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ORIGINAL PAPER

Aerobiological dynamics of potentially pathogenic fungi in a rice agroecosystem in La Habana, Cuba

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Abstract The aerobiology can play a key role in protecting the rice crop since many fungi can cause serious damage to agricultural areas. In this way, the ideal time to implement different security measures can be identified. To determine the presence of potentially pathogenic fungi in the air of the rice agroecosystem, a weekly monitoring of viable fungi was carried out using a volumetric sampler. Collected fungi were quantified, isolated, and identified based on their morphological characteristics. The results obtained demonstrated that the annual average concentration of filamentous fungi in the atmosphere of rice agroecosystem studied was 1,225 cfu m^{-3} levels ranging between 115 cfu m^{-3} (April) and 2,865 cfu m^{-3} (August). *Pyricularia grisea* was detected in the air for 5 months, since the second week of June until the first week of October, and highest average concentration (25 cfu m^{-3}) was observed in August. Of the meteorological factors evaluated, temperature and relative air humidity influence the concentration of propagules of *P. grisea* in the air. Besides, other fungi were detected such as *Curvularia*, *Bipolaris*, *Alternaria*, and *Cercospora*, all with relevance to rice cultivation. This is the first characterization of aeromycological biodiversity in the studied region.

Keywords *Pyricularia grisea* · Airborne fungi · Biodiversity · Meteorological factors · Fungal propagules

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1 Introduction

Rice (*Oryza sativa* L.) is an important cereal for human consumption. This cereal is the staple food for over half the world population and is considered the world's largest by the size of the area where it is grown and the number of people who depend on their crop

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(IRRI 2002). In Cuba, rice is an important part in the daily diet of the population, and current per capita consumption is among the highest in Latin America, contributing 20% of daily calories consumed (Cárdenas et al. 2007).

Controlling diseases that may attack the crop is of great importance to increase productivity in rice-growing areas. In particular, fungi have been described as causal agents of various rice diseases more than other groups of pathogens (Herrera 2003). Piriculariosis, caused by the fungus *Pyricularia grisea* Sacc, has been extended to over seventy countries that are highly producers of rice and is one of the most important diseases that affect this cereal (Lanoiselet et al. 2002). In Cuba, the highest incidence occurs in the western region where it is considered a phytopathological problem and is the most widespread and destructive disease affecting the crop, due to its difficult handling and the potential pathogenicity of the causal agent (Cárdenas et al. 2007). Symptoms occur in all parts of plant, which can be affected at any stage of crop development (Ou 1985; Sesma and Osbourn 2004).

Most fungi that affect rice cultivation can spread through the air, which is why aerobiological investigations are useful for understanding the disease epidemiology and its control (Cordero and Rivero 2001). Aerobiological systematic studies provide information to develop databases and predictive modeling, in order to identify the periods of greatest concentration of fungal propagules in the air, which poses a risk to the crop (Kasprzyk 2008). Like this, the ideal time to implement different security measures, such as applying a chemical fungicide or biological product, can be identified (Ponti and Cavanni 1992).

In Cuba, aerobiological studies have been mainly carried out in urban areas (Rojas and Martínez 2000; Llanes et al. 2006; Lopetegui et al. 2006). However, there is no a systematic monitoring on rice-growing areas. These investigations could help to characterize the fungi present in the air and provide additional information (occurrence, concentrations, and their relationship to different environmental factors). The aim of this study was to determine the presence of propagules of pathogenic fungi in the air of a rice agroecosystem with emphasis on the species *Pyricularia grisea* Sacc.

2 Materials and methods

2.1 Study area

The experiments were conducted at Instituto de Investigaciones de Granos during a year (Bauta, La Habana, Cuba). This area has a humid climate, and its vegetation is composed by various graminoids crops and fruit-bearing groves. The geographical location was provided by the Global Positioning Systems, GPS (Leica SR20) (Fig. 1). Experiments were carried out in a plot planted to *Oryza sativa* var J-104, and it has an area of 3,216.0 $m^{2.}$

2.2 Sampling

The collection of air fungal propagules was done with a volume sampler SAS Super 100 (PBI International, Italy), controlled with an air flow 20 L/min. The medium used was potato dextrose agar (Uddin 2004). The study was carried out over a year in the period

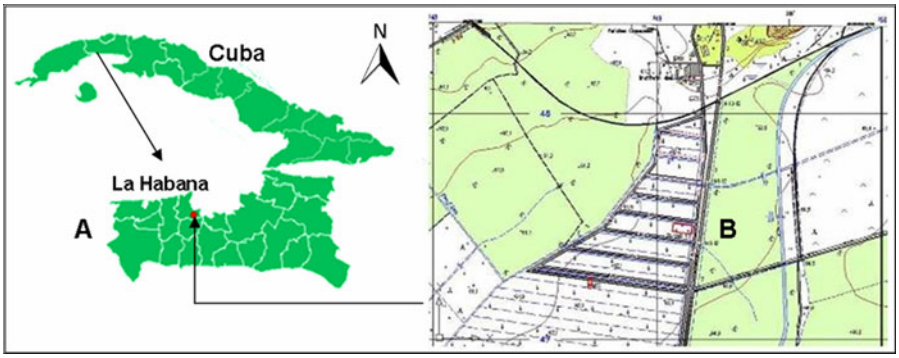

Fig. 1 Geographical location of study area. **a** Instituto de Investigaciones de Granos ($X = 349161.90$ m; $Y = 348154.80$ m), La Habana, Cuba. **b** Terrace 11 ($X = 349158.2$ m; $Y = 347504.1$ m)

between the first week of March 2007 (stage of crop germination) and the last week of February 2008 (6 months after the plot harvested). Sampling was performed once a week at 09.00 h and followed a diagonal design with ten points to the height of two meters above ground level (Rojas et al. 2007). The meteorological variables for mean temperature and relative air humidity were measured at each sampling site, and these data were confirmed with the daily forecasts issued by the Institute of Meteorology website (http://www.insmet.cu), which were taken by the station Meteorological Bauta, La Habana, Cuba.

2.3 Classification and identification of isolates

Petri dishes of each sample were incubated at $30 \pm 1°C$ for 7 days. Emerging colonies were counted after 4 and 7 days of incubation. The concentration of fungi propagules detected in the air was calculated using the formula suggested by the equipment manufacturer (Manual SAS) and was expressed as unit of fungi concentrations in air (cfu m^{-3} air). The identification of isolates genera was carried out by conventional methods, according to the criteria of Barnett and Hunter (1998). Identification of *Pyricularia* Sacc isolates was done following the criteria presented by Ellis (1971).

2.4 Statistical analysis

For statistical analysis, as a starting point to all data were analyzed for normality and homogeneity of variance using the Kolmogorov–Smirnov test and Bartlett, respectively. An analysis of variance of simple classification was applied to monthly average cfu m^{-3} of filamentous fungi, and it was completed with the parametric test for comparison of means of Tukey. Monthly averages of cfu m^{-3} *P. grisea* were analyzed using nonparametric Kruskal–Wallis test supplemented by the Student–Newman–Keuls (SNK nonparametric) test. The relationship between fungal propagules concentration (cfu m^{-3} air) of different fungal groups detected and meteorological factors was found by the coefficient of Spearman's rank correlation (*R*s). The data were analyzed with statistical software STATISTICA 6.0.

3 Results

3.1 Concentration and diversity of fungal propagules

The highest concentration of filamentous fungi propagules was detected in August (3,287 cfu m^{-3}), differing significantly from the rest of the monthly averages (Fig. 2). Mean values below 1,000 cfu m^{-3} were shown in the months of March to June 2007 and January to February 2008, but only statistically different from those observed in the months of August and October 2007. The lowest concentration of viable propagules in the air was detected in the first sampling in April with a value of 115 cfu m^{-3}. In assessing the possible influence of meteorological variables in the concentration of fungal propagules, there was a positive correlation between the concentration of viable and temperature, $R = +0.307535$, with a confidence interval of 95–98.9% and (R value < 0.01 differences are significant). No evident correlation between the relative air humidity and the concentration of fungi in the atmosphere.

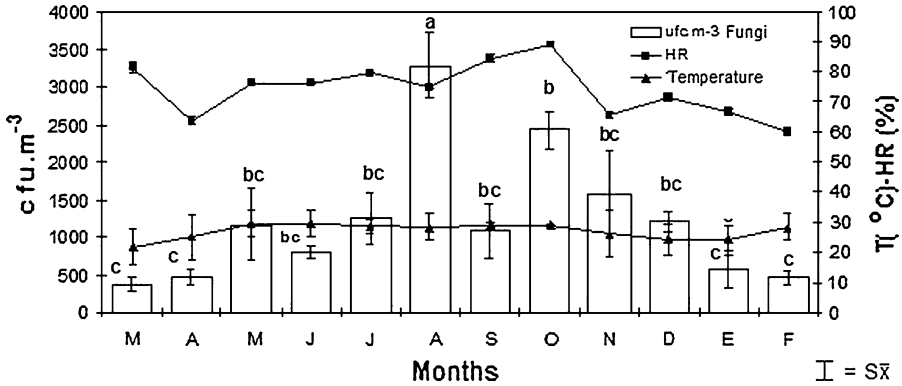

Fig. 2 Variation of the concentration of filamentous fungi (average value ufc m^{-3}) and meteorological variables, relative humidity (*RH*) and temperature (*T*). *Different letters* within months indicate significant differences at $p < 0.05$, according to the Tukey's test

From a qualitative point of view, 39 different genera were detected in the air during the year, which were of phytopathological interest, *Alternaria* Nees ex Fr, Boedijn, *Curvularia*, *Bipolaris* Shoem, *Cercospora* Fres, and *Pyricularia* Sac (Fig. 3), and some species of this genera can cause damage to this crop.

Alternaria was detected between March and June and the months of August, September, and November 2007, with a relative frequency (RF) of 40.9%. The presence of *Curvularia* covered from March to July and September 2007 to January 2008, with a RF of 63.6%. Propagules of *Bipolaris* were isolated in the periods from March to July 2007 and September to November the same year with a RF of 45.5%. The detection of *Curvularia* and *Bipolaris* shows a tendency to appear in two periods during the year of sampling. The genus *Cercospora* was only detected in June with a relative frequency of 2.3%.

When analyzing the incidence of *Pyricularia,* there was a dynamic appearance in the months of June to October 2007, and although the RF was 27.7%, detection is of great importance to the crop, and within this genus is *P. grisea* Sac, which is the most important pathogen affecting rice (Cárdenas et al. 2007). So we proceeded to identify all isolates belonging to this genus.

3.2 Aerobiological dynamic of *Pyricularia grisea* Sacc

Of all the fungi collected, 14 isolates were obtained for the genus *Pyricularia*, which were identified as *P. grisea* Sacc, taking into account the presence of cultural and morphological characteristics for this species, according to Ellis (1971). All colonies formed abundant aerial mycelium and gray–white on PDA and a growth range of 80 mm on the seventh day in this medium. Microscopic observations confirmed the presence of conidiophores that are straight, unbranched, geniculate toward the apex, smooth and pale brown; conidiogenous cells that are polyblastic, integrated and sympodial; conidia that are solitary, acropleurogenous, obpyriform, hyaline, smooth, with two septa and hilum protuberant (Fig. 4). Measurements were made of their conidia, and its dimensions were between 19.5 ± 2 and 26.4 ± 2 µm in length and 7.5 ± 0.9 and 8.4 ± 0.5 µm wide, values characteristic of the species as observed by Ellis (1971).

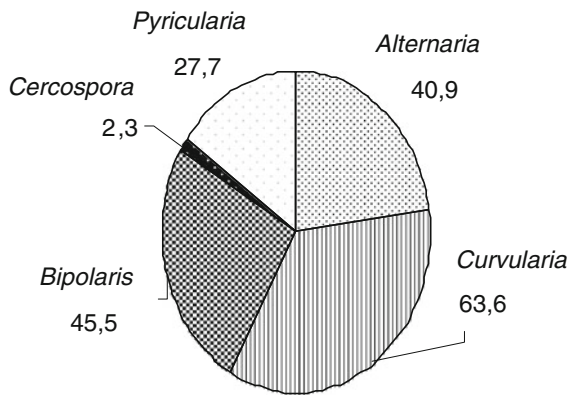

Fig. 3 Relative frequencies (%) of *Taxa* identified during the period monitored

Propagules of *P. grisea* began to be detected from the second sampling in June (20 cfu m^{-3}), coinciding with the development stage of panicles of the plants in the plot (Fig. 5). The maximum concentration of *P. grisea* was detected in August (25 cfu m^{-3}). No significant differences were showed in the following samples (5–15 cfu m^{-3} for sampling from 19 to 23), since the second week of July until the second week in August. In the last sampling in August turned to detect a further increase of 55 cfu m^{-3} and then returns to demonstrate a similar pattern to earlier samplings. This species was detected in the air until the first week of October (5 cfu m^{-3}).

By analyzing the correlation between temperature and relative air humidity, it was determined that these two factors positively influence the concentration of airborne propagules of this fungus, as evidenced by the values of the Spearman's test, $R = 0.3609628$ and $R = 0.342241$, respectively, with a confidence interval of 98.39 and 97.70%. This influence is seen in the detection of the species during the hot humid months of the year.

4 Discussion

The monthly concentration of filamentous fungi propagules in the region studied ranged between 115 and 1,000 cfu m^{-3}. These results are consistent with those in a rice agroecosystem in India by some researchers (Uddin 2004; Adhikari et al. 2004). The tendency to find the greatest concentration of fungal propagules in the air during the warmer and humid months of the year (July and August) has been suggested in other aerobiological outdoor works made

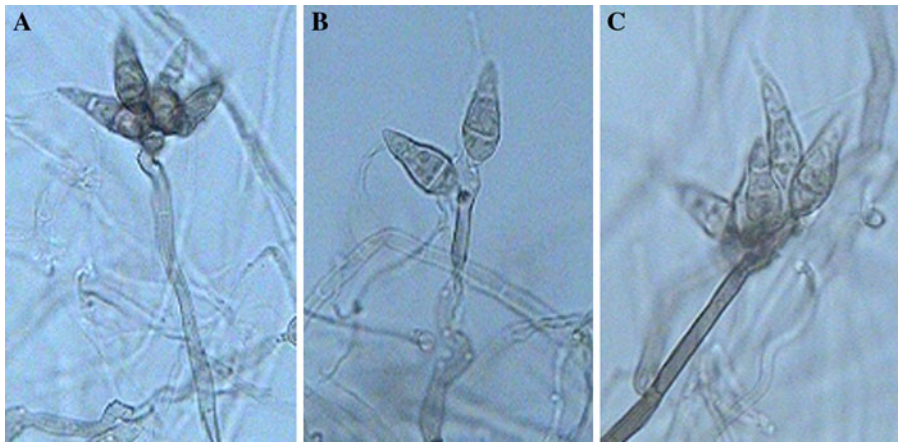

Fig. 4 Photographs of three isolates of *Pyricularia grisea* Sacc. (1,000×): **a** strain AAM-426, **b** strain AAM-455, **c** strain AAM-459

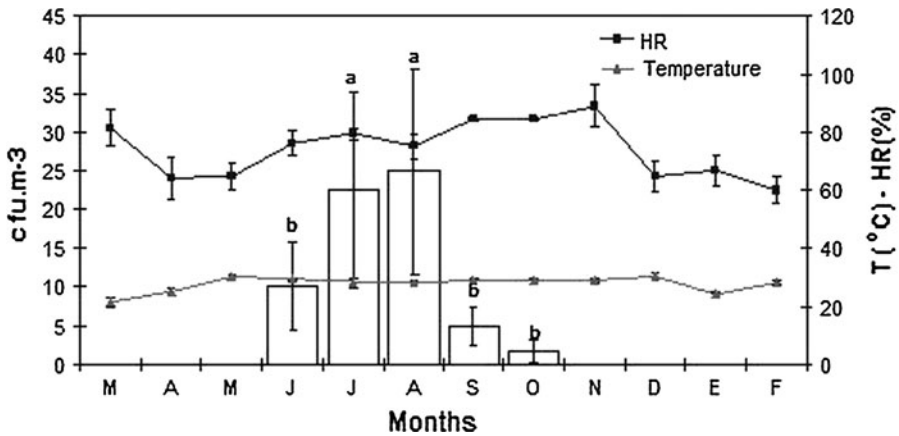

Fig. 5 Monthly average concentration of *Pyricularia grisea* and the weather variables, relative humidity (*RH*) and temperature (*T*). *Different letters* within months indicate significant differences for nonparametric SNK $p < 0.05$

in Havana (Llanes et al. 2006; Rojas et al. 2007). In the present investigation, the highest concentration was detected in August (4,180 cfu m^{-3}), and there was a positive correlation between temperature and air concentration of filamentous fungi. Kasprzyk and Worek (2006) suggested that the concentration of fungal spores in the atmosphere of a rural area of Poland is increased from June to November, and the highest values are detected in July and August. Quintero et al. (2010) suggested that the concentration of fungal spores in the atmosphere of Puerto Rico is increased from August to September. However, the comparison of aerobiological results is very difficult due to geographical, meteorological, and biological differences between the areas that are studied, as well as the impact of humans on ecosystems (Randon et al. 2002; Pyrri and Kapsanaki-Gosti 2007). These results could also be affecting the wind speed, variable not recorded in this work and as the temperature and relative air humidity can vary from year to year (Agrios 2005).

All the genera detected in this research have been detected in other aerobiological studies around the world (Corden et al. 2003; Kasprzyk and Worek 2006; Rao et al. 2009). The genus *Curvularia* was observed in more than half of the sampling, and detection is of importance since this genus is related to diseases in rice grains, which can cause considerable losses in the cultivation of rice (Hassikou et al. 2003; IIA 2006). Species of *Alternaria* are also related to this disease, and this genus is also detected in this study. *Bipolaris* is another genus of importance for rice cultivation, as the species *B. oryzae* causes brown spot (Neninger et al. 2003; Ojeda and Subero 2006), which is the second most important disease associated with this crop (Picco and Rodolfi 2002). This genus has been

reported in aerobiological studies conducted in the atmosphere of rice agroecosystem from Italy and Spain (Picco et al. 2004; Lara-Alvarez et al. 2004). Although the presence of *Cercospora* in our study was very low, no longer of interest and that within this genus is the species *C. oryzae* that can cause the linear patch of rice cultivation, and is another disease that can affect the (Cárdenas et al. 2003).

The monthly aeromycological dynamic of *Pyricularia grisea* Sacc corresponds (period of detection of the fungus and concentration) with studies made in the United States by Kaminski (2006) and made in Spain by Lara-Álvarez et al. (2005). These studies found the maximum of spores in the air from the middle of August and September, respectively. Literature indicates that relative air humidity and temperature influence positively various stages of the life cycle of this fungus and this favor sporulation and release of conidia (Lara-Álvarez et al. 2005). The fact that it is also demonstrated this trend in a agricultural area located in the west of our country, is important because the tropical characteristics allows to reach very high temperature and relative air humidity in most of the year in this geographical area.

However, in these results, it was not noticed a high incidence of *Pyricularia grisea* on the plants of the parcels for which surveys were conducted. This behavior might be that during the study period, the concentrations of *P. grisea* were not high and that the field over the study took place has an appropriate agronomic management (Mishra et al. 2006). However, in the first week of October, lesions were detected in the aerial parts of rice plants from experimental plots of the variety INCA LP-5 nearby the study area. In this month also found the presence of *Pyricularia grisea* Sacc in the remains of harvest of the plots on which the study was done. There is evidence that this fungus can be detected in the remains of the harvest for more than 2 years, and this may be a route for maintenance and dissemination in the fields, which favors the infection of new crops (IIA 2006).

Considering that many propagules of fungi that affect crops spread through the wind, this research could provide information on the mode of distribution in the air, the spores of these fungi. The continuity of this type of research and evaluation of the influence of different climatic parameters would explain the ecology of the fungus with potential to affect different crops and a better understanding of the epidemiology diseases they cause.

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