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Airborne concentrations of *Botrytis*, *Uncinula* and *Plasmopara* spores in a vineyard in Leiro-Ourense (N.W. Spain)

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Abstract

Three important phytopathogenic spores which cause serious fungal diseases on vines (*Botrytis*, *Uncinula* and *Plasmopara*) were monitored from June 2, 1994 to September 21, 1994, in vineyards belonging to 'Estación de Enología e Viticultura' of Ponte San Clodio-Leiro (Ourense), one of the most important vine growing areas of northwest of Spain. The concentrations for each of the genera were correlated with rainfall, humidity, maximum, minimum and average temperatures. These meteorological parameters are currently used to determine chemical sprays to be applied to the vines. The aim was to establish the behaviour of airborne concentrations of spores of these taxa during the period from the flowering to harvest because these are the periods of greatest susceptibility to attack by these pathogens. © 1997 Elsevier Science Ireland Ltd.

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1. Introduction

Air is probably the most important agent for the transport of spores of pathogenic fungi and monitoring spore concentrations provides information on temporal and spatial patterns of dispersal from different sources. Aerobiology in Galicia started in 1945 (Viéitez, 1946, 1947), and in the last few years it has been used to study airborne pollen (Ferreiro and Fontán, 1984; Iglesias, 1990; Iglesias et al., 1988; 1993; 1995). However, some airborne fungi have been little studied in Galicia. Only spore concentrations of *Alternaria*, *Cladosporium*, *Aspergillus* and *Penicillium* have been reported from Ourense (Méndez, 1994). The province of Ourense, in the NW Iberian Peninsula, belongs to the Autonomous Region of Galicia (Fig. 1) and vines are of major agricultural importance, principally on both sides of the rivers Miño, Sil and Tamega. Over 12 909 ha are currently grown (Xunta de Galicia, 1992).

Diseases caused by fungi (grey mould, powdery mildew and downy mildew) can cause economic losses and chemical treatments are applied depending on meteorological conditions or plant development stages. Until now there have been no aerobiological studies of airborne inoculum to find the potential risk of disease during the season. The aim of this work was to measure the spore concentrations of three vine fungal pathogens: *Botrytis*, *Uncinula* and *Plasmopara*, during the vine production season.

Grapes which have suffered surface damage accidentally or by disease are vulnerable to infection by a wide range of moulds (Parry, 1990). But undamaged grapes can become mouldy in a few days just before vine harvest when rain and temperature conditions are suitable. In this case, the prevailing disease is the grey mould *Botrytis cinerea* Pers. ex Pers., conidial form of *Sclerotinia fuckeliana* (de Bary) Fuckel (Marcilla, 1968; Smith et al., 1992), and it is fruits close to maturity that are most affected.

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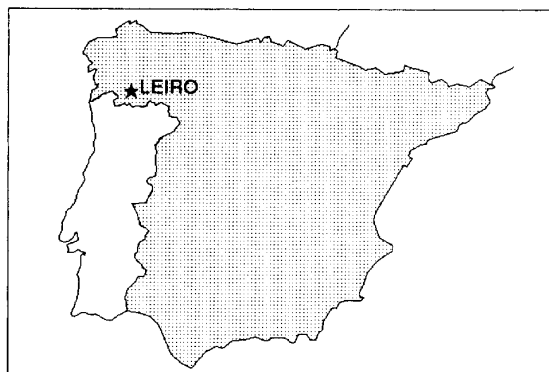


Fig. 1. Geographic location of the sampling area.

Uncinula is a ectoparasitic ascomycete genus, in which the asexual reproductive stage produces conidia with granular and vacuolated content, which allows them to resist drying and to germinate without water. *Uncinula necator* (Schw.) Burr. is responsible for powdery mildew in grape vines (*Vitis vinifera*). Temperature and humidity, without the necessary presence of water, are the meteorological factors that mainly affect conidial germination. The symptoms may appear either on the upper or lower surface of the leaves and on buds, petioles, teasels and young shoots and grapes. Flowers are not frequently attacked, but before fertilization it can be common and infection can cause failure of fruit set (Hidalgo, 1991).

Plasmopara is an endoparasitic oomycete mould (*Plasmopara viticola* Berlese y De Toni) responsible for downy mildew in *Vitis vinifera*, and is endemic to wet regions. The most important cause of failure of the fruit set in Spanish vines is early infection by downy mildew during the flowering stage. When this occurs the loss of yield is irremediable. The fungus can affect all varieties, but serious epidemics occur only sporadically (Hidalgo, 1991). Symptoms may appear on leaves, buds, flowers and grapes. They are most likely to appear on leaves but flowering or fruiting racemes and buds may be attacked; however, young shoots are scarcely infected. The typical symptom of downy mildew is a white dust covering the infected organs.

2. Materials and methods

Sampling was carried out in a 5 ha vineyard in Ponte San Clodio-Leiro (Ourense). In Leiro, there are 480 ha of vines, representing 10.82% of the total crop area. This area belongs to the Ribeiro Mark of Origin which has an area of 55 852 ha, of which 3011.82 ha grow vines. The climate is Mild Mediterranean, according to Papadakis Classification (Papadakis, 1966), with an annual temperature range of 14.95°C (Carballeira et al., 1983).

Spore concentrations were measured using a Hirst-type volumetric spore-trap (LANZONI VPPS 2000) located at a height of 1.5 m above the ground and 0.5 m above the maximum height of the vines, in the vineyard of the 'Estación de Enología e Viticultura' of the Consellería de Agricultura of the Xunta de Galicia. The study period was from June 2, 1994 to September 21, 1994. Measurements were not made from July 7, 1994 to July 19, 1994 because summer storms disrupted the electricity supply preventing the spore trap from operating.

Melinex impregnated with silicon 2% solution applied using a brush was used as spore-trapping surface. Identification and counting of spores of *Botrytis*, *Uncinula* and *Plasmopara* were done using an Olympus BX50 microscope with a 100X/1.30 oil-immersion lens. Of the sampling surface, 7.71% was examined by sweeping five passes along the length of the slide. The guides used for spore identification were: Grant Smith (1990), Ellis (1971, 1976) and Barnett and Hunter (1972). Spore counts were converted to concentrations and data were expressed as spores/m³.

The correlations between meteorological factors and spore concentration of each type, were examined using Spearman's Correlation Test. Meteorological factors included were: maximum, minimum and average temperatures, humidity and rainfall. The spore data were not transformed because Spearman's test is valid for non-normal distributions.

3. Results

3.1. *Botrytis*

Botrytis cinerea conidia are hyaline or ash-grey, unicellular, ellipsoidal, ovoid, spherical or subspherical in shape, with a protruding hilum, 11–25 µm in diameter with smooth walls. Fig. 2 shows the daily average concentration of *Botrytis* spores for the season. Spores were present during the period and concentrations fluctuated periodically, but rarely exceeded 600 spores/m³. The highest concentrations were found on June 22 and 23, 1994, reaching 1009 and 1004 spores/m³, respectively. There were other peaks with values close to 700 spores/m³. Minimum concentrations were observed at the beginning of August and in the middle of September.

3.2. *Uncinula*

The conidia of this species are ovoid in shape with truncated tips and measure 16–28 µm and have hyaline membrane and granular vacuolated content (Bulit and Lafon, 1978). *Uncinula* were found throughout the studied period (Fig. 3). The highest concentrations

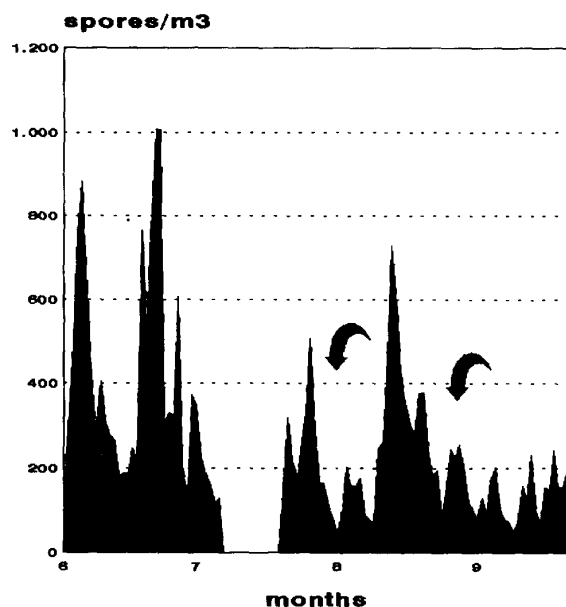


Fig. 2. Concentration of *Botrytis* spores during the sampling period. Fungicide application dates are marked with arrows.

occurred on June 5, 1994 reaching 152 spores/m³. The second highest concentration was 134 spores/m³ (June 12, 1994). Concentration values fluctuated from day to day but tended to decrease gradually, reaching a minimum concentration on August 11, 1994. After that, concentrations were below 30 spores/m³.

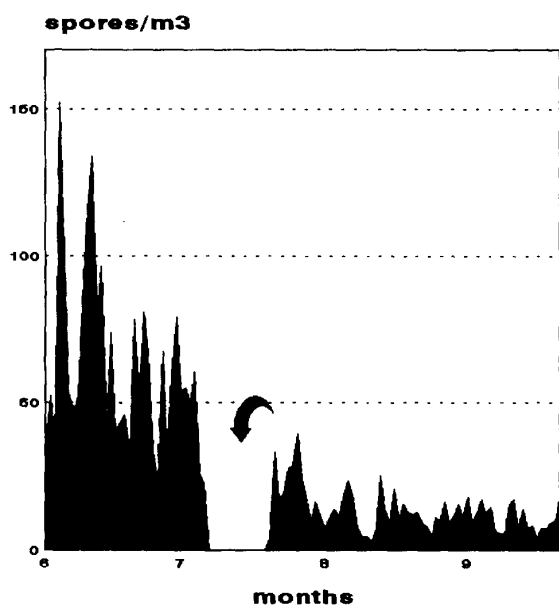


Fig. 3. Concentration of *Uncinula* spores during the sampling period. Fungicide application date is marked with an arrow.

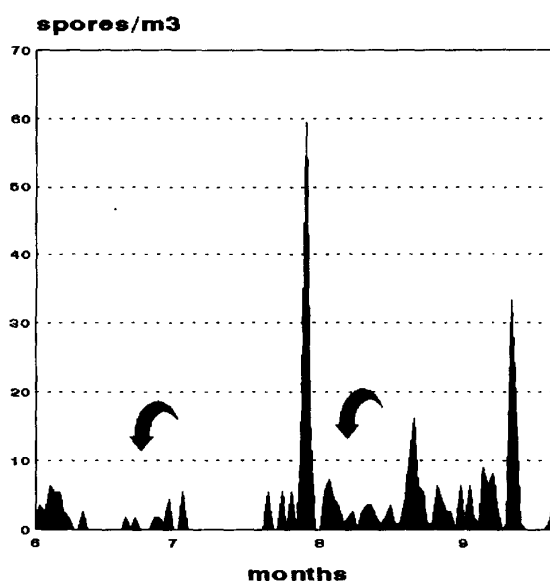


Fig. 4. Concentration of *Plasmopara* spores during the sampling period. Fungicide application dates are marked with arrows.

3.3. *Plasmopara*

Conidia of *Plasmopara* are ovoid tip-stretched, hyaline and measure $16\text{--}40 \times 10\text{--}20 \mu\text{m}$. Conidia were caught only occasionally (Fig. 4); they were absent more frequently during the first weeks of sampling. The highest concentration found was 59 spores/m³ (August 29, 1994), much higher than the values found during the rest of the period, which never exceeded 20 spores/m³. From the middle of September there was a slight increase in spore concentration.

3.4. Relationship between spores and meteorological factors

To examine the relationship between different types of spores and meteorological factors, we applied Spearman's Correlation Analysis (Table 1). In all cases, meteorological parameters were considered as independent variables and spore counts as dependent variables (Comtois and Padilla, 1995). For *Botrytis*, humidity and rainfall showed negative correlations while maximum temperature showed positive correlation, at 99% significance in all three cases. For *Uncinula*, humidity and rainfall were negatively correlated at 99% and 95%. For *Plasmopara*, only minimum and average temperatures showed positive correlation at 99%.

4. Discussion

Of the studied genera, *Botrytis* reached the highest

Table 1
Correlation of spore concentrations with meteorological factors

	Humidity	Rainfall	Max T	Min T	Average T
<i>Botrytis</i>	–0.444**	–0.418**	0.343**	–0.146†	0.186†
<i>Uncinula</i>	–0.386**	–0.261*	0.142†	–0.152†	0.032†
<i>Plasmopara</i>	0.007†	–0.063†	0.145†	0.291**	0.274**

*Significant value, 95%.

**Significant value, 99%.

†Not significant.

concentrations, with values above 150 spores/m³ found frequently during the period. This suggests that disease risk exists for most of the vine growing period, increasing when there is a decrease of humidity combined with an increase in maximum temperatures. For *Uncinula*, the most risky period appears to be June, after that, spore concentrations remain constantly below 50 spores/m³. *Plasmopara* had the lowest concentrations: values occasionally exceeded 10 spores/m³, with 50 spores/m³ exceeded only once.

These results were obtained under crop conditions where fungicides were used which, without doubt, would have altered the reproductive cycles of the fungi. Currently, fungicide applications are based on meteorological conditions and the real disease risk measured by airborne spore counts is never taken into account. The applications of fungicides are marked on the graphs, but they do not coincide with the highest spore concentrations in the air. The reproductive life-cycles and the dispersal mechanisms of fungal inoculum through the atmosphere are related to meteorological factors, but the influence of the different factors varies according to the fungal type under consideration (Palmas and Cosentino, 1990; Rantio-Lehtimäki et al., 1991a,b; Hasnain, 1993).

In this study, the highest correlations were found between the spore concentrations and the selected meteorological parameters (mainly humidity and rainfall). Relative humidity directly affects the spread of cryptogamic diseases through space (López et al., 1992). There have been many studies of the effects of humidity on disease reported in the literature (Hamilton, 1959; Fitt and McCartney, 1986). For *Uncinula*, our results agree with Mansilla et al. (1991).

Interpreting the effects of rainfall is difficult because the period of study included summer time (from June to September) when the precipitation was low. Further years data will be needed to confirm or not the correlations found.

Maximum, minimum and average temperatures can play a very important role in the development of many moulds (López et al., 1992). *Botrytis* correlated positively with maximum temperature, according to Hamilton (1959), who points out that concentrations increase with increasing temperature. *Plasmopara* was positively

correlated with minimum and average temperatures (significance 99%), which agrees with the findings of Mansilla et al. (1991).

In summary, monitoring spore concentrations may give additional information to aid fungicide application decision making when combined with meteorological measurements.

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