Fungal Associations in Corn Kernels and Effects on Germination

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ABSTRACT

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Fusarium moniliforme was negatively correlated with both *F. graminearum* and *F. subglutinans* in kernels in a 2-yr study of naturally infected kernels of 10 corn cultivars at eight locations in South Africa. Environmental effects accounted for these significant correlations. No significant correlations occurred in the incidence of pathogens within corn samples. The most significant associations were recorded by examining the mycology of individual kernels, the most prominent being negative between *F. moniliforme* and *Diplodia maydis*, but negative associations were also found between *D. maydis* and *D. macrospora* and between *F. moniliforme* and *F. graminearum*. This is the first report of a significant negative association between *F. moniliforme* and *D. maydis*. Seed germination was negatively associated with *Diplodia* spp., whereas there was relatively little influence on germination by *Fusarium* spp. and other fungi tested.

Additional keywords: antagonism, biocontrol agent, *Zea mays*.

Associations between *Fusarium moniliforme* Sheld. and other fungi have been reported (1,6,9,13,16,18,19). Nyvall and Kommedahl (13) noted that *F. moniliforme* was never isolated in association with other fungi from colonized plant material in soil. Kommedahl et al (6) found that the presence of *F. moniliforme* in corn (*Zea mays* L.) stalks may be influenced by other *Fusarium* spp. because it decreased as other *Fusarium* spp. increased. The converse has also been suggested, where the incidence of other *Fusarium* spp. increased as *F. moniliforme* decreased (18). Further, *F. moniliforme* protected corn seedlings against infection by *F. graminearum* Schwabe (18). The incidence of *F. graminearum* and other *Fusarium* spp. has been reported to be low in corn seed samples in which *F. moniliforme* predominated (9–11,16). A negative correlation between the isolation frequencies of *F. graminearum* and *F. moniliforme* also was reported by Blaney et al (1). They attributed this to competition for substrate, production of antagonistic substances, or environmental conditions that differentially influenced corn ear infection by these two fungi.

A negative association or interaction between *Diplodia maydis* (Berk.) Sacc. (synonym *D. zeae* (Schw.) Lev., also known as *Stenocarpella maydis* (Schw.) Sutton) and *D. macrospora* Earle (also known as *S. macrospora* (Earle) Sutton) has been reported

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TABLE 1. Total isolations of *Fusarium moniliforme, F. subglutinans, F. graminearum, Diplodia maydis*, and *D. macrospora* from corn kernels produced at eight locations in South Africa during the 1985/86 and 1986/87 seasons

	Total number of isolations[a]									
	F. moniliforme		*F. subglutinans*		*F. graminearum*		*D. maydis*		*D. macrospora*	
Location	1986	1987	1986	1987	1986	1987	1986	1987	1986	1987
1	1,449	1,909	209	61	0	3	68	92	0	0
2	1,196	1,572	52	56	2	3	24	63	0	0
3	571	403	444	360	46	1	7	94	0	0
4	958	1,034	321	329	23	18	32	158	0	0
5	978	1,806	11	18	0	0	7	0	0	0
6	695	1,222	221	32	0	0	935	781	0	0
7	59	178	440	796	301	246	293	527	0	0
8	314	159	661	692	171	372	188	397	263	882

[a]Each value represents the number of isolations from 3,000 plated kernels.

(4). This interaction was evidenced by the way in which these two pathogens rarely were isolated from the same kernels and the particular aversion between colonies of these two fungi.

The percentage of visibly diseased corn kernels has been inversely related to percent germination (8). Both *F. graminearum* (5) and *D. maydis* (2) are well known to cause embryo death. The role of *F. moniliforme*, the most prevalent seedborne fungus in corn, in influencing germination, emergence, and seedling growth is, however, uncertain (18).

The aim of our study was to establish the extent of the relationships between *F. moniliforme* and *F. graminearum, F. moniliforme* and *D. maydis*, and *D. maydis* and *D. macrospora* in corn kernels and to establish which of the ear-rot pathogens and other seedborne fungi had the most significant effect on seed germination.

MATERIALS AND METHODS

Corn samples. Random samples of harvested, decobbed kernels representing five yellow and five white corn cultivars were obtained from eight geographical locations throughout the major corn-producing areas of South Africa: Potchefstroom (irrigation), Potchefstroom (dryland), Bethlehem (irrigation), Bethlehem (dryland), Vaalharts (irrigation), Viljoenskroon (dryland), Ermelo (dryland), and Cedara (dryland). These are designated as locations 1–8. There were three replicate samples from each location during each of two seasons (1985/86 and 1986/87), thus, a total of 480 corn samples (15).

Isolation and identification of fungi. A subsample of kernels (±100 g) from each sample was surface-disinfested for 1 min in 3.5% NaOCl containing one drop of detergent (15). Kernels were then rinsed twice in sterile water, and 100 kernels per subsample (a total of 48,000 kernels) were transferred to plates (five kernels per plate) of 1.5% malt extract agar containing novobiocin at 150 mg/L. Plates were incubated at 25 C for 5–7 days. All cultures that developed from the kernels were identified directly on the plates.

Fungal associations based on locations. The total numbers of isolations of *F. moniliforme, F. subglutinans* (Wollenweb. & Reinking) Nelson, Toussoun & Marasas, *F. graminearum, D. macrospora*, and *D. maydis* from kernels grown at the different locations during 1985/86 and 1986/87 were used to determine any associations between these fungi. Analysis was performed by use of the Pearson product moment correlation coefficients (PPMC) (17).

Fungal associations based on samples. A covariance analysis (17) was performed on data (per individual sample and across cultivars) for both seasons to detect possible fungal interactions in the isolation frequencies of *F. moniliforme, F. subglutinans, F. graminearum*, and *D. maydis*. The pairing of *D. maydis* and *D. macrospora* was also compared on a sample basis for location 8 and both seasons with PPMC coefficients.

Fungal associations based on kernels. Fungal associations between *F. moniliforme* and *F. graminearum, F. moniliforme* and *D. maydis*, and *D. maydis* and *D. macrospora* were determined (2,800, 2,900, and 2,400 kernels examined, respectively) across cultivars on a kernel basis by means of a chi-square test for independence in a 2 × 2 contingency table (14). Data were taken only from those locations where the respective pairs of fungi occurred at their highest frequencies during 1986/87, because many incidence levels for 1985/86 and at other locations were too low for valid analysis.

TABLE 2. Correlation coefficients for certain fungal associations in corn kernels based on the total isolations for locations[a]

	Correlation coefficients (r)[b]	
Associations	1986	1987
F. moniliforme vs. *F. graminearum*	−0.843*	−0.734*
F. moniliforme vs. *D. maydis*	−0.308	−0.433
F. moniliforme vs. *F. subglutinans*	−0.730*	−0.911*
F. subglutinans vs. *F. graminearum*	0.673*	0.862*

[a]Total isolation figures given in Table 1.
[b]* = Significant correlation at $P < 0.05$, with the critical r-value = 0.6215.

Effect of seedborne fungi on germination. Kernel germination was assessed directly on malt extract agar plates after incubation and therefore not according to standard methods. Positive germination was recorded where both the coleoptile and hypocotyl had emerged without being killed by a fungus or without necrotic tissue in the seedling. Germination was expressed as a percentage of 100 kernels plated per sample. Germination percentages were correlated across cultivars on a sample basis during both seasons, with incidences of *F. moniliforme, F. subglutinans, F. graminearum, D. maydis, D. macrospora*, and total other fungi. Data for locations 5–8 (a total of 240 samples) were analyzed by means of PPMC coefficients (17), and a stepwise regression procedure (17) was performed on data for all locations except 5 and 8 (a total of 360 samples). *Fusarium* spp., *D. maydis*, and total other fungi served as parameters.

RESULTS

Fungal associations based on locations. *F. moniliforme, F. subglutinans, F. graminearum*, and *D. maydis* varied in their distribution among locations, whereas *D. macrospora* occurred at location 8 only (Table 1). Correlations between pairs of these fungi based on the total isolation figures for locations indicated that some were significantly associated (Table 2). Negative correlations were recorded between *F. moniliforme* and *F. graminearum* and between *F. moniliforme* and *F. subglutinans*, whereas *F. subglutinans* and *F. graminearum* were positively correlated. *D. macrospora* could not be included in the analysis because it occurred at one location only.

Fungal associations based on samples. No significant associations were found among the tested fungi based on a covariance analysis. The isolation frequencies of *D. maydis* and *D. macrospora* from location 8, however, showed a marginally significant negative correlation ($r = -0.438$, $P < 0.05$) during 1986/87, but not during 1985/86 ($r = 0.191$, $P < 0.05$).

TABLE 3. Effect of seedborne fungi on germination of corn kernels based on the correlation coefficients between the incidence of different fungi and germination

Location and year	Correlation coefficient (r)[a]					
	F. moniliforme	*F. subglutinans*	*F. graminearum*	*D. maydis*	*D. macrospora*	Total other fungi
5 1986	−0.491**	...[b]	...	...	...	0.079
1987	−0.248	...	...	...	...	0.021
6 1986	0.088	...	...	−0.887**	...	0.413*
1987	−0.149	...	...	−0.862**	...	0.244
7 1986	−0.129	0.070	0.087	−0.766**	...	0.299
1987	−0.402*	−0.390*	−0.446*	−0.694**	...	−0.124
8 1986	0.228	0.433*	0.171	−0.250	−0.704**	0.516**
1987	−0.114	−0.026	0.009	−0.741**	−0.519**	0.349

[a]* = Significant at $P < 0.05$; ** = significant at $P < 0.01$.
[b]Not analyzed because fungi were absent or were present only at low levels.

Fungal associations based on kernels. All three fungal pairs (*F. moniliforme* and *F. graminearum, F. moniliforme* and *D. maydis*, and *D. maydis* and *D. macrospora*) were negatively associated. The most significant negative association involved *F. moniliforme* and *D. maydis* ($\chi^2 = 421.6$). In this example, *F. moniliforme* and *D. maydis* both grew from 77 (2.7%) of the 2,900 kernels examined, considerably fewer than expected (319 = 11%). Each species also occurred in kernels apart from one another (*F. moniliforme* = 1,070, *D. maydis* = 732), and neither species grew from 1,021 of the kernels. Significant negative associations also were found between *F. moniliforme* and *F. graminearum* ($\chi^2 = 12.5$) and between *D. maydis* and *D. macrospora* ($\chi^2 = 156.6$). *F. moniliforme* and *F. graminearum* both grew from 15 (0.5%) of the 2,800 kernels examined, significantly fewer than the number expected (33 = 1.2%). The same pattern was followed by *D. maydis* and *D. macrospora*, which both grew from 6 (0.25%) of the 2,400 kernels examined, significantly fewer than the number expected (107 = 4.5%).

The influence of seedborne fungi on germination. All the tested fungi had significant effects on kernel germination at one or more locations during one or both seasons (Table 3). *D. maydis* and *D. macrospora* had the largest negative effects as displayed by their highly significant correlation coefficients. *F. moniliforme, F. subglutinans*, and *F. graminearum* also were negatively correlated with germination at some locations, but only for one of the two seasons at the respective locations. Germination was positively correlated with *F. subglutinans* at one location and with total other fungi at two locations during 1986.

The stepwise regression procedure indicated the relative importance of the various fungal parameters and the roles they play in affecting kernel germination (Table 4). *D. maydis* again had the largest negative effect on germination and is included in the model for all six locations examined. At two locations, *Fusarium* spp. were negatively associated with germination, though not to the same degree as *D. maydis*, as judged by the R^2 values. The only positive effect on kernel germination in the model was that of other fungi at location 7.

TABLE 4. Effect of seedborne fungi on germination of corn kernels (1986 and 1987) based on a stepwise regression procedure

Location	Fungal parameter	No. of parameters included in model[a]	Partial R^2[b]
1	*Diplodia maydis*	1	−0.6060
	Fusarium spp.	2	−0.0421
2	*D. maydis*	1	−0.0951
3	*D. maydis*	1	−0.2190
4	*D. maydis*	1	−0.5973
	Fusarium spp.	2	−0.0405
6	*D. maydis*	1	−0.4575
7	*D. maydis*	1	−0.5949
	Other fungi	2	+0.0293

[a]Fungal parameters included in the model are significant in their effect on kernel germination ($P < 0.05$).
[b]R^2 = Coefficient of determination, which determines the percentage variation in kernel germination (Y) that can be attributed to the effect of the fungal parameter (X).

DISCUSSION

The significant correlations between different *Fusarium* spp. over locations should not be seen as fungal interactions, but rather as the influence of environmental conditions. Previous reports from South Africa (9,15) indicated that *F. moniliforme* was adapted to a warm, dry climate, *F. subglutinans* to a cooler, more temperate climate, and *F. graminearum* to an intermediate climate. Fungal interactions should therefore be sought at a sample level or, preferably, on an individual kernel basis. The lack of a significant relationship between the isolation frequencies of *F. moniliforme* and *F. graminearum* from the same samples contrasts with other reports of a negative relationship between these species in corn (1,9,10,16). The lack of significance could be attributable to the low incidence of both fungi in the same corn samples. The presence of a significant interaction, however, could still have reflected the influence of environmental conditions rather than antagonistic effects between the fungi themselves.

The most significant results were obtained by analyzing the presence of certain fungi in individual kernels. By this method, a significant negative association was demonstrated between *F. moniliforme* and *F. graminearum*. This was, however, less significant than the negative associations between *F. moniliforme* and *D. maydis* or between *D. maydis* and *D. macrospora*. Initial kernel infection by *F. moniliforme* may serve as an important deterrent to subsequent kernel invasion by other seed-infecting fungi (3,19) and may have a "protective effect" against infection by other pathogens (18). The significant negative association between *F. moniliforme* and *D. maydis* is reported here for the first time. The nature of this association is unknown, as these two fungi did not display any visible signs of antagonism toward each other in culture.

The highly significant interspecific aversion between *D. maydis* and *D. macrospora*, which was first reported by Hoppe in 1936 (4), was the only interaction visibly noticeable in culture, as seen by sharp lines of differentiation between colonies of these two fungi.

The large negative effect of *D. maydis* on germination of kernels placed on an agar medium is clearly evident and agrees with the report that this pathogen attacks and kills the embryo (2). In contrast, *Fusarium* spp. had a relatively smaller negative effect on germination. The positive correlations of *F. subglutinans* and total other fungi with germination at locations 6 and 8 indicate that kernel germination was stimulated by the presence of these fungi. This, however, remains an unexplained observation. The lack of larger adverse effects caused by *F. moniliforme* is in agreement with the results of Naik et al (12), who found no significant effect on germination, seedling emergence, growth, and yield. This finding, together with the fact that *F. moniliforme* may serve as a deterrent to kernel invasion by other seed-infecting fungi including *F. graminearum* and *D. maydis*, would make it

a possible choice as a biocontrol agent against these fungi. However, the toxicity and carcinogenicity of toxins produced by *F. moniliforme* (7,10) would have to be considered carefully in the development of such a disease control strategy.

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