Resistance of Maize Hybrids and Inbreds Following Silk Inoculation with Three Isolates of *Fusarium graminearum*

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ABSTRACT

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Maize hybrids and inbred lines, including six quality protein maize (QPM) inbreds, were evaluated for resistance to ear rot after silk inoculation with three *Fusarium graminearum* isolates. Conidial suspensions were injected into the upper silk channel to approximate natural infection. Although significant isolate effects and genotype × isolate interactions were observed, the rankings of maize genotypes were similar across isolates. One of the isolates appeared to be less aggressive, and this may account for the observed isolate effects and interactions. Each of the three isolates identified two inbreds (CO272 and CO325) and one hybrid (Pride K127) as the most resistant. All of the QPM material tested was susceptible. It was concluded that, in breeding programs, one sufficiently aggressive isolate or a mixture of isolates can be used to screen for resistance.

The development of ear rot resistant maize or corn (*Zea mays* L.) hybrids is an important goal in plant breeding. An ear-rotting pathogen of great concern in Canada, some parts of the United States, and other countries is *Fusarium graminearum* Schwabe (sexual state: *Gibberella zeae* (Schwein.) Petch) (11). Infected grains may be contaminated by mycotoxins produced by this pathogen.

Insects and birds can be vectors of this pathogen, and wounds created by feeding may predispose the ear to fungal invasion (2,12). *F. graminearum* also can enter the ear via the silk and/or silk channel, with infection spreading down the ear from the tip (4,5). Genotypic differences in resistance to silk infection have been detected (9), but in most years natural infection is too low to identify such differences. Thus, screening for silk resistance involves inoculation of the silk, usually by injection of a conidial suspension into the silk channel.

Since host resistance to pathogens is sometimes isolate- or race-specific (3), host–isolate interactions could complicate the identification of resistant genotypes and reduce the effectiveness of screening and selection programs. The ability to successfully screen germ plasm with only one isolate would be a distinct advantage in breeding programs through the lowering of resource inputs in the screening process.

Mesterhazy and Kovacs (6), when testing for resistance to *F. graminearum* infection by wounding and inoculating maize ears with a colonized toothpick inserted through the husk and kernels into the rachis, found significant genotype × isolate interactions for lines with intermediate resistance but not for lines with either high or low resistance. They concluded that the use of one isolate should be sufficient for germ plasm screening. Atlin et al (1) tested six isolates on six hybrids by inserting a colonized toothpick into the silk channel, and they also concluded that genotype × isolate interactions were not large enough to warrant the use of more than one isolate for resistance breeding. However, neither of these inoculation methods reflected natural infection by the invasion of conidia through the silk or silk channel.

The purpose of this study was to evaluate the necessity of using more than one *F. graminearum* isolate in screening for resistance by a silk channel inoculation technique.

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MATERIAL AND METHODS

Three isolates of *F. graminearum* (DAOM194276, DAOM180378, and DAOM212678), obtained from the National Agriculture Canada culture collection, were used. All three isolates were originally isolated from naturally infected maize ears, and all produce mycotoxins (deoxynivalenol and zearalenone) in contaminated grain. Slight morphological differences were found to exist among the three isolates, but sporulation on low sugar medium (0.2% dextrose) was similar. Inoculum was prepared as previously described (9).

Individual plants were inoculated by injecting 2 ml of a 5×10^5 conidia per milliliter suspension into the center of the silk channel (the region between the rachis/kernel tip and the husk tip where the silks emerge) of the primary ear. All inoculations were made when silks were elongated, pollinated, and had some tip browning (approximately 6 days after silk emergence), because this stage of plant growth was found to give the greatest differentiation between genotypes (8). After inoculation, plots were overhead sprinkler irrigated 2–5 mm twice daily for 4 wk. Evaluations for spread of infection were made at normal harvest time (average moisture content of 24%) in mid-October using a 7-class rating scale where 1 = no infection, 2 = 1–3%, 3 = 4–10%, 4 = 11–25%, 5 = 26–50%, 6 = 51–75%, and 7 = more than 75% of the kernels being infected.

Kernels exhibiting symptoms of infection and symptomless kernels from randomly selected ears were used to reisolate the pathogen to verify that *F. graminearum* was the causal agent of the infection. Kernels were surface sterilized in 10% Javex for 1 min, plated on potato-dextrose agar with 2% dextrose, and incubated at 25 C. Visual identification of *F. graminearum* was made 7 days later according to Nelson et al (7).

Experiment 1—inbreds. In 1989 and 1990, a 12 × 4 factorial experiment arranged in a split-plot design with four replicates was conducted at the Central Experimental Farm, Agriculture Canada, Ottawa, Ontario. Each main plot unit consisted of four single-row subplot units (3.8 m long, 76 cm between rows) of 12 plants each. Twelve public inbreds (A641, CK44, F2, F7, Mo17, CO265, CO266, CO267, CO272, CO282, CO317, and CO325), previously shown to range from highly resistant to highly susceptible (9), were randomized to the main plot units. The subplot units consisted of one control row inoculated with sterile water and three rows inoculated with one of the three *F. graminearum* isolates per row. The center 10 plants of each row were inoculated and evaluated for resistance. Ears were rated individually, and a mean rating was calculated for each row.

Experiment 2—hybrids. In 1990 and 1991, a 5 × 5 factorial experiment arranged in a split-plot design with four replicates was conducted at the Central Experimental Farm in Ottawa. Five hybrids (Cargill SX108, Dekalb DK-415,

Pioneer 3902, Pioneer 3953, and Pride K127) were randomized to the main plot units. The experiment was carried out as above except that an additional subplot unit was added in which the ears were inoculated with a mixture of all three *F. graminearum* isolates.

Experiment 3—QPM inbreds. In 1990 and 1991, an 8 × 4 factorial experiment arranged in a split-plot design with three replicates was conducted at McGill University, Macdonald Campus, Ste-Anne-de-Bellevue, Quebec. Six Quality Protein Maize (QPM) inbreds (QPM-3, QPM-19, QPM-23, QPM-37, QPM-43, and QPM-50), a normal-endosperm check (A619), and a soft-endosperm high-lysine (opaque) check (A619*o2*) were randomized to the main plot units. Due to limited seed supply, QPM-23 was replaced by QPM-26 in 1991. The QPM lines used here were developed from two gene pools, Northern Temperate Region-1 (NTR-1) and NTR-2, that were converted to QPM at CIMMYT in Mexico and selfed to the S_5 generation at Ottawa. Each main plot unit consisted of four single-row subplots to which the three isolates and a sterile-water control were randomized as above. The inbred A619 was detasseled prior to anthesis to prevent pollen contamination of QPM and *o2* germ plasm.

Statistical analyses. Average ratings on a per-plot basis were analyzed separately by year for each experiment using standard analysis of variance procedures. These analyses were conducted twice, once using the data from all inoculation treatments and once using data from the *F. graminearum* inoculation but not the sterile water. Residual error terms were generated and tested for normal distribution using the Kolomogorov D statistic (10).

RESULTS

F. graminearum was reisolated from all kernels exhibiting symptoms of infection. Both *F. graminearum* and some *Fusarium moniliforme* J. Sheld. also were isolated from infected kernels of the control plants, especially of the more susceptible genotypes, indicating that some infection occurred in noninoculated plants.

In each experiment, data were analyzed separately for each year because error mean squares were not homogeneous ($P < 0.05$). Residual error terms were found to be normally distributed ($P > 0.05$), as tested by the Kolomogorov D statistic.

Analyses of each experiment, including the water controls, indicated highly significant inoculation–treatment effects for both years. All genotypes remained relatively free of infection (ratings between 1 and 2.8) when water alone was used, yet ranged in ratings between 1.2 and 6.7 when inoculated with the different *Fusarium* isolates.

Experiment 1—inbreds. Genotype effects and isolate effects were significant in both years at the 0.01 probability level (Table 1). Genotype × isolate interactions were significant at the 0.01 and 0.05 probability levels in 1989 and 1990, respectively, but this source of variation represented only a small proportion of the total sum of squares.

The most resistant inbreds were CO272 and CO325 in both years (Table 2). The inbreds CK44 and F2 were moderately resistant, and the remaining eight inbreds were susceptible. The DAOM-212678 isolate appeared to be the least aggressive of the three isolates; on several inbreds, the disease ratings obtained with this isolate were lower than those obtained with one or both of the other isolates. For some inbreds, such as Mo17, A641, and CO265, this resulted in a significant lowering of the disease rating. The most resistant inbred, CO272, consistently ranked as the most resistant to all isolates in both years.

Experiment 2—hybrids. Genotype and isolate effects were significant in both years (Table 1). Genotype × isolate interactions were not significant in 1990 but were significant in 1991. As in experiment 1, the interaction sum of squares was small in relation to the sum of squares for genotypes.

Table 1. Analysis of variance for three experiments in which maize genotypes were inoculated with three isolates of *Fusarium graminearum*

	Expt. 1: inbreds			Expt. 2: hybrids[y]			Expt. 3: QPM inbreds		
		Mean squares			Mean squares			Mean squares	
Source	df	1989	1990	df	1990	1991	df	1990	1991
Replicates	3	0.19	0.02	3	0.15	0.03	2	0.17	1.49
Genotypes	11	20.23**[z]	16.01**	4	16.96**	28.73**	7	7.41**	5.13**
Error a	33	0.30	0.73	12	0.74	0.11	14	0.61	0.55
Isolates	2	14.12**	15.67**	2	4.18**	0.95**	2	1.31	5.90**
G × I	22	0.71**	1.13*	8	0.93	0.87**	14	0.10	0.78*
Error b	72	0.30	0.58	30	0.62	0.10	32	0.41	0.30

[y] A mixture of three isolates was also included in this experiment, but data from that treatment were not included in this analysis.
[z] * = Significant at the 0.05 probability level; ** = significant at the 0.01 probability level.

Table 2. Mean disease ratings and rankings in 1989 and 1990 for 12 inbreds inoculated with *Fusarium graminearum*

	Mean disease rating[y] and ranking[z]											
Isolate	CO317	CO282	CO266	CO267	Mo17	A641	CO265	F7	F2	CK44	CO325	CO272
1989												
DAOM194276	4.9a	5.5b	5.4b	4.9ab	5.0b	4.9b	4.2ab	4.1 a	4.2b	2.2ab	3.0b	1.8a
	(8)	(12)	(11)	(8)	(10)	(8)	(5.5)	(4)	(5.5)	(2)	(3)	(1)
DAOM180378	6.0b	5.8b	5.9b	5.5b	4.9b	4.4b	4.9b	4.0a	3.5b	2.5b	2.7b	1.6a
	(12)	(10)	(11)	(9)	(7.5)	(6)	(7.5)	(5)	(4)	(2)	(3)	(1)
DAOM212678	5.5ab	4.1a	4.2a	4.6a	3.3a	3.5a	4.0a	3.7a	2.3a	1.5a	1.6a	1.3a
	(12)	(9)	(10)	(11)	(5)	(6)	(8)	(7)	(4)	(2)	(3)	(1)
1990												
DAOM194276	5.5a	6.0b	5.6b	5.7b	6.1b	5.2b	4.4b	4.7b	4.1b	4.2a	2.6a	1.8a
	(8)	(11)	(9)	(10)	(12)	(7)	(5)	(6)	(3)	(4)	(2)	(1)
DAOM180378	5.1a	5.1ab	4.2a	5.4b	5.6b	5.0b	4.7b	4.0ab	3.5b	3.9a	2.1a	1.9a
	(9.5)	(9.5)	(6)	(11)	(12)	(8)	(7)	(5)	(3)	(4)	(2)	(1)
DAOM212678	5.2a	4.6a	5.1ab	4.4a	3.4a	3.9a	2.7a	3.1a	2.2a	3.4a	2.3a	1.9a
	(12)	(10)	(11)	(9)	(6.5)	(8)	(4)	(5)	(2)	(6.5)	(3)	(1)

[y] Mean ratings are on a scale of 1–7, where 1 = no infection and 7 = more than 75% of the kernels infected. Means followed by the same letter for a given inbred within a given year are not significantly different at the 0.05 probability level based on the Fisher (protected) LSD test.
[z] Numbers in parentheses are ranks of genotypes within each isolate with average ranks assigned to ties.

The most resistant hybrid was Pride K127, followed by Pioneer 3953, Pioneer 3902, Dekalb DK-415, and Cargill SX108, with ratings ranging from 1.2 to 5.6 (Table 3). The DAOM194276 and the DAOM180378 isolates appeared to be equally aggressive in both years, and the DAOM212678 isolate tended to be the least aggressive of the three. Very little change in genotypic rankings occurred. Pride K127 consistently ranked first (most resistant) and Pioneer 3953 second. When the hybrids were inoculated with a mixture of the three isolates, ratings tended to be higher but in most cases were not significantly different from one or more of the ratings with a single isolate.

Experiment 3 - QPM inbreds. Genotype effects were significant in both years at the 0.01 probability level (Table 1). Isolate effects and genotype × isolate interactions were not significant in 1990 but were significant in 1991 at the 0.01 and 0.05 probability levels, respectively. Again, the interactions were a minor source of variation.

All inbreds were susceptible, with ratings as high as 6.5 (more than 50% of the kernels infected). The o_2 version of A619 was more susceptible than normal-endosperm A619. As in experiments 1 and 2, the DAOM212678 isolate was the least aggressive of the three isolates. Within a given year, rankings of genotypes were fairly consistent across isolates, except that in 1991, QPM-26 and QPM-37 were ranked lower with DAOM212678 than with the other isolates (Table 4). In contrast to what was observed in experiments 1 and 2, where rankings were fairly consistent between years, some rankings in experiment 3 differed between 1990 and 1991. The two largest rank reversals were for QPM-19, which ranked fourth in 1990 and first in 1991, and QPM-43, which ranked third in 1990 and an average of seventh in 1991.

Table 3. Mean disease ratings and rankings in 1990 and 1991 of five hybrids inoculated with *Fusarium graminearum*

	Mean disease rating[w] and rankings[x]				
Isolate	Cargill SX108	Dekalb DK-415	Pioneer 3902	Pioneer 3953	Pride K127
1990					
Mean[y]	5.0	4.6	4.2	2.9	2.2
	(5)	(4)	(3)	(2)	(1)
Mixture[z]	4.4	5.6	3.0	2.9	2.4
	(4)	(5)	(3)	(2)	(1)
1991					
DAOM194276	5.3b	4.6b	3.0ab	2.9ab	1.4a
	(5)	(4)	(3)	(2)	(1)
DAOM180378	5.5b	3.8a	3.3b	3.1b	1.2a
	(5)	(4)	(3)	(2)	(1)
DAOM212678	3.9a	4.3b	2.7a	2.6a	1.3a
	(4)	(5)	(3)	(2)	(1)
Mixture	4.3	5.0	4.1	3.5	1.8
	(4)	(5)	(3)	(2)	(1)

[w] Mean ratings are on a scale of 1–7, where 1 = no infection and 7 = more than 75% of the kernels infected. Means followed by the same letter for a given inbred within a given year are not significantly different at the 0.05 probability level based on the Fisher (protected) LSD test.

[x] Numbers in parentheses are ranks of genotypes within each isolate with average ranks assigned to ties.

[y] Genotype means across all isolates since genotype × isolate interactions were not significant in 1990.

[z] Mixture of the three isolates.

Table 4. Mean disease ratings and rankings in 1990 and 1991 of QPM inbreds inoculated with *Fusarium graminearum*

	Mean disease rating[x] and ranking[y]								
Isolate	QPM 3	QPM 19	QPM 23	QPM 26	QPM 37	QPM 43	QPM 50	A619 *o2*	A619
1990									
Mean[z]	5.3	4.9	3.8	...	6.5	4.4	5.2	5.9	4.1
	(6)	(4)	(1)		(8)	(3)	(5)	(7)	(2)
1991									
DAOM194276	4.7b	3.1a	...	4.1b	5.8b	5.8ba	3.8a	5.3a	4.5a
	(5)	(1)		(3)	(7.5)	(7.5)	(2)	(6)	(4)
DAOM180378	5.9b	3.6a	...	4.7b	5.9b	5.8a	4.6a	5.2a	4.9a
	(7.5)	(1)		(3)	(7.5)	(6)	(2)	(5)	(4)
DAOM212678	4.2a	3.4a	...	2.7a	3.8a	5.1a	4.1a	4.9a	4.6a
	(5)	(2)		(1)	(3)	(8)	(4)	(7)	(6)

[x] Mean ratings are on a scale of 1–7, where 1 = no infection and 7 = more than 75% of the kernels infected. Means followed by the same letter for a given inbred within a given year are not significantly different at the 0.05 probability level based on the Fisher (protected) LSD test.

[y] Numbers in parentheses are ranks of genotypes within each isolate with average ranks assigned to ties.

[z] Genotype means across all isolates since genotype × isolate interactions were not significant in 1990.

DISCUSSION

Significant isolate effects were observed in all experiments except for 1990 in experiment 3 (QPM inbreds). Significant genotype × isolate interactions were observed in both years for experiment 1 but only in one year (1991) for experiments 2 and 3. However, it was rare for a genotype to be classified as resistant with one isolate and susceptible with another. In each case, the relative lowering of disease rating was due to DAOM212678, which appeared to be the least aggressive of the three isolates. This also was reflected in the sometimes different rankings of genotypes obtained with this isolate. All three isolates consistently ranked the more resistant genotypes and the more susceptible genotypes. Inconsistencies in ranking tended to be restricted to those genotypes with moderate resistance or moderate susceptibility (ratings of 3.8–5) in a given group of germ plasm. This finding agrees with that of Atlin et al (1) and Mesterhazy and Kovacs (6).

Although the finding of significant isolate and genotype × isolate interactions in resistance to *F. graminearum* has been reported previously, it has not been tested for resistance to silk infection or on such a wide range of germ plasm. Our study confirmed that genotypic differences in silk resistance can be detected with different isolates. The three isolates acted consistently with respect to their relative degrees of aggressiveness in each experiment. In experiments 1 and 2, two inbreds (CO272 and CO325) and one hybrid (Pride K127) appeared to possess high resistance and are thus potential sources of resistance in future breeding programs. However, the QPM material of experiment 3 possessed very little resistance, with the least susceptible inbreds having average ratings of 3.8.

All of these studies, covering a broad range of genetic backgrounds, indicated that if a sufficiently aggressive isolate (in comparison to other isolates) is used, a plant breeder–geneticist will be able to identify genotypes with useful resistance and those with severe susceptibility. Also, the use of the most aggressive isolate (i.e., that which results in the greatest disease severity) available would allow for a greater range in disease ratings and thus more differentiation between geno-

types and more reliable rankings, especially in years when the environment is not conducive to fungal growth.

Although these results indicate that one isolate should be sufficient to screen for resistance, in nature and different environments or locations, plants may be exposed to many different strains. In experiment 3, inoculation with a mixture of the three isolates tended to increase the amount of infection without changing the relative ranking of the hybrids. A mixture of isolates of various levels of aggressiveness would closely model natural conditions and would lower the probability of improper evaluation of resistance. In conclusion, in breeding programs, the use of either a sufficiently aggressive isolate or a mixture of isolates should give adequate evaluation of resistance to silk infection by *F. graminearum*.

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