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Survival of *Colletotrichum sublineolum* and Other Seed-borne Fungi in Sorghum Seeds after Twenty Months of Storage

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ABSTRACT

Investigations were conducted to determine the longevity of *Colletotrichum sublineolum* in sorghum seeds. Sorghum grains of fifteen exotic, one local resistant and one susceptible (to anthracnose), genotypes were used for this study. The agar plate and blotter methods were used to isolate pathogens belonging to the species *Alternaria, Aspergillus, Cladiosporium, Colletotrichum, Dreschlera, Penicillium* at the end of eight months storage period. Depending on the level of contamination, the presence of mycotoxin producing fungi, *Alternaria, Aspergillus and Penicillium* may reduce the quality of the grains as food and feed materials. Only pathogens belonging to the species *Colletotrichum, Curvularia, Dreschlera* and *Penicillium* were isolated at the end of twenty months. Warwarabashi was the only genotype from which *Colletotrichum* pathogen was isolated, making it an inferior seed material. Warwarabashi had the highest mean seed infection rates of 87 and 56% after eight and twenty months storage, respectively. Initial moisture content of 15% significantly declined to an average of 7 to 8% throughout the storage periods. The result of Blotter test revealed higher seed viability between 52 to 100% when stored for eight months; this decreased significantly (to > 20%) when the storage period is extended to 20 months. One thousand grain weight was also highest (13.6 to 38.4 g), when stored for eight months. The result of this investigation indicates that twenty months storage did not completely eliminate *C. sublineolum* from sorghum seeds rather it may substantially reduce the pathogen. Long storage of the sorghum grain, however, renders the grains un-suitable as seeds and may also reduce the nutritional qualities. To ensure good establishment of seedlings from such infected seeds, treatment with seed protectants will be essential.

Key Words: *Colletotrichum sublineolum;* Sorghum seeds; Longevity; Seed viability; One-thousand grain weight and moisture content

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important world cereal and an important native cereal in Africa (FAO, 1995; FAO & ICRISAT, 1996; Murty & Renard, 2001). Total world production of sorghum in the year 2002 was estimated at about 54 million tonnes (FAO, 2004) and total annual production of about 70 million metric tons of grains from 50 million hectares of land (National Academic Science, 1996). Sorghum is also the dietary staple of more than 500 million people in more than 30 countries (National Academic Science, 1996).

In Nigeria, sorghum is an important staple cereal grown in more than twenty states of the federation including Akwa Ibom and Enugu states (Wudiri & Fatoba, 1992). It accounts for about 50% of the total cereal crops grown in Nigeria. The estimated annual production is about 9 million tons produced on about 6 million hectares (National Academic Science, 1996). Average grain yield of subsistent farmers in the West African sub-region are generally between 500 to 800 kg ha^{-1} (Sharma *et al.*, 1992).

Sorghum is also a principal source of energy, protein, vitamins and minerals to the poorest people of the semi-arid tropics (National Academic Science, 1996). It is used to prepare stiff porridge, thin porridge or fried dumpling. It is also used in the brewing of local beer. The leaves provide fodder for farm animals and the stalk are used in fencing, roofing, weaving baskets and mats and also as fuel wood (Obilana, 1995). Sorghum grains are used industrially in the manufacture of items such as wax, starch, syrup, alcohol, dextrose agar, edible oils and gluten feed. In addition, it is used to manufacture gypsum lath, paper and cloth sizing and adhesives (Onwueme & Singh, 1991; Komlaga *et al.*, 2001; Murty & Renard, 2001).

The major constraints of sorghum production are insects, birds and diseases. Fungi and bacteria are important sorghum disease pathogens. Anthracnose is a seed, soil and trash borne fungal disease of sorghum caused by *Colletotrichum sublineolum (*formerly called *Colletotrichum graminicola).* It is the most destructive foliar disease of sorghum, most prevalent in warm humid regions. Anthracnose affects all plant parts including the, stem, leaf, peduncle, inflorescence and grain (Gwary *et al.*, 2003). Various reports have shown yield losses of up to 67% (Pande *et al.*, 1991; Marley, 1996; Gwary *et al.*, 2002). Little or no information exists about the longevity of *C. sublineolum* on the sorghum seed. It is therefore imperative to investigate the longevity of seed borne anthracnose in sorghum seeds under the local storage conditions in Maiduguri, Nigeria.

MATERIALS AND METHODS

Seventeen sorghum genotypes from different sorghum zones of the world were obtained from the International Crop Research Institute for Semi-Arid Tropics (ICRISAT), India. These genotypes consist of fifteen exotic and two local materials namely IS 854, IS 1006, IS 2508, IS 3552, IS 3758, IS 6928, IS 6958, IS 8354, IS 12467, IS 17141, IS 18442, IS 18758, IS 18760, IRAT 204, ICSV 247, KSV 4 (local resistant) and Warwarabashi (local susceptible). They were grown in the anthracnose screening fields of the Department of Crop Protection, University of Maiduguri. Seeds from infected plots were harvested and dried to 15% moisture content at 37ºC for six days. The seeds were stored in polythene bags at ambient laboratory temperature of about 32 to 36ºC and relative humidity between 40 to 67% for eight and twenty months separately.

Isolation of internally borne seed fungi. The agar plate method was used to isolate fungi associated with the seeds of different sorghum genotypes. One gram of seeds were randomly obtained from each sorghum genotype several times and pooled to obtain samples used. Ten seeds taken at random from each sorghum genotype were plated on Acidified Potato Dextrose Agar (APDA) medium after surface sterilization with 10% sodium hypochloride solution (NaOCl) for five minutes to eliminate all surface contaminants. Each genotype was replicated five times. Plates were incubated at room temperature (25ºC) for seven days.

Blotter test. The blotter method was used to isolate the fungal pathogens associated with the sorghum seeds as well as to determine the viability of seeds after the storage periods. With this method, sterile Whatman's filter papers were placed in sterile 9 cm Petri dishes and moistened with sterile distilled water to provide moist condition. Twenty five seeds of each genotype were placed in each petri dish. Each genotype was replicated four times and incubated at room temperature under for seven days. Daily observations were made to count the number of germinated seeds and fungal colonies that appeared. From both tests resulting fungi were isolated and pure cultures were prepared and used to identify them.

One-thousand grain weight. One hundred grains were counted randomly from each sorghum genotype and weighed. This was multiplied by ten to give the weight of one thousand grains of each genotype. Each genotype was replicated ten times.

Moisture content. Grain moisture content was determined by oven dry weight method using the formula;

Moisture content = dry seed weight – oven dry seed weight/oven dry seed weight x 100.

Data analysis. Data collected was subjected to analysis of variance ANOVA using SPSS version 10 Software.

RESULTS AND DISCUSSION

Fungal pathogens isolated after eight months of storage of sorghum grains in Maiduguri belong to the species *Alternia*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Drechslera* and *Penicillium* and (Table I). After twenty months storage, only pathogens belonging to the species *Colletotrichum*, *Curvularia*, *Drechslera* and *Penicillium* were isolated from sorghum seeds (Table I). Results indicate that the anthracnose pathogen *C. sublineolum* has the ability to survive up to twenty months of storage, internally borne in sorghum seeds. The isolation of *C. sublineolum* and *Drechslera* species confirms the findings of Mazzanii (1988) that both species are highly associated with sorghum seeds. *Colletotrichum sublineolum* normally infects seeds during early stages of seed development and may colonise the seed coat causing necrotic spots, dark brown to black in colour. Infections may be extra or intra embryonic and mycelium may be present in the pericarp, endosperm and/or embryo. The pathogen then becomes active on germination of the seeds (Prasad *et al.,* 1985). Furthermore, infection may also occur during grain harvest or threshing. Mechanical damage in seeds i.e. cracks, breaks or scratches in seed coat or pericarp permits invasion by xerophilic fungi when seed moisture was relatively high between 20 to 25%. Xerophilic fungi differ in longevity essentially due to their capacity to endure desiccation. Longevity of seeds generally depends on their moisture content, temperature and relative humidity in storage. This is because temperature and moisture content are the primary factors that determine the development of storage fungi within seeds. *Colletotrichum sublineolum* was recovered (Fig. 1 & 2) from the most severely infected cultivar, Warwarabashi with the mean percentage seed infection of 87 and 56% after eight and twenty months of storage, respectively (Table I). This supports the understanding that fungal pathogens survive longer in seeds that are heavily infected. It is suggested that *C. sublineolum* interferes with seed development, which result in the production of small sized seeds. In addition, pathogens belonging to the species *Curvularia*, *Drechslera* and *Penicillium* have also been reported to cause seed rot in grains, thereby, lowering seed weight. Table II shows significantly ($p < 0.05$) lower one thousand grain weights after a longer storage period (twenty months) ranging between 13.1 g in IS18760 to 31.7 g in Warwarabashi than after a shorter storage period (eight months), for example 13.6 g ICSV 247/IS17141 to 38.4 g in IS 18758. The moisture content of stored seeds was not statistically significant ($p < 0.05$), showing a decline from the initial 15% to an average 7 to 8% at the end of twenty months storage periods (Table II). Significantly higher seed germinability was recorded between 52% in IS 18442 to 100% in IS 18758 at the end of shorter storage periods (i.e. eight months). Seed germinability significantly declined varing from 12% in IS3758 to 88% IS3552 after a twenty months storage period according to genotype. Warwarabashi seeds on which the highest fungal infection of 56% was recorded (Table I) fungal mycelia can be seen

Table I. Effect of storage period on the type and number of fungi identified from sorghum seeds stored for eight and twenty months

Genotypes	Eight months storage		Twenty months storage	
	Fungi identified	Seed infection (%)	Fungi identified	Seed infection (%)
IS 854	*Penicillium sp.*	43	*Curvularia sp.*	16
IS 1006	None	-	*Drechslera sp; D. ellisii*	26
IS 2508	*Aspergillus sp.*	43	None	-
IS 3552	*Rhizopus; Penicillium sp.*	20	None	-
IS 3758	None	-	*Curvularia sp*	20
IS 6928	None	-	None	-
IS 6958	None	-	None	-
IS 8354	*Drechslera sp*	67	*Drechslera sp*	36
IS 12467	None	-	None	-
IS 17141	None	-	None	-
IS 18442	*Aspergillus sp; Penicillium sp*	93	*Drechslera sp*	36
IS 18758	*Cladosporium sp*	17	None	-
IS 18760	*Drechslera sp*	53	*Drechslera sp*	26
ICSV 247	*Rhizopus sp; Aspergillus sp.*	53	*Penicillium sp.*	13
IRAT 204	*Alternia sp.*	23	None	-
KSV 4	None	-	None	-
Warwarabashi	*Colletotrichum sublineolum*	87	*Colletotrichum sublineolum*	56
Mean		29 ± 8.3		13 ± 4.9

Table II. Mean seed viability, one-thousand grain weight, seed moisture content and % fungal incidence on different sorghum seeds stored for 8 or 20 months

Genotypes	Seed viability (%)		One-thousand grain weight (g)		Seed moisture content (%)		% Fungal incidence on seeds	
	8 mths	20 mths	8 mths	20 mths	8 mths	20 mths	8 mths	20 mths
IS 854	84	20	19.6	18.7	7.64	9.9	43	16
IS 1006	88	28	22.2	20.1	7.28	7.2	50	26
IS 2508	96	36	15.8	15.1	7.73	4.3	43	13
IS 3552	96	88	18.7	20.1	7.42	7.7	20	13
IS 3758	84	12	29.7	18.7	7.42	7.3	43	20
IS 6928	76	84	26.3	13.3	7.42	7.2	27	3
IS 6958	96	44	20.6	20.1	7.63	7.6	53	3
IS 8354	92	56	23.5	21.2	7.42	7.5	67	36
IS 12467	96	20	20.6	17.8	7.64	7.5	27	16
IS 17141	72	56	13.6	15.6	7.42	7.2	37	0
IS 18442	52	28	19.0	25.0	7.42	7.4	93	36
IS 18758	100	52	38.4	21.3	7.42	7.2	17	10
IS 18760	92	52	16.7	13.1	7.87	7.6	53	26
ICSV 247	96	40	13.6	18.9	7.42	7.8	3	13
IRAT 204	84	16	21.5	24.0	7.42	6.8	23	6
KSV 4	84	40	25.7	16.0	7.73	7.1	40	16
Warwarabashi	96	60	27.9	31.7	7.42	7.4	87	56
Mean ± SE	87.2 ± 3.8	43.0 ± 0.2	21.9 ± 1.5	19.4 ±1.1	7.5 ± 3.8	7.3 ± 0.2	42.7 ± 5.7	18.1 ± 3.4

*Mths refers to months of grain storage

covering the emerging seedling emergence from the seed affecting the normal germination both of which can be seen in Fig. 3.

The isolation of mycotoxin producing fungi, *Aspergillus, Penicillium and Alternaria* from the grains especially after 8 months of storage suggests the safety risk in the use of the grains for human or animal consumption. Similar concerns have been expressed when Gachomo *et al.* (2004) reported that 70% of peanut samples collected from Nairobi market were infected with various moulds.

The fungi reported here have earlier been reported to be sorghum contaminants and are capable of producing afflatoxins and other carcinogenic substances (Christensen, 1965 & 1974; Stoloff, 1976; Sharma & Salunkhe, 1991). The fact that most of them are not recovered from the grains after 20 months of storage (Table II) due to decreasing moisture content of the grains appears good if grains are intended for consumption but not so if they are to be used as seed materials since Table II also shows lower seed viability with longer storage period.

In conclusion, storage for twenty months may substantially reduce but not completely remove the anthracnose pathogen from sorghum seeds. The pathogen may be inactivated during storage and may be incapable of producing viable inoculum. The implication of this is that sorghum seeds, particularly such as the anthracnose susceptible variety Warwarabashi will need to be treated with seed protectants before sawing to ensure good establishment. Not a single fungal pathogen was recovered from the genotypes IS 6928, IS 6958, IS 12467, IS 17141 and KSV 4. The use of these genotypes should therefore be encouraged to prevent or reduce fungal diseases in sorghum

Fig. 1. Young culture of *Colletotrichum sublineolum* isolate on oatmeal agar

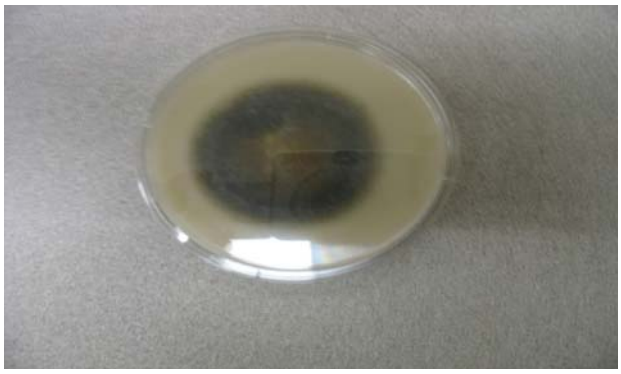

Fig. 2. Sporulating culture of *Colletotrichum sublineolum* isolate on acidified potato dextrose agar

Fig. 3. Healthy and fungus-infected Warwarabashi germinating seeds after 8 months of storage

seeds in the Sudan Savanna region of Nigeria and similar regions.

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