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Mycoflora of stored rice in Portugal

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Abstract

Rice (*Oryza sativa* L.) is a staple food for over half of the world's population and is grown on approximately 146 million hectares, more than 10 percent of the total available land. In the tropics, rice is the primary source of human nutrition, and is one of the cheapest sources of food energy and protein. In Portugal, rice is grown on 18,500 hectares and the average per capita consumption is the highest in Europe, around 15 kg per year per person.

In the work we have collected rice samples from different origins within Portugal and these samples were analysed for fungal infection. Several fungi were isolated, mainly *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Alternaria* sp. and *Trichothecium* sp. Some of them are known to be mycotoxin producers. This information about fungal mycoflora is essential to describe the status quo and to establish programs to prevent mycotoxin formation if necessary.

Fungus–feeding insects were also collected and identified: *Ashverus advena*, *Coninomus* spp., *Cryptophagus* spp., *Typhae stercorea* and *Litargus balteatus*.

Key words: rice fungi, storage fungi, rice insects, storage insects

Introduction

Rice (*Oryza sativa* L.) is a staple food for over half of the world's population and is grown on approximately 146 million hectares, more than 10 percent of the total available land. In the tropics, rice is the primary source of human nutrition, and is one of the cheapest sources of food energy and protein (Mejia, 2003).

Portugal is the biggest consumer of milled rice in Europe (15 kg/person/year). The annual paddy production in Portugal is about 129,000 tonnes of *japonica* rice (short-grain) and 26,000 tonnes of *indica* rice (long-grain) distributed among three main regions: Sado Valley, Tejo Valley and Mondego Valley. Paddy rice is a seasonal crop in Portugal and, as a consequence, the storage of paddy and milled rice is very important for year-round availability. Besides national production, 98,000 tonnes of rice are imported to reach consumers needs.

In storage, the development of fungi especially *Aspergillus*, *Fusarium* and *Penicillium* is an unresolved problem. They are responsible for quantitative and qualitative losses and under

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certain conditions these species can develop toxic metabolites called mycotoxins. The mycotoxins are hazardous to animal and human health and constitute a factor for economic losses in food products worldwide (Christensen and Saucer, 1992).

During storage, paddy is susceptible to insects and their infestations disseminate the fungi and create entry points in the grain for fungal infection (Pitt and Hocking, 1997).

The aim of the present work was to isolate and identify the fungi associated with stored rice in Portugal and also the insects that develop in the same ecosystem.

Material and methods

Climatic data collection

In relation to On-Farm storage, for the determination of temperature and relative humidity, data loggers were placed in one flat warehouse. In the case of rice mill, for grain humidity and temperature, data loggers were used inside brown rice.

Mycoflora analysis

Most paddy is initially stored on farms and is periodically delivered to the mills over the course of the storage period (October to March). At the rice mill, paddy is stored prior to processing. Both on-farm storage and rice mill presented two storage facilities and had the equipment for cleaning, weighing, drying and aeration.

In relation to On-Farm storage, paddy rice samples were collected on three stores, belonging to the rice farmers association of Sado Valley region, from November 2005 to March 2006. Additionally, paddy rice, brown rice, and processed rice samples were also collected in the rice mill located in Santiago do Cacém, from October 2005 to date. This facility usually processes the previous paddy from the rice farmers association of Sado Valley. These samples were taken to laboratory.

Rice samples were disinfected at surface with 1 % sodium hypochlorite, during 2 minutes, as describe by King et al. (1986).

One gramme of rice was placed on Potato Dextrose Agar (PDA) medium. For each sample, three replicates were made. These grains were incubated at 28 ºC for 8 days and then examined under a light stereomicroscope for fungal growth. Isolation of the colonies was made to obtain pure cultures. Slides of fungal growth were prepared and observed under a compound microscope for fungal morphology study. The identification was carried out using identification keys (Carmichael et al., 1980, Domsch et al., 1980, Onions et al., 1981, International Mycological Institute, 1991, Samson et al., 1995, Hanlin, 1997, Malloch, 1997, Pitt and Hocking, 1997, Barnett and Hunter, 1998).

Insect analysis

Since 2002 to date, trials were carried out in seven on-farm stores and one rice mill to monitor stored rice insects species: in stored paddy, brown and milled rice using Stogard WBII probe and PC traps without lures; in the rice mill facility using Dome traps with kairomone.

The traps were observed weekly or every 2 weeks, and the insects counted and identified.

Results and discussion

Climatic data

Temperature and relative humidity data registered on on-farm storage are present in Table 1. Data of grain temperature, ambient temperature and relative humidity in the grain, registered in rice mill, are presented in Table 2.

Mycoflora identification

All the 30 samples analyzed contained fungi and a wide variety of genera was found, with a high prevalence of storage fungi. In Table 3 fungi identified are indicated.

Table 1. Paddy rice stored in bulk in a flat warehouse: grain temperature (surface and 2 meters depth), ambient temperature and relative humidity in the grain, using data loggers sensors from 20 November 2005 to 30 April 2006

	Temp. ºC						r.h.. (%)	
	Data logger 1		Data logger 2		Data logger 3			
	Surface	Depth	Surface	Depth	Surface	Depth	Ambient	Surface
Mean ± SE	6.94 ± 0.09	11.52 ± 0.05	14.27 ± 0.04	12.71 ± 0.05	10.09 ± 0.05	16.05 ± 0.05	9.94 ± 0.09	76.50 ± 0.44
Minimum	-2.55	6.55	11.33	9.60	5.77	13.22	0.24	64.01
Maximum	18.52	19.79	19.46	17.46	17.87	20.88	21.57	84.20

Table 2. Brown rice stored in bulk in a flat warehouse: grain temperature (surface and 2 meters depth), ambient temperature and relative humidity in the grain using data loggers sensors from 20 November 2005 to 30 April 2006

	Temp. ºC						r.h.. (%)	
	Data logger 1		Data logger 2		Data logger 3			
	Surface	Depth	Surface	Depth	Surface	Depth	Ambient	Surface
Mean ± SE	14.21 ± 0.06	23.36 ± 0.04	13.48 ± 0.05	23.36 ± 0.04	12.59 ± 0.04	21.52 ± 0.04	9.78 ± 0.07	54.31 ± 0.13
Minimum	5.03	20.65	6.54	20.65	6.46	17.83	0.27	26.51
Maximum	28.12	28.47	23.0	28.47	27.54	25.76	18.46	65.10

Table 3. Fungi identified from samples of paddy, brown and processed (parboiled and long grain) rice.

	Sample	Fungi Identification
On-farm stores	Paddy rice	*Alternaria* spp. *Aspergillus* spp. *A. flavus* *A. niger* *Fusarium* spp. *Penicillium islandicum* *Stemphylium* spp. *Trichothecium* spp.
Rice mill	Paddy rice	*Aspergillus* spp. *Penicillium* spp. *P. islandicum*
	Brown rice British Guiana	*Aspergillus* spp. *A. candidus* *A. flavus* *A. niger* *A. sydowii* (Bain. & Sart.) Thom & Church *Fusarium* spp. *Penicillium* spp. *P. islandicum*

Continue...

Table 3. Continue

	Sample	Fungi Identification
Rice mill	Parboiled Rice	*Aspergillus* spp. *A. flavus* *A. niger* *A. sydowii* *Penicillium* spp.
	Long grain rice	*Aspergillus* spp. *A. candidus* *Fusarium* spp. *Penicillium* spp.

Fungi genus detected were: *Alternaria, Aspergillus, Fusarium, Penicillium, Stemphylium* and *Trichothecium*.

Paddy rice on-farm showed the presence of several field fungi as a result of the favourable temperature and relative humidity conditions registered on the stores. In the case of paddy rice on rice mills, those conditions were not so favourable and only storage fungi were detected (*Aspergillus* sp.and *Penicilium* sp.).

The brown-rice samples show higher fungi diversity than the parboiled and the long grain rice, as a result of the processing procedures which reduced the fungi presence.

Insects associated with fungi

On-farm stores most of the insects collected were fungus-feeders (Figure 1). Many species of stored-product Coleoptera are exclusively fungus-feeders as Cryptophagidae, Lathridiidae and Mycetophagidae families and others may supplement their diet by feeding on mould on the surface of grains in humid conditions (*Ashaverus advena* (Waltl) and *Alphitobius piceus* Olivier) (Table 4). When fungi are present, spores may become attached to the insect body surface and are therefore considered vectors of fungi (Haines, 1991). The presence of large numbers of fungus-feeders in paddy stores is an indicator of fungal presence and of appropriate ecological conditions (temperature and moisture) for insect development and reproduction. This type of information should be utilized to initiate actions such as drying, aeration or turning of the grain.

Most of the insect species associated with fungi were found in the farm storage and illustrates some grain management issues. The husk of paddy rice likely carries a great deal of fungal spores, many of which are not killed during drying. The husk is also porous and easily absorbs moisture which in turn allows for the fungal development when temperatures are appropriate. For example, in one farm store with paddy, a large number of fungus-feeders were reported and *Aspergillus niger* van Tieghem, *A. flavus* Link, *A. candidus* Link and *Penicillium islandicum* Sopp were identified from paddy samples taken near the probe traps (Carvalho et al., 2004).

The main commodity-feeders species in stored rice is *Sitophilus zeamais* Motsch followed by *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst), *Cryptolestes* spp. and *Rhyzopertha dominica* (F.) (recorded in one on-farm storage). At the rice mill, more than 90 % of the total of insects caught was commodity-feeders species (Figure 2). High infestations of *Sitophilus* spp. may increased moisture content and allow fungi development.

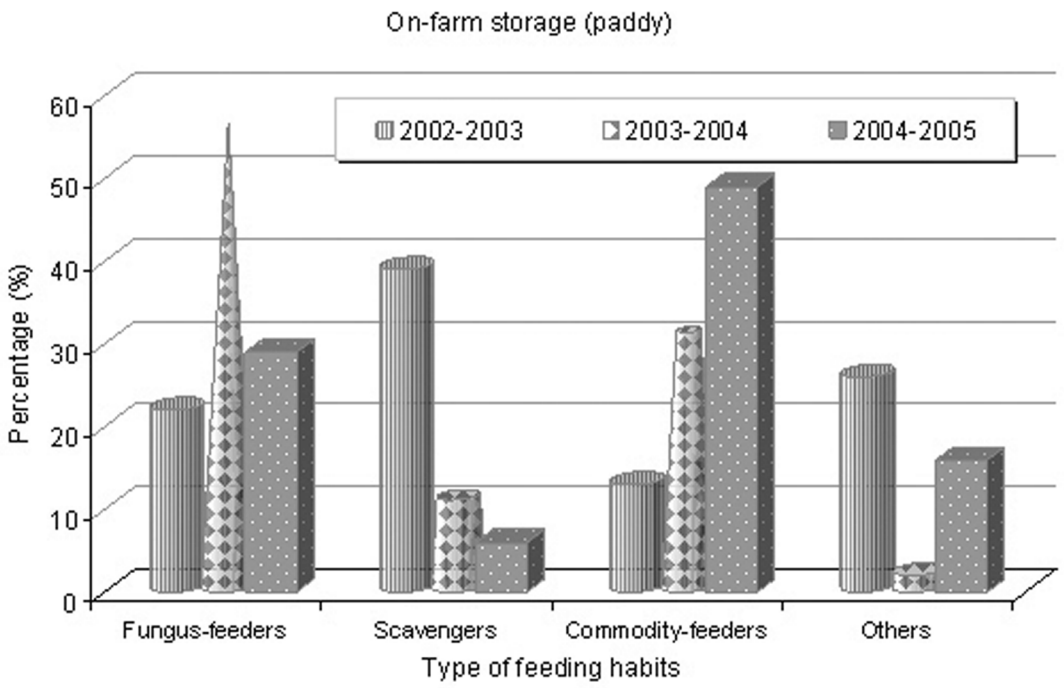

Figure 1. Percentage of the total of fungus-feeders, scavengers, commodity-feeders insects caught in on-farm storage.

Table 4. Insect species: fungus-feeders, commodity-feeders and scavengers caught in the traps placed at the farm storage (Probe and PC traps) and rice mill (Dome traps)

Feeding habit	Species	Farm storage Paddy	Rice mill Paddy	Rice mill Brown rice	Rice mill White Rice	Rice mill Factory
	Bostrichidae					
Ck	*Rhyzopertha dominica* (F.)	+				
	Carabidae					
P	*Harpalus rufipes* (Degeer)	+				
	Cryptophagidae					
F	*Cryptophagus cellaris* (Scopoli)	+				
F	*Cryptophagus saginatus* Sturm	+				
F	*Cryptophagus perrisi* Brisson	+				
	Cucujidae					
Ck	*Cryptolestes turcicus* (Grouvelle)	+				
Ck	*C. ferrugineus* (Stephens)		+	+		+
	Curculionidae					
Ck	*Sitophilus oryzae* (L.)	+	+	+		+
Ck	*Sitophilus zeamais* Motsch.	+	+	+		+
	Lathridiidae					
F	*Coninomus constrictus* (Gyllenhal	+				
F	*Coninomus nodifer* (Westwood)	+		+		

Continue...

Table 4. Continue

Feeding habit	Species	Farm storage Paddy	Rice mill Paddy	Rice mill Brown rice	Rice mill White Rice	Rice mill Factory
F	*Coninomus bifasciatus* (Reitter)	+				
	Mycetophagidae					
F	*Litargus balteatus* LeConte	+				
F	*Typhaea stercorea* (L.)	+		+		+
	Ptinidae					
S	*Ptinus raptor* Sturm	+				
	Ptinus spp.	+				
	Silvanidae					
C,F	*Ashaverus advena* (Waltl)	+				
C	*Monotoma* sp.	+				
Ck	*Oryzaephilus surinamensis* (L.)	+		+		
	Tenebrionidae					
Ck	*Tribolium castaneum* (Herbst)	+		+		+
C	*T. confusum* Duval					+
C	*Gnathocerus cornutus* (F.)	+				
C,F	*Alphitobius piceus*	+				

C = commodity-feeders; Ck = key-pest; F = fungus-feeders; S = Scavengers.

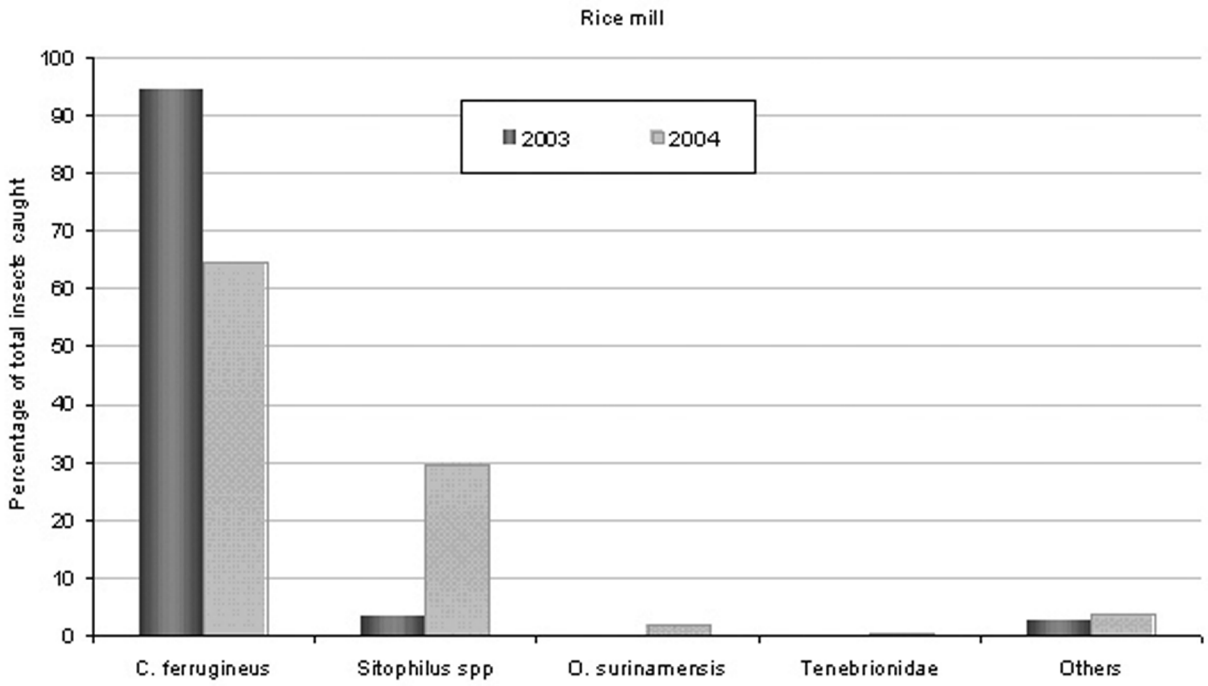

Figure 2. Percentage of the total of commodity-feeders insects caught at the rice mill.

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