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The effect of temperature and relative humidity on the airbone concentration of *Pyricularia oryzae* spores and the development of rice blast in southern Spain

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Abstract

The rice (*Oryza sativa* L.) cultivars Puntal and Thaibonnet were used in a field trial designed to investigate how temperature and relative humidity affect the airborne concentration of *Pyricularia oryzae* spores and the development of rice blast. A relative humidity of 95% and an average temperature of 26-27°C were optimum for infection and substantially favoured spore release. The first symptoms of infection were detected on the leaves when the crop was at the mid-tillering stage, the number of lesions increasing as the plant developed. After the onset of heading the number of leaf lesions decreased while the number of node and panicle base lesions increased. A temperature increase of 1°C in early August (mid-tillering stage) led to an increase in the mean intensity of disease. The presence of spores in the air from July 15th predicted the appearance of leaf lesions a few days later. The peak spore concentration in August could be used to forecast panicle blast. Assessing the airborne concentration of *P. oryzae* could help in understanding the population dynamics of this pathogen.

Additional key words: AUDPC, epidemiology, phytopathogenic fungus, pore content, *Pyricularia oryzae,* relative humidity, temperature.

Resumen

Evolución de la seca de arroz en el suroeste de España afectada por el estado de crecimiento del cultivo, condiciones ambientales y contenido de esporas en el aire

Los cultivares de arroz Puntal y Thaibonnet fueron sembrados en campo para analizar el efecto de la temperatura y la humedad relativa media sobre el contenido de esporas de *Pyricularia oryzae* en el aire y el progreso de la enfermedad. Valores de humedad relativa del 95% y temperatura media entre 26-27°C propician condiciones óptimas para la infección del patógeno y favorecen sustancialmente la expulsión de esporas. Los primeros síntomas aparecieron sobre hojas, cuando el cultivo se encontraba en estado de ahijado medio. La cantidad de lesiones infecciosas en hoja incrementó conforme la planta aumentaba su desarrollo. Después del inicio de espigado estos valores decrecieron, mientras que los valores de infección en nudo y base de la panícula se incrementaron paulatinamente. Un incremento de temperatura de 1°C a principios de agosto (estado de ahijado medio) causó un incremento de la intensidad media de la enfermedad. La presencia de esporas en el aire desde el 15 de julio ayuda a predecir la aparición de manchas en hojas varios días más tarde. El pico de esporas registrado en agosto puede ser usado para predecir la infección en panícula. La evaluación del contenido de las esporas de *P. oryzae* en el aire ayuda a comprender la dinámica de la población del patógeno.

Palabras claves adicionales: área bajo la curva de progreso de la enfermedad, contenido de esporas, epidemiología, hongos fitopatógenos, humedad relativa, *Pyricularia oryzae*, temperatura.

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Introduction[1]

The EU has about 450,000 ha devoted to rice (*Oryza sativa* L.) production, of which 111,000 ha are located in Spain. In Andalusia (southern Spain), the rice-growing area is mainly located between Seville and the mouth of the River Guadalquivir (*Marismas del Guadalquivir*). The entire area has a typical Mediterranean climate, which is suitable for rice cultivation, and is flat with clayey, saline soils of sedimentary origin. Some 38,000 ha of this area are devoted to rice, making it Spain's most important rice-growing area; indeed, some 40% of the country's crop is grown here. Andalusia is in fact home to half of all the area in the EU given over to long grain rice production. The cultivar Doongara (known as Puntal in Spain), from Australia, makes up about 75% of all the rice grown in the *Marismas*; L-202 from California (known as Thaibonnet in Spain) makes up a further 15%, and short grain cultivars make up about 10%. Both Puntal and Thaibonnet belong to the Indica (long grain) group, have an early growth cycle, and are adapted to the Mediterranean climate (Aguilar Portero, 2001). Both are of great agronomic value, provide high yields and show medium susceptibility (level 20-30) to *Pyricularia oryzae* (Castejón-Muñoz *et al.*, 2007).

Rice blast, caused by the fungus *Magnaporthe grisea* (Herbert) Barr [anamorph, *Pyricularia grisea* (Cooke) Sacc, usually known in rice as *P. oryzae* Cav.] (Rossman *et al.,* 1990), is the most important of all rice diseases, a consequence of its wide distribution (Anonymous, 1968; Pans, 1976) and the damage it causes (Ou, 1972, 1980). Its great destructive power can cause serious yield losses (Padmanabhan, 1965) and its control is a major goal of rice farmers in temperate areas prone to high levels of relative humidity. It can damage any aerial organ of rice plants, although the leaves and panicles are usually the most affected. Leaf infection reduces the photosynthetic area of the plant and may even lead to its death. However, panicle infection —which causes yield losses— inflicts the greatest economic injury (Roumen, 1992).

The presence of the disease in Spain was first mentioned in 1968 (Anonymous, 1968; Benlloch, 1975), but not until 1978 was it reported to affect the paddies of the *Marismas* (Marín-Sánchez and Jiménez-Díaz, 1981). In 1979, Marín-Sánchez reported *P. oryzae* infections throughout the area, with a mean incidence of over 90%. These infections affected all plant organs and caused serious yield losses when the climatic conditions where favourable to the pathogen. By 1997, rice blast seriously affected all the cultivars used in the *Marismas*, with yield losses reaching 15% – an economic loss of some €10,000,000 (information provided by rice growers).

The climate has a strong influence on the appearance of blast epidemics (Suzuki, 1975), as does the susceptibility of the different varieties planted and the crop management practices employed (such as the application of nitrogen fertilizer). The weather is clearly an important factor in the variability of disease development; certainly, when there are no fluctuations in relative humidity and temperature there appears to be no modification to the number of disease (Asai *et al*., 1966). Certainly, the risk of blast epidemics is no small concern for the future of global rice production (Luo *et al*., 1977, 1998).

The aim of the present study was to determine how temperature and relative humidity affect the airborne concentration of *P. oryzae* spores and the development of rice blast in southern Spain.

Material and Methods

Field trials were undertaken at the Sartenejales (La Puebla del Río, Sevilla) and Casudis (Los Palacios, Sevilla) estates during the 2002 and 2003 growth seasons. The Sartenejales estate (37° 22' N, 6° 10' W) occupies some 850 ha while the Casudis estate (37° 20' N, 6° 13' W) occupies some 2,400 ha; both estates are at an altitude of just 3 m above sea level. The tillage and agronomic practices used in these trials were those that favoured *P. oryzae* infection: late seeding date, high plant density and high N-fertilisation rate). This allowed disease intensity (see Teng and James, 2002) to be studied.

The cultivars Puntal and Thaibonnet were seeded in independent seedbeds between the 4th and 10th of June. Seedlings were transplanted into experimental plots 30 days later. Each plot consisted of 12 furrows (6 m long) spaced 25 cm apart; the distance between plants was 20 cm. Four additional furrows were included with 'Baixet' (a cultivar highly susceptible to *P. oryzae* in the conditions of the *Marismas*) and two more with 'Maratelli', which is also very susceptible to this pathogen (Roumen *et al*., 1997). The trial was surrounded by a border of

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[1] Abbreviations used: AUDPC (area under the disease progress curve), RPA (rice-polish agar).

five furrows containing Baixet; these plants, together with this cultivar's representatives in the plots, acted as an inoculum source (the Maratelli plants did not seem to be so susceptible under the conditions of the *Marisma*). The experiment had a random block design with three replicates.

Fertiliser was applied on two occasions: on May 10th, when 100 kg ha^{-1} of nitrogen (46% urea = 115 U N_2) were machine-applied before seeding, and on August 1st, when granulated ammonium sulphate (21%) was hand-applied to complete the 250 UN ha^{-1} required.

The airborne concentration of *P. oryzae* spores was measured using collectors with two adhesive traps consisting of microscope slides covered with glycerine (Ono, 1963) placed 35 and 70 cm above the ground. The total area of the glycerine sampling surface was 56.25 cm^2 (three slides at each height). Spore counts were first performed two days after transplanting, and then once every two days. The weather conditions were recorded using digital thermohygrographers (one per experimental plot) placed 90 cm over the ground. These continuously recorded the absolute humidity, relative humidity, and maximum and minimum temperatures. Both the thermohygrographers and the spore traps were placed at the centre of the experimental plots.

Since in the study area the first signs of infection usually appear around the middle of July, samples of 20 plants per cultivar and replication were taken for examination once every 10 days from the 15th of July onward. Once in the lab, small pieces of affected tissues (leaves, nodes, panicle bases and ligules, depending on the phenological stage of the plant at the moment of sampling), were surface disinfested in a 1:9 (v:v) solution of household bleach (5.25% sodium hypochlorite). Disinfested pieces of the affected tissues were then placed on damp filter paper in a humid environment chamber and incubated at 25 ± 1°C with 12 h alternating periods of fluorescent LUXLINE Plus and Gro-Lux light of about 2,500 lux for two days. Spores of *P. oryzae* from sporulating spots on these samples were aseptically streaked onto a thin layer of rice-polish agar (RPA) (Tuite, 1969) in Petri dishes. The growth and sporulation of the pathogen was determined by incubating these dishes for seven days under the same conditions as above. Finally, the percentage of infectious lesions was calculated with respect to the total number of lesions (60) sampled from each part of the rice plant (one plant has an average of 15 tillers) at each sampling date. The node zone included the ligule as well as the node, while the panicle zone included the panicle neck, the rachis, pedicels and grains. Disease progress was evaluated by infectious lesion production, and represented as the percentage of infectious lesions per affected plant part (see Figs. 3 and 4 below).

Disease intensity was recorded in terms of the area under the polygonal epidemic curve [area under the disease progress curve (AUDPC)] since no adjustments were made to the experimental data. The AUDPC was calculated by accumulating the daily disease index (percentage of infectious lesions) for the whole growing season using the formula:

$$\text{AUDPC} = \sum_{i=1}^{n-1} [(x_i + x_{i+1}) / 2]\ (t_{i+1} - t_i)$$

in which x_i is the percentage of infectious lesions, n is the total number of observations, and t_i the date. This provides information regarding the dynamics of disease progress that can be used as a measure of disease intensity. The term «disease intensity» is often used to describe the incidence (percentage of infected plants) or severity (area of plant tissues affected) of disease.

Results

The first symptoms of disease appeared on the leaves when the crop was in the mid-tillering stage. Since in previous work the incidence and severity of disease were found to be very similar in the Puntal and Thaibonnet cultivars (51.98% and 48.58% and 21.10 and 20.75 respectively) at the end of the crop cycle (Castejón-Muñoz *et al*., 2007), means were calculated for the two cultivars as whole.

Airborne spore concentration

Pyricularia oryzae spores were trapped from the middle of July until the end of the season. Figures 1 and 2 show the mean temperature, relative humidity and mean numbers of spores trapped per cm^2 for the 2002 and 2003 seasons respectively. Both figures record two increases in relative humidity over the crop cycle due to cold, damp, southwesterly winds coming off the Atlantic. Coupled with the fall in temperature in mid August, this allowed the appearance of dew and fog, which provided optimum conditions for infection. Since the first leaf symptoms appeared at the end of July, when the crop was in the mid-tillering stage, the

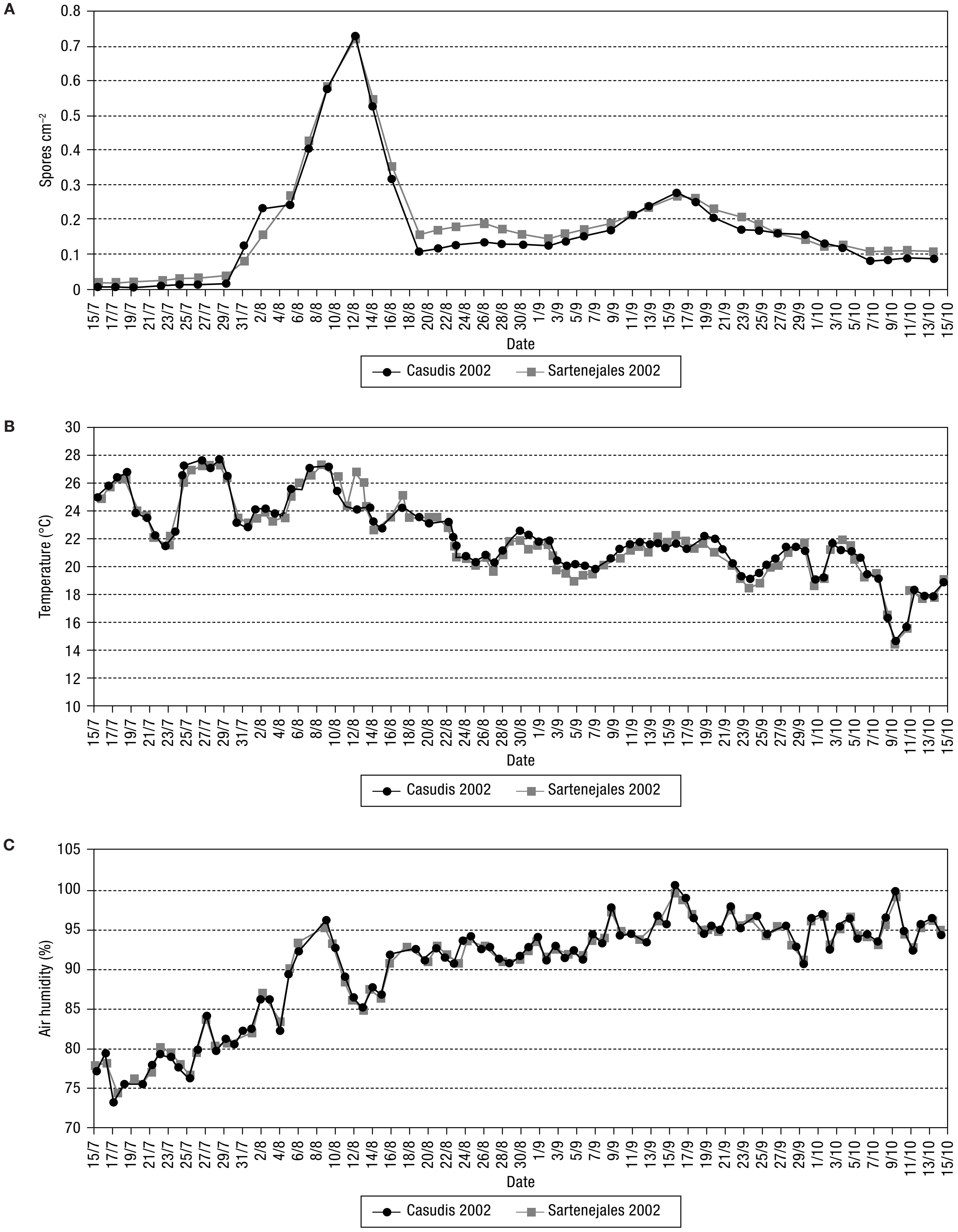

Figure 1. Change in the number of spores cm⁻² (A) with mean temperature (B) and relative humidity (C). Year 2002.

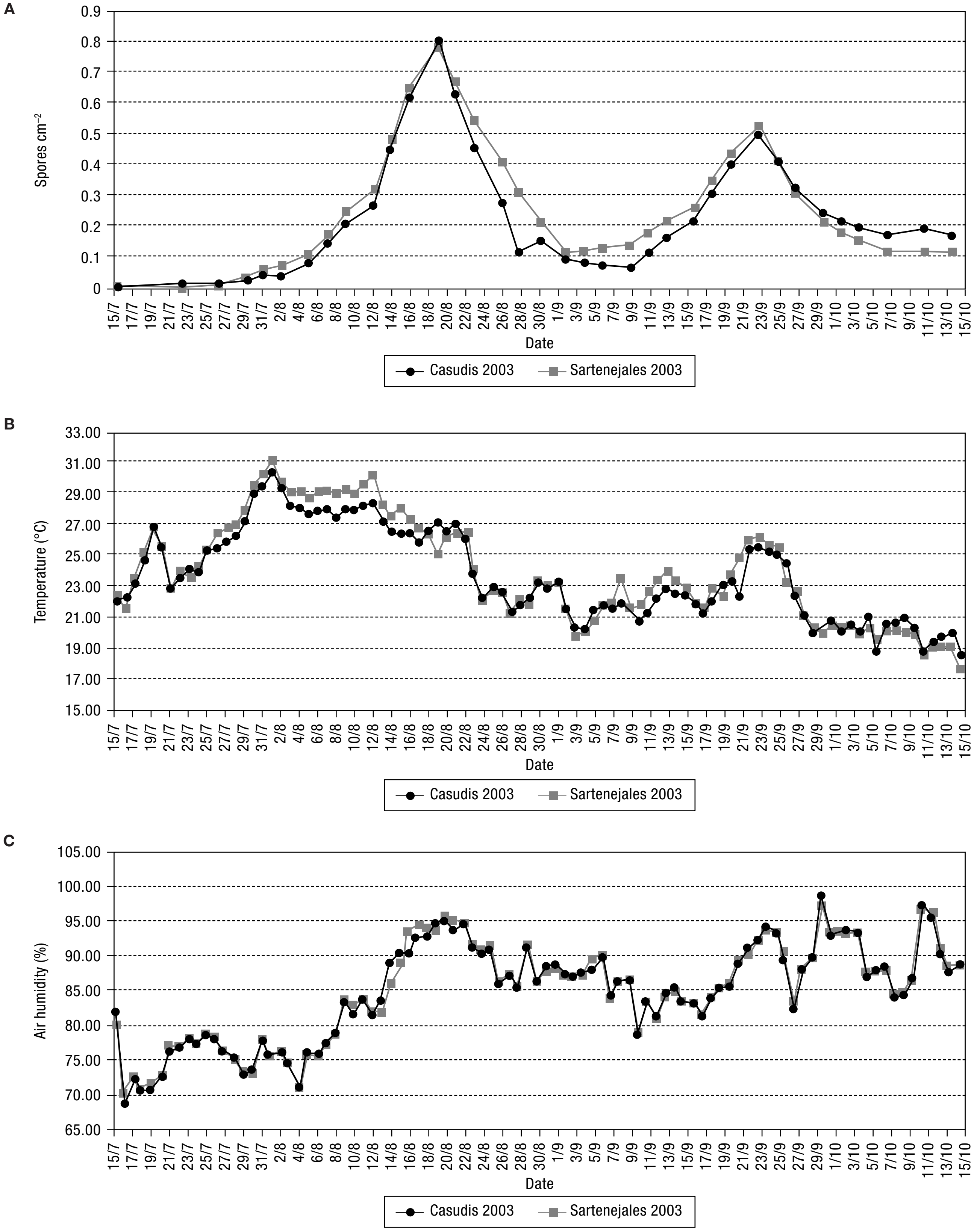

Figure 2. Change in the number of spores cm^{-2} (A) with mean temperature (B) and relative humidity (C). Year 2003.

primary infection and these favourable climatic conditions were responsible for the rise in the concentration of airborne spores.

Figure 1B reflects a period (5th-12th August 2002) in which there was a rise in relative humidity to over 95%. The average temperature (Fig. 1A) was around 27°C at both trial sites during this time. As reported by Leach (1980), these conditions substantially favoured the expulsion of spores, with values of over 0.7 per cm^2 recorded for this period (Fig. 1C). The number of airborne spores then fell as the season progressed, but reached a second maximum of about 0.3 spores cm^{-2} in the last two weeks of September. At this time, some 80-90% of the rice had headed.

In 2003 (Fig. 2), the maximum concentration of airborne spores was 0.8 per cm^2, recorded between the 20th and 25th of August (Fig. 2C). Before this period the relative humidity increased to 95% (Fig. 2B) and the average temperature was around 26°C (Fig 2B). In 2002, the number of spores was smaller during this period, but a second maximum of 0.5 spores cm^{-2} occurred between the 25th and the 30th of September (in 2003 the numbers reached in this second peak were smaller). This period was preceded by optimal conditions for infection. These spore concentrations, together with the favourable climatic conditions, were responsible for the maximum severity of disease seen in late September (during the ripening stage), with damage seen on different aerial parts of the plants.

The numbers of spores recorded over the growth cycle are similar to those reported by Marín-Sánchez and Jiménez-Díaz (1981). In both this and the present study the two spore concentration maxima were favoured by earlier periods of 90-95% humidity. However, the only maximum spore concentration recorded by the latter authors that agrees in time with the present results was that registered in September. The first peak recorded by Marín-Sanchez and Jiménez-Díaz (1981) was seen at the beginning of June; in the present work this was seen in the last fortnight of August.

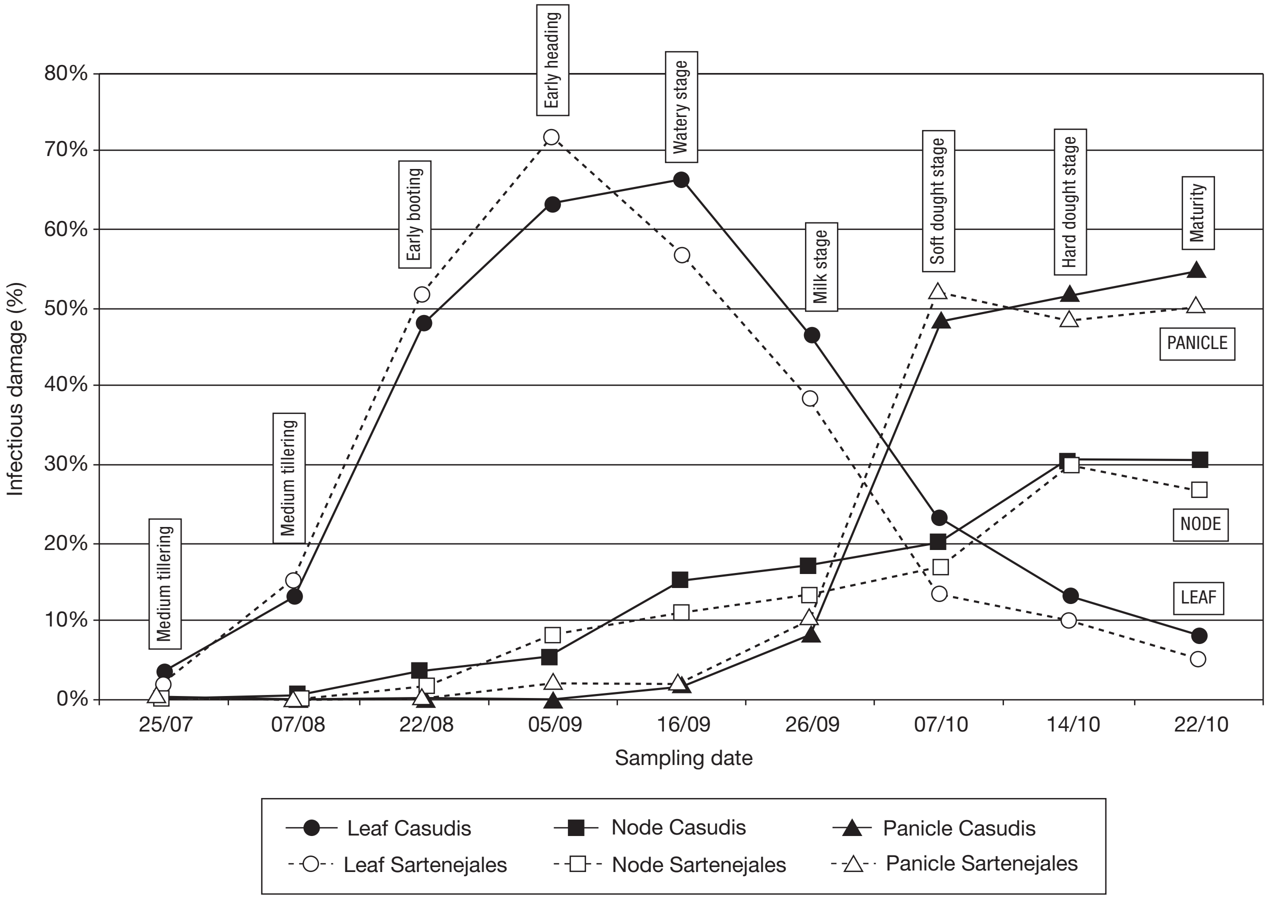

Figure 3. Disease progress during the rice growing season. Year 2002.

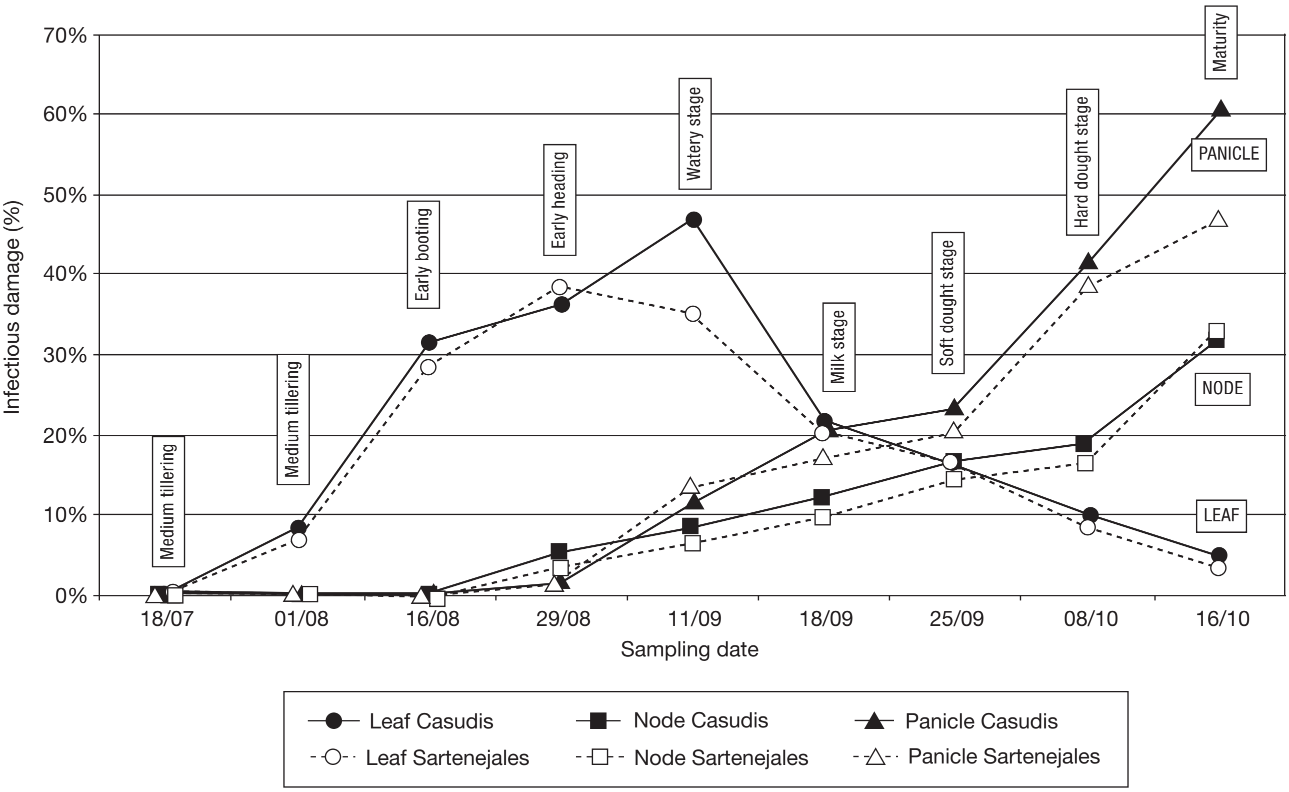

Figure 4. Disease progress during the rice growing season. Year 2003.

Lesion evaluation

The percentage infection per plant part over the crop cycle was similar in both seasons (Figs. 3 and 4). Leaf lesions were first detected when the crop was at the mid-tillering stage. Thus, 72% and 39% infectious leaf lesions were recorded at early heading in 2002 and 2003 respectively; these values became smaller as the plants developed. Infection of the node zone was first detected in late August (early heading stage), reaching values of 7% and 3% in 2002 and 2003 respectively (Fig. 5). These values increased until mid October. Panicle zone lesions were first detected in late August; at first, values of < 2% were recorded in both years, increasing to 45-60% for the rest of the crop cycle.

Under the trial conditions, a reduction in temperature of just 1°C resulted in less disease. As a consequence of the 27°C reached in 2002 compared to the 26°C recorded for 2003, the leaf AUDPC values in 2002 and 2003 were 3,006 and 1,749 respectively at the Sartenejales estate, and 3,234 and 1,888 at the Casudis estate. The AUDPC values for the rest of the plant organs were more similar in both seasons. Nevertheless, a large number of lesions was recorded in 2002 at the mid-tillering stage as a consequence of a rise in temperature. The yield losses caused were not measured in this work since earlier studies have confirmed that a difference of 2°C does not affect the yield (Lou *et al.*, 1998). Any greater temperature increase would, however, likely have serious effects on disease load and yield.

Discussion

Spores were trapped in the air from mid July to the end of the crop cycle. The first leaf symptoms appeared at the end of July, when the crop was in the mid-tillering stage. In both seasons and at both estates there were two periods of maximum spore production; these both followed increases in relative humidity to around 95% and average temperatures of over 26°C; such conditions favour the production of spores and their dispersal (Ono, 1963).

Spore detection from July 15th predicted the appearance of leaf blast within a few days; spore level surveillance may therefore be useful in the forecasting of leaf blast (Picco and Rodolfi, 2002). The high concentration of spores detected on August 16th-24th may have been a

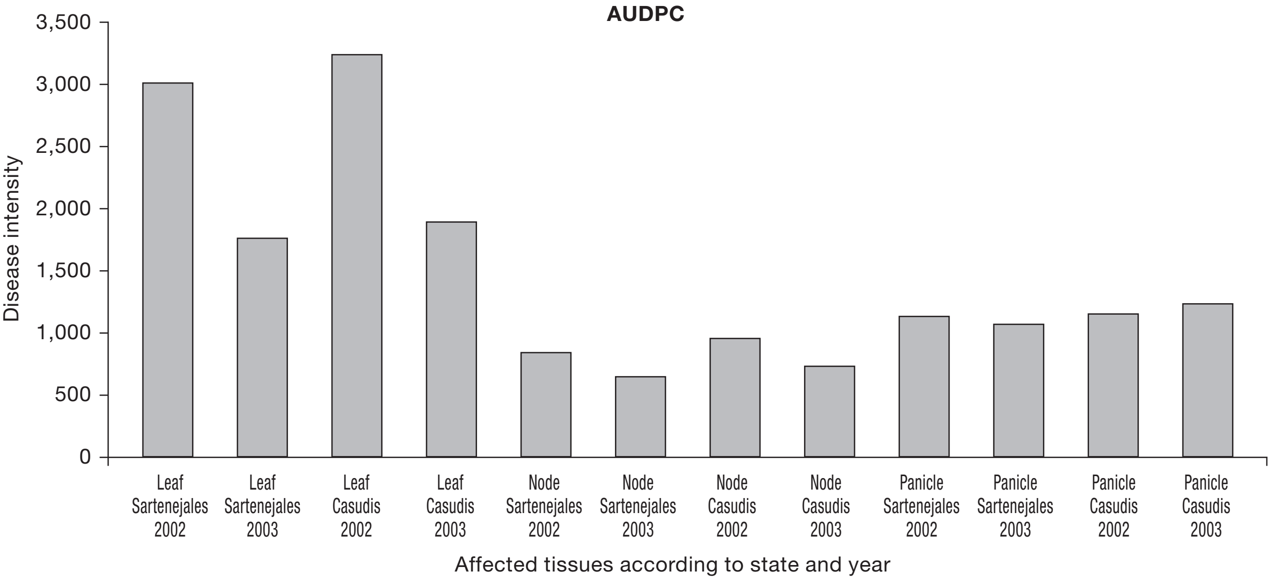

Figure 5. Blast intensity in terms of area under the polygonal epidemic curve (AUDPC) in all affected rice plant tissues. Years 2002 and 2003.

consequence of the primary infection and the climatic conditions favourable to the fungus. The incubation period varies depending on the temperature, relative humidity and humectation period (Maheswari Amma and Sam Raj, 1973), and the time elapsed between August 16th and September 29th ought to have been sufficient for the penetration of spores, the invasion of the host and sporulation. The peak spore concentration recorded in August may be used to forecast panicle blast problems.

In both seasons the number of infectious leaf lesions increased as the plants developed. After early heading, the number of leaf lesions decreased while the number of node and panicle base infections increased. Thus, the development of the disease is influenced by the crop growth stage, the climatological conditions and the airborne spore concentration.

A rise in temperature of 1°C in early August (mid-tillering stage) may have caused an increase in mean leaf blast intensity in terms of the AUDPC.

According to earlier work (Dhua, 1986; Castejón-Muñoz *et al*., 2007), an accurate examination of the colour and size of blast lesions can be sufficient to determine the severity of disease. In this work we mainly found grey spots on affected leaves, a type of lesion well known to release larger numbers of spores than brown spot lesions (Abumija, 1955). Certainly, leaf, auricle and ligule lesions can provide sufficient spores to infect the panicle (Thomas, 1930; Ono and Suzuki, 1960; Hori, 1963). A rapid increase in the number of spores was recorded during the heading stage, as reported by Kato *et al.* (1970). Thus, continuous air sampling for spores plus the assessment of disease severity in the field might be helpful in predicting forthcoming rice blast epidemics.

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