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IMPORTANT PHYTOPATHOGENIC AIRBORNE FUNGAL SPORES IN A RURAL AREA: INCIDENCE OF *BOTRYTIS CINEREA* AND *OIDIUM* SPP.

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Abstract: The effects of the climatic changes more and more frequently, favour the emergence and the development of plant diseases. *Botrytis cinerea* and *Oidium* spp. spores are often responsible for enormous productivity losses in cultures with high commercial interests such as the grapevine. This work aims to detect these airborne spores, before the emergence of lesions in *Vitis vinifera*. In the rural area of Amares, the seasonal distribution of the concentration of the 2 spore types, was continuously studied between 1 March–31 October (2005–2007), using a 7-day volumetric Hirst-type spore trap. These data was compared with phytopathological data. *B. cinerea* sporulation occurs in March-April while *Oidium* spp. occurs in April–May. Fluctuations were observed due to the influence of different meteorological factors. The emergence of the first signs of grey mould and powdery mildew were preceded by increments of *B. cinerea* and *Oidium* spp. spore concentration. The precocious detection of increasing trends in airborne spore concentration of *B. cinerea* and *Oidium* spp. can notify the probable onset of grey mould and powdery mildew leading to application of lower quantities of phytopharmaceutical products in the most favourable developmental stage.

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Key words: airborne fungal spores, Entre Douro e Minho, grey mould, phytopathogenic fungal spores, powdery mildew, rural area, *Vitis vinifera*.

INTRODUCTION

Botrytis cinerea spores are the causal agent of the disease commonly known as "grey mold". The fungus is usually referred by the anamorphic form (asexual form) since the telemorph (sexual form, the *Botryotinia cinerea*) is rarely observed.

During the winter months, the fungus survives in its resistant form – sclerotia – or as mycelia that germinate in the spring giving origin to grey ramified conidiophores where hyaline conidia grow. Afterwards, the conidia are disseminated by wind and rain, unchaining new infections. The optimal temperature for the germination is 18ºC while high temperatures stop the development of the fungus, thus, this species can be essentially found in areas with temperate and subtropical climates. The meteorological factors, such as precipitation and relative humidity and, to a significant lesser degree, temperature, also interfere with the infection conditions. Sclerotia and mycelia are formed during autumn and the spores' liberation begins in the following spring, when more intense rains and mild temperatures are registered. The maximum of the sporulation happens from the middle of April to the end of May [1, 4].

B. cinerea is a pathogenic or saprophytic fungus of numerous vegetable species (horticultural, ornamental, fruit trees, extensive cultures), especially annual cultures, that can also attack plantlets of forest species. In Portugal, from the economic point of view, the grapevine constitutes the most prominent host of this fungus, not only for the extension of this culture but also due to the high losses

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induced by the presence of this fungi. In the grapevine, the infection of *Botrytis* affects not only flowers and fruits, but also, any green parts of the plant in any phase of development (steams, young branches and leaves). The fungus removes the water of the berries, resulting in a more intense, concentrated final product, due to a higher concentration of solids (sugars, fruit acids and mineral salts). The presence of this fungus in the grapes renders more complex the fermentation process which is interrupted before the wine accumulates a sufficient level of alcohol due to the presence anti-fungal substances capable of destroying the enzymes. This fact results in high losses to the viticulture industry [1, 4].

B. cinerea spores are able to infect other plants, such as soft fruits (strawberries). To reduce the infection in strawberry plants, good ventilation is recommended to avoid the moisture accumulation between leaves and fruits which is achieved by the elevation of the strawberry plants in relation to the soil, by using straw [2].

Finally, in human health, *B. cinerea* is responsible for the emergence of a disease usually designated as "winegrower's lung", a rare form of hypersensitivity pneumonitis (a respiratory allergic reaction in predisposed subjects). In fact, these fungal spores are frequently present in wine cellars [16]. An aerobiological study performed in the Netherlands showed that *B. cinerea* may be found in considerable numbers both outside and inside the dwellings of asthmatic patients, about 7% of whom presented positive skin tests with this fungus [10]. In fact, in Europe, the prevalence of positive reaction to this fungus is similar to that of *Aspergillus* spp. and greater than *Cladosporium* spp. and *Penicillium* spp. Also, in sera of sensitized patients, the binding of anti-*B. cinerea* IgE and IgG was observed, highlighting the need for further characterization of these allergens [7].

Some of the most important phytopathogenic species belong to the Order Erysiphales, commonly obligate parasites, being responsible for the diseases known as powdery mildews, one of the most widespread and easily recognized plant diseases. They affect virtually all kinds of plants: cereals and grasses, vegetables, ornamental plants, weeds, shrubs, fruit trees, broad-leaved shade trees and forest trees [1, 4].

The members of this order present a superficial mycelium which extracts nourishment from the host plant through specialized hyphae that penetrate the epidermal cells of the host by means of absorbing organs as haustoria. In the minute cleistothecia (0.1 mm in diameter), the asci are arranged in a hymenial layer, resembling a perithecia. The number of asci per ascoma varies, constituting an important discriminating criteria between genera [1, 4].

Infection of the host plant starts with the germination of the ascospore (sexual propagation) or of the conidia (asexual propagation) in the epidermal cells of the stem or leaves, creating a septate mycelium of uninucleate cells. The external mycelium produces short and erect conidiophores, bearing a single row of barrel-shaped spores, the youngest being at the base (the affected parts thus becomes covered with abundant conidiophores, assuming a white powdery appearance). The ripe spores are separate and quickly dispersed by the wind, provoking new infections. During the autumn, sexual cleistothecia, containing the latent stage of the pathogen, are produced. The ascospores remain dormant all winter. During spring, in cloudy mornings of high relative humidity (superior to 25%), followed by sunny afternoons with temperatures higher than 25ºC, the cleistothecia germinate and the ascospores liberation occurs. After transportation, the ascospores are deposited on the green organs of the grapevine, under favourable climatic conditions, provoking the primary contaminations [1, 4].

Table 1. Scientific classification of *B. cinerea* and *Oidium* spp.

Kingdom	Fungi	Fungi
Phylum	Ascomycota	Ascomycota
Subphylum	Pezizomycotina	Pezizomycotina
Class	Leotiomycetes	Leotiomycetes
Order	Helotiales	Erysiphales
Family	Sclerotiniaceae	Erysiphaceae
Genus	*Botryotinia*	*Oidium*
Species	*B. cinerea*	

The grapevine is highly susceptive to this disease from the budding to the bunch closing/bunch ripening. It can attack leaves, bunches and branches. The early manifestation is the presence of a powdery and thick mycelium of white yellowish color on the green organs of the grapevine. The attacked leaves present a twitched aspect and they bend in leak. In the bunches, the attacks are more severe: the bunches are covered by a thick mycelia in which are formed the visible cleistothecia, its skin stops growing and the berries can break open and even suberize, exposing the seeds, and constituting an open door for the grey mould attacks. In the pruning season, the vine-twigs present a white color with typical brown spots. At the end of the grapevine cycle it is possible to notice cleistothecia in the leaves, bunches and the vine-twigs. As preventive measures, the removal of the primary focus of infection is recommended during the pruning season, accomplished by treatment with phytopharmaceutical products in officially ratified conditions. These products should be used together with the correct technology to protect the bunches from the flowering to the bunch closing [1, 4].

Some other species of medical importance previously classified as *Oidium* include *Trichosporon cutaneum* (formerly *Oidium cutaneum*), responsible for some human fungal diseases, and *Oidium pulmoneum* and *Geotrichum candidum* (formerly *Oidium lactis*), present in soil, water and air, but also belongs to normal human flora.

For more than 25 years, the *Direcção Regional de Agricultura e Pescas do Norte – Divisão de Protecção e Controle Fitossanitário* has been developing a warning system in order to assist farmers from the northwest region of Portugal in the prevention of diseases of different cultures, based on the observation of the first signs of pathology.

This work aims to detect the presence of *B. cinerea* and *Oidium* spp. spores in the atmosphere, before the emergence of lesions in *Vitis vinifera* in order to avoid decreases in productivity, and to reduce the amount of applied fungicides, thereby resulting in the better execution of good agricultural practices (Tab. 1).

MATERIAL AND METHODS

Spore trap location. The air sampler was located on the roof of a farm in the rural area of Amares (41º38'N 8º23'W). The sampler is surrounded by kiwi orchards, orange and lemon trees, grapevines, greenhouses of seasonal crops, pine and *Eucalyptus* trees. This area, located close to the city of Braga, presents a temperate climate with an Atlantic influence, an annual level of precipitation of 1,515 mm and a medium temperature of 14.0ºC.

Daily spore counts. Daily spore concentrations were sampled between 1 March–31 October (2005–2007) using a Hirst-type 7-day volumetric trap (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, UK) with a flow rate of 10 liters per minute. Spores were trapped onto a melinex adhesive tape which was cut into daily segments. The slides, with adhesive segments, were covered with fucsin-stained glycerol jelly and a cover glass. The daily mean concentration of the number of fungal spores was carried out using an optical microscope at ×400 magnification along 2 full lengthwise traverses. Spore counts were then converted to spores per cubic meter of air sampled per day.

To verify the degree of association among the atmospheric spore concentration and the meteorological data (temperature, relative humidity and rainfall), the Pearson correlation coefficient was employed with the significance levels of 1 and 5%.

Warning system. Three times a week (every Monday, Wednesday and Friday), a technician from *Direcção Regional de Agricultura e Pescas do Norte – Divisão de Protecção e Controle Fitossanitário* went to the area and evaluated the vineyards in the study (experimental field) in terms of percentage of infected leaves or bunches, and percentage of infected area per leaves or bunches. These results were matched with meteorological data, resulting in the publication of a new warning journal when a high probability of secondary infections was predicted.

The warning journals are usually sent, by post or e-mail to subscribers on order. The aim of these journals is to advise about the application (dates and quantities) and handling of the phytopharmaceutical products in several cultures.

Table 2. Distribution of main spores types present in the atmosphere of Amares during the studied period (March–October, 2005 to 2007; in percentage).

	2005	2006	2007
Agaricus	1.6	6.3	2.6
Alternaria	1.7	1.5	1.6
Aspergillaceae	1.4	1.3	0.9
Botrytis	1.0	0.7	1.1
Cladosporium	88.8	83.5	89.8
Oidium	0.1	0.1	0.1
Rusts	0.6	1.2	0.6
Smuts	1.0	1.0	0.7
Others	3.8	4.4	2.5

RESULTS

Airspora components. In the rural atmosphere of Amares, from 1 March–31 October (2005–2007), 42 types of fungal spores were identified. Among these, *Cladosporium* spp. was the most abundant spore type; nevertheless *Alternaria* spp., *Aspergillus* spp., *Penicillium* spp., *Botrytis cinerea*, *Oidium* spp., rusts and smuts also stand out for their pathogenic roles as of causal agents plant disease (Tab. 2).

B. cinerea spores were present during almost the entire sampled period (89% of the days), constituting around 1% of the fungal spore spectrum (Tab. 2). The total of spores of this species ranged between 1,375–2,270 spores/m^3 of air, the highest value was registered in 2007 and the lowest value in 2006 (Tab. 3). In the first 2 years, the maximum values of spores were observed in March and April; in 2006, high concentration of *B. cinerea* spores was also found in October. In the latter year, a delay until July and August occurred (Fig. 1 and 2). The symptoms of grey mould in grapevines in the northwest region of

Table 3. Distribution of *B. cinerea* and *Oidium* spp. spores in the atmosphere of Amares (2005–2007).

	Botrytis cinerea				*Oidium* spp.			
	2005	2006	2007	Average	2005	2006	2007	Average
Total (spores/m^3)	2,086	1,375	2,270	1,910	53	138	138	110
Maximum (spores/m^3)	64	45	79	63	7	18	14	13
Date	24-Mar	26-Mar	18-Jun	22-Apr	6-Jun	29-May	25-Jun	9-Jun
Days of occurrence	225	213	215	218	30	73	73	59
Days of occurrence (%)	92	87	88	89	12	30	30	24

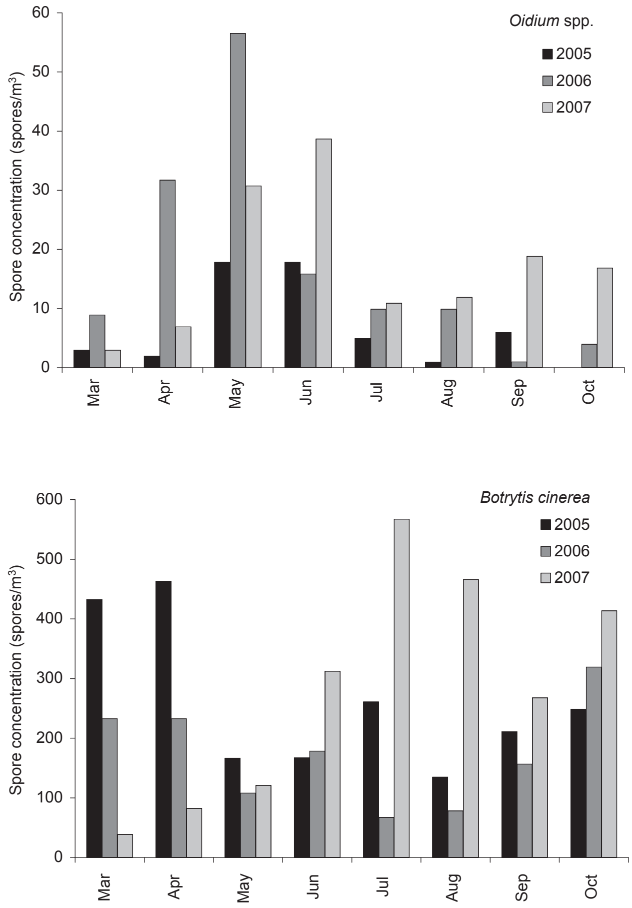

Figure 1. Total monthly concentration of *B. cinerea* and *Oidium* spp. spores in the atmosphere of Amares (2005–2007).

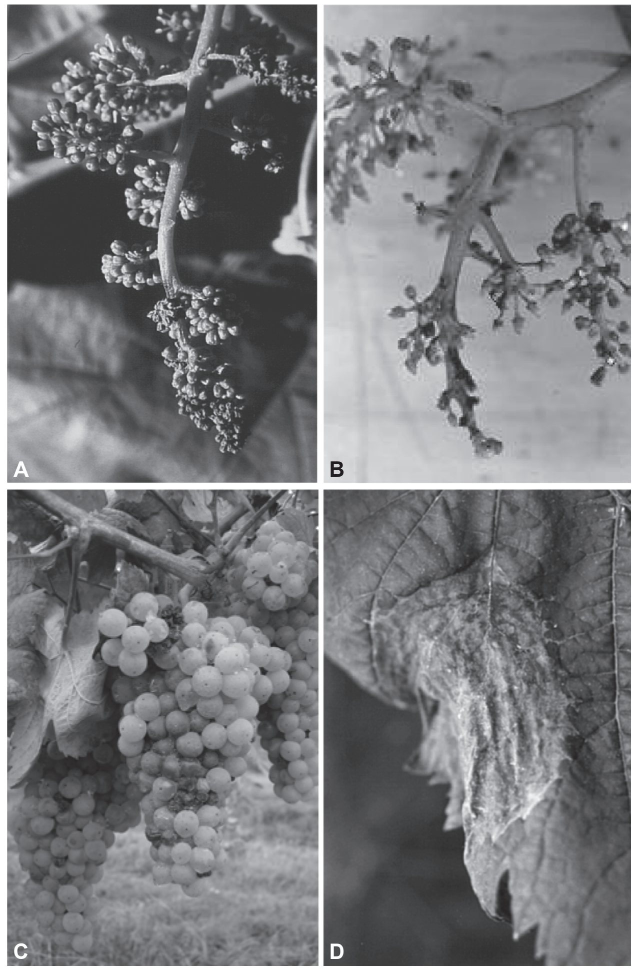

Figure 2. Grapevine at several phenological phases attacked by grey mould: A: inflorescence before pollination; B: inflorescence during pollination; C: grapes; D: leaf.

Portugal were reported from May to the end of October (Fig. 3 and Tab. 4).

Although present in nearly 24% of the days, *Oidium* spp. spores were much less abundant, constituting only 0.1% of the fungal spore spectrum in the same period (Tab. 2). The total of spores of this species oscillated between 53–138 spores/m^3 of air, the highest values being registered in 2006, while the lowest value was observed in 2005 (Tab. 3). The peak concentration occurred in May (2005 and 2006) and June (2007) (Fig. 1). The symptoms of powdery mildew in grapevines in Amares region were reported from April to the end of October (Tab. 4).

When meteorological data was concerned, the temperatures increased from March to June, with the following three months being the warmest, and in October a decline of thermal parameters was registered. The relative humidity was rather constant during this period, the drier summer months being characterized by the absence of rain (Fig. 4 and Tab. 5).

In 2005 and 2006, *B. cinerea* spore concentration was positively correlated with mean relative humidity and total rainfall. Moreover, a negative correlation with mean temperature was also registered in 2006. In 2007, an inversion

Table 4. Reports of grey mould and powdery mildew in grapevines from the Entre Douro e Minho region (2005–2007).

Year	Disease	March	April	May	June	July	August	September	October
2005	Grey mould								
	Powdery mildew								
2006	Grey mould								
	Powdery mildew								
2007	Grey mould								
	Powdery mildew								

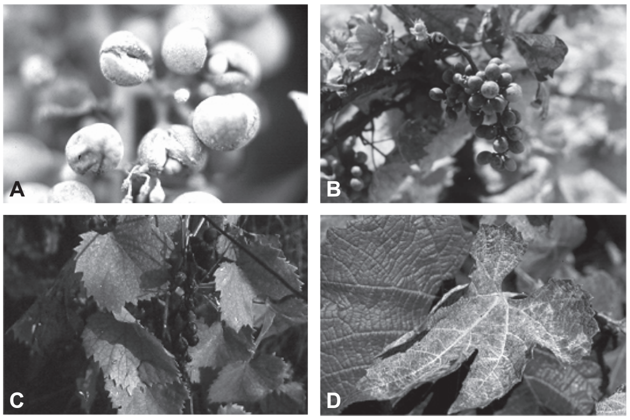

Figure 3. Grapevine at several phenological phases attacked by powdery mildew. A–C: grapes at different developmental stages; D: leaves.

in the correlations was observed. Thus, positive correlations with mean temperature and negative correlations with mean relative humidity and total rainfall were obtained. In general terms, this fungal type presented significative positive correlations with mean relative humidity and total rainfall (Tab. 6).

In 2005, *Oidium* spp. presented significative positive correlations with mean temperature and, in the following years, negative correlations with mean relative humidity. In general terms, this fungal type presented significative positive correlations with mean temperature, and significant negative correlations were obtained with relative humidity and total rainfall (Tab. 6).

Table 5. Resumé of main meteorological factors (2005–2007).

	2005	2006	2007
T_{mean} (ºC)	18.6	19.2	18.0
Rh_{mean} (%)	74.8	79.7	78.9
Rainfall (mm)	639.8	1292.4	382.8

Warning system. From February–October 2005, a total of 14 warming journals were published. The first recommendations about the preventive application of phytopharmaceutical for powdery mildew dated from 13 April. The first signs of grey mould attacks were observed on 27 April, 7 and 29 June, and finally on the 20 October. Powdery mold attacks were detected on 10 July, 9 August and 18 October. Powdery mould symptoms were detected on the 20 May, 7, 22 and 29 June.

Table 6. Correlation between *B. cinerea* and *Oidium* spp. spores and meteorological factors (2005–2007).

			T_{mean}	Rh_{mean}	Rainfall
2005	*Botrytis*	Correlation Coefficient	-0.113	0.144*	0.241**
		Sig. (2-tailed)	0.077	0.024	0.000
		N	245	245	245
	Oidium	Correlation Coefficient	0.152*	-0.124	-0.104
		Sig. (2-tailed)	0.017	0.053	0.103
		N	245	245	245
2006	*Botrytis*	Correlation Coefficient	-0.318**	0.380**	0.355**
		Sig. (2-tailed)	0.000	0.000	0.000
		N	245	245	245
	Oidium	Correlation Coefficient	0.016	-0.168**	-0.104
		Sig. (2-tailed)	0.806	0.008	0.103
		N	245	245	245
2007	*Botrytis*	Correlation Coefficient	0.442**	-0.054	-0.036
		Sig. (2-tailed)	0.000	0.400	0.579
		N	245	245	245
	Oidium	Correlation Coefficient	0.115	-.134*	-0.075
		Sig. (2-tailed)	0.073	0.036	0.244
		N	245	245	245
2005–2007	*Botrytis*	Correlation Coefficient	-0.007	0.143**	0.171**
		Sig. (2-tailed)	0.854	0.000	0.000
		N	735	735	735
	Oidium	Correlation Coefficient	0.080*	-0.122**	-0.087*
		Sig. (2-tailed)	0.030	0.001	0.019
		N	735	735	735

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

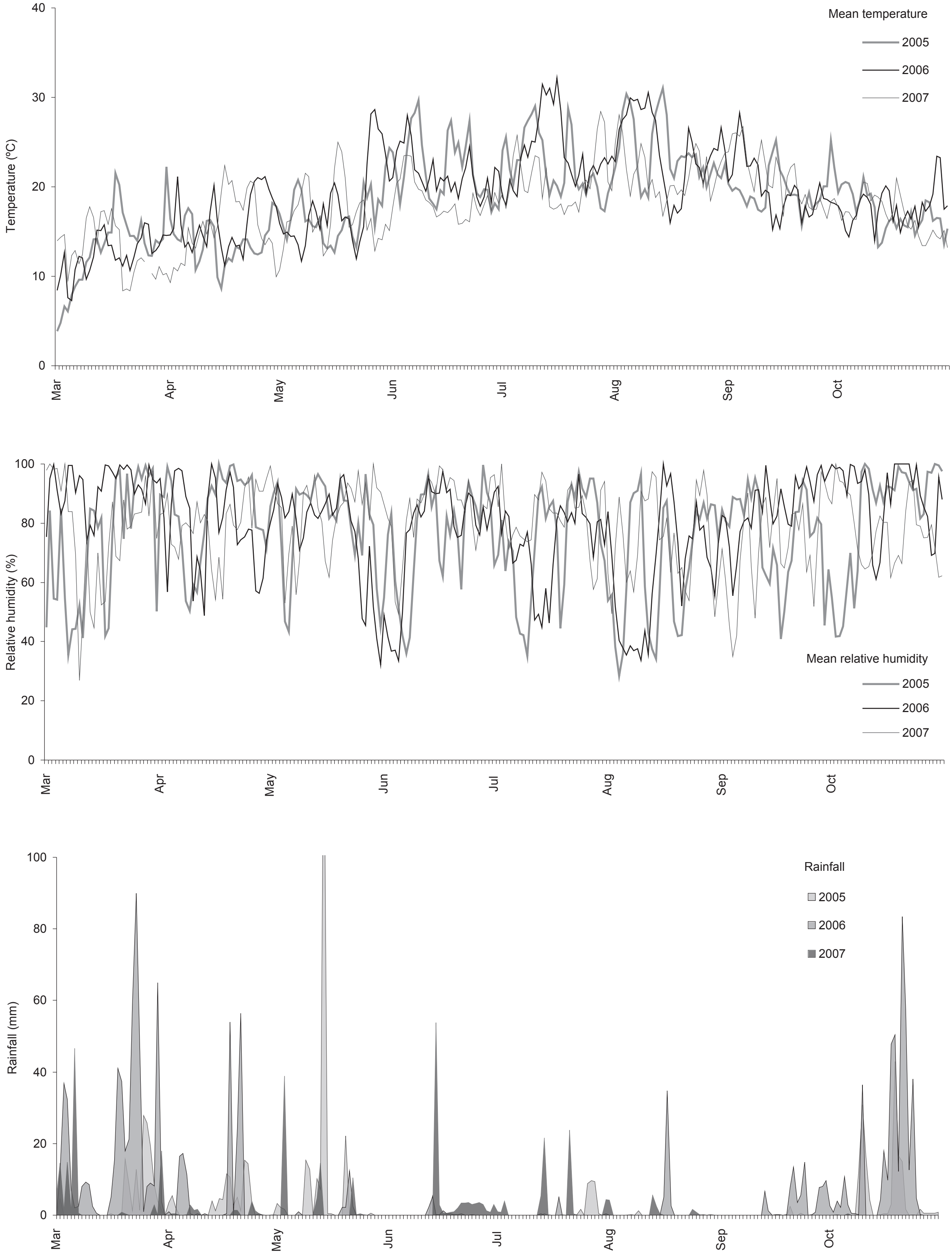

Figure 4. Meteorological conditions registered in Amares.

In 2006, in the studied period, a total of 13 warming journals were published. The first recommendations about the preventive application of phytopharmaceutical for powdery mildew dated from 21 April. The first signs of grey mould attacks were observed on the 8, 25 and 31 May, on 9 and 26 June, on 9 August, and finally on the 18 October. Powdery mould attacks were detected on 10 July, 9 August and 18 October.

In 2007, from February–October, a total of 17 warming Journals were published. The first recommendations about the preventive application of phytopharmaceutical for powdery mildew was dated 10 May, while the application of these products to prevent grey mould attacks was only published on 19 June. Signs of grey mould and powdery mould attacks were detected on 22 May, 11 July (strongest attack) and 8 August.

DISCUSION

In the atmosphere of Amares, the spores of *B. cinerea*, constituting around 1% of the fungal spore spectrum, oscillated between 1,375–2,270 spores/m^3 of air. These values were significantly lower than those found in Valdeorras, a rural area near Ourense, where values of nearly 12,500 spores/m^3 of air were described [15]. Also, in Leiden, a semi-urban area, values of nearly 32,000 spores/m^3 of air were found [11]. An aerobiological study performed in the atmosphere of Porto, an urban area, during 2003 and 2004 refers to an average of 1,500 spores/m^3 of air of *B. cinerea* [14]. The differences between spore concentration in rural and urban areas has already been described elsewhere, and the average concentrations of *B. cinerea*, an important plant pathogen, was significantly higher in the rural environment [9].

The maximum values of *B. cinerea* spores were registered from March–June. The same annual distribution has been described previously [9, 11, 12, 13], while other works refer to later sporulation seasons [8, 14, 17]. The differences found in the distribution of the spores of *B. cinerea*, among the studied years, can be due to the different conditions climatic registered. Thus, in 2005 and 2006, the low temperatures observed in January and February were followed by an increase of the temperature of the air, registering values significantly higher than the average values of the reference period (1961–1990). When rainfall is concerned, the winter of 2004–2005 was the driest for the last 75 years and the spring of 2005 the fourth driest in the same period. These meteorological conditions favoured the sporulation peak in March and April, when a progressive increase of the temperature and of the precipitation was registered. In the winter of 2006–2007 and spring of 2007, the medium temperatures were similar to those of the reference period, but higher rainfall levels were observed. The medium temperature decreased, delaying until May and June.

In general, significant positive correlations were observed between the daily concentrations of the spores of *Botrytis* and all meteorological factors analyzed (temperature, relative humidity and rainfall). In fact, the highest concentrations occurred during spring and the beginning of summer, when a progressive increase of the temperatures accompanied of sporadic rains were registered, contributing to the increase of the relative humidity. The importance of these meteorological factors for the development of grey mould has been demonstrated in previous works, in that an increase of the number of spores of this fungus was verified on hot days with rain periods [6, 17]. The increase in the size of the lesions of grey mould has also been related with high temperatures and relative humidity [5].

Once demonstrated, the influence of the meteorological factors in the development of the fungus and in the propagation of infection, the differences found among the different sporulation periods can be due to the different temperature and humidity conditions in the places where the studies were conducted. Also, the phenological phases of the different cultures that surround the sampler and the organic matter supply can change the beginning and end dates of the sporulation period.

B. cinerea spore concentrations showed significant positive correlations with mean relative humidity and rainfall, and significant negative correlations with mean temperature. In fact, unlike other diseases provoked by fungus (powdery mildew, rusts, etc.), the spores of this species proliferate in the climates with a relative humidity of between 70–80%, which occurs frequently in May. Another important factor is the thermal amplitude between diurnal and night temperatures.

Contrarily to the previous species, *Oidium* spp. spore concentrations showed significant positive correlations with mean temperature and significant negative correlations with mean relative humidity and rainfall. The year 2007 was particularly dry, which can explain the different correlations found during 2005–2006 and 2007.

In 2005, signs of grey mould attacks were observed on 27 April, 7 and 29 June and finally on the 20 October. The first signs of this disease were preceded by a *B. cinerea* spore concentration peak of 41 spores/m^3 (22 April, 5 days before the attack), prior to this, continuous sporulation was registered since 17 March with a daily average of 20 spores/m^3. Before the second attack, another peak of 22 spores/m^3 (12 May, 25 days before the attack) was observed. Previous to the third attack, two peaks of 15 and 14 spores/m^3 (16 June, 13 days before the attack; 19 June, 10 days before the attack), and a daily average of 6 spores/m^3 were registered. Finally, on 25 September and on the 12 October, a *Botrytis* spore peak anticipated the attack in the vineyards (21 spores/m^3, 25 days before the attack; 30 spores/m^3, 8 days before the attack). Powdery mould attacks were detected on 10 July, 9 August and 18 October. Powdery mould symptoms were detected on 20 May, 7, 22 and 29 June. The first signs of this disease were preceded by a *Oidium* spore concentration peak of 6 spores/m^3 (4 May, 16 days before the attack). Before the second attack, 3 peaks of 4 and 3 spores/m^3 (23 May, 15 days before the attack; 28 May, 11 days before the attack; 29 May, 11 days before the attack) were observed; and previous to the third attack, a value of 2 spores/m^3 (12 June, 10 days before the attack).

In 2006, the first recommendations about the preventive application of phytopharmaceutical for powdery mildew dated from 21 April. Signs of grey mould attacks were observed on the 10 July, 9 August and 18 October. The signs of the first attack were preceded by two peaks in *Botrytis* spore concentration of 22 and 14 spores/m^3 (16 June, 24 days before the attack; 29 June, 11 days before the attack) followed by several days of continuous sporulation with an average of 7 spores/m^3. Before the second attack, 2 peaks of 8 and 6 spores/m^3 (1 August, 8 days before the attack; 2 August, 7 days before the attack) were observed. Previous to the third attack, a peak of 40 spores/m^3 (6 October, 12 days before the attack) was registered.

Powdery mildew attacks were detected on 10, 8, 25 and 31 May, on 9 and 26 June, on 9 August, and finally on 18 October. The higher concentrations of *Oidium* spores were detect on 29 April (4 spores/m^3, 9 days before the attack), 19 (7 spores/m^3, 6 days before the attack) and 29 (18 spores/m^3, 11 days before the attack).

In 2007, signs of grey mould and powdery mould were detected on 22 May, 11 July (strongest attack) and 8 August. Comparing these dates with the spore concentration results it was interesting notice that since 2 May *B. cinerea* spores were continuously present in the atmosphere of Amares and *Oidium* spore concentration presented small peaks, on 9, 10, 19 and 20 May.

The strongest attacks were registered on 11 July. Prior to this attack, peaks of *Botrytis* spores were registered on 18 June (79 spores/m^3; 23 days before the attack) and on 5 July (65 spores/m^3; 6 days before the attack), being the average daily spores concentration of 24 spores/m^3 during this period. When powdery mildew was concerned, an *Oidium* spp. spore peak was detected on 25 June (14 spores/m^3; 17 days before the attack). Finally, the attacks of 8 August was preceded by a *Botrytis* spores peak on 14 July (31 spores/m^3; 25 days before the attack), followed by several days of continuous sporulation with an average of 11 spores/m^3.

As preventive measures for the mildew control in *V. vinifera*, despite intensive use of fungicides, low disease control has sometimes been achieved in the field due to a misalignment between fruit susceptibility and timing of fungicide applications [3]. With additional years of air spores, meteorological and culture monitoring, further works should be conducted to development a predictive model in order to improve the warming system and decrease the high productivity losses observed in this culture.

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