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# QUALITY PRESERVATION OF STORED RICE USING MODIFIED ATMOSPHERES IN PORTUGAL

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#### **ABSTRACT**

Portugal is the largest consumer of white rice in Europe, and consequently a large number of farmers and industries are associated with rice production, transportation and processing.

During five years, studies were conducted in six on-farm stores, and three rice industries in Portugal in order to identify the most important noxious agents (insects and fungi associated to stored rice) and implement IPM strategies, such as sanitation, tools for risk assessment, sampling programs, and non-chemical alternatives like ventilation/refrigeration and modified atmospheres as sustainable technologies to replace conventional chemical treatments.

The implementation of these strategies and the dissemination of the results, illustrates an advancement in stored rice protection in Portugal. Currently, many of the rice processing companies apply monitoring programs to assist in decision-making and in the use of strategies such as modified atmospheres (MA) on stored rice.

MA based CO<sub>2</sub> was tested to control *Sitophilus zeamais* and *S. oryzae* in bulk stored milled rice. The trials were conducted in a silo containing 40 tonnes of polished rice and four hermetic big bags of 1 tonne capacity; two with paddy and two with polished rice. The composition of the atmosphere was 90-95% CO<sub>2</sub> and 0.7-2.1% O<sub>2</sub>. Three trials were carried out using different temperature and different treatment times: stored rice in the silo at 30°C for 26 days (first trial) and at 34°C for 10 days, (second trial) and in big bags at 22°C during 26 days (third trial).

The exposure of eggs and adults of *Sitophilus* spp. to modified atmospheres showed mortality close to 100% and no F1 emergency was recorded after each treatment. This was the first time that a Portuguese rice mill used modified atmospheres.

This paper presents some of the most preeminent strategies and results on stored rice in Portugal.

**Key words:** stored rice, insect, fungi, IPM, traps, refrigeration, modified atmospheres

#### 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. Rice production is a seasonal crop in Europe. In Portugal, rice is grown on 18500 ha and the average per capita consumption is the highest in Europe, around 15 kg per year and person (Magro et al., 2006), and consequently around 2000 farmers and eight industries are associated with rice production, transportation and processing. Rice is stored as paddy in on-farm structures or in co-operatives in horizontal warehouses or vertical silos, until the end of winter when the remaining paddy is transported to processing facilities (Carvalho et al., 2004, 2010; Pires et al., 2008; Passarinho et al., 2008. In Europe, among the pest species of stored paddy Sitophilus orvzae (L.), Sitophilus zeamais Motschulsky and Rhyzopertha dominica (F.) are the main weevils present in rice (Trematerra 2009: Lucas and Riudavets, 2000; Pascual-Villalobos et al., 2006). In Portugal, the maize weevil S. zeamais, is the key-pest of stored rice, followed by S. orvzae (Barbosa et al., 2011). A common practice to control hidden infestation in stored paddy is the use of chemical fumigants to prevent insect development. The development of insect resistance to insecticides and consumer concern over the use of pesticides in food has resulted in the search for alternative methods of insect control. Consumers today expect a food product that is pesticide free or with much reduced residue levels (Carvalho et al., 2012). This is a general tendency that industry finds difficult to conform with because insecticides are often necessary to prevent economic damage. In addition, in many countries insects have been developing resistance to contact insecticides and to the fumigant phosphine. The most common non-chemical alternative identified in the rice storage and processing industry was using aeration to reduce the temperature of stored paddy rice (Barbosa et al., 2011; Barbosa et al., 2011). During these studies, the rice mills, for the first time, applied modified atmospheres as alternative control methods. The gas used (CO<sub>2</sub>) is comparatively safe and environmentally friendly and showed to be effective against key pests on both paddy and polished rice (Carvalho et al., 2012). The implementation of these strategies was reflected in the significant decrease of the number of rejected units of polished rice from 111 tonnes of packaged polish rice before the project (2006) to 7 tonnes until the end of the project (January 2009) and continued decreasing to reach only 500 kg in June 2009, which may be interpreted as an increase in consumer satisfaction (Barbosa et al., 2011).

This paper pretends to be a compilation of the strategies and results along these studies.

## MATERIAL AND METHODS

## Monitoring of storage to perform risk assessment

a. Field studies

Experiments were carried out in rice fields in Tejo and Sado valleys, two main rice producer regions in Portugal. The objective of this study was to clarify the origin of stored rice pests. Pests were looked for in rice fields of Sado and Tejo valleys, during the maturation of rice until harvest (during August until the end of September - beginning of October). Different types of traps were used: Moericke, light, adhesive and cromotropic traps. Samples of rice panicles were taken and incubated at the laboratory (Mateus et al., 2008).

## b. From on-farm storage to the rice mill

After harvest, paddy is cleaned and dried to 13-14% moisture. The majority of 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 mills, paddy is stored prior to processing. Both, rice farms and the rice mills can have two types of storage, horizontal warehouses and silos, and have the equipment for cleaning, weighing, drying and aeration.

*Insects:* Studies were conducted in three rice fields, nine on-farm warehouses with stored paddy, and storage units and mills of three rice plants, in order to determine the insect species associated with stored rice and their abundance, distributed through two of the three main rice production regions: Mondego, Tejo and Sado valleys.

For rice in bulk, Storgard WB Probe II traps, without lures, were used and for structures, Storgard Dome traps containing standard attractant oil with or without pheromone (for *Tribolium* spp or for *Sitophilus* spp.) were placed on the floor below rice mill equipments and silos for grain drying. The traps were observed weekly and insects were counted and identified (Pires et al., 2008; Passarinho et al., 2008). Visual inspection and samples of paddy, brown and polished rice were taken to collect psocids and fungi (Kucerova et al., 2006, 2007; Magro et al., 2006).

Fungi: One gram of rice was placed on Potato Dextrose Agar (PDA) medium. For each sample, three replicates were made. These grains were incubated during a week to available fungal growth. Isolation of the colonies was made to obtain pure cultures. The identification was carried out using identification keys (Carmichael et al., 1980; Domsch et al., 1980; Onions et al., 1981; International Mycological Institute, 1991;; Hanlin, 1997; Malloch, 1997; Pitt & Hocking, 1997; Barnett & Hunter, 1998).

## c. Monitoring environmental conditions

Data loggers were used to measure the temperature and relative humidity of the cereal and of the environmental conditions of the rice plant. The grain moisture was measured mainly using two methods: by heating and by the Trime GW sensor device, that measures the values of grain temperature and moisture, in real time, using TDR technology. The rice temperature was monitored at several locations using temperature probes and data loggers placed on the surface and at several depths of rice in bulk in warehouses and silos.

d. The susceptibility of different varieties of rice produced in portugal to the attack of Sitophilus zeamais

The grain samples of 20gr, of four rice varieties (Gládio, Albatros, Thaibonnet and Eurosis) under three types of post-harvest treatment (polished, brown and paddy) were artificially infested with 20 *S. zeamais* adults and kept in laboratory conditions at 27° C and 70% relative humidity. Ten replications of each treatment were experimented including ten replications for control. At the end, the following resistance parameters were evaluated: Dobie index, mean developmental time, percentages of weight loss and damaged grains (Antunes, 2011).

e. Examining the use of modified atmospheres to control Sitophilus zeamais and S. oryzae on stored rice

The trials were conducted in a silo containing 40 tonnes of polished rice and in four hermetic big bags of 1 tonne capacity each: two with paddy and two with polished rice. Gas was supplied through a battery of food grade CO2 contained in 13

cylinders of 30 kg each The composition of the atmosphere ranged 90-95% CO<sub>2</sub> and 0.7-2.1%O<sub>2</sub>. Three trials were carried out using different temperature and different treatment times: stored rice in the silo with 29.6±0.1°C during 26 days (first trial) and 34.1±0.2°C during 10 days, (second trial) and in big bags at 22°C during 26 days (third trial).

To evaluate the efficacy of the treatment, metal cages with 16 g of infested rice were placed at bottom, middle, top and surface of the polished rice in the silo. Four replications of infested brown and polished rice containing one-week-old of *S. zeamais* adults or eggs of *S. zeamais* were incubated at laboratory at the same temperature as in the silo, served as control (Carvalho et al., 2012).

## RESULTS AND DISCUSSION

## Monitoring of storage to perform risk assessment

#### a. Field studies

Traps placed in the fields did not catch insects associated to storage and no insects emerged from the incubated panicles. Nevertheless, traps placed outside the stores, which were empty by that time, and next to the fields, caught *Sitophilus* sp. (Coleoptera, Curculionidae), *Cryptolestes* sp. (Coleoptera, Laemophloeidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), indicating that the storage environment may constitute a source of pests infestation. Measures must be taken in order to avoid rice infestation between harvest and storage, and to avoid insect entrance into the stores while rice is stored there (Mateus et al., 2008).

## b. From on-farm storage to the rice mill

The list of insect species collected from harvest until package is shown in Table 1.

In on-farm storage moisture content was the major factor that affects paddy storage. Given that the relative humidity determined from this study (during paddy storage) ranged from 75% to 85%, this may explain the presence of *Cryptophagidae* and *Mycetophagidae* spp as the main insect species that are fungus-feeders. Mycotoxin producing fungi were also detected. At the rice mills the main insects caught were commodity feeders and the key-pest in Portugal is *Sitophilus zeamais* Motschulsky followed by *S. oryzae* (L.) (Coleoptera, Curculionidae), *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae) and Cryptolestes *ferrugineus* (Stevens) (Arthur et al., 2007, Barbosa et al 2011, Carvalho et al., 2004, 2005; Magro et al., 2006; Pires et al., 2008).

At the rice mill, the main insects caught were commodity feeders, especially *Sitophilus* spp. (mainly *S. zeamais* followed by *S. oryzae*). Predators and parasitoids were recorded but most of them were occasional. During trials, when *Sitophilus* spp and *Cryptolestes ferrugineus* populations were high, their associated parasitoids, *Anisopteromalus calandrae* and *Cephalonomia waterstoni*, were also reported with some abundance. Fungus–feeding insects were also collected and identified: *Ahasverus advena*, *Coninomus* spp., *Cryptophagus* spp., *Typhae stercorea* and *Litargus balteatus* but at much lower populations when compared with commodity-feeders.

Psocids records from Portuguese rice stores and comparison with worldwide psocids occurrence in stored rice and other cereals are given. Five psocid species (Psocoptera, Liposcelididae) were recorded as new stored product species for Portugal found in stored rice. Three of them (*Liposcelis entomophila* (Enderlein), *L. rufa* Broadhead, *L. tricolor* Badonnel) are new species for Portugal fauna as well (Kucerova et al., 2006, 2007).

Table 1. List of insect species of stored products caught in stored rice and rice mills

FH	Species	On-farm storage			Rice plant				
		Rice							
		Paddy		Brown	Polished	Facility			
	Anobiidae								
С	Stegobium paniceum (L.)	+							
	Anthicidae								
С	Anthicus floralis (L.)	+		+					
C	Anthicus quadriguttatus Rossius	+		+					
	Bostrichidae								
С	Rhyzopertha dominica (F.)	+				+			
	Carabidae								
P	Harpalus rufipes (Degeer)	+							
	Laemostenus complanatus (Dejean)	+							
	Cryptophagidae								
F	Cryptophagus cellaris (Scopoli)	+							
F	Cryptophagus saginatus Sturm	+							
F	Cryptophagus perrisi Brisson	+							
	Cucujidae								
С	Cryptolestes turcicus (Grouvelle)	+							
С	C. ferrugineus (Stephens)		+	+		+			
	Curculionidae								
Ck	Sitophilus oryzae (L.)	+	+	+		+			
Ck	S. zeamais Motschulsky.	+	+	+		+			
	Lathridiidae								
F	Coninomus constrictus (Gyllenhal)	+							
F	C. nodifer (Westwood)	+		+					
F	C. bifasciatus (Reitter)	+							
	Mycetophagidae								
F	Litargus balteatus LeConte	+							
F	Typhaea stercorea (L.)	+		+		+			
•	Nitidulidae								
С	Carpophilus dimidiatus (F.)	+							
	Ptinidae								
S	Ptinus raptor Sturm	+							
	Silvanidae								
C,F	Ashaverus advena (Waltl)	+							
C	Monotoma sp.	+							
С	Oryzaephilus surinamensis (L.)	+		+					

FH	Species	On-farm storage			Rice plant			
		Rice						
		Paddy		Brown	Polished	Facility		
	Staphylinidae							
P	Aleochara sparsa Heer			+				
P	Leptacinus linearis (Grav.)	+		+		+		
	Tenebrionidae							
C,P	Tribolium castaneum (Herbst)	+		+		+		
C,P	T. confusum Duval					+		
C,P	Gnathocerus cornutus (F.)	+				+		
F	Alphitobius ovatus (Herbst.)	+						
F	Alphitobius piceus Olivier	+						
	Pyralidae							
C	Ephestia kuehniella Zeller	+						
C	Plodia interpunctella (Hubner)	+						
	Gelechiidae							
С	Sitotroga cerealella (Olivier)	+						
	Pteromalidae							
Pc	Lariophagus distinguendus (Förster)	+						
Pc	Anisopteromalus calandrae (Howitz)			+				
	Braconidae							
Pl	Braconinae sp.	+				+		
	Bethylidae							
Pc	Cephalonomia waterstoni Gahan	+		+		+		
	Anthocoridae							
P	Lyctocoris campestris F.					+		
P	Xylocoris flavipes(Reuter)	+				+		
	Acaridae							
С	Glycyphagus domesticus (De Geer)	+				+		
P	Cheyletus spp	+		+		+		
F	Psocoptera	+	+	+	+	+		

C=Commodity -feeder; Ck=Key-pest; F=fungus-feeder; P=predator; Pc=Coleoptera parasitoid ; Pl=Lepidoptera parasitoid

Insect infestations are likely to occur relatively soon after conditions become appropriate. The insect pest infestations (especially *Sitophilus* spp.) create damage and consequently allow for more fungal production. Those managing rice should consider the damaged grain, from these two factors, seriously as it results in direct economic loss (Carvalho et al., 2004, 2005).

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.

#### c. Risk assessment

The insects' populations fluctuations followed the temperature oscillations inside the rice plant while the stored rice can be at risk all over the year because temperatures are always above the lower developmental threshold of some 13°C-18°C depending species (Barbosa et al., 2011; Fields, 1992). Hidden infestation of *S. zeamais* can surpass all the process until package.

Studies on the susceptibility of different varieties of rice produced in Portugal showed that grain temperature of stored rice was different among varieties and among paddy and brown rice which may confer different susceptibility to grain weevil attack (Pires et al., 2008). Laboratory experiments demonstrated that the physical characteristics were not directly related with the number of emerged insects by female and with the biological cycle. The chemical composition of the rice appears to influence rice susceptibility to *S. zeamais* attack. Brown rice varieties showed the highest susceptibility, followed by polished and finally paddy. The polished rice varieties more resistant were Eurosis and Gladio and the more susceptible was Thaibonnet (Antunes, 2011).

## d. Examining the use of modified atmospheres to control Sitophilus zeamais and S. oryzae on stored rice

Figures 1 and 2 show that the exposure of eggs and adults of *Sitophilus* spp. to modified atmospheres showed mortality close to 100% and no  $F_1$  emergency was recorded after each treatment.

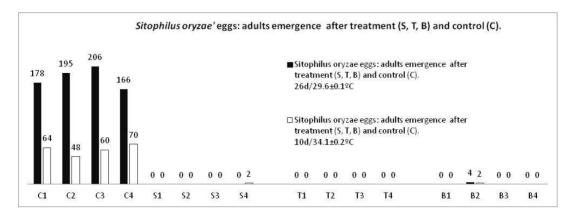


Fig. 1- *Sitophilus oryzae* eggs: adults emergence after treatment with CO<sub>2</sub> (S, T, B) and control (C) during 26 days with the rice temperature of 29.6±0.1°C and during 9 days under 34.1±0.2°C. (S= surface, T= Top, B= Button)

The treatment suppressed eggs, early larvae and adults of *Sitophilus* spp. The exposure time to the gas depended on grain temperature. Increasing rice temperature could lead to

decrease in exposure time to the gas and could thus suppress development of insect pests (Barbosa et al., 2011, Carvalho et al., 2012).

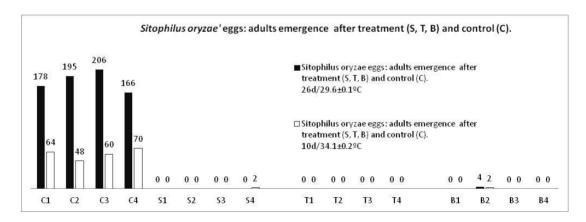


Fig. 2- Sitophilus zeamais: adult mortality after treatment with CO<sub>2</sub>(S, T, B) and control (C) during 26 days with the rice temperature of 29.6±0.1°C and during 9 days under 34.1±0.2°C. (S= surface, T= Top, B= Button)

#### CONCLUSIONS

The implementation of monitoring programs to identify the noxious agents sounds fundamental for risk assessment and to help to decision-making. Together with the application of alternative control methods more environmental friendly and innocuous to consumer to protect the stored rice, applied by the rice mills in Portugal was reflected in the increase rice quality and the significant decrease of the number of rejected package units of polished rice, which may be interpreted as an increase in consumer satisfaction (Carvalho et al., 2011, 2012). These strategies can be classified as social, economical, and environmentally sustainable

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