



***Pyricularia grisea* and *Bipolaris oryzae*: a preliminary study on the occurrence of airborne spores in a rice field**

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Received 7 September 2000; accepted in final form 7 December 2001

Key words: *Bipolaris oryzae*, fungal airborne spores, *Pyricularia grisea*, rice field

Abstract

Qualitative and quantitative analysis of fungal air spores in a rice field located around Pavia (North Italy) were made from 10th June to 7th October 1996. Quantitative data were analyzed for the two rice pathogens *Pyricularia grisea* (Cooke) Sacc. and *Bipolaris oryzae* Shoem., causal organisms of blast and brown spot. Results showed that *B. oryzae* was detected at the end of June, reaching its peak in July. Brown spot symptoms in-field were detected six-seven days after the aforementioned peak. *Pyricularia grisea* was monitored later than *Bipolaris* as it was detected for the first time on July 21st, reaching its peaks on July 27th and August 6–7th. Field evaluation of the disease showed the presence of blast starting from the first week of July.

The knowledge of the atmospheric concentration of *Pyricularia* and *Bipolaris* airborne spores together with a correct examination of the crops can yield information about the risk of infection during the vegetative season, thus allowing for a more accurate use of fungicides on rice crops, according to the modern conception of integrated control.

1. Introduction

The actual number of distinct rice diseases is not clear; over 80 biotic and abiotic diseases have been well described (Ou, 1980). Unfavourable weather conditions, nutritional disorders, weeds, together with bacteria, algae and virus are the most common causes of interference and debilitation of rice plants. In particular, fungi have been reported to cause more diseases of rice than any other group of pathogens. In addition to stem rot, sheath blight and bakanae, epidemics of blast and brown spot are continually reported (Ou, 1985). The causal organisms are respectively *Pyricularia grisea* (Cooke) Sacc., anamorph of *Magnaporthe grisea* (Hebert) Barr, and *Bipolaris oryzae* Shoem., anamorph of *Cochliobolus miyabeanus* (Ito and Kurib.) Drechs..

Rice blast is one the most serious fungal diseases in rice growing areas and it is most severe in irrigated rice fields of temperate regions and drought-prone environ-

ments (Bonman, 1992). Its destructiveness may infect panicles and completely kill plants when conditions are favourable.

Brown spot infection reduces the number of grains per panicle and kernel weight and it occurs in all the major rice-growing regions of the world. Brown spot is more of an indicator of nutritional or physiological disorders than a pathological disease; climatic factors and soil abnormalities, such as deficiency of one or more nutrient elements, are correlated with it.

Despite the attempts and studies of scientific research, defence of rice crops is, at the moment, still based on chemical control, not being varietal resistance for a long time. The fungicides used to control the infection are effective but they can't prevent the multiplication of the pathogen. According to the correct interpretation of the rules about the use of fungicides, the problem of the maximum residue allowed poses a set of complex problems that require close examination. Moreover, this kind of strategy doesn't

coincide with the modern concept of foodstuff free from any chemical treatment.

The idea of a possible integrated control has become increasingly consolidated in recent years; in our opinion, aerobiology is becoming important in this context. By an aeromycological monitoring it is possible to evaluate the concentration of fungal spores in the air in order to identify both the saprophytic and pathogenic fungi of rice.

This one-year survey on the atmospheric concentration of *Pyricularia* and *Bipolaris* spores in the air of a rice field is the first effort in Italy. We suggest the possibility to evaluate atmospheric concentration of airborne spores, meteorological data and field examination in order to predict disease infection periods.

2. Materials and methods

From June 10th to October 7th 1996, a qualitative and quantitative analysis was carried out on the fungal air spores of a rice field located near Pavia (North Italy). The quantitative analyses enabled the fluctuation of spore presence in the air to be established, while the qualitative investigation made observation, isolation and identification of fungal colonies possible.

2.1 Quantitative sampling

Quantitative air sampling was carried out using an automatic volumetric spore trap (Hirst, 1952), VPPS Lanzoni, placed about 1.5 m above the ground, in the centre of the rice field; airborne spores were sampled daily and the tape used for catching spores was replaced every week; transparent plastic tapes, coated with glycerine jelly, were used as an adhesive. Air was sucked at 10 l/min through an orifice of 14×2 mm; the tape was moved through the orifice at a constant rate of 2 mm/hour. After the exposure the plastic tape was cut into 48 mm long segments (each segment representing one day); each segment was mounted in one or two drops of molten jelly to which 1% of acid fuchsin was added. Four longitudinal bands on each slide were scanned under an optical microscope ($\times 400$) and results were expressed as the number of spores/m³ per day.

2.2 Qualitative sampling

Qualitative sampling was made using the gravity settling culture method; 5 Petri dishes (16 cm diameter) containing Potato Dextrose Agar (Oxoid),

spaced out 10 m apart diagonally in the field, were exposed for 5 minutes every week. Exposed dishes were incubated at 25 °C in natural day/night period and examined after 7 and 14 days; fungal colonies were counted, sub cultured onto PDA or other appropriate media and identified. Sterile mycelia were described as "mycelia without fructification".

Rain, temperature and relative humidity (R.H.) data were collected (Figure 1).

The direct visual estimation of stress on leaves was made using the Standard Evaluation System for rice (INGER-IRRI, 1996).

3. Results

Quantitative aerobiological data were analysed for the two rice pathogens *Pyricularia grisea* and *Bipolaris oryzae*. The qualitative analysis enabled identification of all the colonies grown.

3.1 Quantitative data

The observation of the microscope slides showed the incidence and the concentration of *Pyricularia* and *Bipolaris* spores during the study period (Figure 2).

B. oryzae was the first pathogen detected on June 26–27th with 2 daily spores/m³; it reached its highest level on July 8th (26 spores/m³). The direct field examination showed the appearance of the typical circular to oval brown spots caused by *B. oryzae* six–seven days after the aforementioned maximum; 10% of the leaf area was observed as diseased. From mid–July onwards, the incidence of *Bipolaris* spores was steady, i.e. two or three spores daily; a further increase in the presence of *Bipolaris* in the last week of August was detected, followed by an almost total lack of spores during September and October.

The behaviour of *Pyricularia* was significantly different. *P. grisea* appeared later than *Bipolaris*: the fungus was detected on July 21st with 2 spores/m³ of air and its concentration quickly increased in the following days up to the value of 42 spores/m³ which was observed on July 27th. The high concentration of *P. oryzae* spores observed on August 6–7th (42–40 spores/m³ respectively), on August 28th (48 spores/m³) and on September 24th (31 spores/m³) are worth mentioning.

Field evaluations of the disease showed the appearance of grey and lanceolate necrotic spots produced by *P. grisea* from the first week of August. The exami-

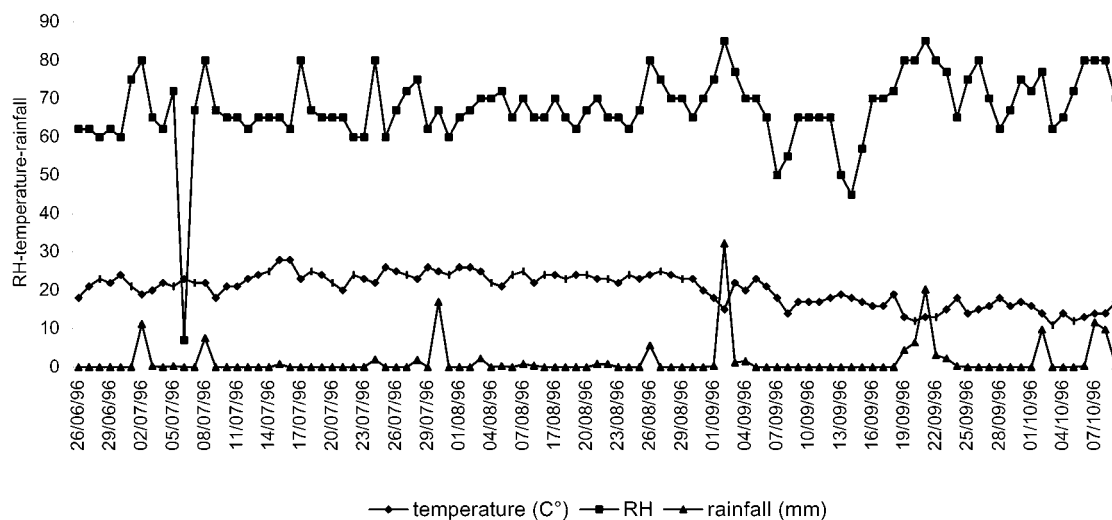


Figure 1. Weather conditions over the period June–October 1996.

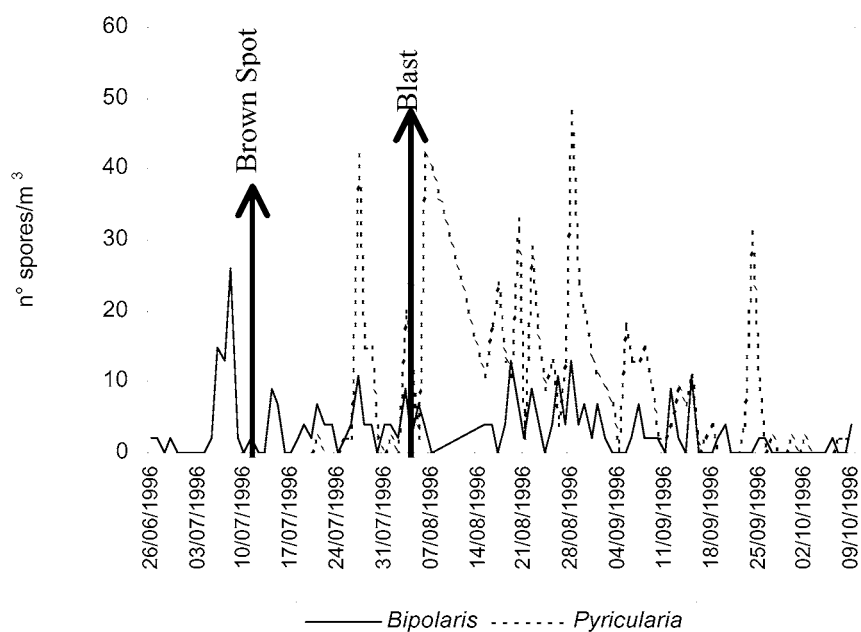


Figure 2. Atmospheric concentration of *Bipolaris oryzae* and *Pyricularia grisea* spores.

Table 1. Fungal taxa isolated from air by the gravity settling culture Petri dish method

	June	July	August	September	October
<i>Acremoniella atra</i> (Corda) Sacc.	50	/	/	/	/
<i>Acremonium fusidioides</i> (Nicot) Gams	/	/	/	/	3
<i>A. kiliense</i> Grutz	1	/	/	/	/
<i>Alternaria alternata</i> (Fr.) Keissl.	102	96	37	31	4
<i>Aspergillus fumigatus</i> Fresen.	/	/	/	1	/
<i>A. niger</i> van Tiegh.	/	/	/	1	/
<i>Aureobasidium pullulans</i> (De Bary) Arnaud	/	1	/	/	/
<i>Botrytis cinerea</i> Pers.	/	3	/	/	/
<i>Cladosporium</i> spp.	567	612	515	1169	448
<i>Cunninghamella elegans</i> Lendn.	20	/	/	/	/
<i>Curvularia lunata</i> (Wakker) Boedijn	/	2	/	/	/
<i>Epicoccum nigrum</i> Link	184	218	56	196	54
<i>Fusarium avanaceum</i> (Fr.) Sacc.	4	8	30	23	2
<i>F. moniliforme</i> Sheld.	5	37	9	38	3
<i>F. oxysporum</i> (Fr.) Schlechtend.	5	4	/	88	/
<i>F. solani</i> (Mart.) Sacc.	/	1	/	/	/
<i>F. tricinatum</i> (Corda) Sacc.	22	/	/	/	/
<i>Gliocladium</i> sp.	/	1	/	/	/
<i>Monocillium mucidum</i> Gams	4	/	/	/	/
<i>Mortierella isabellina</i> Oudem. and Koning	/	2	/	/	/
<i>Nigrospora oryzae</i> (Berk. and Br.) Petch	/	2	/	4	/
<i>Penicillium citrinum</i> Thom	/	/	/	/	3
<i>P. funiculosum</i> Thom	/	1	/	/	/
<i>P. glabrum</i> (Wehmer) Westling	/	/	10	/	/
<i>P. janthinellum</i> Biourge	/	1	/	/	/
<i>P. oxalicum</i> Currie and Thom	/	/	/	3	/
<i>P. purpurogenum</i> Stoll	/	1	/	/	/
<i>Penicillium</i> sp.	/	2	2	/	/
<i>Pithomyces chartarum</i> (Berk. and Curt.) Ellis	/	/	/	1	/
<i>P. maydicus</i> (Sacc.) M.B. Ellis	/	2	/	/	/
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier	1	/	/	/	/
<i>Trichoderma viride</i> (Fr.) Pers.	52	50	/	1	2
Micelia without fructification	1	3	/	34	5

nation of stress incidence detected lesions corresponding to 5% of the affected leaf area. Blast symptoms were observed when R.H. was about 70% and temperature between 20 and 25 °C (Figures 1–2).

3.2 Qualitative data

A total of 4,838 colonies, which represented 19 genera and 32 species, were isolated (Table 1). These data highlight the absence of *Bipolaris oryzae* and *Pyricularia grisea*.

Data show the presence of five dominant genera: *Alternaria*, *Cladosporium*, *Epicoccum*, *Fusarium* and *Trichoderma*. In particular, the species *A. alternata* (Fr.) Keissl., *F. avanaceum* (Fr.) Sacc., *F. moniliforme* Sheld., *F. oxysporum* Schlech. and *Trichoderma viride* (Fr.) Pers. were constantly sampled. Within the dominant genera, *Cladosporium* spp. comprised more than half of the sample, representing 68% of the isolated colonies.

4. Discussion

Knowledge of the atmospheric concentration of *Pyricularia* and *Bipolaris* spores in the air of a rice field and its correlation with the environmental factors could make it possible to forecast blast and consequently a more rational use of fungicides. In Japan, many studies have been made to determine the optimum time at which to apply chemicals (Ono, 1965; Yamaguchi, 1970; Suzuki, 1969, 1974); in Italy, climatic factors, different cultivars of rice and the existence of strains of *P. grisea* differing in pathogenicity make Japanese tests inapplicable.

Analysing our results, the considerable disagreement between the two methods of examination stands to reason. It is beyond doubt that the quantitative analysis is the most reliable. The unsuccessful isolation of the two pathogens from the exposed dishes was probably caused by the great facility of growth

of *Alternaria*, *Cladosporium* and *Epicoccum*; they are common saprophytes of plants, lacking particular nutritional requirements and their confluent development has probably interfered with the growth of *Bipolaris* and *Pyricularia*. As we suppose that some of the 43 mycelia without fructification could conceal *Bipolaris* or *Pyricularia* colonies, different media containing plant tissue (Ou, 1985) could be tested to stimulate the pathogen's growth.

The quantitative evaluation highlights a very interesting behaviour of *Pyricularia* in the air: the peak of its spores (July 27th), noticed a few days before the appearance of leaf blast, may be useful for leaf blast forecasting. The high concentration of spores noticed on August 6–7th could be a consequence of the infection which had taken place; the incubation period varies with temperature, humidity and wetting period of leaves (Hemmi et al., 1936; Maheswari Amma and Sam Raj, 1973) and the lapse of time occurring from July 27th up to August 6–7th should be enough for the penetration of spores, the invasion of the host and the sporulation. The peak of spores, registered on August 28th, may be used to forecast panicle blast. The value reached on September 24th (31 spores/m³) could be interpreted as related to harvesting.

According to Dhua, 1986, the careful examination of colour and size of blast lesions indicates the severity of the disease. We mainly found grey-purple spots on rice leaves monitored; it's known that this kind of lesion releases a greater number of spores than the brown one (Abumija, 1955). Therefore, a continuous air sampling connected with a direct field examination could indicate an epidemiological pattern of blast disease.

The optimum temperature for sporulation of *Pyricularia* is 25–28 °C both in the tropics and in temperate regions, while the level of relative humidity necessary for the release of conidia seems to be different; in Japan, no sporulation occurs below 89% relative humidity (Hemmi and Imura, 1939), while in this study blast developed with about 70% of R.H. and a spores peak of 42 spores/m³.

The maximum presence of *Bipolaris*, noticed one month before *Pyricularia*, coincides with the appearance of brown spot on rice crops. The oval brown lesions were observed during the whole rice season but yield losses due to brown spot in Italy are inconspicuous.

Although this infection is not as heavy as blast, it is a mistake to neglect its appearance as *B. oryzae*

produces a non-host-specific toxin called ophiobolin (Nakamura et al., 1960), able to suppress the defence mechanism of rice. In our opinion, the monitoring of airborne fungal spores may be an effective diagnostic method to forecast the diffusion of the disease, useful to obtain an early etiological diagnosis and also to reduce chemical treatment and foliar applications of fungicides.

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