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# Effects of meteorological factors on the levels of *Alternaria* and *Cladosporium* spores in the atmosphere of Palencia, 1990–92

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This study examines the daily levels of *Alternaria* and *Cladosporium* spores in the atmosphere in Palencia city (Spain) for three consecutive years 1990–92. *Alternaria* and *Cladosporium* represented 55% of all the identified spores. Their annual distributional pattern reached its maximum values in summer. Multiple regression analyses show that *Cladosporium* spore concentrations have a positive correlation with minimum temperature, whereas *Alternaria* spore concentrations have a positive correlation with maximum temperature. *Alternaria* spore concentrations were also negatively correlated with precipitation. Duncan's multiple range test of *Alternaria* data show that winds blowing from a northeasterly direction were significantly related to higher levels of these spores.

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*Alternaria* and *Cladosporium* species have been shown to live as parasites or saprophytes on a large number of plants (Hjelmroos 1993), and are well represented in temperate areas. Spore dispersion of both moulds in the atmosphere is considered to be responsible for a large number of respiratory diseases (allergy, asthma, persistent cough). Some *Alternaria* species have been reported as being responsible for the diseases otomycoses, onychomycoses, cutaneous alternarioses and keratomycoses (Srivastava & Wadhwani 1992). A knowledge of the pattern of distribution in the atmosphere of these spores may help health programs to prevent, or be prepared for, the outbreak of these diseases.

In spite of the importance of these studies, none has been carried out in the north of Spain. The objective of the present study is to gain a first insight into the influence of meteorological factors on the dispersion and concentration of *Cladosporium* and *Alternaria* spores in the atmosphere of Palencia city.

## MATERIAL AND METHODS

Spore concentrations in the atmosphere of Palencia were studied for three years, from January 1990 through to December 1992. An active filtration device CAP2 (Suárez-Cervera & Seoane-Camba 1983) was used. This device consists primarily of a filtration chamber with a vane. The filter is 7 cm in diameter filter with a 5 µm pore size. They were prepared for light microscopy by placing on slides and using immersion oil. Three preparations were obtained daily from each filter without further manipulation. A full analysis of every filter was recorded, and the results expressed as the number of spores/m<sup>3</sup> of air. All those airborne particles with a diameter greater than 5 µm were collected on the filter. The collecting device was placed 12 m above ground level on a flat roof of the building of the Agricultural Engineering School, in the southeast part of the city.

Spore identification was made using several atlases (Nilsson 1983,

Smith 1984; 1986). The results of daily concentrations of *Alternaria* and *Cladosporium*, and total spores, are given as weekly means, to make the interpretation of the yearly tendencies easier.

*Alternaria* and *Cladosporium* spores were expressed as daily concentration: spores/m<sup>3</sup>. To establish the Main Spore Season (MSS; Pathirane 1975), weekly mean concentrations of the three years sampled, daily percentages, weekly percentages per year, weekly mean percentages of the triennium sampled and the accumulated spore percentages were obtained by adding up day by day the percentages belonging to these types in relation to the total spores gathered throughout the year.

Statistical analyses were made with the SPSS software system (Statistical Package for the Social Sciences, 1990), using the procedures for multiple regression and one-way anova test.

The variable selection method was stepwise regression, when all variables are in the model. The variables included were maximum temperature, minimum temperature, precipitation, relative humidity and wind speed, with the dependent variable being the daily concentration of *Cladosporium* and *Alternaria* spores. The anova test was made, where the dependent variable was the concentration of these spores and the explanatory variable was the wind direction. To assign the wind direction for the day, 70% of the frequencies must have had the same component, and the following code was used: 1=N to E, 2=E to S, 3=S to W, 4=W to N, 5=calm. The confidence interval was fixed at 95%, and a Duncan multiple range test of means was done when the differences among groups were significant.

## Area of study

The sampling station in the current study was located in Palencia city, in the northwest of the Iberian Peninsula. The city is located in the middle Duero basin at 743 m above sea level, on Tertiary sedimentary material set in a continental lake environment. The original vegetation was a mediterranean oak forest: *Quercus rotundifolia* and *Quercus faginea*. Forest clear-cutting and land cultivation has continued since Roman times and now most of the territory has been transformed into extensive cereal and irrigated land (lucerne and beet).

The climate of this area is mediterranean with a clear dry season in summer, and 351 mm average annual precipitation. However, as Palencia is located on the northern Iberian high plateau the climate has a strong continental influence and is classified more precisely as supramediterranean (Rivas-Martínez, 1987). Average annual temperature is 11,7°C, average January temperature is 4,2°C, average July temperature is 13,7°C, and the growing season lasts from about 10th May to 20th October. Meteorological data are gathered from the Meteorological Center of Duero basin, at the Autilla-Palencia station, placed 1 km away from the collecting device for airborne particles. The dominant winds are from the southwest and occur 42,6% of the time. The yearly mean relative humidity is 69,9%, and the city receives more than 2600 hours of sunlight per year.

## RESULTS

*Cladosporium* and *Alternaria* spores have been described as the most abundant in the atmosphere (Kotimaa 1977, Herrero 1994). In the atmosphere of Palencia city they represent more than 50% of the total identified spores for the period studied. Both form-genera are present throughout the year, although they show a clear seasonal pattern. Following Infante (1991), the presence of the fungi are classified as follows: occasional (when a spore type is present between 1 and 19 weeks per year), frequent (from 20 to 39) and very frequent (from 40 to 52). *Cladosporium* spores were very frequent and *Alternaria* frequent.

### *Cladosporium*

This genus is the main component of the airborne mycoflora of the city. The mean of the three years sampled represents 46% of the spore total (Table I), which is similar to previous results observed by other researchers (Goodman et al. 1966, Kotimaa 1977, Nilsson 1983, Linares-López et al. 1985, Nogales et al. 1985, and Infante & Domínguez 1988).

As has been mentioned before, *Cladosporium* is present in the air of Palencia throughout the year, but the main sporulation period occurs from the 19th to 39th week, as expressed by the accumulative percentages of daily spore frequencies following Pathirane's criteria (1975), see Fig. 1. The mean weekly concentrations of the three years sampled (Fig. 2) show that during winter and early spring, the spore concentrations are very low. In April, concentrations start to increase, reaching a maximum of about 380 spores/m<sup>3</sup> in September of 1991. This maximum was due to the combination of high temperatures together with abundant precipitation, circumstances which have been shown to favour sporulation (Govi 1992).

In our study, these fungi reach a maximum concentration with mean temperatures around 25°C and relative humidities of 50–60%. Multiple regression analyses of spore

Table I. Annual percentages of *Cladosporium* and *Alternaria* spores.

|                     | 1990<br>% | 1991<br>% | 1992<br>% | Mean<br>% |
|---------------------|-----------|-----------|-----------|-----------|
| <i>Alternaria</i>   | 8.30      | 7.43      | 10.51     | 8.91      |
| <i>Cladosporium</i> | 51.54     | 45.49     | 43.42     | 47.75     |

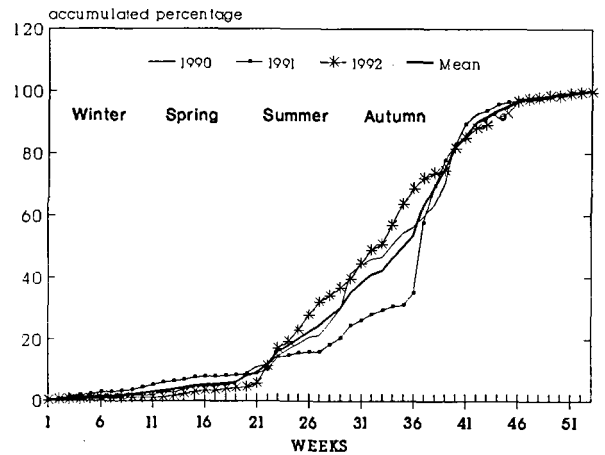


Fig. 1. Accumulated percentages of *Cladosporium* spores during the years 1990, 1991 and 1992.

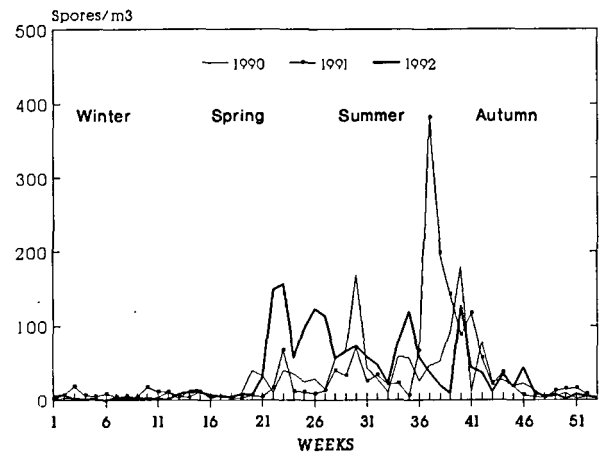


Fig. 2. Mean weekly concentrations of *Cladosporium* spores, during 1990–1992.

concentrations and several meteorological parameters have shown only a significant relationship with minimum temperature (Table II; Fig. 3). An increase in minimum temperature is related with a significant increase in the concentration of *Cladosporium* spores.

Table II. Effect of meteorological factors on *Cladosporium* spore concentrations.

\* significance of  $\alpha$ ,  $P < 0.05$ .

|                     |           |
|---------------------|-----------|
| $R^2$               | 0.108     |
| n                   | 589       |
| Source              | t-student |
| Maximum temperature | 0.591     |
| Minimum temperature | 3.109*    |
| Relative humidity   | 1.865     |
| Precipitation       | 1.146     |
| Speed wind          | −0.708    |

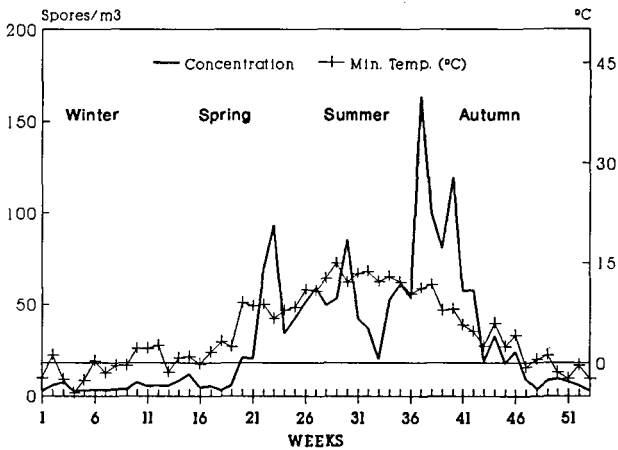


Fig. 3. Mean weekly concentrations of *Cladosporium* spores and mean weekly minimum temperature.

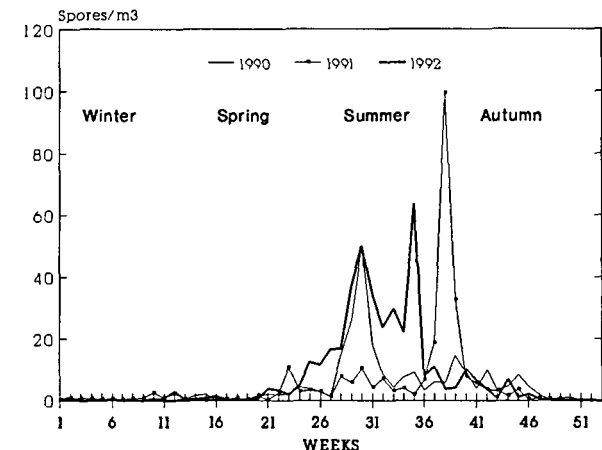


Fig. 5. Mean weekly concentrations of *Alternaria* spores, from 1990 to 1992.

Alternaria

This is a saprophytic form-genus whose optimal development has been shown to be reached at temperature ranges of 22–28°C, with rarely any growth at 0°C (Hjelmroos 1993). *Alternaria* was present in the Palencia atmosphere throughout the year but its main sporulation period was shorter than that of *Cladosporium* (weeks 22 to 38, Fig. 4). Its weekly mean concentration in the three years analyzed was lower than *Cladosporium*. *Alternaria* spore concentrations were never in excess of 95 spores/m<sup>3</sup> per week (Fig. 5). *Alternaria* represented only 9% of the total spores identified, although it was the second most frequent spore group. *Alternaria* reaches highest concentrations in the summer months, with maximum levels in September.

The results obtained from the multiple regression analysis (Table III), show that the concentration of this type of spore is significantly related to maximum temperature and precipitation. An increase in maximum temperature and precipitation produced an increase in *Alternaria* spores (Fig. 6). Likewise, the values calculated for analyses of variance between spore concentration and wind direction are signifi-

cant (Table IV). *Cladosporium* spores are not significantly related to wind direction (Table V). Duncan's multiple range test (Table VI), indicated that *Alternaria* concentration was only significantly higher when the wind blew from the first quadrant (NE), versus the third quadrant (SW).

Table III. Effect of meteorological factors on *Alternaria* spore concentrations.

\* significance of  $\alpha$ ,  $P < 0.05$ .

|                     |           |
|---------------------|-----------|
| R <sup>2</sup>      | 0.132     |
| n                   | 435       |
| Source              | t-student |
| Maximum temperature | 2.642*    |
| Minimum temperature | 0.676     |
| Relative humidity   | 0.968     |
| Precipitation       | 2.121*    |
| Speed wind          | 1.110     |

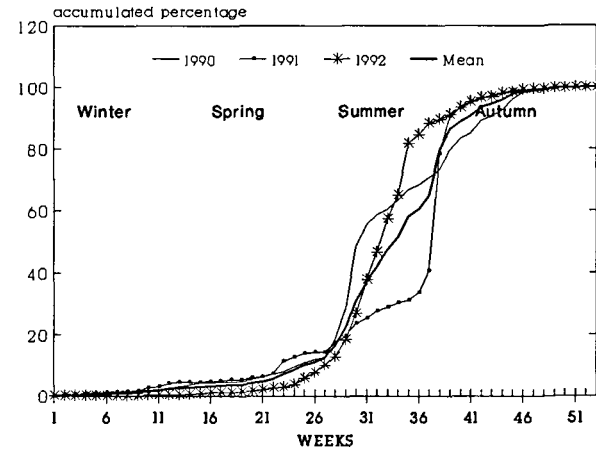


Fig. 4. Accumulated percentages of *Alternaria* spores during 1990, 1991 and 1992.

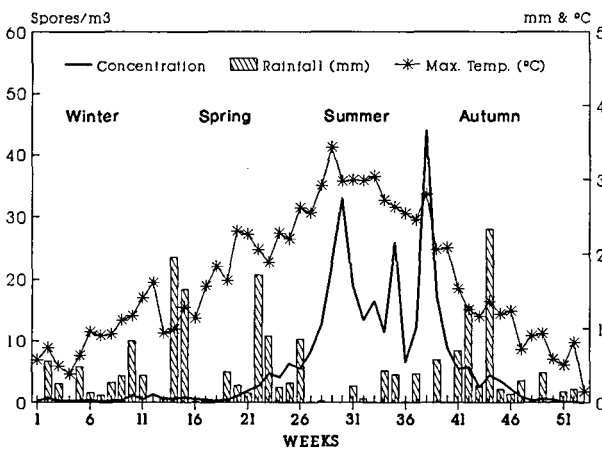


Fig. 6. Concentration of *Alternaria* spores and weekly means of maximum temperature and precipitation.

Table IV. One-way analysis of variance for *Alternaria* spore concentrations versus wind direction.

\* significance of  $\alpha$ ,  $P < 0.05$ .

| Source         | D.F. | S.S.     | M.S.    | F      |
|----------------|------|----------|---------|--------|
| Wind direction | 4    | 2425.099 | 606.274 | 1.791* |
| Error          | 435  |          |         |        |

Table V. One-way analysis of variance for *Cladosporium* spore concentrations versus wind direction.

\* significance of  $\alpha$ ,  $P < 0.05$ .

| Source         | D.F. | S.S.      | M.S.     | F     |
|----------------|------|-----------|----------|-------|
| Wind direction | 4    | 13349.716 | 3337.429 | 0.656 |
| Error          | 589  |           |          |       |

Table VI. Effect of wind direction on *Alternaria* spore concentrations.

(1) Group 1, wind direction quadrant N to E; group 2, E to S; group 3, S to W; group 4, W to N; group 5, calm.

(2) Means with the same letter are not significantly different.

(3) Means separation by Duncan's multiple range test, 5% level.

| Wind direction (1) | Mean spore concentrations (2)<br>(spores/m <sup>3</sup> /week) |
|--------------------|----------------------------------------------------------------|
|                    | 11.1089 a                                                      |
|                    | 7.8590 a, b                                                    |
|                    | 6.9814 b                                                       |
|                    | 4.5063 a, b                                                    |
|                    | 3.8865 a, b                                                    |

## DISCUSSION

A review of the current literature shows a lack of agreement for the main season of *Cladosporium* sporulation. Pady (1957), Kramer et al. (1959) and Collins-Williams et al. (1973) have stated that spring and summer are the main seasons of *Cladosporium* sporulation. Nevertheless, Goodman et al. (1966) report that the main season for *Cladosporium* sporulation is autumn. According to Davies et al. (1963), the concentration of *Cladosporium* in the atmosphere has been shown to increase in summer. On other hand, Infante and Domínguez (1988) have found a decrease in the aerial content of this form-genus during this period. In Palencia, as in the nearby regions (Fernández-González et al. 1993), summer is the most favourable season for the development of this taxon. The same has been found for many other temperate regions of the world (Goodman et al. 1966) where the *Cladosporium* spores have a summer periodicity.

The growth, sporulation and dispersal of *Cladosporium* are very sensitive to changes in weather. There are several meteorological factors that have been shown to be favourable to sporulation, such as high temperatures and relative humidity, as well as precipitation before spore dissemination (Ebner et al. 1989). According to Hjelmroos (1993) daily precipitation together with daily mean temperatures higher

than 15°C can cause an increase in relative air humidity that will contribute to an increase in the concentration of *Cladosporium*. This verifies the existence of significant correlations between mean temperature and precipitation. Srivastava and Wadhvani (1992) have added to these factors an increase in the number of hours of sunshine to support the hypothesis that in summer time the concentration of these spores is at a maximum.

The summer pattern in the *Alternaria* spores has been shown to be characteristic of temperate zones located more than 1000 m above sea level (Ebner et al. 1989) together with high air temperature (Gregory 1973, Fernández-González et al. 1993).

*Alternaria* has been found to be particularly sensitive to changes in precipitation and wind direction, a result which coincides with that obtained by Bashan et al. (1991).

The results obtained with the present collecting device (CAP2), can be qualitatively compared to data obtained from other types of equipment for collecting biological particles.

## CONCLUSION

This three year study in Palencia shows that *Cladosporium* and *Alternaria* spores were the most abundant, comprising more than 50% of the total studied. The mean sporulation period for *Cladosporium* was longer than for *Alternaria*, being aestival in both cases.

These two form-genera have a similar pattern with a clearly seasonal periodicity determined by meteorological factors. The results of multiple regression analysis showed a positive correlation between increase in minimum temperature and increase in *Cladosporium* concentration.

For *Alternaria*, a positive correlation with maximum temperature and precipitation was found. When these factors increased, the concentrations of this form-genus also increased. Similarly, Duncan's test showed that winds coming from the first quadrant were significantly associated with higher concentrations of *Alternaria*, as opposed to the third quadrant.

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