Seedborne Infection of *Eleusine coracana* by *Bipolaris nodulosa* and *Pyricularia grisea* in Uganda and Kenya

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ABSTRACT

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Seed samples of finger millet (*Eleusine coracana*) collected from Uganda and Kenya had mixed infections with *Bipolaris nodulosa* (4–49%) and *Pyricularia grisea* (7–55%). Most samples showed poor germination and were unfit for sowing because of a high percentage of seedborne infection. We obtained similar seed infection counts with the standard blotter, deep-freezing blotter, and agar plate methods, which suggests that any of these methods could be used for routine seed health testing. However, sporulation was better on frozen seeds. Gray or black discolored seeds had higher levels of infection by *P. grisea* than apparently healthy, normal reddish brown seeds. Plating of seed components showed that both fungi were present in the pericarp and endosperm but not in the embryo. Seed transmission tests demonstrated the ability of these fungi to kill young seedlings.

Finger millet (*Eleusine coracana* (L.) Gaertn.), locally known as *wimbi* (in Kiswahili), is widely cultivated as a subsistence rain-fed crop in Kenya and Uganda. The grain is used exclusively for human consumption. Grain yields are low in these countries (8,14), primarily because of two important diseases: blast, caused by *Pyricularia grisea* (Cooke) Sacc.; and blight, caused by *Bipolaris nodulosa* (Berk. & M. A. Curtis) Shoemaker. Both pathogens are seedborne in finger millets (3,10–13) and may cause heavy losses. McRae (9) and Govindu (2) reported more than 50% reduction in grain yield caused by blast.

Several fields in Kenya and Uganda were surveyed during late 1991 and early 1992. Most of the fields had severe incidence of blast and blight. Relatively little is known about the seedborne phase of blast and blight diseases of finger millets grown in Uganda and Kenya. We sampled seeds of finger millet grown in these two countries for seedborne infection by *B. nodulosa* and *P. grisea* and attempted to identify an effective method for routine seed health testing for these two pathogenic fungi.

MATERIALS AND METHODS

Twenty seed lots were collected from fields, village markets, and farmers' storage bins and analyzed for infection by *B. nodulosa* and *P. grisea*. Five randomly selected samples were later assessed by three methods to establish a routine testing procedure for these pathogens. A component plating technique was used to locate these fungi in the seed parts. Disease transmission from seed to plant was studied by the water agar seedling symptom test.

Seed samples were collected during November and December 1991 and January and February 1992. Samples were stored in a refrigerator at 5 ± 1 C. Seeds were tested at the plant pathology laboratory of the Kenya Agricultural Research Institute at Muguga, following standard procedures of the International Seed Testing Association (5). Three methods were used: the blotter method, deep-freezing blotter method, and agar plate method.

Blotter method. Twenty-five seeds were placed on two layers of moistened filter paper and one layer of folded tissue paper in a plastic petri dish. Seeded petri dishes were incubated in a chamber at 22 ± 2 C with alternating 12-hr cycles of near-ultraviolet light (NUV) and darkness. After 6 days, seeds were examined for fungal infection under a stereoscopic binocular microscope. One hundred seeds of each of the 20 samples were tested. Another 200 seeds of each of the five randomly selected samples were tested to compare the efficacy of the blotter method with that of the other two methods.

Deep-freezing blotter method. Seeds of the five selected samples were plated on blotters and tissue paper as in the standard blotter method. After incubation in the dark at 22 ± 2 C for 24 hr, petri dishes were placed in a freezer at −4 C for the second and third days, followed by 5–6 days at 22 ± 2 C with 12-hr alternating cycles of NUV and darkness. Two hundred seeds of each sample were tested. Fungal infection counts were determined as in the standard blotter method.

Agar plate method. Fifteen seeds per plate were sown on potato-dextrose agar (PDA) (Difco Laboratories, Detroit, MI) after treatment with an NaOCl solution (2% available chlorine) for 8–10 min. Incubation conditions were similar to those used in the blotter method. After 6 days, colonies were examined for mycelial growth, color, pigmentation, and sporulation. Two hundred seeds of each of the five selected samples were tested by this method.

Component plating. Component plating (7) was used to study the location of the pathogens in seed tissues. Twenty seeds of each of the five selected samples were soaked individually overnight (18–20 hr) in sterilized distilled water and then dissected aseptically with sterilized needles and forceps under a stereoscopic binocular microscope. The pericarp, endosperm, and embryo from each seed were surface-sterilized with 1% NaOCl for 1–2 min, rinsed four or five times in sterilized distilled water, and then plated either on sterilized wet blotters or on PDA in petri dishes equidistant from each other. Incubation conditions were the same as described for the blotter method.

Discolored versus apparently healthy seeds. The blotter method was used to examine 200 gray to black discolored seeds and 200 normal reddish brown seeds of each of two seed lots for the associated fungal pathogens.

Seed transmission. We used the water agar seedling symptom test suggested for the detection of *Septoria nodorum* in wheat grains (6) for our seed transmission studies. The test was conducted in test tubes, with one seed per tube on 20 ml of 1% water agar. Symptoms were recorded after incubation for 10–12 days. To confirm transmission of disease from seeds to seedlings, isolations on PDA were made from the root, attached seed,

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collar, stem, and leaves of the diseased seedlings.

Germination test. Germination of all seed samples was assessed by the between-paper method of the International Seed Testing Association (5). Substrate, temperature, and light conditions were similar to those recommended for *Setaria italica,* since no procedure has yet been given specifically for *E. coracana* in the international rules for seed testing.

Statistical analysis. Data were analyzed by analysis of variance to determine the effects of treatments, and the standard error of the means and/or least significant difference (LSD) was computed at $P < 0.05$.

RESULTS

Growth characteristics on seed surface. *B. nodulosa* produced grayish to dark gray to black or olivaceous brown colonies on the seed surface. Colonies frequently covered the whole seed and extended to the blotter. Mycelium was scanty. The olivaceous brown conidiophores were straight or flexuous and bore dark brown to olivaceous brown conidia acropleurogenously. Conidia were generally four- to nine-septate (six septa were most common), subcylindrical, and slightly curved or straight; they tapered toward the rounded ends and measured 54.5–89.5 × 14.5–21.4 μm.

P. grisea produced effuse growth of whitish gray mycelium. Conidiophores arose singly or in groups and covered usually part of the seed and in a few cases the whole seed. Conidiophores were slender, straight, and grayish or pale brown and bore clusters of conidia. Conidia were typically obpyriform or obclavate, hyaline, and two-septate, with the central cell larger than the two terminal cells. Conidia measured 18.5–29.5 × 7.5–10.5 μm.

Growth characteristics on agar. On PDA, colonies of *B. nodulosa* were fast-growing and spreading and ashy gray to dark gray, and mycelium was fluffy, with abundant conidial production. The reverse sides of colonies were dark gray to sooty black, with a brownish tinge and wavy margins. Colonies of *P. grisea* were comparatively slow-growing and whitish gray to ashy gray, with flat mycelial growth. The reverse sides of *P. grisea* colonies were dark gray, and margins were smooth with a whitish tinge.

Samples tested by the blotter method. All 20 samples were infected with *B. nodulosa, P. grisea,* and other fungi (Table 1). Seed samples collected from different locations differed significantly in the incidence of these fungi. In 50% of the samples, more seeds were infected by *P. grisea* than by *B. nodulosa.* Often both pathogens were found growing together. In such cases, *B. nodulosa* overgrew *P. grisea* so that identification of the latter became difficult. Similarly, in several cases, species of *Aspergillus, Curvularia, Fusarium,* and *Phoma* overgrew *B. nodulosa* and *P. grisea* and made their identification difficult.

Many of the heavily infected seeds did not germinate. The roots and shoots of most of the seeds that did germinate were heavily colonized by *B. nodulosa, P. grisea,* or both. Such seedlings often died during the test period, and the two fungi sporulated profusely on the dead tissue. Germination in samples 16 and 17 was 90 and 88%, respectively (Table 1). Four samples did not germinate, and two had less than 10% germination. Many germinated seeds produced seedlings with decayed roots and shoots, no primary roots, poorly developed shoots, and spirally twisted and stunted plumules.

Table 1. Seed infection of *Eleusine coracana* by *Bipolaris nodulosa, Pyricularia grisea,* and other fungi in 20 samples tested by the blotter method

Sample number	Country Location	Source[a]	Seedborne fungi (%)[b]			Germination[d] (%)
			B. nodulosa	*P. grisea*	Other fungi[c]	
	Uganda					
1	Mwizi-1	FF	22	19	48	38
2	Busibo-1	FF	28	40	32	2
3	Rusibo-1	FF	27	24	49	32
4	Rubare-Bushenyi	FF	21	34	48	45
5	Rwentobo	FF	19	25	56	75
	Western Kenya					
6	Kericho-Kisii	FF	33	55	12	8
7	Nandi	SB	33	35	31	18
8	Irimis	SB	21	21	57	76
9	Kadongo	VM	26	18	53	63
10	Kadongo	VM	31	14	45	70
	Eastern Kenya					
11	Mutunga	FF	28	26	45	52
12	Ciothirai	FF	35	38	27	0
13	Ruiri	FF	40	43	17	0
14	Nkubu	FF	40	33	27	73
15	Kiboko	FF	22	15	55	0
16	Kiboko-90	RS	13	19	68	90
17	Kiboko-91	RS	4	7	12	88
18	Kiboko-92	RS	33	37	23	23
19	Kiboko-92-1	VM	22	29	49	60
20	Karatina	VM	38	39	23	0
	Mean		26.8	28.6	38.8	40.6
	SE[e]		±2.04	±2.64	±3.70	±7.23

[a] FF = farmer's field, SB = storage bin, VM = village market, RS = research station.
[b] One hundred seeds per sample were tested for fungi by the blotter method.
[c] Mainly species of *Aspergillus, Curvularia, Fusarium,* and *Phoma.*
[d] One hundred seeds per sample were tested by the between-paper method (5) for germination.
[e] Standard error of the mean.

Table 2. Analysis of variance mean squares and *F* test for percentages of *Bipolaris nodulosa, Pyricularia grisea,* and other fungi in seed samples tested by three methods

Source of variation	df	Mean squares		
		B. nodulosa	*P. grisea*	Other fungi
Method of seed health testing	2	94.47[a]	56.47[a]	33.07[a]
Error	12	100.70	56.03	196.46

[a] *F* value not significant at $P < 0.05$.

Table 3. Percentage of infection[a] by *Bipolaris nodulosa, Pyricularia grisea,* and other fungi in five samples of *Eleusine coracana* tested by the blotter method, deep-freezing blotter method, and agar plate method

Sample number	*B. nodulosa*			*P. grisea*			Other fungi[b]		
	Blotter	Freezer	Agar	Blotter	Freezer	Agar	Blotter	Freezer	Agar
1	22	26	48	19	28	32	11	46	20
4	21	26	27	34	27	36	48	30	33
7	33	49	22	35	35	22	31	16	31
9	26	47	31	18	35	19	53	18	27
20	40	35	22	37	40	23	12	21	40
Mean[c]	28.4	36.6	30.0	28.6	33.0	26.4	31.0	26.2	30.2

[a] Data are means of 200 seeds per sample and method.
[b] Mainly species of *Curvalaria, Aspergillus, Fusarium,* and *Phoma.*
[c] Least significant difference analysis showed that the methods were not significantly different in detection of fungi ($P < 0.05$).

Comparison of seed evaluation methods. Results from the three methods used to detect seedborne microflora in five samples of *E. coracana* did not differ significantly (Table 2). Infection counts of *B. nodulosa* and *P. grisea* were marginally higher in the deep-freezing blotter method than in the standard blotter and agar plate methods (Table 3). The blotter method and the agar plate method gave comparable results for most samples. Sporulation of these two fungi was generally higher in the deep-freezing blotter method than in the blotter and agar plate methods.

Location of seedborne inoculum. Significant differences were observed in the occurrence of *P. grisea* and other fungi on the pericarp and endosperm of seeds (Table 4). *B. nodulosa* and *P. grisea* were found in the pericarp of 10–45% of the 20 seeds tested from each of the five samples and in the endosperm of 10–20% of the seeds, while all embryos were free from infection (Table 5).

Discolored versus normal seeds. Gray to black, shriveled seeds were often found mixed with normal reddish brown seeds. When 200 discolored seeds and 200 normal-looking seeds from each of two samples were examined by the blotter method, the two seed types did not differ significantly in the occurrence of *B. nodulosa* and *P. grisea* (Table 6). However, the percentage of seeds infected by *P. grisea* was two to nine times higher in discolored samples than in normal seeds (Table 7). Although *B. nodulosa* also was present in discolored seeds, it occurred at similar levels in the healthy, normal-looking seeds.

Transmission of pathogens from seeds to seedlings. We used a seed sample with high levels of infection by *B. nodulosa* and *P. grisea* in our seed transmission test. Symptoms produced by the two pathogens separately and in combination were recorded. Most infected seeds (≥80%) did not germinate. Several germinated seedlings (≥90%) were heavily colonized by *B. nodulosa* and died soon after emergence. In both cases, the fungus sporulated heavily on ungerminated seeds and dead seedlings. In less severe infections, rot developed as browning of the coleoptile, which slowly progressed upward. The main roots of such seedlings were discolored with a tinge of brown on the root hairs.

Seedlings that emerged from seeds infected by *P. grisea* had discolored, dull brown coleoptiles with some etiolation at the tip. No seed rot or quick death of the seedlings was observed; however, with time, browning and etiolation of the coleoptile increased, and seedlings collapsed after 9–12 days. *P. grisea* was isolated from the brown, discolored seedling parts, particularly root and coleoptile, but never from etiolated leaves.

Table 4. Analysis of variance mean squares and *F* test for percentages of *Bipolaris nodulosa, Pyricularia grisea*, and other fungi observed on seed components (pericarp and endosperm)

Source of variation	df	Mean squares		
		B. nodulosa	*P. grisea*	Other fungi
Pericarp vs. endosperm	1	302.50[a]	490.00[b]	1,562.50[b]
Error	8	68.75	83.75	232.50

[a] *F* value not significant at $P < 0.05$.
[b] *F* value significant at $P < 0.05$.

Table 5. Percentage recovery of *Bipolaris nodulosa, Pyricularia grisea*, and other fungi from pericarp and endosperm of *Eleusine coracana* seeds[a]

Sample number	*B. nodulosa*		*P. grisea*		Other fungi[b]	
	Pericarp	Endosperm	Pericarp	Endosperm	Pericarp	Endosperm
1	10	10	10	15	80	75
4	30	10	45	15	25	75
7	15	15	30	15	55	70
9	30	20	30	10	40	70
20	35	10	30	20	35	70
Mean	24	13	29	15	47	72
LSD[c]	NS[d]		13.4		22.3	

[a] Data are means of 20 seeds of each sample. Pericarp and endosperm were obtained from the same seed. No fungi were recovered from embryos.
[b] Mainly species of *Aspergillus, Curvularia, Fusarium*, and *Phoma*.
[c] Least significant difference ($P < 0.05$).
[d] NS = not significant at $P < 0.05$.

Table 6. Analysis of variance mean squares and *F* test for percentages of *Bipolaris nodulosa, Pyricularia grisea*, and other fungi observed on normal-looking, healthy seeds and shriveled, grayish black seeds

Source of variation	df	Mean squares		
		B. nodulosa	*P. grisea*	Other fungi
Normal vs. discolored seeds	1	25.52[a]	1,892.25[a]	2,505.00[a]
Error	2	9.61	114.97	248.55

[a] *F* value not significant at $P < 0.05$.

Table 7. Percentage of infection by *Bipolaris nodulosa, Pyricularia grisea*, and other fungi in normal-looking seeds and discolored seeds tested by the blotter method[a]

Sample number	*B. nodulosa*		*P. grisea*		Other fungi[b]	
	Normal seeds	Discolored seeds	Normal seeds	Discolored seeds	Normal seeds	Discolored seeds
9	17.7	22.3	6.7	63.7	81.5	14.3
20	21.5	27.2	26.7	56.5	50.1	17.2
Mean[c]	19.6	24.7	16.7	60.1	65.8	15.7

[a] Data are means of 200 apparently healthy, normal reddish brown seeds and 200 small, gray-black seeds from each sample.
[b] Mainly species of *Aspergillus, Curvularia, Fusarium*, and *Phoma*.
[c] Least significant difference analysis showed that means were not significantly different at $P < 0.05$.

DISCUSSION

All 20 seed samples of *E. coracana* had mixed infections with *B. nodulosa* and *P. grisea*. In 11 samples, a greater percentage of seeds was infected with *P. grisea* than with *B. nodulosa*. Low seed germination was associated with high seed infection by these two fungi. Only two of the 20 samples examined would qualify for quality certificates for germination (above 80%), and even these samples had 4–13% and 7–19% infection by *B. nodulosa* and *P. grisea*, respectively. These data support previous reports on the seedborne nature of these fungi (12). Seed infection by species of *Aspergillus, Curvularia, Fusarium, Phoma*, and other fungi suggests that detailed investigations are warranted to establish the role of these fungi in the health of finger millet seeds. Because of the high incidence of seedborne pathogens, seeds of *E. coracana* should be treated with effective fungicides before sowing. Discolored gray-black seeds had a higher incidence of infection by *P. grisea* than by *B. nodulosa*. Similar observations were recorded on seed samples collected from diseased fields in India (12). Grewal and Pal (4) reported a definite spotting on finger millet grains

infected with *B. nodulosa,* but we did not observe such spots on diseased grains of *E. coracana.*

Our comparison of three seed health testing techniques indicated that these two pathogens can be successfully detected by any of the methods. However, the standard blotter method is economical and less cumbersome than the other two methods. Furthermore, it restricts the growth of *B. nodulosa* and *P. grisea* to the seed surface, which helps in identification. Sporulation of the two fungi was greater on incubated seeds tested by the deep-freezing blotter method than by the blotter method and the agar plate method, as has been reported previously (12), and this facilitated their detection and identification (12). Furthermore, in both blotter methods, roots and shoots from germinating seeds did not complicate the observations. In addition, developing symptoms on the seedlings infected by *B. nodulosa* and *P. grisea* were more prominent in the blotter method than in the other methods.

Plating of seed components suggested that most of the infection by these two fungi is in the pericarp and endosperm. Embryos were free of infection. However, we did not observe 100% infection of pericarps by these fungi, as reported by Ranganathaiah and Mathur (12). Our results on embryo infection support their findings.

Transmission tests indicated that both *B. nodulosa* and *P. grisea* are seed-transmitted. Both pathogens sporulated on ungerminated, infected seeds and on rotten or necrotic tissues of seedlings and were able to kill the seedlings. Mitra and Mehta (10) and Ranganathaiah and Mathur (12) also reported seedling mortality.

Our results show that *B. nodulosa* and *P. grisea* are seedborne, are confined mostly to the pericarp, and can cause seedling mortality. Seed treatment with effective fungicides will reduce the initial seedborne inoculum and eventually help in obtaining a healthy crop (1,14). The standard blotter test is effective for routine seed health testing to detect these two fungi.

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LITERATURE CITED

1. Dutta, A. K., and Jha, D. K. 1983. Seedborne fungi of ragi (*Eleusine coracana* L.) in Chotanagpur and their control. Pesticides 17(4):28.
2. Govindu, H. C. 1973. Some of the recent significant contributions on three major diseases of *Eleusine coracana* with particular reference to current package of practices. Ext. Note, Univ. Agric. Sci., Bangalore, India.
3. Govindu, H. C., Shivanandappa, N., and Renfro, B. L. 1970. Observations on diseases of *Eleusine coracana* with special reference to host resistance to the *Helminthosporium* disease. Pages 415-424 in: Plant Dis. Prob. Proc. Int. Symp. 1st. Indian Phytopathological Society, Indian Agricultural Research Institute, New Delhi.
4. Grewal, J. S., and Pal, M. 1965. Seed microflora. 1. Seedborne fungi of ragi (*Eleusine coracana*), their distribution and control. Indian Phytopathol. 18:33-37.
5. International Seed Testing Association. 1976. International rules for seed testing. Seed Sci. Technol. 4:3-49.
6. Khare, M. N., Mathur, S. B., and Neergaard, P. 1977. A seedling symptom test for detection of *Septoria nodorum* in wheat seed. Seed Sci. Technol. 5:613-617.
7. Madan, S., Singh, D., Mathur, S. B., and Neergaard, P. 1975. Detection and location of seed-borne inoculum of *Ascochyta rabiei* and its transmission in chickpea (*Cicer arietinum*). Seed Sci. Technol. 3:667-681.
8. Mburu, C. 1989. Importance, genetic resources and breeding of small millets in Kenya. Pages 149-154 in: Small Millets in Global Agriculture. A. Seetharama, K. W. Riley, and G. Harinarayana, eds. Oxford and Indian Book House, New Delhi.
9. McRae, W. 1922. Report of the Imperial Mycologist. Scientific Report, Agricultural Research Institute, Pusa, India.
10. Mitra, M., and Mehta, P. R. 1934. Diseases of *Eleusine coracana* G. & E. and *E. aegyptiaca* D.C. caused by species of *Helminthosporium.* Indian J. Agric. Sci. 4:943-975.
11. Ramakrishnan, T. S. 1963. Diseases of Millets. Indian Council of Agricultural Research, New Delhi.
12. Ranganathiah, K. G., and Mathur, S. B. 1978. Seed health testing of *Eleusine coracana* with special reference to *Drechslera nodulosa* and *Pyricularia grisea.* Seed Sci. Technol. 6:943-951.
13. Ranganathiah, K. G., and Rao, A. N. S. 1982. Seed treatment of finger millet against Helminthosporiose and blast disease. Indian J. Mycol. Plant Pathol. 12:319-320.
14. Zake, V. M., and Khizzah, B. W. 1989. Finger millet improvement in Uganda. Pages 137-148 in: Small Millets in Global Agriculture. A. Seetharama, K. W. Riley, and G. Harinarayana, eds. Oxford and Indian Book House, New Delhi.