Ability of Fungal Isolates from Maize and Sorghum to Infect Roots and Reduce Seedling Emergence of Two Maize Hybrids

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ABSTRACT

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One hundred fourteen isolates in more than 20 species of fungi isolated from roots of both maize and sorghum and from maize seed were tested for their ability to infect the roots and reduce seedling emergence of two maize hybrids. Isolates differed significantly ($P = 0.01$) in their ability to enter maize roots, and only a *Penicillium* sp. significantly ($P = 0.05$) reduced seedling emergence. Of the fungi found to be pathogenic, the ability to infect young plants was an isolate rather than species attribute.

Additional key word: molding

Fungi infecting maize (*Zea mays* L.) roots are facultative parasites occurring in the soil and under the seed coat. Some appear to be directly involved in causing root rot, e.g., *Bipolaris pedicellata* (A. W. Henry) Shoem., *Fusarium moniliforme* Sheldon, and *F. oxysporum* Schlecht. (5,10,15). Other fungi like the mold types, e.g., *Penicillium* and *Aspergillus* spp. (1), cause seed rot under conditions of high moisture.

In South Africa, sorghum is sometimes grown in rotation with maize but more often in fields adjacent to maize. In a preliminary investigation, the same fungi were isolated from maize and sorghum roots and maize seed. Only the frequency of occurrence differed.

In this study, the ability of the fungi associated with maize roots and seed and sorghum roots to penetrate maize roots and cause a reduction in seedling emergence was investigated.

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MATERIALS AND METHODS

The fungi were isolated from under the seed coat of surface-sterilized (3 min of 3% NaOCl) commercially available maize seed, infected roots of maize plants at various growth stages, and infected roots of sorghum seedlings. Isolations were made onto potato-carrot agar (PCA; 20 g each of freshly peeled potatoes and carrots per liter of distilled water). The isolates were kept in culture for a maximum of 4 wk in incubators at 25 C and 70% relative humidity. A modified method of Rao (12) was used to test their ability to infect maize roots. Fries-Bosal liquid medium [5 g of $(NH_4)_2C_4H_4O_6$, 1 g of NH_4NO_3, 1 g of KH_2PO_4, 0.05 g of $MgSO_4 \cdot 7H_2O$, 0.03 g of $CaCl_2$, 0.1 g of NaCl, 30 g of dextrose, and 0.1 g of yeast extract per liter of distilled water] was used to moisten a 5% (v/v) cornmeal-sand mixture in Erlenmeyer flasks, which were then autoclaved for 30 min at 121 C. After sterilization, the flasks were inoculated with the various test fungi. When the fungi had grown through the medium, the inoculum was mixed with steam-sterilized field soil (clay-loam with 25–30% clay content) to give inoculum levels of 1, 3, and 5% (v/v). Five-liter pots filled with this mixture were planted with two seeds of each of the maize hybrids PNR493 and A471W, both widely planted in South Africa. Each pot contained only one test fungus isolate, and the experiment had six replicates of 24 plants each.

The pots were kept on a greenhouse bench with a minimum night temperature of 15 C and maximum day temperature of 25 C. Day length was about 13 hr. They were watered once a day with tap water. At the five- to six-leaf stage, the number of emerged seedlings was recorded. The seedlings were then removed from the pots and adherent soil was carefully washed from the roots, which were cut into 1-cm lengths and surface-sterilized for 3 min in 3% NaOCl. Ten pieces of randomly selected root lengths from each plant were plated onto each of PCA, Difco cornmeal agar, and Difco water agar and incubated at 25 C in the dark. After 3–7 days, depending on the rate of emergence of the test fungus, the number of positive reisolations was recorded.

Mold damage was investigated by placing four seeds of each hybrid onto the surfaces of petri-dish cultures of each test isolate. Each isolate-hybrid combination was replicated 12 times. The petri dishes were incubated at 25 C in the dark. Percent seed germination was recorded after 7 days.

Variance ratios were calculated by analysis of variance, and isolate aggressiveness was determined by Student's *t* test.

RESULTS

One hundred fourteen isolates, categorized into 23 genera-species groups, were tested. Reisolation of the fungi from plant roots ranged from 0 to 100%, depending on the isolate. Seedling emergence ranged from 100 to 33.3% and from 100 to 38.9% for A471W and PNR493, respectively (Table 1).

The fungi isolated with overall highest frequencies were *B. pedicellata* (77.3%), *Curvularia clavata* Jain (77.0%), and *C. brachyspora* Boedijn (71.4%) (Table 1). There were significant intergeneric and intraspecific differences between reisolation frequencies of isolates. The following ranges were obtained for isolates of the succeeding fungi: *Bipolaris bicolor* (Mitra) Shoem. (50–70%), *F. graminearum* Schwabe (3.4–96.1%), *F. moniliforme* (0–100%), *F. oxysporum* (7.8–84.5%), *F. solani* (Mart.) Snyd. & Hans. (16.9–40%), *Phoma sorghina* (Sacc.) Boerema, Dorenbosch, & van Kesteren (0–75.8%), and *Stenocarpella maydis* (Berk.) Sutton (0–40%) (Table 1).

There were no significant differences between hybrids for reisolation of all isolates, and the correlation for reisolation between A471W and PNR493 was highly significant ($r = 0.7898$, $P = 0.001$).

Only one isolate, a *Penicillium* sp., significantly ($P = 0.05$) reduced seedling emergence. It reduced emergence of A471W by 66.7% and of PNR493 by 61.1%. This fungus was not reisolated from the roots. There was no significant difference between hybrids for seedling emergence recorded over all isolates. Correlation between emergence of the two cultivars for all isolates was highly significant ($r = 0.4804$, $P = 0.001$).

The effect of isolate source on reisolation and seedling emergence is presented in Table 2. There were highly significant differences ($P = 0.01$) between reisolation of isolates from seed vs. roots but not between maize and sorghum. A highly significant ($P = 0.01$) interaction occurred between isolate reisolation and source of isolation (seeds vs. roots), but no significant interaction was found for the fungi isolated from maize or sorghum. Whether the isolates came from maize roots or seed or from

Table 1. Effects of fungal isolates on maize hybrids grown to the five- to six-leaf stage in infested cornmeal-sand and clay soil in pots or on seed placed on the surfaces of culture isolates

Fungus (isolates tested)	Mean percent isolates reisolated	No. aggressive isolates[a]		Mean percent seedling emergence	Isolates reducing emergence (no.)	Mold damage to seed by isolates[b] (no.)
		A	HA			
Acremonium spp. (4)	41.2	2	2	91.6	0	2
Alternaria alternata (2)	19.0	2	0	98.1	0	2
Aspergillus niger (1)	47.1	0	1	75.2	1	1
Bipolaris bicolor (2)	62.5	0	2	87.5	2	2
B. pedicellata (1)	77.3	0	1	86.1	0	0
B. zeicola (1)	27.5	1	0	88.3	0	0
Chaetomium spp. (12)	18.4	5	4	83.6	5	2
C. dolicotrichum (1)	21.5	0	0	80.5	1	1
C. globosum (1)	11.8	0	0	86.0	1	1
Curvularia brachyspora (1)	71.4	0	1	95.2	0	1
C. clavata (1)	77.0	0	1	89.3	0	1
Fusarium spp. (11)	48.2	4	7	91.0	4	6
F. graminearum (7)	42.0	4	2	87.8	3	3
F. moniliforme (20)	43.7	17	3	87.8	7	7
F. oxysporum (13)	37.8	12	1	91.4	1	2
F. solani (2)	24.4	1	0	87.1	1	0
Macrophomina phaseolina (1)	0.0	0	0	100	0	1
Nigrospora oryzae (1)	11.6	1	0	88.3	0	1
Penicillium spp. (18)	26.6	11	7	89.4	4	9
Phoma sorghina (2)	39.0	0	2	87.2	0	0
Rhizoctonia spp. (4)	13.4	0	0	88.8	2	0
Stenocarpella maydis (4)	11.6	0	0	86.0	1	3
Trichoderma spp. (4)	52.0	0	4	93.8	0	3
Control (no fungus)	0	...	...	100	...	...

[a] Data combined for A471W and PNR493 maize hybrids by Student's *t* test; A = aggressive isolate ($P = 0.05$) and HA = highly aggressive isolate ($P = 0.01$).

[b] Number of isolates able to cause mold damage to maize seed of both hybrids after 7-day incubation on the surface of a growing culture of each isolate.

sorghum roots had no significant effect on seedling emergence (Table 2).

Variance ratios were used to test the effect of hybrid and inoculum levels on the pathogenicity of four well-known pathogens of maize, namely *F. moniliforme, F. oxysporum, F. graminearum*, and *S. maydis* (Tables 3 and 4). Significant ($P = 0.01$) isolate effects were recorded in both analyses. Cultivar effects were only significant ($P = 0.05$) for *F. graminearum* and *F. moniliforme*. No significant interactions were recorded between isolates and cultivars or inoculum levels.

Few isolates caused mold damage of the seed (Table 1). Most mold damage was caused by *Penicillium* spp. *F. moniliforme* isolates caused slight mold damage. Isolate source did not affect mold damage and neither did mold damage affect seedling emergence.

DISCUSSION

Du Toit (5) and Rouhani et al (14) found *B. pedicellata* to be weakly pathogenic on seedlings but regularly inducing symptoms on secondary roots of field plants. Miller (10), however, found it to be highly pathogenic on maize roots. The results of this study agree with Miller's findings (Table 1). Rouhani et al (14) recorded *F. graminearum* as irregularly aggressive, whereas Ho and Melhus (7) described its aggressiveness as moderate. Miller (10) considers this fungus of prime importance as a pathogen of maize roots. Hornby and Ullstrup (9), however, only occasionally isolated it from maize roots. At least two distinct pathogenic forms of this fungus are known (2,11). They are separated by their ability to infect either maize or other graminaceous hosts. Similarly, Gordon (6) identified a number of strains on the basis of their physiological ability to parasitize specific hosts.

Rao et al (13) found *F. moniliforme* to be strongly pathogenic on maize roots, causing marked root lesions, pruning, and root rot (more so than both *B. pedicellata* and *F. graminearum*). Reisolation in the current investigation, similar to du Toit (5), indicated *F. moniliforme* was weakly pathogenic. Several strains of *F. moniliforme* undoubtedly differing in their ability to parasitize maize have been reported (8). The evidence favoring the existence of distinct strains in the case of root infection as presented in the literature is not always convincing, however.

Ho and Melhus (7) divide root rot fungi into three groups based on their "disease-inducing capacity." *Rhizoctonia solani* Kühn was classified as severe, *S. maydis* and *F. graminearum* as moderate, and *A. niger* Van Tieghem, *F. moniliforme*, and *Fusarium* species as weak to nonpathogenic. These workers, however, do not define "disease-inducing capacity."

Reports on the relative importance of the different fungal species as root pathogens of maize appear contradictory. The reason for this can be explained by the findings of this study. Significant generic differences among isolates were found. This variation would account for these authors ascribing different levels of importance to these fungal species as root pathogens. It appears from this study, therefore, that the ability of a fungal species to cause root rot of maize is an isolate rather than a species attribute.

Although this investigation has shown that many fungal species are involved in maize root rot and that they are able to cause disease during early plant stages, further research is still needed on the ability of these fungi to penetrate deeper into the root than the cortical cells and thus establish themselves as major pathogens. This might be done in the light of the findings of Deacon (3), who found a significant relationship between the position of runner hyphae of *Phialophora radicicola* Cain var. *graminicola* Deacon and root cortical cells and root cortex death of cereals and grasses. In addition, Deacon and Scott (4) observed vascular damage during early root development in the case of *P. zeicola* Deacon & Scott. They regard this fungus as a weak pathogen.

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Table 2. Effects of isolate source on reisolation from maize roots and on seedling emergence

Test	Isolate source	Variance ratios		
		Isolate (I)	Source (S)	I × S
Reisolation from roots	Seed vs. roots	33.608**[a]	0.021	14.275**
	Maize vs. sorghum	1.825	0.723	2.086
Plant emergence	Seed vs. roots	2.144	1.229	1.910
	Maize vs. sorghum	1.244	2.865	0.900

[a] ** = Significant at $P = 0.01$.

Table 3. Effects of isolates of four fungal species inoculated onto the roots of two maize hybrids

Fungal species	Variance ratios		
	Isolates (I)	Hybrids (H)	I × H
Fusarium oxysporum	8.456**[a]	0.431	1.782
F. graminearum	15.355**	6.949*	3.140
F. moniliforme	11.585**	5.858*	0.364
Stenocarpella maydis	51.005**	0.394	0.394

[a] * = Significant at $P = 0.05$ and ** = significant at $P = 0.01$.

Table 4. Effects of isolates of four fungal species inoculated onto the roots of two maize hybrids

Fungal species	Variance ratios		
	Isolates (I)	Inoculum level (L)	I × L
Fusarium oxysporum	6.132**[a]	0.491	0.813
F. graminearum	19.760**	0.832	1.106
F. moniliforme	6.109**	1.056	0.674
Stenocarpella maydis	51.667**	3.000	2.667

[a] ** = Significant at $P = 0.01$.

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