Plant Pathology Journal

ISSN 1812-5387

science alert

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Plant Pathology Journal 7 (1): 98-103, 2008
ISSN 1812-5387

Seed-Borne Infection of Farmer-Saved Maize Seeds by Pathogenic Fungi and Their Transmission to Seedlings

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Abstract: Seed-borne infection of maize (*Zea mays* L.) by fungal pathogens was studied using 22 seed samples of maize collected from different locations in Burkina Faso. The moist blotter test was used to detect fungi on seeds. Experiment was also performed on seedlings raised from naturally infected maize seed samples in order to evaluate the transmission of fungi from seeds to seedlings. Ten pathogenic fungi were recorded on seeds and consisted of *Acremonium strictum* (infection ranging from 2 to 96%), *Bipolaris maydis* (1 to 30%), *Botryodiplodia theobromae* (1 to 17%), *Colletotrichum graminicola* (2 to 8%), *Curvularia* sp. (1 to 39%), *Exserohilum rostratum* (1 to 13%), *Fusarium moniliforme* (38 to 99%), *F. equiseti* (1 to 15%), *F. pallidoroseum* (1 to 23%) and *Phoma* sp. (2 to 50%). *Aspergillus flavus* (1 to 99%), *A. niger* (1 to 99%), *Cladosporium* sp. (1 to 93%), *Penicillium* sp. (12 to 100%) and *Rhizopus* sp. (1 to 51%) were the saprophytic fungi detected. Rates of seedling infection by fungal pathogens were low. Seed samples with 30% infection by *B. maydis*, 8% infection by *C. graminicola* and 26% infection by *Phoma* sp. resulted in 2, 2 and 16% leaf infections, respectively.

Key words: Maize, seed-borne fungi, seed transmission, seed infection, seedling infection

INTRODUCTION

Maize (*Zea mays* L.) is the third most important crop in Burkina Faso regarding the production. It is an important staple for the population. Worldwide, maize is attacked by many pathogens which are seed-borne (Richardson, 1990). Leaf blights and spots caused by different species of *Bipolaris*, *Curvularia*, *Exserohilum* and *Colletotrichum graminicola* (Ces.) Wilson are endemic in maize growing areas (Carson, 2000). Southern leaf blight caused by *Bipolaris maydis* (Nisik. and Miyake) Shoem. (teleomorph *Cochliobolus heterotrophus* (Drechs.) Drechs.), is one of the most damaging diseases in Burkina Faso (Sanou, 1996). Efforts have been made by the breeders to develop high yielding hybrids of maize with high level of tolerance to this disease.

Despite the production of certified seeds of improved varieties of maize, small-scale farmers continue to produce and use their own seeds without knowing the health status of the seed lot. Moreover, so far no work has been done in Burkina Faso to evaluate the health status of maize seeds from formal and informal seed production sectors. Since seed could play an important role in the epidemics in fields, good quality and healthy seed of maize should be released to farmers in order to secure their outputs.

Systemic transmission of fungi from seeds to seedlings is well-documented (Maude and Humpherson-Jones, 1980; Chung and Lee, 1983; Manandhar *et al*., 1998; Shrestha *et al*., 2000). Transmission of *Alternaria brassicae* (Berk.) Sacc. from *Brassica oleracea* L. seeds to seedlings has been demonstrated (Maude and Humpherson-Jones, 1980; Shrestha *et al*., 2000). In many pathosystems, seedling phase of the diseases is very important in the development of epidemics.

The aim of this study was to examine the incidence of seed-borne fungi in maize seeds collected from different locations of Burkina Faso and to evaluate the rates of seed-to-seedling transmission of some fungal pathogens.

MATERIALS AND METHODS

Seed infection

Collection of seed samples: Twenty two seed samples of varieties and landraces of maize (Table 1) were collected

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from different locations in the Houet province, West region of Burkina Faso. The samples were obtained from farmers and breeders at the Institute of Environment and Agricultural Researches (INERA) at Farakô-Ba, Bobo-Dioulasso, Burkina Faso. They were stored at 5°C until use.

Seed health testing: Seed-borne fungi were detected using the blotter method (ISTA, 1999; Mathur and Kongsdal, 2003). Two hundred seeds were randomly taken from each sample. The plates were incubated 24 h at 21-22°C, then deep-frozen 24 h before final incubation at 21-22°C under cycles of 12 h darkness and 12 h near UV-light (Philips TLD 36W/08, Philips Lighting B.V., Roosendaal, The Netherlands). After 5 days of incubation under alternating light conditions, the seeds were examined for fungal growth under stereomicroscope. Identification of fungi was based on their habit character on seeds and microscopic examinations of conidia following Mathur and Kongsdal (2003). According to the authors, seed bearing a fruiting body (conidia, sclerotia, pycnidia or dictyochlamydospores) is considered as infested by the given fungus. Infestation levels were recorded as percentage of infested seeds in each sample.

Seed-to-seedling transmission

Seed samples tested: Three seed samples (Die1, Pal2 and Mat4-2b) having 8, 26 and 30% natural infestation by *Colletotrichum graminicola*, *Phoma* sp. and *Bipolaris maydis*, respectively, were used (Table 2).

Detection of seedling latent infection by pathogenic fungi: Maize seedlings were cultivated under greenhouse conditions at 28±2°C. Five seeds were sown in each 11×11 ×12 cm plastic pot containing sand previously autoclaved at 120°C for 30 min. In total, 100 seeds were sown per sample tested. Leaves and stems of seedlings were collected, respectively 10 and 14 days after sowing. These plant parts were cut in pieces approximately 2 cm long. Leaf pieces were directly plated, whereas stem pieces were surface-disinfected in 70% ethanol for 30 s and in 1% sodium hypochloride for 1 min and dried with sterile blotter papers. Leaf and stem fragments were placed on three layers of moist blotter paper placed in 90 mm Petri dish. The dishes were incubated at 22-25°C under cycles of 12 h darkness and near UV-light as described for seed health testing. Stem and leaf fragments were recorded, respectively 5 and 10 days after incubation, under stereomicroscope for the presence or absence of fungi on each sample. Infection levels were recorded as percentage of infected leaves and stems in each sample.

Table 1: Characteristics of the seed samples collected in December 2003 from different locations of the Houet and Bougouriba provinces, Burkina Faso

Location	Accession	Variety	Destination	Type
Dafinso	Daf4	Obatanpa	Food production	White
	Daf5	ND	Food production	White
Karankasso Sambla	Ksb1	KPB	Food production	White
Kiri	Kir4	ND	Food production	Yellow
Koro	Kor4	FBC 6	Food production	Yellow
Lena	Len 5-2b	SR 21	Seed production	White
Nasso	Nas 3-2r	Local	Food production	Red
Séguéré	Seg 2	Local	Food production	Yellow
	Seg 5	Obatanpa	Seed production	White
Padéma	Pad 3-1b	ND	Food production	White
Samaga	Sam 1	SR 21	Food production	White
	Sam 2	Local	Food production	Yellow
	Sam 3	Local	Food production	Yellow
	Sam 4	SR 21	Food production	White
Vallée du Kou	VDK 3	SR 21	Food production	White
	VDK sb	SR 21	Seed production	White
Matourkou	Mat 2	Hybrid	Seed production	White
	Mat4-2b*	SR 21	Seed production	White
Palla	Pal 1	Local	Food production	Yellow
	Pal 2*	Local	Food production	White
Yéguéresso	Yeg 5	SR 22	Food production	White
Diébougou	Dié1*	ND	Food production	Yellow

*: Seed samples used for testing seed-to-seedling transmission; ND: Not determined

Table 2: Characteristics of the seed samples used for the experiment on seed-to-seedling transmission

	Seed samples[a]		
Fungi	Mat4-2b	Pal2	Dié1
Bipolaris maydis	0	1	30
Colletotrichum graminicola	0	8	0
Curvularia sp.	0	0	39
Exserohilum rostratum	0	0	13
Fusarium moniliforme	97	82	38
Phoma sp.	26	3	7

[a]: The seed samples were selected based on the infection levels of target fungi

RESULTS

Seed infestation

Frequency of infestation: All seed samples were infested at different levels of infestation (Table 3). Ten pathogenic and five saprophytic fungi were recorded. Among the pathogenic fungi, *Acremonium strictum* W. Gams and *Fusarium moniliforme* Sheldom were the most frequent (100% infected samples, respectively), followed by *Phoma* sp., *Fusarium equiseti* (Corda) Sacc., *F. pallidoroseum* (Cooke) Sacc., *Botryodiplodia theobromae* Pat. and *Curvularia* sp. The less frequent pathogens were *Colletotrichum graminicola*, *Exserohilum rostratum* (Drechsler) Leonard and Suggs and *Bipolaris maydis* (Table 3). *Aspergillus niger* van Tieghem and *Penicillium* sp. were the saprophytic fungi which occurred on all samples tested (Table 3).

Table 3: Percent infestation by fungi recorded in 22 samples of maize collected from different locations of the Houet and Bougouriba provinces, Burkina Faso (200 seeds tested per sample)

	Percent infestation of maize seed samples collected from different locations[a]																						
Fungi	Kir 4	Kor 4	Pal 1	Pal 2	Sam 1	Sam 2	Sam 3	Sam 4	Daf 4	Daf 5	Mat 4-2b	Mat 2	Nas3-2r	VDK 3	VDK sb	Yeg 5	Ksb 1	Len 5-2b	Seg 5	Seg 2	Pad 3-1b	Dié 1	Infected seed samples (%)
Pathogenic fungi																							
Acremonium strictum	46	96	39	50	2	5	9	10	37	50	4	16	15	74	29	80	14	31	79	28	74	47	100
Bipolaris maydis	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	30	18
Botryodiplodia theobromae	0	0	0	0	0	4	6	0	2	1	4	0	2	17	1	1	1	12	2	0	9	6	68
Colletotrichum graminicola	0	0	0	8	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	9
Curvularia sp.	4	0	1	0	6	0	1	0	1	0	0	0	0	4	3	1	2	2	0	0	0	39	50
Exserohilum rostratum	0	0	0	0	1	0	0	0	0	0	0	0	0	3	0	0	0	0	4	0	0	13	14
Fusarium equiseti	0	0	6	5	6	0	0	0	15	9	2	1	2	1	0	0	3	1	6	6	2	2	68
Fusarium moniliforme	67	94	90	82	95	95	92	93	98	99	97	95	99	92	88	94	98	89	97	98	96	38	100
Fusarium pallidoroseum	0	0	5	1	3	0	4	0	1	3	1	0	1	2	10	0	5	1	23	0	1	3	68
Phoma sp.	7	2	3	3	50	11	31	5	3	2	26	0	0	3	3	3	6	0	8	0	0	7	77
Saprophytic fungi																							
Aspergillus flavus	65	8	24	37	19	4	8	1	0	21	0	1	0	85	90	26	99	30	11	71	8	6	86
Aspergillus niger	42	3	11	3	43	10	34	3	1	26	1	62	1	57	91	2	99	9	14	53	1	13	100
Cladosporium sp.	0	0	1	1	93	0	6	0	15	5	0	2	0	3	22	2	9	0	8	2	2	25	68
Penicillium sp.	18	12	100	94	90	91	76	82	98	97	93	98	99	74	83	28	98	93	43	84	41	15	100
Rhizopus sp.	1	1	1	0	3	0	1	1	0	2	0	1	5	51	43	2	47	1	0	1	1	7	77

[a] Kir = Kiri, Kor = Koro, Pal = Pala, Sam = Samaga, Daf = Dafinso, Mat = Matourkou, Nas = Nasso, VDK = Vallée du Kou, Yeg = Yéguéresso, Ksb = Karankasso Sambla, Len = Lena, Seg = Séguéré, Pad = Padéma, Dié = Diébougou

Table 4: Detection of latent infection of pathogenic fungi 10 and 14 days after sowing on stems and leaves of maize seedlings raised from naturally infected maize seeds

	Percent infection of plant materials by pathogenic fungi [a]								
	Mat4-2b			Pal2			Diél		
Fungi	Seed (200)[b]	Stem (77)	Leaf (75)	Seed (200)	Stem (93)	Leaf (93)	Seed (200)	Stem (94)	Leaf (93)
Acremonium strictum	4	13	11	50	49	10	47	72	15
Bipolaris maydis	0	0	0	1	0	8	30	1	2
Botryodiplodia theobromae	4	0	0	0	0	0	6	0	0
Colletotrichum graminicola	0	0	0	8	2	2	0	0	0
Curvularia sp.	0	0	16	0	6	34	39	7	30
Exserohilum rostratum	0	0	0	0	1	12	13	0	6
Fusarium equiseti	2	0	3	5	2	1	2	0	3
Fusarium moniliforme	97	23	51	82	53	62	38	64	61
Fusarium pallidoroseum	1	17	3	1	0	4	3	0	0
Phoma sp.	26	1	16	3	3	29	7	5	17

[a]: Based on the total number of plant organs tested; [b]: Total number of plant organs tested

Percent seed infestation: All 22 samples were heavily infested by *F. moniliforme* (38 to 99%) and *A. strictum* (2 to 96%) (Table 3). The percent infestation by *Phoma* sp. ranged from 3 to 50%. Infestation levels of 1 to 23% of *F. pallidoroseum*, 1 to 15% of *F. equiseti* and 1 to 17% of *B. theobromae* were detected in 15, 14 and 14 samples, respectively. In samples collected in Houet province, *B. maydis*, *E. rostratum* and *Curvularia* sp. were found in very low amounts (1% in 2 samples, 1 to 4% in 3 samples and 1 to 6% in 10 samples, respectively), while sample Diél from Diébougou exhibited the highest level of infestation by *B. maydis* (30%). Two seed samples were infested by *C. graminicola* (2 and 8% infestation rates, respectively). Samples VDK3 from Vallée du Kou and Diél from Diébougou were the most infested regarding infestation rates and number of pathogens detected (Table 3). In general, infestation rates by saprophytic fungi were very high. *Penicillium* sp. infested all samples with percent infestation ranging from 12 to 100% (Table 3). *A. niger* and *A. flavus* Link ex Fr. were found with highly variable infestation levels (1 to 99%). High infestation by *Cladosporium* sp. was detected in sample Sam1 (93%) while *Rhizopus* sp. was encountered in moderate levels of incidence in samples VDK3 (51%), Ksb1 (47%) and VDK sb (43%).

Detection of seedling latent infection by pathogenic fungi: Latent infection was confirmed when 14 days old seedlings, raised from naturally infected seeds under greenhouse conditions, were analysed for pathogenic fungi using the blotter method (Table 4). Across samples, 10 pathogenic fungi detected on maize seeds were recovered on seedlings. Apart from *Botryodiplodia theobromae*, seed infestation by other pathogenic fungi generally resulted in plant infection. In some instances, plants raised from apparently healthy seeds were infected by *Curvularia sp.* and *Exserohilum rostratum*. The most frequent fungal species found on plant materials, across samples, were *A. strictum* (10 to 72%), *F. moniliforme* (23 to 64%), *Curvularia* sp. (6 to 34%) and *Phoma* sp. (1 to 29%). Results revealed that pathogens are encountered on

stems and leaves with variable percentages according to fungal species, plant material and seed sample. *A. strictum* (72% of stem infection) and *F. moniliforme* (64%) were most transmitted to stems of seedlings raised from sample Diél. Although encountered on seeds of two samples, *Botryodiplodia theobromae* was not detected on both stems and leaves (Table 4).

Across samples, *Phoma* sp., *Exserohilum rostratum*, *Curvularia* sp. and *Bipolaris maydis* were prevalent on leaves. Low levels of seedling infection by *C. graminicola* and *B. maydis* were observed.

DISCUSSION

Twenty two maize seed samples collected from 14 locations in the Houet and Bougouriba provinces of Burkina Faso were examined for incidence of seed-borne fungi using the deep-freeze blotter method. All samples were infested by pathogenic and saprophytic fungi regardless of their geographical origins. Percent infestation was variable according to locations and seed samples.

Fusarium moniliforme was present in all samples and its infestation percentages were very high. This pathogen is known to attack a large number of plant species. In Burkina Faso, Kini *et al.* (2002) and Somda *et al.* (Unpublished data) detected *F. moniliforme* in seed samples of 15 crops and parkia tree (*Parkia biglobosa*). It is responsible of seed, stalk and ear rots and seedling blight (Dodd and White, 2000). Furthermore, toxins like fumonisins are produced by this fungus in maize kernel (Champion, 1997; Payne, 2000). Owing to high infection level of maize seeds by *F. moniliforme*, there is a clear need to define appropriate measures for maintaining maize

seed and grain quality. Chemical seed treatment programmes and better storage conditions seem justified although further research to work out efficient and affordable control measures to be used in conjunction with fungicides is needed. Attention should be paid to avoid the build-up of seed-borne inoculum of this pathogen in certified maize seed. *Acremonium strictum* was also present in all locations and samples tested. It is reported to cause stalk rot called black bundle disease (White, 2000). Seed of maize can rot when heavily infected by *A. strictum* (Richardson, 1990). Although abundantly detected in all seed samples tested, this fungus has not yet been reported to cause any disease in field in Burkina Faso. As *A. strictum* infection percentages were high, it is important to perform field observations in maize-growing areas to evaluate the incidence of black bundle disease caused by this fungus.

Apart from one seed sample from Bougouriba province which was heavily infected, seed samples from Houet province were free of *Bipolaris maydis*, the causal agent of southern leaf blight. Being the most damaging fungal disease of maize in Burkina Faso according to Sanou (1996), a survey should be undertaken to evaluate the relative incidence of southern leaf blight in the different maize growing areas of Burkina Faso. Although some samples were infected in trace amounts, the explosive nature of the pathogen and widespread epidemics of the disease in 1970 throughout maize-growing areas of the USA indicate that using infected seeds should be avoided (Fatima *et al*., 1974). *B. maydis* has been known to be seed-borne exclusively in maize but it has been shown to be borne on seeds of non-susceptible crops such as lettuce, watermelon, cowpea, sorghum and pearl millet (Fatima *et al*., 1974; Somda *et al*., unpublished data). Sorghum, millet and cowpea are either sown mixed or in rotation with maize in farming system in Burkina Faso. Therefore, management of southern leaf blight should include control of seed health status of other crops.

The abundant growth of saprophytic fungi on maize seeds implies that storage problems should also be studied. It is now imperative that efforts should be made to continuously evaluate the seed health of maize seeds produced in Burkina Faso.

Experiment on seed transmission was conducted under greenhouse conditions in an attempt to prevent contamination from others sources. Infection of seedlings must have started from the infected seeds since seedlings were protected from air-borne inoculum. The present investigation shows that the extent of seed-to-seedling transmission of most of the pathogens is low. Low transmission rates of *Pyricularia oryzae* were also reported by Chung and Lee (1983) who found that only 7 to 8% of seedlings were infected, even though they were raised from seed samples having 65% infection. Furthermore, the absence of infected seedlings also may have been due to lower rate of seed-to-seedling transmission when incidence of infected seeds is low (Michener *et al*., 2002)

Pathogens causing foliar diseases are more transmitted to leaves than stems. Transmission of *A. strictum* from seeds to stems is higher than its transmission to leaves. This could be because this fungus is known to be responsible of stalk rot disease named black bundle disease. Infections of seedlings raised from naturally infected seeds have also been reported in several other pathosystems such as maize/*Rhizoctonia solani*, maize/*F. moniliforme* and rice/*Pseudomonas avenae* (Michail *et al*., 1985; Shakya *et al*., 1986). Owing to the role of seed-borne pathogens in epidemic of field diseases, controlling the health status of maize seed lots and their treatment with adequate products should be part of the management strategies of seed-borne diseases. Work is currently undertaken to seek for environmentally-sound methods to control seed-borne fungal pathogens of maize in Burkina Faso.

ACKNOWLEDGMENTS

The authors are grateful to Mr Palé Ollo for his helpful contribution to the laboratory work. The research was financed by International Foundation for Science (IFS), Sweden.

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