

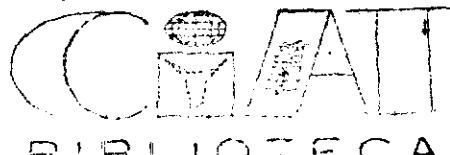


~~SEED BORNE PATHOGENS OF RICE~~

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Rice (Oryza sativa L.) is the most widely grown cereal crop of the world, the average production accounting to about 250 millions tons annually (98, 99). About half of the production is grown in the tropics (66) and it serves as the staple food for more than one-half of the world's population (104). Since rice is not propagated vegetatively the perpetuation of the crop depends on the sexual seed production. The importance of rice in world food production and the significance of seed transmission of several important diseases of this host is, therefore, of concern of man in his unending quest for more and better food. Heavy losses of rice are due to parasitic diseases of which the principal are blast (Pyricularia oryzae), brown leaf spot or seedling blight (Cochliobolus miyabeanus; conidial stage Helminthosporium oryzae), leaf scald (Metasphaeria albescens; conidial stage Rhynchosporium oryzae), bacterial leaf blight (Xanthomonas oryzae) and rice leaf or white tip nematode (Aphelenchoides besseyi). All are seed borne. Fungi cause the largest number of plant diseases and these microorganisms occurs more commonly in seeds than do bacteria or nematodes. So far, no virus disease is known to be seed-borne. The mycoflora may consist of saprophytic fungi and/or pathogenic fungi. The saprophytes are non-host specific species and may be found on seeds of various plants, whereas each pathogenic fungal species is generally confined to a limited host-range. Both may be borne superficially attached to the

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outer seed surface in cracks, or inside the seed coat, but the pathogens may also be present within the cotyledons and other tissues of the embryo (73). Considering just rice and wheat, the cereals of which most of the harvest goes for human food, the losses are about 30 million metric tons. This represents enough food for keep 150 million people alive for a year. The most important part of this picture, however, is that these losses are more severe in those countries which can least afford them; the poorer and under developed or emerging countries of the world. Although the losses are 5% on a world wide basis, in India, and in parts of Africa and South America the losses may reach 30% of the harvested crop. In all of these losses fungi play a major and important role, although this was not fully realized until recently.

The damage caused by fungi varies from spots invisible to the naked eye to complete rot of the endosperm. Further damage may occur during storage. Generally, the same fungi cause heavy spotting or discoloration of the hulls. Kernel spotting increases in damp or warm, rainy weather and in rice that matures late in the season. The presence of spotted or stained kernels reduces the grade of rice. Kernels that are spotted severely and therefore chalky break into pieces in the milling process; thus, kernel spot reduces yield of head rice (5).

Today that agriculture has reached a very high level of technification it is imperative to obtain a good quality of seed. Seeds are both victims and efficient vehicles of diseases. Seed transmission of pathogens is now recognized as the method for excellence by which plant pathogens (a) are introduced into new areas, (b) survive periods when

the host is lacking, (c) are selected and disseminated as host specific strains, and (d) are distributed through the plant population as foci of infection (8). Seeds that harbor pathogenic fungi are important to agriculture because: (a) infected seeds may not germinate and the resulting decreased seedling stand may lead to a reduction in yield; (b) infected seeds can provide inoculum that, under suitable conditions may initiate an epiphytotic that can lower yields; (c) as it was mentioned above, infected seeds can introduce pathogens into areas that are free of the pathogens; (d) infected seeds, even though they are treated with a chemical may still harbor enough viable pathogens to result in any of the above situations and, (e) infection of seed by pathogenic microorganisms before harvest may cause a reduction in seed quality and yield.

An idea of the importance of microorganisms on seeds can be achieved by looking at the number of genera which have been reported from rice seeds. Padwick (105) mentioned about 17 genera which inhabit seeds; the United States Department of Agriculture (152) listed about 24 genera; Richardson (117, 118) prepared a list of some 40 genera comprising the most important disease agents of this cereal. More recently, I made an extensive literature review of seed borne pathogens of rice and I accounted more than 50 genera of microorganisms that inhabit rice seeds (Table 1). From this table it can be observed that more than 100 species of fungi and a reduced number of bacteria and nematodes are associated with the rice grains.

The fungi may be divided into two major groups: one group consists of field fungi which are more or less parasitic and infect the grains before

TABLA 1. LISTA PARCIAL DE MICROORGANISMOS ASOCIADOS CON LA SEMILLA DE ARROZ

ORGANISMOS	REFERENCIAS
<u>Hongos</u>	
<i>Acrocylindrium oryzae</i> , syn. <i>Sarocladium oryzae</i>	138
<i>Alternaria longissima</i>	114,117
<i>A. tenuis</i> , syn. <i>A. alternata</i>	69,78,135,152
<i>Ascochyta oryzae</i>	152
<i>Balansia oryzae</i> , syn. <i>Ephelis oryzae</i>	53,55,92,93,113,152
<i>Botrytis</i> sp.	10,11
<i>Brachysporium</i> spp.	117
<i>Cephalosporium</i> spp.	114
<i>Cochliobolus miyabeanus</i> , syns. <i>Helminthosporium oryzae</i> , <i>Drechslera oryzae</i> , <i>Ophiobolus miyabeanus</i> , <i>Septosphaeria rostrata</i>	4,7,10,11,25,27,40,47,50,52,65,68,69,72,78,98,114,135,143,152
<i>Cochliobolus lunatus</i> , syn. <i>Curvularia lunata</i>	2,4,12,39,44,68,75,78,85,102,112,114,135,152,153
<i>C. spicifer</i>	131
<i>C. bicolor</i> , syns. <i>Helminthosporium bicolor</i> , <i>Drechslera bicolor</i>	27
<i>Chaetomium</i> spp.	80,114,152
<i>Cladosporium cladosporioides</i>	114,117
<i>Cl. herbarum</i>	152
<i>Claviceps oryzae</i> , syn. <i>Ustilaginoidea virens</i> .	45,55,152
<i>Curvularia affinis</i>	4,112
<i>C. brachyspora</i>	152
<i>C. clavata</i>	12
<i>C. cymbopogonis</i>	12
<i>C. eragrostidis</i>	12,26

Cuadro 1. (Continuación)

ORGANISMOS	REFERENCIAS
<i>C. falcata</i>	152
<i>C. geniculata</i>	13,112,135
<i>C. inaequalis</i>	12
<i>C. intermedia</i>	12
<i>C. maculans</i>	152
<i>C. oryzae</i>	12,67,135
<i>C. pallescens</i>	12,84,112,114,135,156
<i>C. siddiquii</i>	12
<i>C. spicifera</i>	117
<i>C. trifolii</i>	12,78
<i>C. tuberculata</i>	67
<i>C. verruculosa</i>	6,12
<i>C. uncinata</i>	12
<i>Diplodia oryzae</i>	105
<i>Drechslera australiensis</i>	16,78
<i>D. hawaiiensis</i>	16,27,78
<i>D. longirostrata</i>	109
<i>D. maydis</i>	27
<i>D. monoceras</i>	27
<i>D. neergaardii</i>	117
<i>D. sorokianiana</i> , syns. <i>Helminthosporium sativum</i> , <i>Bipolaris sorokiniana</i>	27
<i>D. tetramera</i>	27
<i>Epicoccum neglectum</i>	152
<i>E. oryzae</i>	117

Cuadro 1. (Continuación)

ORGANISMOS	REFERENCIAS
<i>E. purpurascens</i>	68,78
<i>Fusarium arthrosporioides</i>	154
<i>F. avenacearum</i>	108
<i>F. culmorum</i>	108
<i>F. chlamidosporum</i>	108
<i>F. decemlellulare</i>	117
<i>F. dimerum</i>	95,96,97,108,135
<i>F. equiseti</i>	108,114,135,154
<i>F. heterosporum</i>	88
<i>F. lateritium</i>	154
<i>F. longipes</i>	108
<i>F. nivale</i>	103,108
<i>F. oxysporum</i>	117
<i>F. poae</i>	108
<i>F. semitectum</i>	2,68,114,135,152
<i>F. solani</i>	63
<i>Giberella fujikuroi</i> , syn. <i>Fusarium moniliforme</i>	2,4,10,11,114,135,154,156
<i>G. zeae</i> syn. <i>Fusarium graminearum</i>	88,103,108
<i>Helicoceras nymphaearum</i>	105
<i>H. oryzae</i>	152
<i>Hendlersonia oryzae</i>	105
<i>Khuskia oryzae</i> , syn. <i>Nigrospora oryzae</i>	4,25,78,135,143,152
<i>Macrophoma</i> spp.	135

Cuadro 1. (Continuación)

ORGANISMOS	REFERENCIAS
Magnaporthe salvinii, syns, Leptosphaeria salvinii, Helmithosporium sygmoideum Sclerotium oryzae	10,36,89,101,137
Melanomma glumarum	105
Memnoniella spp.	114
Metasphaeria albescens, syn. Rhynchosporium oryzae	19,107
Monascus purpureus	105,152
Mycosphaerella danubialis	117
M. shiraiana	105
Myrothecium verrucaria	18,100
Ophiobolus oryzinus	1
Oospora oryzetorum	117
Phaeotrichoconis crotalariae	74,155
Phoma indianensis	22
Ph. glomerata	22
Ph. jolyana	22
Ph. necatrix	152
Ph. glumarum syn. Phyllosticta glumarum	119,152
Ph. sorghina, syn. Ph. glumicola, Phyllosticta glumicola	117,152
Pithomyces chartarum	56,78
Pleosphaerulina oryzae	152
Podoconis sp.	152
Pyrenochaeta oryzae	152
Pyricularia oryzae	40,47,68,77,80,87, 98,135,153

Cuadro 1. (Continuación)

ORGANISMOS	REFERENCIAS
<i>Rhinocladiella</i> sp.	80
<i>Rhizoctonia</i> sp.	10
<i>Sclerotium rolfsii</i>	59
<i>Septoria oryzae</i>	152
<i>Setosphaeria rostrata</i> , syn. <i>Drechelera rostrata</i> , <i>D. halodes</i> , <i>Helminthosporium halodes</i> , <i>H. rostratum</i>	23,27,79,103
<i>Sphaerulina oryzae</i> , syn. <i>Cercospora oryzae</i>	25,40,135,152,154
<i>Stachybotrys</i> spp.	114
<i>Stemphylium</i> sp.	10,11,135
<i>Tilletia barclayana</i> , syn. <i>Neovossia horrida</i> , <i>N. barclayana</i>	54,115,116,129,135, 139,140,152
<i>Trematosphaerella oryzae</i> , syn. <i>Phaeosphaeria oryzae</i>	105
<i>Trichoderma viride</i>	80,152
<i>Trichoconis caudata</i>	152
<i>Trichoconis padwickii</i> , syn. <i>Alternaria padwickii</i>	2,3,4,24,47,52,68, 72,80,86,98,114,131, 135,136,143,154
<i>Trichothecium</i> sp.	2,114
<i>Ulocladium</i> sp.	68
<i>Wolikia decolorans</i> , syn. <i>Protascus colorans</i>	152
<u>Bacterias</u>	
<i>Pseudomonas syringae</i> , syn. <i>Ps. oryzaicola</i>	64,117
<i>Xanthomonas itoana</i> , syn. <i>Erwinia herbicola</i>	117
<i>X. kresek</i>	117
<i>X. translucens</i> f. sp. <i>oryzaicola</i> , syn. <i>X. oryzaicola</i>	15,48

X. oryzae 10,11,20,21,47,125,
128,131,134

Nematodos.

Anguina sp.	117
Aphelenchoides besseyi	60,147,152,159
Ditylenchus angustus	105
Dorylaimus sp.	17
Helicotylenchus sp.	17
Trophurus sp.	17
Hirschmanniella sp.	17
Tylenchorhynchus sp.	17

harvest. The other group contains the storage fungi which usually are saprophytes and develops after harvest. Generally, most of the seed transmitted pathogens are fungi. Some classes or genera are frequent in seed, others occur only occasionally, others do occur but cannot be revealed by conventional testing procedures, and the extent to which fungi occur in seeds depends on their capability to survive under the extreme dry condition of seed as a carrier (99). The fungi have the ability to invade the seed prior to, during, or following germination, either killing the young seedling outright, causing root and crown rots, or becoming established on the cotyledons and from there to the above ground-portions of the plant later on. Fungi may be present as mycelium (e.g. Rhizoctonia sp.) spores (e.g. Helminthosporium oryzae) or fruiting structures (e.g. Phoma glumarum). In addition, there are a great many soil inhabiting plant pathogens which are able to attack the young seed or seedling. Such fungi are species of Sclerotium, Pythium, Achlya, Fusarium, Helminthosporium and Rhizoctonia among others.

FIELD FUNGI

Field fungi invade the seed either while the plant is still growing or after it has been cut before threshing. According to Christensen (32), "it is common to isolate field fungi from close to 100% of freshly harvested surface disinfected kernels of sound rice of the highest grade". In stored rice, field fungi die gradually or rapidly, depending on moisture content and temperature and on the competition of other fungi especially the storage fungi. One of the major environmental factors which determines whether an organism will be adapt as a field fungus or storage fungus is

the moisture requirements of the organism. All plant products are hygroscopic. All hygroscopic materials will tend to come to a steady moisture content condition in equilibrium with the surrounding atmosphere; either gaining or losing water to do so. This steady moisture content is called the equilibrium moisture content (EMC), which depends on both relative humidity (RH) and temperature. Any changes in either or both of these factors will change the EMC of the material. The relationship between the EMC and RH is direct being inverse with respect to temperature. For the starchy cereals such as rice, the field fungi require between 20-25% of moisture content. These moisture contents correspond to the EMC of cereals at about 90% RH. Once the moisture content drops to about 14% the organism die rapidly. Although it was stated that these organisms usually do not cause storage losses, they can occasionally cause such losses. If the seeds or grain is harvested at high moisture contents (above 20-25%) and storage at high RH, these fungi can continue to grow and cause damage. The main reason that storage losses due to field fungi seldom occurs is due to the utilization of seed certification programs, which require seeds to be dried to moisture content closer to those in equilibrium at relative humidity between 70-90%. Christensen and Lopez (34) observed that the percentage of surface disinfected grains of Bluebonnet 50 yielding field fungi decreases with increasing moisture content and with longer time, and the field fungi die in close to 100% of the kernels before germination percentage began to decrease. Death of field fungi in barley and wheat seeds stored at approximately the same moisture contents as those samples of rice has been reported (31,81).

The damage caused by field fungi varies from small brown color spots to complete stained of the glumes. Removing these, the disturb may affects the endosperm and even the embryo; thus reducing germination severely (Figure 1). The effect of field fungi in rice seed germination is evident (4,10,19,22,24,94,131,136,156).

The field fungi may live for years in grain stored at low moisture content (30), their survival like that of the embryos of the seeds themselves, being favored by low moisture content and low temperature. Under the usual condition of storage of food and feed grains, for instance, Fusarium may die within a few months; Helminthosporium may not survive much longer; and Alternaria may not be recoverable from more than a small percentage of seed storage for a year (41,71,148).

STORAGE FUNGI

Pioneering work of the role of fungi in the deterioration of storage grains was not begun until the 1930's. It continued through the 1940's, and full realization of their importance was not achieved until 1950's. Even today, many of the grain merchandasing business do not realize that fungi play a decisive role in their operations, profits and losses. The storage fungi are generally saprophytes or facultative parasites. These fungi do not invade grains to any appreciable degree or extent before harvest (149,150). This is now well established from tests with many different lots of cereal grains, over a long period of time, and with samples from many different sources. Storage fungi invade and grow in grains whose moisture contents are between 14-20% for the starchy cereals.

These are moisture contents in equilibrium with relative humidities of about 70-90%. In those environments storage fungi are among the most abundant and successful of all living things. Almost inevitably they contaminate all seeds. The organisms comprising this group are about twelve species of Aspergillus, several species of Penicillium and less frequently a few other genera of fungi such as Rhizopus, Mucor and Absidia (33,114,124,143). Species of Penicillium are encountered at times, usually in lots of grains stored at low temperatures and with moisture content above 16%. Each of the species of Aspergillus has its own rather sharply defined lower limit of moisture content (or relative humidity) below which it will not grow, and competition with associated species may establish a less sharply defined upper limit. Thus, determination of the number and kinds of fungi in a given lot of seed at times indicates the moisture content and temperature at which the grain has been stored, and sometimes even the approximate length of time it has been stored (33). Although several factors are involved in the selection and succession of microorganisms, the most important by far is the moisture content of the seed. Temperature plays a role in this selection and succession but, since temperature requirements of these organisms are not nearly as restricted in range as are their moisture requirements, the effect of temperature is usually only important at the very low temperatures (Near freezing) or the very high temperatures (70-75°C). Much rice is harvested with a moisture content above 16% (123) and, like other seeds, is subjected to postharvest invasion by storage fungi. This invasion may result in decreased germinability (34,35,49), discoloration of the grains (123) even in the absence of heating, biochemical changes that make grains unfit or unattractive for

food and, production of toxins that constitutes a health hazard for animals and humans (57). According to Christensen and Lopez (34) at moisture contents above about 14.5% invasion of rough rice by storage fungi and decrease in germination percentage of the seeds were proportional to increasing moisture content and the increasing length of storage. In rice, the most obvious economic loss associated with fungal deterioration during storage is loss of grade in white milled rice (121,122). Rice may be downgraded for: (a) damaged Kernels, (b) heat-damaged Kernels, (c) change in general color, and (d) musty or moldy odor. All the other factors are or may be caused by the activities of fungi during storage (124). Losses in storage are a direct result of the activities of various macro and microorganisms made possible by man's failure to understand and follow sound storage principles and practices.

Storage fungi are post harvest invaders. Many common species can invade and grow in seed with little or not free water. Christensen (32) found that many of the common species invade stored grain with moisture contents between 13-18% in equilibrium with relative humidities of 70-85%. However, some species can invade and compete with field fungi, growing rapidly and causing loss in quality in seed with moisture contents above 18%. Since rice is usually harvested with a moisture content between 18-22%, drying or conditioning until the moisture is below 18% must be done promptly. Propagative units of common species of storage fungi are found on the surface of almost every Kernel of rice after harvest (124). Although the fungal flora of rice has been studied in the United States and abroad, a complete listing of all fungi that have been observed associated with stored rice has not been assembled.

The storage fungi are generally saprophytes or facultative parasites and are mainly species of the Aspergillus and Penicillium groups (124). The genus Aspergillus comprises species of A. versicolor, A. sydowi, A. repens, A. parasiticus, A. restrictus, A. ruber, A. amstelodami, A. candidus, A. flavus, A. halophibus, A. niger, A. glaucus, A. ochraceus and A. chevalieri (14,83,124,146). A. glaucus and A. flavus groups, however, appear to constitute the major fungal species that invade stored rice (124). Although insects directly damage grain through penetration and consumption of kernels, they also influence the activities of fungi (29). Serving as carriers and vectors of propagative units they also increase available moisture and rise the temperature, thus fostering and stimulating increased fungal activity. Aspergillus flavus, A. repens and A. restrictus, for instance, have been found associated with the increased moisture content in rice containing weevils (Sitophilus oryza) (62).

Species of Penicillium such as P. puberulum are encountered at times, usually in lots of seed stored at low temperature and with moisture content above 16% (33,117).

CONTROL OF FIELD PATHOGENS

Preventive methods in seed fields.

1. Selection of seed production areas. Seed should be produced in areas where the pathogen populations is unable to establish or maintain itself. The physical environment-especially low relative humidity-markedly restricts incidence of many diseases and prevents infection of the seed produced.

2. Cultural practices. Seed planted in seed fields should be free of pathogens; such seed can be obtained: (a) from areas free of pathogens or where it is unable to infect and (b) by seed treatment to eradicate the pathogens. The life cycle of a plant pathogenic organism can be viewed as consisting of four basic phases, survival, transmission, infection, and disease development. Methods of controlling seed diseases have the objective of interfering with the disease cycle in a way that will reduce subsequent disease development. Seed treatment affects the cycle at the survival and transmission phases by reducing seed-borne or soil borne inoculum. Seed health testing can be used when enough is known about the disease cycle to establish tolerance levels for seed borne pathogens. Some of the chemicals listed in Table 2 have been reported to be effective in reducing the amount of primary inoculum when they are spray at the reproductive stage. Fungicide application to the growing crop usually affects the infection and disease development phases. Cultural practices, such as host resistance, crop rotation, destruction of residues, or alteration of the harvest date, can affect any of the four phases of the cycle. Research is needed to find better methods of control. Some promising results have been reported. It has been demonstrated, for instance, that increasing spacing between rice plants reduce the incidence of seed borne infection causes by Trichoconis padwickii and; decreasing the rate of nitrogen fertilization will reduce the incidence of both T. padwickii and Tilletia barclayana (3,139,140). Additionally, field experiments have showed that early heading decreases the severity of T. barclayana (140) and early or late planting reduce the incidence of Balansia oryzae (92).

3. Point of origin inspection. This method of control is useful in rejecting seed from fields with high incidence of seed transmitted pathogens. However, apparent absence of a disease may not insure freedom from the pathogens in the seed because (a) the pathogen may be present without producing symptoms in the field and (b) pathogen free seed may be contaminated by pathogens during threshing, cleaning, or other handling operations.

Preventive and Control Methods for Harvested Seed.

1. Seed Indexing. The detection of seed borne pathogens is best done after seed has been cleaned and packaged. The best method is to plant the seed in soil, as this approximates actual conditions. The soil used should be: (a) non-treated field soil, to determine the actual field hazard; (b) treated at 100°C for 30 minutes with steam to indicate the maximum potential transmission; (c) treated at 60°C for 30 minutes with aerated steam to give some indication of the extent to which soil antagonists will inhibit transmission (9).

Special direct methods have been devised for microscopic examination of seed on blotter or agar tests. These methods are described at the end of this article. The methods are subject to great variability in comparative tests in different laboratories, and must take into account special requirements of different microorganisms.

2. Seed treatments. Much seed should receive a protection either as oil based dusts, wettable powder slurries or as a direct application of concentrated solution of active ingredients. One purpose of seed treatment by the use of chemical protectants is to destroy seed surface borne microorganisms that cause seedling blights and seed decay and to provide a protective zone around the seed, through which soil borne microorganisms cannot penetrate, thus protecting the developing seedling against attack until it is established on a well developed root system, and has acquired the capability of outgrowing attacks from soil borne pathogens. Hence, the principle of seed treatment is mainly control of primary inoculum in the infection court, although chemical seed treatment may also protect against infection by organisms in the soil. The inorganic and organic mercurials are examples of seed treating materials used to eradicate certain pathogens from the surface of seeds. Organic fungicides, including mercurials, are used as seed protectants to coat the seed and provide protection against damping off and seed-rotting organisms in the soil.

Control or prevention of seed microorganisms depends upon both pre and post harvest measures, including those taken during harvesting, threshing and drying. There exists in the market several chemicals which are reported to be useful to control the most frequent seed-borne pathogens of rice (Table 2). Treated seed, should be stored in a cool dry place and moisture content of the seed maintained below the critical level which will induce germination. Although seed treated with orthocide, dithane M-45 and ceresan do not lose viability for up to 11 months (42),

TAOLA 2 LISTA PARCIAL DE PRODUCTOS QUIMICOS REPORTADOS EFICIENTES PARA CONTROL DE PROBLEMAS FITOSANITARIOS DEL CULTIVO DEL ARROZ

NOMBRE COMERCIAL	INGREDIENTE ACTIVO		Toxicidad (LD 50) en ppm/Kg ²	Helicoverpa oryzae Pyricularia oryzae Cercospora oryzae Phyllosticta spp. Rhizoctonia sp. Tricosepsis palmicallis Alternaria sp. Fusarium sp. Gibberella fujisari Peridermium albescens Coenobolus lunatus Blastocoryza sp. Sclerotinia sp. Botrytis sp. Balanilla oryzae Alternaria tringis Cladosporium chlorocarpum Cochliobolus spicifer Aspergillus sp. Fusarium oryzae Pythium sp. Achlya sp. Xanthomonas oryzae X. oryzae Xanthomonas oryzae Glyptotendipes	Referencias
	NOMBRE COM. OFICIAL	FÓRMULA QUÍMICA			
Antrecol	Propineb	Propileno-bis(ditiocarbamato de zinc	6.000		18
EC-1000	-	2 (Octiloxiflatoz) mas extracto de semilla de toronja	3.500	+	19
Deam	Triclotazolo	S-Metil 1,2,4 - triazol (3,4,5) benzotiazol	250	+	19
Demlate	Bomosal	1 (butilcarbamil) 2 - benzimidazol-carbamato de metilo	9.500	+	20, 142, 151
Brassicol	Quintoceno, PCNB	Pentacloronitrobenzeno	12.000	+	43
Erestan 60	Fentia acetato	Acetato trifenil de estaño	125	+	10
Orthocide	Castan	Cis-7- (triclorometililo) - 4 - ciclohexeno - 1,2 - dicarboximida	9.000	+	10, 19, 50, 90, 125, 158
Orress	Mercurio etílico	Acetato de fenil mercurio	40	+	43, 82, 131, 152
Decenil	Glorotalonil	Tetracloroisoflatozolo	5.000	+	52
Delcene M-200	-	Mancozeb mas carbendazim		+	19
Difolatan	Captafol	Cis-N- (1,1,2,2 - tetraclorometil) tio 4 ciclohexeno - 1,2 dicarboximida	6.200	+	69, 170
Dithane F	Mancozeb	Mancozeb mas zinc ionico.	5.100	+	19
Dilicane M-45	Mancozeb	Etileno-bis(ditiocarbamato de manganeso mas zinc ionico.	7.500	+	50, 69, 106, 157
Dylene	Anilazina	4-6 dicloro - N - (2-clorofenil) 1,3,5 triazina 2 amina	5.000	+	158
Fuji-one	Isopropilolano	Diisopropil 1,3 - ditiolano - 2 tildan malatona	1.340	+	29
Hinosan	Edifenfos	(0 - etil - 5-5- difenil) - fosforoditioato	150	+	19
Maurate D	Mancozeb	Etileno-bis(ditiocarbamato de manganeso mas zinc ionico	6.750	+	158
Pabicide	Tetraclorofalida	4,5,6,7, tetraclorofalida	20.000	+	10
Siracol 50	Piracarbolid	1, metil 5,6 dihidro - 1H - pirrol - 3 - carboxilico	19.000	+	19
Sistane	-	AR6 - butil - alga - fenil - 1 H - imidazol - 1 - propionidulo	1.500	+	52
Tecto 60	Thiabendazole	2-(4- tiazolil) benzimidazol.	3.100	+	93
Terracot L-205	-	PCNB mas 5 etoxi - 1 (triclorometil) 1,2,4 triadiazol		+	29
Arasan 75	Tiram	Bisulfuro de tetrametil - tiram	78	+	76, 120, 151
Tocsin M.	Tiofanato metil	Dietyl 4,4 - 0 - (fonileno) bis 1 - tioalofanato	15.000	+	28
Vitavax	Oxalcarbaxin	5,5 dihidro - 2 - metil - 1,4 - oxaxilinas - 3 - carbaxanilido	3.820	+	19, 43, 52, 93, 158
Vitiguan	Oxalcloruro de cobre	Oxalcloruro de cobre		+	19
Kasumin Plus	Kasugamicina	Kasugamicina mas fosdifen	22.000	+	19
Agrimycin 17	Streptomicina	2,4 Diaminideno 3,5,6 trihidroxi-2,6-dexil -5- deoxi -2-0-10-deoxi -2-3 metiloximino-2C- glucopiranosidil -3- fenil pentahidroquinazolin.	9.000	+	10, 82, 125
Bromuro de metilo	Bromuro de metilo	Monobrometano	-		37, 51
Alusidin	Diazinon	O (2- isopropil -4- metil -6- pirimidinil) fosforotioato de O,O dietilo	100		137
Spectan	Dantón	Beta - etilmercapto etil dietil tiofosfato	2,5		37, 51
Supavan	Triclorfent	12,2,7 - trifenil -1- hidroxil etil fosfonato de dimetilo	450		37, 51

A. Johnson, W.P. 1982. Agricultural chemicals, Books 1-10. Thomson Publications, Fresno, Ca.

Nota: El principal objetivo de esta lista es proporcionar una guía para la identificación apropiada de los productos y en algunos casos se debe considerar como una recomendación de ellos por parte del IIAF.

* Familias también reportados eficientes para el control de enfermedades en los diferentes estados de desarrollo de la planta de arroz.

treated seed should not be stored for long periods of time.

Seed borne fungi. Helminthosporium oryzae and Pyricularia oryzae, two of the most devastating pathogens of rice can be controlled by a wide range of chemicals. Fungicides such as vitavax-maneb, vitavax-orthocide, dyrene and ceresan may retain their effectiveness against both fungi on rice seeds after 6 months of storage (157). Care should be taken, however, with organic fungicides such as arasan 75, brestan 60, beam and hinosan which are classified as intermediate toxic compounds, or ceresan which is classified as highly toxic compound (Table 2). Although other chemicals have moderate to low toxicity, chemically treated seed should NEVER be used for food, for livestock or food for animals or human consumption even though it may have been stored for months or years.

Although a lot is know about seed treatment, we still do not have good chemical seed treatments for certain plant pathogens. This is particularly true since the alkyl organic mercuric compounds have been banned from use. According to Rangaswami and Ramalingam (111) the seed treatment with culture filtrates of Bacillus mycoides increased germination of naturally infected seed with Helminthosporium oryzae, restricted the spread of the fungus in the seedlings, and improved the growth of these. This type of biological control offers an exciting field of research and as long as new seed problems come out this king of control are going to have a rewarded practical application.

Seed borne Bacteria. Bacteria are often present as dry bacterial ooze either on the glumes or in the micropyle. Seed treatment reduces infection by Xanthomonas oryzae, antibiotics being more effective than fungicides (82). Seed treatment with antibiotics such as aureofungin and agrimycin and, fungicides such as ceresan and orthocide give good control of P.oryzae (110) and X. oryzae improving germination and seedling growth (10,82). There are, however, other efficient and less dangerous methods to control this bacteria. Kauffman and Reddy (70), for instance, reported that bacteriophages may play a good role in reducing the bacterial population in germinating seed. X. oryzae may be also controlled by treatments used to break dormancy. According to Subramoney and Abraham (133), soaking the seed in dilute nitric acid for 16-24 hours washed and sun-dried, then soaking again in water and germinated is obtained a good control of the bacteria. It has been reported that Xanthomonaas oryzicola can be controlled efficiently by soaking the seed for 12 hours in a mixture of agrimycin and ceresan and later hot water treatment at 52°C for 30 minutes (125). By irradiating the seed, packing it into polyethylene bags and stored under suitable environmental conditions it is possible to obtain a good control of Pseudomonas spp.(64).

Seed borne nematodes. The leaf or white tip nematode Aphelenchoides besseyi can remain in the rice kernels as long as 2 years (5). The presence of more than 200 nematodes per 100 seeds of rice warranty control measures (51). Some nematicides have been reported to control this nematode efficiently. Chemicals such as Methyl bromide, neguvon and systox among others have shown to reduce the population of A. besseyi significantly (37,51). According to Templeton et al. (142) seed treatment with benlate eradicate the nematode

from infested seeds. Although the application of nematicides is very expensive, it has been reported that the soil treatment with basudin gives satisfactory results in controlling the stem nematode Ditylenchus angustus (132).

3. Thermotherapy. Heat treatment is effective against all classes of seed borne pathogens, but usually is used against pathogens so situated as to be protected from chemicals. There are three types of such treatment: hot water, aerated steam, and hot dry air. Water has twice the thermal capacity of saturated aerated steam, and five times that of dry air. Temperature or time of treatment therefore increases from 49-57°C for 30 minutes for water, through 54-60°C for 30 minutes for aerated steam, to a range of 54°C for 5 hours, to 95-100°C for 12 hours for dry air. Since temperature and time are more or less mutually compensating, it is desirable to standardize on a 30 minute period (9). Hot water treatment has long been used and is very effective in controlling seed borne pathogens of rice. Suryanarayan et al (136), for instance, reported that the treatment of seed infected by Trichoconis padwickii with hot water at 52°C for 10 minutes increases the germination significantly. Although the increase of germination in many cases is not very high, many of the seeds may have lost their viability as a result of infection before the treatment. Excellent control of Balansia oryzae has been also obtained with hot water at 54°C for 10 minutes and by solar treatment (92,93). According to Sinha and Nene (130), soaking the seed in water at room temperature for 12 hours, then at 53°C for 30 minutes can eradicate Xanthomonas oryzae from rice seeds. The seed treatment with hot water at 52-53°C for 15

minutes gives good control of Aphelenchoides besseyi (38). Following any thermotherapy treatment the seed should be treated with a mild fungicide. Since seed germination is somewhat retarded and weakened, this protection from soil microorganisms is beneficial.

CONTROL OF STORAGE PATHOGENS

There are three basic direct methods utilized to control losses by storage pathogens; a) drying to a moisture content safe for storage, b) aeration to maintain low and uniform temperatures and thereby prevent moisture transfers and, c) refrigeration.

Drying. The process of drying is somewhat complicated and involves more than just drying to a moisture content necessary to meet grade standards and for protection from storage losses. The methods used must also maintain quality such as germination, minimum breakage, etc. Sometimes this becomes difficult because of the economics of drying. The longer it takes to dry a load, the more it costs. Thus rapid drying becomes desirable from an economic point of view. Rapid drying, however, requires high temperature (60°C) and this temperature results in low seed germination and chemical alteration. If the grain is to be used for seed it must germinate and a high temperature decreases germination resulting in poorer quality and price for use as seed. If the grain is to be used as feed the importance of high temperature drying is lessened.

If the moisture content is maintained at a uniformly low level, rice can be stored for several years with little deterioration, even when other factors are unfavorable (124).

Aeration. The most general way to maintain low and uniform moisture content, as well as uniform temperatures, is aeration. The main functions of aeration are to a) reduce the temperature of stored grain to about 5-10°C, and b) maintain a uniform temperature, which in turn prevents moisture migration.

Aeration is better and cheaper than other ways of maintaining temperature and moisture. It also has the advantage in that not only does it prevent moisture migration and water from being absorbed into the grain, but it often results in additional reduction of initial moisture content, sometimes as much as 1%. This reduction becomes important for two reasons. First, most of the water removed is lost from the outer layers of the seed, while the inner layers may stay at a high level. Thus, while the inner parts of the seed may have moisture levels conducive to fungal growth or infection, the outer layers may be too dry to allow initial infection to occur. Secondly, such a moisture condition in the seed allows one good protection from fungi under a favorable economic condition since one does not have to reduce the moisture content of the entire seed below the grade requirement.

According to Christensen (35) temperatures below 25°C are preferable, since low temperatures usually decrease the activity of most storage fungi. However, some fungi grow well in the range of 5-10°C. Many Penicillium species grow at low temperatures in the presence of sufficient moisture. He also observed more kernels invaded by species of Aspergillus glaucus group in rice adjusted to an 18.5 percent moisture content and stored at 12°C than in similar rice stored at 27°C. The relation, however, was

reversed for species of the A. flavus and A. candidus groups. Farnse and Christensen (49) stored rice with moisture contents of 12,14 and 16 per cent at 5°C for 465 days without a reduction in germinability, although they observed an increase in the rate of invasion by storage fungi in rice with 16 per cent of moisture.

Refrigeration. This process is usually one of storing grains at 0°C with a moisture content of 20 per cent or more. Cooling to too low a temperature results in large differences between grain temperature and air temperature, leading to moisture migration. When this occurs, organisms such as Penicillium, yeasts and bacteria, which can grow at low temperatures provided that moisture content is about 20-25 per cent, can invade and cause slow deterioration. Both Penicillium and bacteria can grow at or below freezing provided that moisture content is about 22 per cent. Then if the temperature rises, such as when the grain is removed for transport, rapid colonization can occur, resulting in incipient deterioration within 24 hours. So in essence, while refrigeration gets rid of the Aspergilli and other fungi, we may often simply be selecting a new group of organisms as the cause of storage losses. Much more information needs to be obtained about refrigeration with high moisture content before it becomes established as a predominant form of storage and control of microbial deterioration.

Fungicides. In general, the fungicides have little utilization in the control of storage fungi. First, at present, they are generally ineffective and secondly, governmental regulations prevent many potential control fungicides from being used in grain destined for consumption. More recently, however, the use of organic acids in high moisture storage is

becoming more prevalent. One interested in the possibility of chemical control of storage fungi should consult both a specialist in seed pathology and a recent pesticide manual for recommendations.

A P P E N D I X

LABORATORY METHODS FOR DETECTION OF SEEDBORNE MICROORGANISMS

- A. Agar plate test. Two sets of four-hundred seeds per each cultivar are seeded on petri dishes containing 2% agar. Four-hundred seeds are seeded with glumes, the other ones are seeded without them. Seeds are pretreated by soaking 5-10 minutes in a 1% hypochlorite solution. The solution is poured off and the seeds rinse with sterile water prior to planting onto the agar surface. Ten seeds are placed per plate. The seeds are well distributed over the plate and incubated at 20-25% for a period of 4-8 days (Sheppard, 1979). Readings of seed germination and number of seeds from which bacteria or fungi or both have grown after 4 and 8 days are recorded. The different types of bacteria are transferred to nutrient agar plus calcium carbonate tubes and the fungi to potato-dextrose-agar (PDA) plus lactic acid and V-8 vegetable juice agar for later identification and pathogenicity test. The isolates are stored in tubes containing sterilized distilled water.
- B. 2,4-D blotter test. The blotter test is actually a modification of a germination test as the seeds are placed on the surface of moistened filter paper discs. The blotters are saturated with sterile water and the excess water is poured off. As in the agar plate test, two sets of 400 seeds with and without glumes are tested. Seeds with glumes are sown onto the blotter with a pair of forceps. As in the agar plate test, ten seeds are placed per blotter. Since in the blotter

test the growth of seedlings often makes observation of fungi difficult and determination of percentage infection impossible, a 0.2% solution of 2,4-Dichlorophenoxy acetic-acid (2,4-D) to prevent growth of seedlings is used (Neegaard and Saad, 1973; Sheppard 1979). The petri dishes are wrapped in paper in order to reduce evaporation of the 2,4-D in the incubator. They are incubated at 20-25°C with a 12 hour day/night NUV light cycle for a period of 4-8 days.

BIOASSAY OF FUNGICIDES ON TREATED SEED

Test agar. A suspension of a given pathogen washed from the surface of PDA (or other media) petri dish with sterile distilled water is diluted to approximately 150,000 spores/ml. This suspension is mixed with either PDA or V-8 vegetable juice agar in the ratio of 1:4, giving a final spore concentration of 37,500 spores/ml of seeded agar. Twenty ml of the seeded agar are poured into each plate. Five plates with four seeds are used for each chemical and each standard.

Standard curve. A standard curve is prepared to relate the diameter of the zone of inhibition to the concentration of chemical present. Five lots of seeds are treated at five different concentrations, i.e. 0.50, 100, and 1000 ppm. These seeds are planted out on test agar, and the diameter of the resulting zone of inhibition is measured. The average zone for each treatment is plotted on the linear scale of semilog paper against the concentration of treatment (on the log scale). By entering the curve with the zone of inhibition, the concentration of "equivalent effectiveness" for each chemical can be determined.

Testing seeds. The seeds to be tested are pressed into the seed agar to the bottom of the plate so the entire surface of the seed will be in contact with the agar. The plates are incubated for 40-48 hours at 20-25°C. The diameter of inhibition zone around each seed is measured and the average determined. This diameter is converted to equivalent concentration of seed treatment chemical by reference to the standard curve (Mitchell, 1967).

Damage assessment. A lot of seeds is used to study the effect of the given pathogens on seed germination and disease transmission. The seeds are artificially infested in the following manner: Twenty ml of sterile distilled water are placed on each petri dish culture and the mycelium and spores from the agar are separated. The resulting suspensions are combined and enough water is added to bring the concentration at approximately 150,000 spores/ml. Two-hundred seeds are dipped into it and they are placed to dry on a piece of filter paper at room temperature.

One-half of each lot of infested seeds is treated by placing approximately 20 at a time in a small beaker with a small amount of the best chemical from the bioassay test. The other half of the seed will serve as untreated controls. Another lot of seeds is reserved that is neither infested nor treated to serve as germination controls. Also several seeds that have not been infested are treated. These seeds will serve to show any phytotoxic effects the chemical alone may have on the seeds or seedlings.

Small plastic pots are filled with soil or sand autoclaved for 1 hour at a pressure of 15 lbs/sq. in. The seeds are planted (five of the same treatment per pot) in the greenhouse in labeled pots and watered as needed. Every other day observations are made. The number of seeds that germinate and the number of seedlings showing disease symptoms are recorded (Stevens, 1967).

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