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Resistance breeding strategy for *Stenocarpella maydis* and *Fusarium graminearum* cob rots in tropical maize

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With 8 tables

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Abstract

Maize cob rot caused by *Fusarium graminearum* and *Stenocarpella maydis* affects grain yield and quality. The objective of this study was to investigate the appropriateness of multiple infection as a selection and breeding strategy for multiple resistance to *F. graminearum* and *S. maydis*. Twelve tropical inbred lines with varying resistance to either or both pathogens were mated in a full diallel and the progeny and their parents evaluated for reaction to single or multiple infection. Under multiple inoculation, *S. maydis* suppressed colonization of cobs by *F. graminearum*. GCA effects indicated that inbred WL 110–18 effectively transmitted resistance to both diseases. Hybrids' resistant to *S. maydis* was also resistant to *F. graminearum,* but the reverse was not true. Therefore, efficient screening should initially involve screening for *S. maydis* followed by *F. graminearum.* Overall, the suppression of *F. graminearum* by *S. maydis* shows that multiple infection cannot be used as an appropriate breeding strategy to obtain multiple resistance. The use of *F. graminearum* and *S. maydis* separately is, therefore, the best breeding strategy.

Key words: combining ability — cob rot — resistance — tropical maize — *Zea mays*

The mycotoxin-producing fungi that infect maize cobs such as *Fusarium graminearum, Fusarium verticillioides* and *Stenocarpella maydis* are endemic, widespread and are the major causes of cob rots in sub-Saharan Africa, as well as in other tropical and temperate agroecologies (Kapindu et al. 1999, Munkvold 2003, Bigirwa et al. 2007, Mukanga et al. 2010b). Infection by these fungi causes yield losses and grain quality deterioration, which pose a health hazard to humans and animals consuming cereals products, contaminated with mycotoxins (Odriozola et al. 2005). To control cob rots, a combination of crop sanitation, good agronomic practices and timely harvesting has been used, but with limited success (Munkvold 2003). For cereals, deployment of resistant varieties remains the most cost effective and efficient approach (Agrios 2005). In general, resistance breeding to cob rots in maize has been based on single pathogen screening systems, but recently, multiple pathogen challenge infection has been advocated for (Mukanga et al. 2010a). However, the suitability and reliability of multiple pathogen challenge infection to obtain genetic information essential for resistance breeding is limited.

Previous studies have indicated that resistance to *S. maydis* is conditioned by additive gene action, while resistance to *F. graminearum* involves both additive and non-additive gene action (Reid et al. 1992, Dorrance et al. 1998, Rossouw et al. 2002). In a related pathosystem, resistance in maize when singly infected by *Aspergilus flavus* is conditioned by epistasis and additive gene action (Walker and White 2001). However, resistance to multiple infection of maize cobs by *A. flavus, F. verticillioides* and *S. maydis* is conditioned by both additive and non-additive gene action with non-additivity being predominant (Mukanga et al. 2010a). These similar and to some extent different mechanisms of resistance to maize cob rot are indicative of the complex nature of resistance to highly variable and virulent pathogens. Whereas multiple challenge infection screening methods have been proposed, the differences in pathogen species and their pathogenicity properties may influence the suitability of such a system. The aim of this study was to investigate the appropriateness of multiple infection as a selection and breeding strategy for multiple resistance to *F. graminearum* and *S. maydis* cob rots.

Materials and Methods

Experimental sites: The experiment was conducted in three sites in Uganda: Bulindi (1°25 N, 31°21 E; altitude 1 140 m), Masaka (0°20 S, 31°44 E, altitude 1 315 m) and Namulonge (0°32 N, 32°35 E; altitude 1 150 m). These sites are located approximately 184 km north-west, 118 km south-west and 30 km north of Kampala, respectively. All the three locations experience a bimodal rainfall pattern, with the first season, A, from March to August and the 2nd season, B, from September to December/January.

Germplasm used in the study and field layout: Twelve tropical inbred lines, selected based on previous assessment at the National Cereals Programme located at Namulonge in the National Crop Resource Research Institute (NaCRRI), were used (Table 1). These materials, with varying resistance to both pathogens, were mated in a 12 × 12 full diallel during the first cropping season of 2010 (2010A) at NaCRRI. The main and reciprocal crosses together with their 12 parents and two checks were evaluated in all three test sites during the second cropping season of 2010 (2010B). The experiments were laid out following an alpha lattice design with two replications. Plants were established in two rows/plot at a spacing of 75 × 30 cm in 5-m long rows with 17 plants per row. Two seeds were initially planted per hill but were subsequently thinned to one plant per hill, 4 weeks after germination. In all the experiments, standard cultural practices such as weeding and appropriate fertilizer applications were followed.

Pathogen culture and inoculation: The pathogens were initially isolated from infected cobs obtained from NaCRRI fields. Inoculum was prepared

Table 1: Inbred lines used in the full diallel including their reaction responses to *Stenocarpella maydis* and *Fusarium graminearum* evaluated previously at NaCRRI in Namulonge

Parent	Inbred line	Grain type	Reaction to *Stenocarpella maydis*	Reaction to *Fusarium graminearum*
1	WL 429–35	Flint	R	R
2	CKL 05017	Flint	R	R
3	CML 506	Semi flint	M	R
4	WL 118–10	Flint	R	R
5	WL 118–22	Flint	S	M
6	CKL 05018	Flint	M	M
7	CKL 05004	Flint	M	R
8	NML 166	Dent	M	R
9	CML 384	Flint	S	M
10	NML 89	Dent	S	S
11	NML 56	Dent	S	M
12	CZL 8	Flint	S	S

S, susceptible; R, resistance; M, moderate; NaCRRI, National Crop Resource Research Institute.

using the modified procedure of Chambers (1998). Infected grains were first sterilized in 10% commercial bleach of the JIK brand that contains 0.39% sodium hypochlorite (NaCIO) (Reckitt Benkiser East Africa Limited, Nairobi, Kenya) solution for three minutes and then rinsed thrice in distilled water. The seeds were subsequently blotted on sterilized filter paper to dry and then 2–3 seeds were plated on 3% potato dextrose (Becton Dickinson, Sparks, MD, USA) agar plates and then incubated at 28–30°C. The fungal growth on plates was sub-cultured after 4 days and was ready for transfer to toothpicks after 5 –7 days. Toothpicks were used as the inoculation tool because they are sharp, can trap culture media and be used to injure and inoculate test maize cobs at the same time. These toothpicks were initially sterilized by boiling in water for 20 min and air-dried. They were then placed upright in bottles measuring 6 cm in diameter and 11 cm in height, containing 100–150 ml of potato broth (prepared by infusion of 20 g freshly skinned potato in 1 l of water) to coat the toothpicks, autoclaved for 15 min and left to cool to room temperature. Each bottle had approximately 600–650 toothpicks. In order for the fungal culture coated toothpicks to be used as carrier for inoculum, culture plugs from pure cultures of either *F. graminearum* or *S. maydis* were placed in each bottle containing the sterile toothpicks and allowed to colonize them for 10 days. After the toothpicks were fully colonized, they were air-dried before inoculating the test genotypes. Inoculation was performed by piercing through the middle of developing maize cobs, using colonized toothpicks at approximately 20 days after mid-silking (R3) stage (Chambers 1988). For the multiple inoculation, two separate toothpicks colonized singly with each of the two pathogens was inserted 5 mm from each other at the middle of the growing cobs. Whilst for the single inoculation, only one pathogen colonized toothpick was used to inoculate test plants. Due to the severity and obvious symptom development on cobs when artificially infected by cob rots, a maximum of five plants was deemed sufficient for disease assessment. Single inoculations were performed on test plants in the first row of the plot, with each treatment bordered by non-inoculated plants. With multiple inoculation, it was performed on the second row of the plot and other plants were left as border and control treatment.

Scoring of genotypes: At harvest, each inoculation treatment per genotype was harvested separately, the plot number noted and the inoculated cobs bulked. For each of the three treatments and the control, visual estimate of disease severity, using percentage of cob colonized by the pathogen from the point of infection, was made and mean severity ratings computed. Severity ratings were subsequently converted to a qualitative form using 1–5 or 1–6 scale for *S. maydis* and *F. graminearum,* respectively. The qualitative scale for *S. maydis* severity ratings was 1 = 0–25%; 2 = 26–50%; 3 = 51–75%; 4 = 76 –99%; and 5 = 100% (completely rotten) (Kapindu et al. 1999). For *F. graminearum,* it was 1 = 1–3%, 2 = 3–10%, 3 = 11–25%, 4 = 26 –50%, 5 = 51–75% and 6 = 76–100% (Reid et al. 1992). Inspection of the plot of residuals (data not shown) revealed violation of ANOVA preconditions, and transformation of the data using appropriate methods did not solve the problem. But conversion of the data to qualitative form addressed this problem, accounting for the use of qualitative data in subsequent data analysis.

Data analysis: The genotype responses were evaluated using analysis of variance (ANOVA) and restricted maximum likelihood (REML) approaches. The relationship between disease severity under single and multiple inoculations of (*S. maydis* and *F. graminearum*) was assayed using correlation analysis. Heritability estimates for resistance to *S. maydis* and *F. graminearum* were performed using mid-parent offspring regression and was computed as $b_{po} = V_a/V_p = h,^2$ where b_{po} is the regression coefficient, V_a and V_p are additive and phenotypic variance components, respectively, and h^2 is the narrow sense heritability estimate. Diallel analysis was performed using Griffing's (1956) Method 1 fixed model. The relative contribution of GCA and SCA was estimated using Baker's ratio (Baker 1978), computed as $2\delta^2gca/(2\delta^2gca + \delta^2sca)$, where δ^2gca and δ^2sca are the variance components of GCA and SCA, respectively. All data analysis were performed using GenStat (Payne et al. 2010).

Results

Disease development under single and multiple infection

Highly significant differences ($P < 0.001$) were found in disease severity among genotypes in all locations for all three treatments (single inoculation using *F. graminearum*, *S. maydis* and multiple infection). Genotype by location interactions was similarly highly significant for all treatments ($P \leq 0.001$), but was of lesser magnitude than the genotype effects themselves. The grand mean across all locations were 2.61, 3.87 and 3.99 for *F. graminearum, S. maydis* and multiple infection, respectively (Table 2). Infection of non-inoculated test plants was very rare and random, with the infected cobs showing very low severity suggesting late infection. Severity ratings under single infection with *S. maydis* and with multiple infection by pathogens revealed a strong correlation ($r = 0.80$). Visual inspection of multiple inoculated cobs revealed that in most cases, *S. maydis* colonized larger areas than *F. graminearum.* The correlation between severity ratings for single infection with *F. graminearum* and multiple infection was low ($r = 0.39$). Based on across location means, cross-tabulation of genotype reaction to single infection of the pathogens across locations revealed that genotypes with resistance to *S. maydis* were also resistant to *F. graminearum,* but the reverse was not

Table 2: Mean disease severity scores across genotypes for all treatments evaluated in three locations during the 2010 second (B) season

Location	*Fusarium graminearum*	*Stenocarpella maydis*	[1]Multiple infection
Namulonge	2.48	3.55	3.63
Masaka	2.69	3.96	4.02
Bulindi	2.66	4.10	4.33
Mean	2.61	3.87	3.99
LSD	0.057	0.059	0.059

LSD- Fishers Protected Least Significant Different test performed at $P \leq 0.05$, Severity ratings scores, *S. maydis*: 1 = 0–25%; 2 = 26–50%, 3 = 51–75%, 4 = 76–99% and 5 = 100, *F. graminearum*: 1 = 1–3%, 2 = 3–10%, 3 = 11–25%, 4 = 26–50%, 5 = 51–75%, and 6 = 76–100%.

[1] = Multiple infection involved inoculation of the same maize cob with both *F. graminearum* and *S. maydis.*

true (Table 3). Twenty-six genotypes had scores of ≤ 3.5 (about 65% infection) for *S. maydis*, with the same genotypes having scores of ≤ 3.0 for *F. graminearum*. Genotypes falling under the resistant class (1.6–3) to *F. graminearum* were 114 in total, all with scores of ≤ 3.0 (about 25%infection). Interestingly, 88 of these *F. graminearum* resistant materials were susceptible to *S. maydis* with scores of >3.5 (about 65% infection) infection (Table 3).

Heritability and combining abilities for resistance to single or multiple infection

Narrow sense heritability (h^2) for *S. maydis* and *F. graminearum* under single infection was 0.18 and 0.35, respectively ($P < 0.01$), based on the mid-parent offspring regression of across location means. The general combining ability (GCA) was highly significant ($P < 0.001$) for each treatment within and across locations (Tables 4–6). The GCA effects showed that inbred lines WL 118–10 (Parent 4) and CZL-8 (Parent 12) were highly significant ($P < 0.001$) in each location and across locations, contributing to resistance and susceptibility, respectively (Table 7). In the same way, their mean parental score in and across locations was highest and lowest, respectively, when compared to other inbreds (data not shown). Specific combining ability (SCA) was highly significant within and across locations only for the *F. graminearum* treatment ($P < 0.001$) (Table 4).

Averaged across locations, reciprocal effects were not significant for any of the three treatments, due to the magnitude of the reciprocal by location interactions for all three treatments (all significant), generally involving both maternal and non-maternal interactions (Tables 4–6). For *F. graminearum,* the reciprocal differences were significant in each location and were equally attributed to both maternal and non-maternal effects except that maternal effects were not significant in Namulonge (Table 4). *Stenocarpella maydis* reciprocal differences were significant only for Bulindi and were entirely due to maternal effects (Table 5). Under multiple inoculation, significant differences among reciprocals, primarily due to maternal effects, were found in Namulonge and Masaka (Table 6).

Variance components and relative importance of GCA to SCA

The variance component for GCA was at least more than twice the component for GCA × location for all treatments (Table 8). Furthermore, the variance components, using Baker's ratio ($2\delta^2gca/(2\delta^2gca + \delta^2sca)$, indicated that additive effects were important for resistance to *S. maydis* and to multiple

Table 3: Cross-tabulation of genotype responses to single infection for *Fusarium graminearum* and *Stenocarpella maydis* (scores averaged across locations) evaluated in 2010 second (B) season

	F. graminearum										
Score	1–1.5	1.6–2	2.1–2.5	2.6–3	3.1–3.5	3.6–4	4.1–4.5	4.6–5	5.1–5.5	5.6–6	Total
S. maydis											
1–1.5											
1.6–2		1									1
2.1–2.5											
2.6–3		1	3[1]								4
3.1–3.5		9	7	5							21
3.6–4		13	32	21	10	2	1				79
4.1–4.5		5	10	7	12	2	2				38
4.6–5										1	1
Total		29	52	33	22	4	3			1	144

Example: [1]Three genotypes out of 144 evaluated across locations had a score of between 2.1–2.5 for *F. graminearum* and a score of 2.6–3 for *S. maydis*.

Table 4: Mean squares for the 12 × 12 maize diallel analyses for *Fusarium graminearum* cob rot disease severity across and in three experimental sites (location) evaluated in 2010 second (B) season

	Across multi-locations		Individual sites (locations)			
Source of variance	df	Across Location	df	Namulonge	Masaka	Bulindi
Location	2	2.05***				
Rep/location	3		1			
Block/replicate	54		18			
GCA	11	6.42***	11	1.67***	2.88***	2.55***
SCA	66	1.14***	66	0.53***	0.54***	0.85***
Reciprocal differences	66	0.38	66	0.28***	0.41***	0.43***
Maternal	11		11	0.22	0.39***	0.46***
Non maternal	55		55	0.29***	0.42***	0.42***
GCA × location	22	0.34***				
SCA × location	132	0.39***				
Rec × location	132	0.37***				
Maternal × location	22	0.29***				
Non maternal × location	110	0.39***				
Error	375	0.12	125	0.15	0.099	0.10

***F-test significant at $P \leq 0.001$, MS, mean square.

Table 5: Mean squares for the 12 × 12 maize diallel analyses for *Stenocarpella maydis* cob rot disease severity across three sites (locations) evaluated in 2010 second (B) season

	Across multi-locations		Individual sites (locations)			
Source of variance	df	Across location	df	Namulonge	Masaka	Bulindi
Location	2	11.95***				
Rep/location	3		1			
Block/replicate	54		18			
GCA	11	4.41***	11	3.27***	0.70***	0.68***
SCA	66	0.28	66	0.38***	0.20***	0.20***
Reciprocal differences	66	0.16	66	0.25	0.13	0.18***
Maternal	11		11			0.45***
Non maternal	55		55			0.13
GCA × location	22	0.41***				
SCA × location	132	0.24***				
Reciprocal × location	132	0.16*				
Maternal × location	22	0.94***				
Non maternal × location	110	0.01				
Error	375	0.13	125	0.20	0.095	0.089

*,**,*** Data significant at P ≤ 0.05, P ≤ 01, and P ≤ 0.001 respectively, MS, mean square.

Table 6: Mean squares for the 12 × 12 maize diallel analyses for *Fusarium graminearum* and *Stenocarpella maydis* multiple cob rot disease severity across three sites (locations) evaluated in 2010 second (B) season

	Across multi-locations		Individual sites (locations)			
Source of variance	df	Across location	df	Namulonge	Masaka	Bulindi
Location	2	17.60***				
Rep/location	3		1			
Block/replication	54		18			
Genotype	143	0.46***	143	0.56***	0.18***	0.21***
GCA	11	3.65***	11	3.16***	0.89***	0.93***
SCA	66	0.20	66	0.36**	0.10	0.16**
Reciprocals	66	0.20	66	0.33*	0.13*	0.13
Maternal	11		11	0.55**	0.17*	
Non maternal	55		55	0.28	0.13*	
GCA × location	22	0.52***				
SCA × location	132	0.21**				
Reciprocal × location	132	0.18*				
Maternal × location	22	0.25**				
Non maternal × location	110	0.17*				
Error	375	0.13	125	0.22	0.086	0.098

*,**,*** Data significant at P ≤ 0.05, P ≤ 0.01, and P ≤ 0.001 respectively, MS, mean square.

infection, while for *F. graminearum*, non-additive effects were only slightly less important than additive effects. Baker's ratio, based on across location means, was 0.57, 0.94, and 1.00 for *F. graminearum*, *S. maydis*, and multiple inoculation, respectively.

Discussion

A key challenge for plant breeders is how best to select for multi-resistant genotypes in cases of endemic pathogens such as *S. maydis* and *F. graminearum*. The aim of this study was to investigate the appropriateness of multiple infection as an approach to breeding for multiple resistance to cob rots caused by *S. maydis* and *F. graminearum.* Our data show that *S. maydis* is a much faster colonizing pathogen than *F. graminearum* (Table 2), which may have influenced the colonizing ability of developing maize grain by *F. graminearum* under multiple infection. In other studies, it has been shown that multiple infection of the same host determines infection dynamics and produces several effects, including primed resistance against successive infection by the same or different pathogen (Laine 2011). Thus, infection by the more aggressive *S. maydis* may actually elicit resistance reactions that could curtail pathogenesis by *F. graminearum*. In this study, resistance to *S. maydis* under single or multiple infection was mainly due to additive gene action (as shown by Baker's ratio of 0.94 and 1.00, respectively), while resistance to *F. graminearum* was conditioned by both additive and non-additive gene action (Baker's ratio of 0.57). These results suggest that multiple resistance to both pathogens may have similar mechanisms. In other studies, based on single inoculations, additive gene action accounts for resistance to *S. maydis,* while both additive and non-additive gene actions are equally important for *F. graminearum* (Reid et al. 1992, Rossouw et al. 2002). In maize, germplasm resistant to *Aspergillus flavus* is also well correlated with resistance to *F. verticillioides* and the related mycotoxin production resistance (Henry et al. 2009). However, the finding that additive gene action accounts for resistance under multiple infection is contrary to what has been reported for multiple infection of maize cobs by *A. flavus, F. verticillioides* and *S. maydis,* in which non-additive gene

Table 7: General combining ability (GCA) effects for parental lines used across three sites (locations) evaluated during the 2010 second (B) growing season

	Fusarium graminearum				*Stenocarpella maydis*				[2]Multiple infection			
[1]Parent	Across locations	Namulonge	Masaka	Bulindi	Across locations	Namulonge	Masaka	Bulindi	Across locations	Namulonge	Masaka	Bulindi
P1	−0.08	0.02	−0.05	−0.21**	0.10	0.18	0.08	−0.19**	0.10	0.22*	−0.01	0.09
P2	0.004	0.11	0.03	−0.12*	−0.08	−0.19	−0.01	0.03	−0.03	0.08	−0.01	−0.16**
P3	−0.16**	−0.14	−0.37***	0.03	−0.20**	−0.38***	−0.12*	−0.06	−0.22**	−0.38***	−0.17**	−0.14*
P4	−0.52***	−0.39***	−0.57***	−0.58***	−0.61***	−0.77***	−0.46***	−0.39***	−0.55***	−0.76***	−0.47***	−0.48***
P5	−0.08	−0.16*	−0.08	0.004	−0.05	−0.19*	0.07	0.00	−0.03	−0.22*	0.10	0.05
P6	0.07	−0.10	0.11	0.19**	0.02	−0.02	0.05	0.15*	0.03	−0.004	0.10	−0.02
P7	−0.06	0.005	−0.11	−0.10	−0.06	−0.11	−0.04	0.07	−0.02	−0.15	−0.007	0.09
P8	−0.01	−0.12	0.04	0.07	0.07	0.06	0.02	0.13*	0.03	0.22*	−0.03	−0.10
P9	−0.12	−0.14	−0.15*	−0.06	0.26***	0.43***	0.14*	0.02	0.19**	0.26**	0.18**	0.17**
P10	0.20**	0.23**	0.30***	0.06	0.19**	0.27**	0.10	0.11	0.08	0.04	−0.01	0.19**
P11	−0.03	0.02	0.01	−0.12*	0.03	0.12	−0.04	−0.10	0.02	0.01	−0.01	0.09
P12	0.78***	0.67***	0.84***	0.84***	0.32***	0.60***	0.22***	0.23***	0.39***	0.68***	0.33***	0.23***
SE	0.07	0.08	0.06	0.06	0.07	0.09	0.06	0.06	0.07	0.09	0.06	0.06
LSD	0.196	0.22	0.18	0.18	0.20	0.25	0.17	0.17	0.20	0.27	0.17	0.17

*,**,*** GCA significantly different from 0 at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$.
[1] = Letter P denotes parents, corresponding to the inbred lines numbered 1–12 in Table 1.
[2] = Multiple infection involved inoculation of the same maize cob with both *F. graminearum* and *S. maydis*.

Table 8: Significant variance components for disease responses evaluated in three sites (locations) during the 2010 second (B) growing season

	Fusarium graminearum				*Stenocarpella maydis*				Multiple			
Source	Across locations	Namulonge	Masaka	Bulindi	Across locations	Namulonge	Masaka	Bulindi	Across locations	Namulonge	Masaka	Bulindi
GCA	0.08	0.06	0.116	0.1	0.06	0.13	0.025	0.025	0.04	0.12	0.034	0.035
SCA	0.13	0.19	0.221	0.38		0.09	0.053	0.056		0.07	0.007	0.031
Maternal		0.003	0.012	0.01				0.015		0.005	0.0035	
Non maternal		0.07	0.161	0.16							0.22	
GCA × location	0.01				0.01				0.02			
SCA × location	0.14				0.06				0.04			
Maternal × location	0.01				0.03				0.01			
Non maternal × location	0.14								0.02			
Error	0.12	0.15	0.099	0.10	0.13	0.20	0.095	0.089	0.13	0.22	0.089	0.098

action was more predominant (Mukanga et al. 2010a). This dissimilarity might have arisen from differences in pathogen species and their pathogenicity ability. The predominance of non-additive genetic variance has been shown to increase under severe selection pressure, as might have been in the case when three aggressive pathogens infected the same plant tissues (Waldmann 2001). Moreover, natural selection is expected to reduce additive genetic variation for adaptive quantitative characters such as disease resistance and may account for these observations (Falconer and Mackay 1996).

The highly negative GCA effects ($P < 0.001$) of the inbred line WL 118–10 (Parent 4) in and across locations for all treatments suggest that the inbred line can be used as a source of resistance in hybrids combinations for *S. maydis* and *F. graminearum*. On the other hand, the inbred line CZL-8 (Parent 12) had positive significant GCA effects ($P < 0.001$) across all locations suggesting that it had a stable contribution towards susceptibility in hybrid combinations for all treatments. WL 118–10 and CZL-8 had the lowest and highest mean scores, respectively, in and across locations, indicating that these inbred lines transmit their respective resistance/susceptibility genes to the hybrids. Crosses involving these two inbred lines could be suitable for mapping resistance loci to *S. maydis* and *F. graminearum.* The inbred line CML 384 (Parent 9) exhibited consistently significant positive GCA effects (Table 7) in and across locations for single *S. maydis* inoculation treatments and for multiple inoculation treatments. The single *F. graminearum* inoculation treatment had consistently negative GCA effects with the same parental line even though this GCA value was only significant in Masaka. This finding further confirms that differences occur in the way individual cob rot pathogens affect particular genotypes.

Reciprocal differences were more important when data from individual study locations were examined for single *F. graminearum* infection treatments, as compared to single *S. maydis* and multiple infection treatments, respectively, due to both maternal and non-maternal effects (Tables 4–6). However, reciprocal differences based on pooled location means for both single and multiple *S. maydis* inoculation treatments were not significant due to genotype by location interactions. This result corroborates the earlier report of Reid et al. (1992), who found out that reciprocal effects with *F. graminearum* were influenced by study locations with both significant and non-significant reciprocal effects at either location. Because reciprocal differences were relatively small, and not consistent, consideration of reciprocal differences in breeding for resistance to *S. maydis* and *F. graminearum* is not paramount. However, in seed production areas where *S. maydis* and *F. graminearum* cob rots are prevalent, using the resistant parent as the cob parent will reduce the possibility of losses due to low seed viability, poor grain quality and mycotoxin exposure as a result of cob rot.

Indirect selection is a common breeding strategy used for diseases resistance, utilizing associated agronomic traits especially for traits that are difficult to assess and or obtain reliable infection/disease development (Agrios 2005, Roussow et al. 2002). Indeed, for cob rots, it has been proposed that indirect selection for resistance could be based on the assessment of the cob rather than agronomic traits (Hefny et al. 2012, Roussow et al. 2002). We find in this study that indirect selection can be used as a breeding strategy for *S. maydis* and *F. graminearum,* with selection being based on *S. maydis* ratings as suggested by the fact that hybrids resistant to *S. maydis* were also resistant to *F. graminearum,* but the reverse was not true (Table 3). Whereas the heritability estimates for *F. graminearum* ($h^2 = 0.35$) were higher than that for *S. maydis* ($h^2 = 0.18$); the strong association between resistance to *S. maydis* and *F. graminearum,* as well as the faster colonizing ability by *S. maydis,* shows that it is a better candidate to be used as the selection target than *F. graminearum.* The lower values of the variance components (Table 8) for GCA × location as compared to those for GCA across locations indicate consistent responses of genotypes in the different locations and suggest that preliminary selection for genotype resistance to *S. maydis* and *F. graminearum* can be performed in a single environment. Thus, most genotypes can be eliminated based on the initial screening in one environment before embarking on multiple locations. Overall, the suppression and obscuring of *F. graminearum* by *S. maydis* indicates that this form of multiple infection cannot be used as an appropriate breeding strategy to obtain multiple resistance for both pathogens. The use of *F. graminearum* and *S. maydis* separately is, therefore, the best breeding strategy.

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