values were distributed in the summer months in both years of study, reaching a maximum of 10 km/h. In 2015–2016, wind speed values were also low in November (which registered the lowest average speed of that year) and December. In 2014–2015, December recorded the lowest value for that year. Regarding wind direction, it behaved differently each year. A predominance of SW direction was observed throughout the period 2014–2015, with May, July and December being more influenced by NE winds. During 2015–2016, the NE and NW winds were predominant throughout the year. SE and SW winds were predominant in December and January 2015–2016, respectively, while their values were similar in April. Hours of sunshine were similar during the two years of the study; the most considerable difference was found in November, which in 2015–2016 registered more sunshine hours than in 2014–2015 (5.7 h on daily average compared to 3.2). The opposite phenomenon occurred in January, where 2014–2015 averaged 5.8 h of sunshine, while 2015–2016 averaged only 3.0 h.

3.2 Comparison of aerobiological behaviour from the point of view of allergenic potential

During 2014–2015, the *Alternaria* spores concentration registered in both zones was quite similar, while

Table 1 Monthly values of meteorological parameters for the studied periods (2014–2015 and 2015–2016)

2014/2015	F	M	A	M	J	J	A	S	O	N	D	J
Tmax	10.3	15.4	19.8	22.4	26.6	29.0	29.6	26.4	23.7	14.1	8.6	9.3
Tmin	0.1	0.3	5.2	6.5	9.3	11.3	11.1	10.2	7.4	3.8	−1.9	−3.8
Tmean	5.2	7.8	12.5	14.4	17.9	20.2	20.3	18.3	15.5	8.9	3.3	2.7
Rainfall	48.6	24.8	22	20.9	2.6	10.3	0	26.5	32.8	72.9	3.2	9.7
RH	76	67	69	57	57	58	59	65	66	79	81	77
Wind speed	19	12	12	14	11	10	7	7	8	13	6	10
Winds NE	1.6	20.8	12.3	31.6	25.6	33.5	20	19.6	13	13.2	34.6	20
Winds SE	2.6	16	12.1	5.6	10.5	9.6	5.9	13.6	13.3	15	20.6	20.3
Winds SW	65.4	27.7	38.9	25.3	26.6	20.5	14.8	28.7	33.3	42.1	10.5	22.2
Winds NW	20	22.4	26.8	31.2	27	24.7	45.4	16.7	11.5	17.5	12.1	19.1
Calm freq	10	12.9	9.5	5.8	9.6	11.2	13.3	18.7	28.7	12	21.6	17.9
Sunlight	4.1	7.3	8.4	11.2	12.4	11.7	11.8	8.5	8.0	3.2	4.5	5.8
2015/2016	F	M	A	M	J	J	A	S	O	N	D	J
Tmax	9.4	16.1	18.8	24.7	29.2	33.1	30.3	24.8	19.9	15.6	13.6	10.6
Tmin	−1.3	0	5.1	7.5	11.5	12.8	11.3	6.8	5.9	1.3	−0.3	2.3
Tmean	4.0	8.0	12.0	16.1	20.3	23	20.8	15.8	12.9	8.5	6.6	6.4
Rainfall	18.5	2.4	47	4.6	51.2	2.2	2.3	10.5	32.8	24.6	13.6	101.5
RH	74	63	64	57	58	56	58	57	70	71	75	77
Wind speed	15.5	11.6	12.4	14.1	8.9	7.9	8.9	8.9	10.9	6.7	7.1	14.5
Winds NE	20.1	41.4	22.2	40.4	30.5	18.5	21.3	24.1	26.2	22.1	16.9	12.1
Winds SE	7.3	13	25.2	11	16.2	10.5	12.2	12.5	17.4	21.5	31.8	12.8
Winds SW	26.3	13.8	25	23.8	20.4	17.1	21.5	22.8	28.4	15.6	25	49.5
Winds NW	38.5	17.5	18.2	16.6	20.6	39.3	32.2	19.9	11	13.1	6.7	12.9
Calm freq	7.5	14.1	9.1	7.9	11.9	14.3	12.5	17	16.7	27	19.3	10.9
Sunlight	4.9	7.8	8.1	11.9	10.7	12.6	10.8	9.0	6.1	5.7	4.3	3.0

Tmax maximum daily average temperature (°C), *Tmin* minimum daily average temperature (°C), *Tmean* mean daily average temperature (°C), *Rainfall* total daily rainfall (mm), *RH* daily average relative humidity (%), *Wind Speed* daily average wind speed (km/h), *Winds NE* daily average frequency of North-Easterly winds (%), *Winds SE* daily average frequency of South-Easterly winds (%), *Winds SW* daily average frequency of South-Westerly winds (%), *Winds NW* daily average frequency of North-Westerly winds (%), *Calm Freq* daily average frequency of calms (%), *Sunlight* daily average sunshine (hours)

in 2015–2016 we could observe several differences between the areas (Table 2).

In 2014–2015, the spore trap located in the city centre registered an annual integral (ASIn) of 6105 spores/m^3 of *Alternaria*, while the trap located in the Faculty of Pharmacy collected 6650 spores/m^3. The maximum daily concentration (263 spores/m^3 in the central zone and 286 in the semi-urban zone) coincided on the date (9 July). Furthermore, the risk levels, established for those days in which the daily concentration exceeded the limit of 100 spores/m^3, also coincided in number of days exceeding this amount (15) and were distributed in the last three weeks of July in both spore traps.

In the period 2015–2016, the differences were reflected both in the ASIn (4538 spores/m^3 in the city centre and 5560 in the Faculty of Pharmacy) and in the peak day and value, which occurred on 2 September for the spore trap of the centre (with a maximum value of 134 spores/m^3), and 17 June in the trap of the Faculty of Pharmacy (with 291 spores/m^3). The days with risk values (exceeding concentrations of more than 100 spores/m^3) were less in the second period of study; that amount was only exceeded on three days in the centre (17 June, 8 July and 2 September) and five days in the semi-urban (16–20 June).

Concerning the monthly concentrations of *Alternaria* spores present in the atmosphere, similarities between both zones were observed (Table 3). Throughout the first period of study, the spore trap of the Faculty of Pharmacy recorded in most months a higher spore integrals of spores than the one in the centre of the city. However, in September and October, the centre sampler recorded slightly higher monthly spore integrals than the other sampler. In the period 2015–2016, the trap of the Faculty obtained greater monthly spore integrals with respect to the centre for all the months of the year. Comparing both years, we can see that the closest values are located between June and July of both years. In 2014–2015, July was the month of maximum abundance (above 3000 spores/m^3), while in 2015–2016 June registered the highest monthly spore integral. September also showed a rather high monthly spore integral, especially in the city centre, while in the semi-urban values remained high from June to September.

3.3 Comparison of the main spore season (MSS) and seasonality

The 2014–2015 MSS showed greater differences than 2015–2016. During the first period of study, the MSS went from 2 June to 22 October (Table 2), with a duration of 143 days for the city centre while, in the Faculty of Pharmacy, the period began almost a month earlier but ended on similar dates (8 May to 25 October, with 171 days). Regarding the period 2015–2016, the main seasons of both samplers were quite adjusted to the same dates, with a difference of five days at the beginning; the season began on 9 May for the centre, and the 4th of the same month for the semi-urban environment, but ended in both cases on the same date (10 November). Both concluded with a similar number of days (186 for the centre spore trap and 191 for the Faculty spore trap).

The seasonality (Fig. 2) reflects a similar behaviour throughout the year for both spore traps. The graphs show the period from May to November to cover the entire MSS and to observe the beginning of the season with the highest abundance. The values of maximum presence in the atmosphere are found between June and August, and there are differences in the presence of *Alternaria* between both periods. During

Table 2 *Alternaria* data from both spore monitoring sites

	City centre							Faculty of Pharmacy						
	ASIn	Peak value[a]	Peak day date	Days above threshold levels[b]	Start MSS	End MSS	MSS length (days)	ASIn	Peak value[a]	Peak day date	Days above threshold levels[b]	Start MSS	End MSS	MSS length (days)
2014–2015	6105	263	9-Jul	15	2-Jun	22-Oct	143	6650	286	9-Jul	15	8-May	25-Oct	171
2015–2016	4538	134	2-Sep	3	9-May	10-Nov	186	5560	291	17-Jun	5	4-May	10-Nov	191

[a]Spores/m^3

[b]*Alternaria* threshold value: > 100 spores/m^3

2014–2015, two peaks can be observed in July, separated only by a sharp drop around 20 July. The second peak extends until early August and represents the maximum amount of *Alternaria* spores in both spore traps. This behaviour is not repeated in 2015–2016, where the maximum peak is registered in the second half of June with differences between spore traps regarding the number of spores present in the atmosphere, being more abundant in the Faculty of Pharmacy.

3.4 Comparison of intradiurnal values

The bihourly distribution of *Alternaria* spores obtained a reasonably regular pattern throughout the day. During 2014–2015 the bi-hourly mean percentage of spores remained between 3 and 4% (Fig. 3). A period of decrease was found at 8 h, with the lowest spore concentrations and increasing gradually until 12–14 h, from this time until 22 h the sporal levels had similar values, at which time a new increase in concentrations was recorded until midnight. The increases and decreases were more pronounced in the semi-urban area, while the percentages were more stable in the central area.

In the period 2015–2016, the graph indicated some differences between the two spore trap samplers. On the one hand, the trap in the city centre registered variations in the concentrations along the day with peaks and lows, showing decreases from 10 to 12 h and from 22 h to 24, with higher values from 16 to 20 h and a peak around 4 h (close to 5%). On the other hand, the Faculty of Pharmacy's trap obtained similar results to 2014–2015, with a very similar trend.

3.5 Statistical correlations between both spore traps

The correlations between the daily values of *Alternaria* obtained in the two spore traps were analysed over the two periods of the study and monthly (Table 4). The correlation coefficients obtained were high and statistically significant for the two periods together and separately for each period. Analysing the relationships between the two areas for each month of the year, we observed that June and July had the highest correlation coefficients between the two spore traps. In the first period, the highest correlation was obtained in July, whereas in the second one the maximum was reached in June. During 2014–2015, November also contributed quite significantly to the relationship between the two study areas. In contrast, during the first period of study, the months where the relationships were not significant or recorded negative values were the coldest (December and February) and also June. During the second period, May and February obtained a non-significant correlation, while December, January, March and July did achieve a significant value, but a low coefficient. Globally, analysing the

Table 3 Total monthly values of *Alternaria* spores

	2014–2015		2015–2016	
	City centre	Faculty of Pharmacy	City centre	Faculty of Pharmacy
February[a]	7	5	3	9
March	35	70	22	54
April	73	213	99	153
May	157	244	287	305
June	502	567	896	1516
July	3107	3242	864	868
August	705	806	662	711
September	816	717	849	918
October	574	547	491	541
November	70	113	297	348
December	23	64	60	92
January	28	51	6	29
February[a]	3	11	1	15

[a]February was analysed with the last two weeks of the first year considered and the first two weeks of the second one

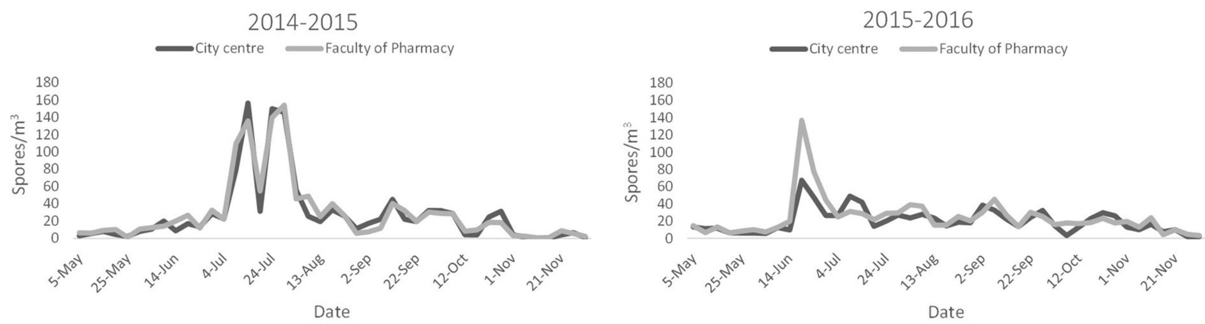

Fig. 2 Seasonality of *Alternaria* between both sampling points in the study period

months of the two periods together, the results show the highest correlations in June, July and November.

3.6 Comparison of correlations between meteorological parameters and daily *Alternaria* spore concentrations

Table 5 shows the values obtained using Spearman's correlation coefficient to see the influence of the meteorological parameters in each of the two study areas, during the MSS of the two periods of sampling, analysed both independently and jointly. Separately, the correlation coefficients were quite similar for the maximum and average temperature, especially during 2015–2016, while the minimum temperature showed differences between the central area and the semi-urban area, recording non-significant values in the centre and significant but low in the Faculty. Rainfall and relative humidity obtained similar correlation coefficients with spore concentration in both samplers. Wind speed did not show positive significance in either case. The winds that had a positive influence on spore concentration were the NE winds and, during 2015–2016, the SE winds, while both the SW and NW winds resulted in a negative significance, not significant in the case of the latter. Sunshine had a positive influence on both areas and resulted in higher coefficients in the central area.

The factors that obtained a significant and positive correlation between both locations were temperatures and total daily sunshine. However, the minimum temperature was not significant in the central area, while in the semi-urban environment there was a significant positive correlation between minimum temperature and spore concentration although it was lower than the correlation coefficients for maximum and average temperature. Another factor that obtained a positive and significant correlation was the winds from the NE and, for the Faculty area, also those from the SE. The factors negatively correlated with the number of spores recorded were both rainfall and relative humidity, with slightly higher coefficients in the semi-urban area. The influence of winds, on the other hand, obtained higher coefficients in the city centre.

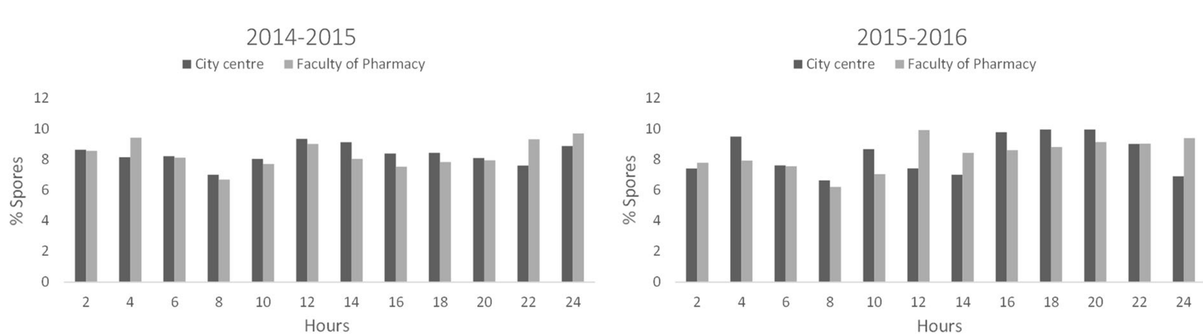

Fig. 3 Intradiurnal patterns of *Alternaria* established during the Main Spore Season

Table 4 Spearman correlation coefficients between both monitoring sites

Alternaria	2014–2015	2015–2016	2014–2016
Annual	.824**	.836**	.833**
Monthly			
February[a]	.414	.255	.285
March	.571**	.438*	.524**
April	.705**	.474**	.556**
May	.580**	.345	.465**
June	.246	.866**	.633**
July	.829**	.370*	.765**
August	.580**	.577**	.595**
September	.608**	.473**	.518**
October	.629**	.493**	.583**
November	.758**	.560**	.721**
December	− .178	.454*	.206
January	.358*	.363*	.477**
February	− 285	.460	− .018

[a]February data were analysed with only two weeks since the design of the sampling cuts the month in half. The column from period 2014–2016 was also performed with two weeks: first row with the 15 days from 2014 (17th to 28th) to 2015 (1st to 16th) and the last row with the last 15 days from 2015 (17th to 28th) and 2016 (1st to 16th)

Significance level: **99%; *95%

4 Discussion

4.1 Comparison of data and threshold levels for both spore trap samplers

Although *Alternaria* is a genus commonly distributed throughout the world and quite abundant in the atmosphere, the spore integral of spores collected in this work is far from other similar areas of the Mediterranean such as Morocco or Italy (El Haskouri et al., 2016; Marchesi, 2020) or other areas of Europe, such as Poland (Grinn Gofroń et al., 2016), where *Alternaria* ASIn usually exceed 10,000 spores per year. In Salamanca, the values obtained for both samplers remain similar to those recorded in nearby cities such as Valladolid (Sánchez Reyes et al., 2009) but are higher than those obtained in other areas to the west such as Portugal (Oliveira et al., 2009b). Despite having captured a high total number of spores, the data obtained in previous works (Fuentes et al., 2019) did not show *Alternaria* as one of the most abundant spore types in the atmosphere of Salamanca. This study shows that fewer spores reach the interior of the city than were registered in a semi-urban area. Other works that relate rural environments with urban environments obtained a higher incidence of *Alternaria* spores in rural areas, due to a more abundant presence of the necessary substrate for its development and release into the atmosphere (Calderón et al., 1997; Oliveira et al., 2010). Although there is a limitation on the number of spores reaching the city centre, either because of the buildings shielding the spore trap or because of the unfavourable environment for the spread of spores (Lin et al., 2018), the maximum values seem to coincide around the dates when the spore peaks are established. Other investigations carried out in various parts of Spain obtained similar values for the peak quantities and the days on which they were collected. They were concentrated between June and July, the months with the highest presence of *Alternaria*, and in some cases were recorded in September and October (Aira et al., 2013; Maya Manzano et al., 2016; Recio et al., 2012).

The risk levels established under Gravesen's criteria (1979) imply that the average daily value of *Alternaria* spores exceeds 100 spores/m^3. Even considering other classification levels such as those established by Frankland Davies (1965), where the risk value was established for a concentration of more than 50 spores/m^3, and even those proposed by Munuera Giner et al. (2001), who established a classification of five levels with respect to the abundance of *Alternaria*, the levels in Salamanca did not involve a large number of days as occurs in other areas where this spore is more abundant, such as northern Europe (Kasprzyk et al., 2015) or southern Spain (Bardei et al., 2017). The number of days with high values seems to be influenced by the characteristics of the year since other studies obtained similar results (Sánchez Reyes et al., 2009). Thus, one month in a year may have several days where the limit of 100 spores/m^3 is barely exceeded and the same month in another year may have a higher number of days with concentrations above the limit.

4.2 Main spore season (MSS) and seasonality

As *Alternaria* is a fungus with such a marked seasonal distribution since its optimum growth temperature is

Table 5 Spearman correlation coefficients obtained between MSS daily concentrations and meteorological factors during two years (2014–2016)

Year	Trap	T_{max}	T_{min}	T_{mean}	R	RH	WS	Wind NE	Wind SE	Wind SW	Wind NW	CF	Sunshine
2014–2015	City centre	.337**	.016	.275**	− .362**	− .281**	− .222**	.314**	.023	− .349**	− .007	.176*	.354**
	Faculty of Pharmacy	.444**	.252**	.425**	− .305**	− .263**	− .187*	.297**	.128	− .264**	− .129	.144	.283**
2015–2016	City centre	.338**	.093	.266**	− .229**	− .169*	− .227**	.233**	.188*	− .181*	− .039	.224**	.254**
	Faculty of Pharmacy	.331**	.162*	.281**	− .179*	− .122	− .285**	.205**	.171*	− .217**	− .022	.262**	.206**
2014–2016	City centre	.333**	.079	.277**	− .286**	− .191**	− .230**	.256**	.086	− .227**	− .007	.205**	.304**
	Faculty of Pharmacy	.442**	.253**	.413**	− .345**	− .248**	− .216**	.224**	.111*	− .195**	− .017	.012	.128*

T_{max} maximum daily average temperature (°C), T_{min} minimum daily average temperature (°C), T_{mean} mean daily average temperature (°C), *R* total daily rainfall (mm), *RH* daily average relative humidity (%), *WS* daily average wind speed (km/h), *Wind NE* daily average frequency of North-Easterly winds (%), *Wind SE* daily average frequency of South-Easterly winds (%), *Wind SW* daily average frequency of South-Westerly winds (%), *Wind NW* daily average frequency of North-Westerly winds (%), *CF* daily average frequency of calms (%), *Sunshine* daily average sunshine (hours)

Significance levels: *95%; **99%

between 22 and 28 °C (Fernández et al., 1998), its abundance is quite limited to the warm season but, as several authors pointed out (Eduard, 2009; Ianovici et al., 2016), temperatures over the optimum growth range could result in a decrease in the spore concentration. This situation could provoke the decrease that occurred during the high temperatures in July 2015–2016 with respect to the previous July (Table 1).

However, in some countries, such as Spain, *Alternaria* conidia are usually present throughout the year (Vélez Pereira et al., 2019), especially in cities with weather conditions that favour its development and release, such as Mérida (Maya Manzano et al., 2012). The lowest concentrations of *Alternaria* are recorded during the coldest months (December-February), with the MSS starting around March–April, depending on the city and weather conditions (Aira et al., 2013) and lasting until October–November. In our study, the MSS was delayed until May in the case of the semi-urban environment, and between May and June in the city centre. The situation of the spore trap of the Faculty likely influences the number of spores collected, which causes an advance at the beginning of the MSS, as it is surrounded by natural environments where *Alternaria* can develop more easily than in the centre of the city (Olsen et al., 2009a). Nevertheless, the work of Fernández Rodríguez et al. (2015) obtained a similar MSS to that of our study, with a similar duration concerning the days of the period and similar peak values, but with a different date, being established in October in two of the three years analysed. This pattern is also repeated in other Northern European countries, like Poland (Rapiejko et al., 2017), where the MSS from six different cities was analysed. In that study, the start of the MSS took place between May and June and the peak day occurred in July in all cases.

Our study's seasonal distribution coincides with the values obtained by researchers from other parts of Spain, both in areas close to Salamanca (Sánchez Reyes et al., 2016) and in more Mediterranean areas (Elvira Rendueles et al., 2019). In these cases, the results obtained by the researchers coincide with the seasonality observed, with the maximum levels of *Alternaria* distributed between June and July, and reaching, in some cases, September and October. Likewise, in countries of the Mediterranean region such as Turkey (Aydogdu & Asan, 2008) or Greece (Pyrri & Kapsanaki Gotsi, 2015), the period of maximum amount of spores collected is also grouped around June, July and August; in some cases, it is displaced until September, with peaks in October (Kasprzyk & Worek, 2006). The decrease observed in July 2014 (Fig. 4) affecting the two locations seems to be caused by the presence of rain during this month, which could have a sweeping effect on these biological particles in the atmosphere (Artaç et al., 2014; Magyar et al., 2009).

4.3 Comparison of intradiurnal values

Alternaria's intraday behaviour showed similar results in both sampling locations, with slight differences during 2015–2016. The results obtained for Salamanca are similar to those obtained in nearby areas such as Valladolid (Sánchez Reyes et al., 2009) and other countries such as Pakistan (Khan et al., 2016). These works coincide in the points where *Alternaria* starts to increase from its minimum values around 8 h, increasing its concentration gradually until 12–14 h. In Valladolid, the second increase in spores occurs from 18 h and remains until 24-1 h when it decreases again. In our research, however, the increase takes place four hours later, from 22 to 24 h, which is similar to that observed in Pakistan. In 2015–2016, the city centre sampler obtained differences both in spore concentration and in distribution hours, since the first peak was shortened from 8 to 12 h and the second was reached from 16 to 20 h and decreased at that point, while in the semi-urban the behaviour was similar to that obtained for the previous period. This behaviour has been reported in northern cities such as Worcester (England) (O'Connor et al., 2014) or Cracow (Poland) (Stępalska & Wolek, 2009). In those cities there is a decrease in the concentration of *Alternaria* spores from 20 to 24 h (Cracow), and there is also a peak around 4 h or even from 16 h (Worcester). The results obtained in another study conducted in southwestern Spain (Fernández Rodríguez et al., 2015) agree with those obtained in our study. An increase is observed from 7 to 12 h, at which time the concentration of *Alternaria* spores begins to decrease. This is due to the fact that spore release into the atmosphere occurs at the highest temperatures, which is associated with vertical wind speed and spore-moving turbulence (Savage et al., 2012).

4.4 Correlations between both spore traps

There are not many papers in the literature that deal with the comparison of spore values using Spearman's correlation coefficient. However, this coefficient has been used in comparative studies, mainly of pollen and meteorological factors, but also comparing data series obtained by different samplers (Gharbi et al., 2017; Sánchez Mesa et al., 2005). Similar studies for pollen were conducted in Salamanca using these same two sampler locations (Rodríguez de la Cruz et al., 2016; Fuentes et al., 2020), where highly significant correlations were obtained for most of the pollen types studied in different periods (2007, 2014–2016).

The results of this study show very similar values between both stations. The difference in sampler height seems not to have an influence on the particle capture as Fuentes et al. (2020) suggested and as other authors pointed out, since the optimal height of a sampler trap could be > 10 m (Rojo et al., 2019), being the pollen and spore concentrations more homogenous above that height. However, more data are needed to a better assessment of this fact. The days with the highest abundance of spores in the atmosphere seem to correlate, although in one area the number is higher than in the other. A similar study by Kasprzyk and Worek (2006) obtained similar results where *Alternaria* showed the highest correlation coefficient when compared to other spore types between two sampling areas. Although rural areas present a higher number of spores (Lin et al., 2018), airborne *Alternaria* spore concentrations show highly positive and significant correlation coefficients, especially when analysing the monthly results for the set of years. If the months are analysed independently, it can be seen that during summer, where *Alternaria* conidia are more abundant, the quantities collected in both areas of the city are similar, especially in 2014–2015 during June, July and August. The high values in September also coincide with similar research results in other Mediterranean areas (Marchesi, 2020). In contrast, the lowest and even non-significant values occur during the winter months, probably due on the one hand to the lack of data during February (sampling start and end at mid-February) and on the other hand due to the scarcity of *Alternaria* spores at this time (Oliveira et al., 2009a). The few spores that arrive at this time may be collected by the spore trap from the Faculty of Pharmacy, while the few spores present in the air barely reach the city centre, possibly due to the distribution of buildings that modify the airflow (Janhäll, 2015), which causes insignificant or negative values.

4.5 Correlations to meteorological parameters

The statistical results when assessing the relationship between *Alternaria* spore concentrations and the main meteorological parameters in our study coincide with the majority of works carried out throughout the world, regardless of whether the location is in Europe (Almeida et al., 2018) or in other countries such as Australia (Stennett & Beggs, 2004). *Alternaria* belongs to the so-called dry spore types, which means that its concentration in the atmosphere increases with low levels of relative humidity and high temperatures (Olsen et al., 2019b). Because of this hot-season distribution, studies are beginning to focus on the analysis of these spore types during the summer (O'Connor et al., 2014). Our results are mainly in line with those of other areas of the Mediterranean (Pyrri & Kapsanaki Gotsi, 2017), where temperatures have a significative influence on the presence of *Alternaria* in the atmosphere. Although the correlation with minimum temperature did not obtain significant results in the city centre, the correlation coefficients with sunshine hours were higher than those obtained at the Faculty of Pharmacy. Similarly, in other studies the correlations with the minimum temperature showed different values of significance, according to the characteristics of the year (Filali Ben Sidel et al., 2015). The parameters that tend to be negatively correlated, rainfall and relative humidity, coincide in most of the research carried out, and a highly significant or non-significant negative trend is observed depending on the city under study (Kasprzyk et al., 2015; Oliveira et al., 2009b). As explained in previous research, rain negatively affects the release of spores into the atmosphere (Hirst & Stedman, 1963; Peternel et al., 2004). However, situations have been reported in which, after abundant rainfall, the release of conidia is favoured (Pérez Gorjón et al., 2003; Sánchez Reyes et al., 2009), a trend that has been confirmed in this study when the maximum peak was recorded on 17 June 2015–2016 in the collector of the Faculty after abundant rainfall during the first 15 days of the month. The winds that had the most significant influence on *Alternaria*'s presence in the two areas of the city were northeast winds and, to a lesser extent, southeast winds in 2015–2016. This relationship could be given by the situation of abundant crops in the area of Valladolid located NE of Salamanca (Fuentes et al., 2019), while the presence of *Alternaria* linked to southeasterly winds could be related to a greater abundance of host plants of the fungus in areas of central Spain (Sabariego et al., 2012).

5 Conclusion

The seasonal behaviour of *Alternaria* spores does not show great differences between the two sampling areas except for the amount recorded, as they were

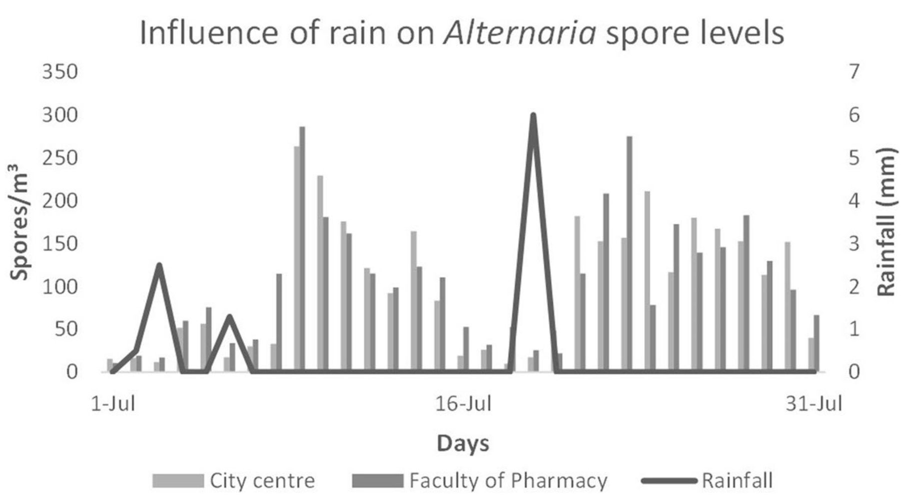

Fig. 4 Daily rainfall (mm) and daily atmospheric spore levels (spores/m^3) in both spore traps during July 2014

more abundant in the semi-urban environment. The farmlands and the vegetation surrounding this area could influence the number of spores that reach the trap. The higher density of buildings in the city centre may have acted as an artificial screen against *Alternaria* spore-carrying winds, as reflected in the lower spore levels of the sampler at this location.

There does not seem to be a notable difference in the intradiurnal distribution in the two study areas, except that there was a more uniform distribution throughout the day in the city centre, whereas the semi-urban showed more pronounced increases and decreases.

Regarding the correlations established between the number of spores obtained in both locations, no differences can be pointed out, since the coefficients obtained show high similarity in the results. Even concerning the days that presented risk levels, the differences between the two zones were not significant, as they showed several days with the same or similar risk concentrations, on almost the same dates.

About the possible influence of meteorological factors, a similar trend is observed, with higher correlation coefficients in the Faculty of Pharmacy; this is probably due to greater exposure, while in the central area, the spore trap is more protected. The factors positively correlated with the number of spores collected were temperature and sunshine, while those negatively related were rainfall and relative humidity. The presence of winds obtained different results, with a positive correlation for the NE winds.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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