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Evaluating commercial maize hybrids for resistance to gibberella ear rot *

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Abstract

An integral component of breeding maize for resistance to *Fusarium graminearum* ear rot is the identification of resistant genotypes. Since natural infection is not consistent from year to year, maize researchers must use manual techniques to inoculate the plant material with fungal spores. Information is presented here on site resistance of commercial maize hybrids to *F. graminearum* over three years and at two locations. Additionally, results of an investigation on the two predominant techniques of inoculating maize, the silk channel and kernel inoculation methods, are reported. Of 61 commercial hybrids tested, only two were ranked as moderately resistant to the fungus by both inoculation methods. These two hybrids also had a stable response to the *F. graminearum* infection across seven environments when the silk channel inoculation method was used. The majority of the hybrids were ranked as either susceptible or highly susceptible and less than 10% of the hybrids had a stable response to fungal infection. In the investigation of methodology, it was concluded that silk browning would be the least laborious way to identify the ideal time to complete silk channel inoculations. It was found that kernel inoculations using the pin inoculation method should take place between 11 and 15 days after 50% silking to achieve proper hybrid discrimination. Mist irrigation increased mold severity ratings and resulted in greater discrimination between hybrids with varying levels of resistance to *F. graminearum* infection.

Introduction

Fusarium graminearum Schw. (teleomorph: *Gibberella zeae* (Schw.) Petch) is a fungal pathogen that causes gibberella ear rot of maize (*Zea mays* L.) and head blight of wheat (*Triticum aestivum* L.). This fungus causes economic damage in Canada (Martin and Johnston, 1982), the United States (Abbas et al., 1988), and elsewhere (Marasas et al., 1979, Tanaka et al., 1988) by reducing grain yield and producing mycotoxins that render the remaining kernels unsuitable for human and livestock consumption. In Ontario, mycotoxins produced by gibberella ear rot continue to be a significant problem for pork producers (Trenholm et al., 1988). Zearalenone causes infertility in male swine and hyperestrogenism in female swine (Mirocha and Christensen, 1974). Deoxynivalenol (DON, vomitoxin) causes feed refusal and vomiting in swine (Prelusky et al., 1994) which leads to a reduction in weight gain and feed conversion.

The fungus gains entry into the maize ear by two routes. One infection route is via the silk. Conidia or ascospores land and germinate on the silk, entering the ear by way of mycelium growing down the silk. The second route is through wounds in the husk created by insects (Sutton, 1982, Miller, 1994), birds (Sutton et al., 1980), or hail (Abbas et al., 1988). The biotic causes of husk wounds probably result in the greater frequency of *Fusarium* spp. infection because insects and birds can be vectors of the fungus. For example, conidia have been isolated from picnic beetles (*Glischrochilus quadrisignatus*) (Attwater and Busch 1983, Windels et al., 1976) and birds (Warner and French 1970). Also, insects such as thrips (*Frankliniella occidentalis*) and the European corn borer (*Ostrinia nubilalis*) have been related to high incidences of disease

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caused by *Fusarium* spp. (Farrar and Davis, 1991; Christensen and Schneider, 1950).

We have developed a standard procedure to evaluate and recommend maize hybrids from varying commercial sources for resistance to this fungus. Previous work has demonstrated some corn hybrids and inbreds that vary in their response to gibberella ear rot (Reid et al., 1992; Reid et al., 1993; Schaafsma et al., 1993). The objectives of this study were to determine the reaction of 61 current commercial maize hybrids to gibberella ear rot and rank their susceptibility to *F. graminearum*, and to evaluate and refine the procedures for inoculating maize ears by silk channel injections (Chungu et al., 1996), and by kernel wounding (Reid and Hamilton, 1996) with the fungus. The first objective was completed to provide current resistance information on hybrids in use in Ontario. The second objective was accomplished by investigating the effects of several factors on the response of different hybrids known to be moderately resistant or susceptible to *F. graminearum*.

Materials and methods

General plot information

The plots were planted during the first week of May on a gravelly loam soil in 1993, 1994, and 1995 at Ridgetown, Ontario, and on a sandy-loam soil in early May in 1995 at Ottawa, Ontario. Plots were single rows spaced 0.76 m apart and 4 m long, with 35 seeds planted per plot, and thinned to 25 plants per plot during the first week of June. The crop was maintained following provincial recommendations for weed control and plant nutrition (OMAFRA, 1993, OMAFRA, 1995). At Ottawa, the plots were 3.8 m long with 14 plants per plot.

Irrigation

At Ridgetown, the entire experiment was irrigated using overhead misting, from the first day of inoculations until 3 weeks after the last inoculation for each year. The misting system consisted of a hollow aluminum sleeve which supported a 2-cm polyethylene pipe suspended above the crop at full height, snap-in nozzles (‘green nozzle’ part number AT 77-930502) spaced 1.32 m apart, and a ‘green kwik spinner’ (part number AT 77-930202). The nozzles and spinner were ordered from Vanden Bussche Irrigation Limited, Milton, ONT. The system was operated at 206 kPa pressure and nozzles delivered approximately 0.98 L min^{-1}. A cycle of 8 seconds on and 30 seconds off from 1000 to 2000 hours delivered approximately 7.5 cm of water per week. The timers were adjusted manually to account for changes in temperature and moisture. Misting was set to keep the ear zone wet without saturating the soil. At Ottawa, plots were irrigated using an overhead rain bird sprinkler system that delivered 4 to 5 mm of water twice daily (morning and early evening).

Inoculations

Ten primary ears were inoculated per plot unless otherwise stated. There were two methods of inoculation. The first was the silk channel inoculation (SILK) method described by Reid et al. (1993) where 2 ml of a macroconidial suspension of *F. graminearum* (Schaafsma et al., 1993), at a concentration of 10^5 conidia ml^{-1} were injected with a blunt needle into the silk channels of individual ears. The second method involved directly damaging the kernels, via penetration of the husk with various numbers of pins previously dipped in a conidial suspension, or with untreated pins followed by injection of conidial suspension (PIN method). For hybrid screening, the PIN method wounding device used at Ridgetown consisted of 25 brass pins embedded in a lead square measuring 2.5 cm $\times$ 2.5 cm. Each ear was wounded on the upper surface at the mid-point of the cob followed immediately by an injection of 2 ml of 10^5 conidia ml^{-1} suspension into the wound area. At Ottawa, the 4 pin bar method described in Reid and Hamilton (1996) was used.

Inoculations were timed according to silking date except for those experiments that tested the effect of various inoculation times. SILK inoculations were timed to silking of each ear, and PIN inoculations were timed to 50% silk for each plot. Plots were examined daily for silking, and silked ears were then marked. SILK inoculations were done when 195 crop heat units (CHU) (Brown and Bootsma, 1993) accumulated after silking (about 7 days), while PIN inoculations were done when 600 CHU accumulated after 50% silking (about 21 days). The first SILK inoculations commenced during the third week of July, and were spread over three weeks to accommodate the range of hybrid maturities. The first PIN inoculations commenced during the second week of August and were again spread over three weeks.

Conidial suspension

Single-spore cultures of *F. graminearum* (isolate DAOM 180378) were generated according to Schaafsma et al. (1993) except for the following changes to the medium: the amount of KH_2PO_4 and KCl was halved, 2.0 g of KNO_3 instead of 8.0 g was used, and the micronutrients $FeCl_3$, $mgSO_4$ and $ZnSO_4$ were added to 1 l distilled water at the levels of 0.2 mg, 1.0 g and 0.2 mg, respectively. In Ottawa, the inoculum was prepared according to Reid et al. (1992) and was a mixture of three strains (DAOM 194276, DAOM 180378 and DAOM 212678).

Mold severity scores and hybrid rankings

Plots were evaluated when hybrids in each maturity block reached 24% moisture or less. Cobs were rated for mold severity using a 1–7 rating system (Reid et al., 1993) where 1 = NIL, 2 = 1–3%, 3 = 4–10%, 4 = 11–25%, 5 = 26–50%, 6 = 51–75%, and 7 = 76–100% of the ear was covered with mold.

Commercial hybrid rankings were made on the basis of the frequency that they were numerically better or worse than the best or worst checks in the mold severity ratings, for each year. The moderately resistant (MR) checks were Ciba G-4106 and Pioneer 3790 (Miller, 1994) dependent upon which hybrid of the two had the lower severity rating in a given year and Pioneer 3737 was used as the highly susceptible (HS) check (Schaafsma et al., 1993). The final ranking was obtained by listing all of the yearly rankings and identifying the median rank.

Maize hybrid screening trial

The screening of hybrids was completed for three years at Ridgetown (1993 to 1995) and one year (1995) at Ottawa, using both the SILK and PIN inoculation methods. At Ridgetown, the four experiments were arranged as randomized complete block designs with three replications. Hybrids were selected on the basis of dominance in the market place, with the selection representing over 90% of market share in Ontario. Hybrids were assigned to three groups of equal size according to maturity: early, 2500 to 2765 CHU, approximately equal to the FAO's 200–325 RM units (Troyer, 1994); mid-season, 2780 to 2941 (350–410 RM); and late, 2950 to 3300 CHU (410–480 RM). Plots were split in two equal sub-plots with one half assigned to SILK inoculations and the other half to PIN inoculations. At Ridgetown, the experiments were surrounded by four guard rows and a wind break (PVC snow fencing with 50% porosity) on the windward side. At Ottawa, the experiments were surrounded by 5 guard rows.

SILK inoculation studies

Effect of inoculation timing

Experiments to determine whether silk browning could be used as a reliable predictor of optimum timing for SILK inoculations were conducted under misting at Ridgetown in 1994 and 1995. Silk browning is defined here as the point when fresh silk just begins to senesce at the tips. In 1994 only one hybrid was used (Pioneer 3573), and the experiment was designed as a randomized complete block with three replications. Ten primary ears were inoculated per plot using the SILK method. Each ear was marked at silk emergence and inoculated when one of three milestones was reached: accumulation of 195 CHU after silk emergence, 7 days after silk emergence or silk browning.

In 1995, the experiment was designed as a 3×10 factorial arranged in a randomized complete block with three replications. The three timing factors that were compared were the same as those used in 1994. The second factor was hybrid including the following maize hybrids: Agriseed 280, and 350, Pioneer 3737, 3790, 3953, Ciba G-4030, G-4106, G-4148, Pickseed 5545 and Pride 6372.

Effect of mist irrigation

Experiments to test the effect of overhead misting on hybrid discrimination under SILK inoculations were planted at Ridgetown in 1994 and 1995. In 1994, the experiment was designed as two randomized complete blocks with four replications. Main plots consisted of misted and non-misted blocks which were divided into four subplots of one row for each of four maize hybrids: Pioneer 3737 and Ciba G-4148 (susceptible hybrids) and Pioneer 3790 and Ciba G-4106 (moderately resistant hybrids). In 1995, two separate experiments were planted with one conducted under mist and one without mist. The maize hybrids were the same except that Ciba G-4030 replaced Ciba G-4148. Ten primary ears were inoculated per plot in 1994 while in 1995 twenty were inoculated.

PIN inoculation studies

Effect of inoculation timing

Experiments were planted at Ridgetown in 1994 and 1995 to determine the optimum timing for PIN inoculations. Two aspects of timing were investigated. First, ratings for each hybrid were plotted against days after silking to discern the best time for obtaining high and uniform ratings. Second, different ratings within individual hybrids over time were statistically analyzed to determine which inoculation time gave the best separation of the hybrids. For both years, the experiment was mist irrigated, and 10 primary ears were inoculated per plot. In 1994, the plots were arranged as a 4 × 5 factorial in a randomized complete block design with three replications. Main effects were hybrid (Pioneer 3737, Ciba G-4030, Pioneer 3790 and Ciba G-4106) and inoculation timing (7, 12, 17, 21 and 26 days after silking). In 1995, the plots were arranged as a 6 × 6 factorial in a randomized complete block with three replications. The six different times compared were: inoculation after 7, 11, 15, 19, 23 or 27 days after silk emergence, and the hybrids used were the same as those in 1994 plus two more, Pioneer 3953 and Ciba G-4148.

Effect of mist irrigation

Experiments were planted at Ridgetown in 1994 and 1995 and were inoculated with the large pin block. For both years the factors and experimental design were the same as described for Silk Inoculation, except that in 1995 one extra hybrid was tested (Pioneer 3953).

Statistical analysis

The results were evaluated using analysis of variance (ANOVA) procedures by way of PROC GLM, or PROC ANOVA procedures (SAS, 1991). Assumptions for ANOVA were tested using PROC GLM and PROC UNIVARIATE. Unless otherwise stated a 5% level of significance was used to separate means. The stability of hybrid response to *F. graminearum* infection was analyzed by regressing individual hybrid mold severity means for each year against an environmental index, in this case, the overall annual mold severity mean for all hybrids (Eberhart and Russell, 1966). Hybrid stability was analyzed by each inoculation method separately. The dependent variable of disease severity is different from that of Eberhart and Russell (1966), who were analyzing crop yields. Stability in this paper is defined as those regressions that have slopes approaching zero rather than one, as the concerns of underestimating and overestimating yields in different environments do not apply here. Hybrids are said to be increasingly unstable as they decrease or increase away from zero. It should be noted that the purpose of this method of stability analysis was not to explain genotype by environment interactions, as this is probably not appropriate (Westcott 1986), but rather to identify which genotypes had a significantly consistent response to fungal infection. This analysis allows the researcher to identify genotypes that have stable mold severity ratings across different environments and coupled with the rankings, should identify the ‘best’ hybrids for *F. graminearum* resistance.

Table 1. Analysis of variance for the effect of hybrid and method of inoculation and environment (year) on mean mold severity of commercial maize hybrids inoculated with *F. graminearum*

Source	*df*	Mean squares	F-ratio	*P*
Hybrid	60	2.9	10.0	0.0001
Method	1	226.2	766.6	0.0001
Year	3	77.2	261.4	0.0001
Hybrid∗Method	60	1.0	3.5	0.0001
Hybrid∗Year	180	1.1	3.7	0.0001
Method∗Year	3	40.0	135.6	0.0001
Hybrid∗Method∗Year	180	0.7	2.3	0.0001
Error	845	0.30		

Results and discussion

Maize hybrid evaluation experiments

The SILK inoculation data from Ridgetown in 1993 was not used for the final hybrid rankings as mean mold severity ratings were generally too low to distinguish resistance levels. Before an ANOVA was completed, a Bartlett’s (1937) test was completed and this revealed that the variances between years were homogenous ($P>0.05$). The analyses of variance showed that all parameters (hybrid, inoculation method and test year) affected mold severity (Table 1). Inoculation method (SILK versus PIN) had the greatest effect, accounting for 64.8% of the error. There were significant interactions between these main effects, but these interactions contributed relatively little to the error except for the

Table 2. Analysis of variance for the effect of hybrid (n=61) on mean mold severity scores, by individual years and methods of inoculation. The corresponding average mold severity for all hybrids within each year is also shown

Inoculation Method	Year[1]	*df*	Mean squares	F-ratio	*P*	Coefficient of variability (CV) (%)	Mean mold severity score (1–7)
SILK Channel	1994-R	60	0.90	3.3	0.0001	15.3	3.0
	1995-R	60	0.90	1.9	0.002	20.7	2.9
	1995-O	60	3.32	21.2	0.0001	10.6	3.2
PIN Block	1993-R	60	0.88	3.2	0.0001	12.5	3.5
	1994-R	60	0.62	1.7	0.008	17.7	2.8
	1995-R	60	0.75	1.8	0.004	11.9	3.9
	1995-O	60	1.39	13.4	0.0001	5.5	4.2

[1] R = Ridgetown, Ontario and O = Ottawa, Ontario.

interaction between inoculation method and year. The effect of hybrid maturity and the interactions of hybrid maturity with method, year, and mold severity were not significant (separate ANOVA not shown). The effect of hybrid on mold severity was significant every year, for each method of inoculation (Table 2). PIN inoculations resulted in higher mold severity scores and a lower standardized variation (coefficient of variability) for the majority of the years compared to SILK inoculations (Table 2).

Table 3 shows the cumulative data used to obtain the final maize hybrid rankings. The majority of commercial hybrids tested were either susceptible or highly susceptible to *F. graminearum* for both inoculation methods (Table 3). For the SILK inoculations, Ciba G-4106 was used as the moderately resistant (MR) check for the three environments (with mean mold severity values of 2.5, 2.6 and 1.6) and was used in one year (1995, Ottawa) as the PIN inoculation MR check (with a mean mold severity of 3.2). Pioneer 3790 was used as the MR check for the PIN inoculations in the remaining environments, and had corresponding severity values of 2.9, 2.3 and 4.1. The highly susceptible (HS) check, Pioneer 3737, had mold severity values of 3.8, 4.4 and 3.7 for the SILK inoculations, and 4.5, 3.4, 6.0 and 5.9 for the PIN inoculations. Only two hybrids (Pick 4990 and Pioneer 3527) were ranked as (MR) for both inoculation methods. None were found to be as susceptible as the highly susceptible check, although one, Pioneer 3787, was HS when SILK inoculated, and susceptible (S) when PIN inoculated. Most hybrids fell between the two ends of the rankings. As hybrids within each final ranking were ordered according to increasing crop heat units (CHU), a changing level of susceptibility was not implied.

All of the tested sources had significant interactions with the year, and in a given year, some of the hybrids known to have a certain level of *F. graminearum* resistance or susceptibility fell outside their expected performance. Environmental factors, such as rainfall and temperature, affect the severity of *Fusarium* (spp.) infections (Sutton, 1982; Tuite et al., 1974) and these are the likely causes of the variation in mold severity scores. Hybrid susceptibility is influenced by environmental conditions, underscoring the need for several years of data to satisfactorily rank maize hybrids.

The significant effects of year and year by hybrid interaction on mean mold severity probably contributed to the lack of hybrids that had a stable response to *F. graminearum* infection. Although there was a significant genotype by environment interaction, this represented only 0.32% of the total error, which allows for the stability analysis to be completed.

The stability analysis revealed that very few hybrids had significant regression slopes approaching zero (and hence were stable). More regressions were significantly unstable rather than stable (e.g. Hyland 2241, Ciba G-4273). Some hybrids had a significantly stable response to *F. graminearum* infection for one of the inoculation methods (e.g. Mycogen 4670, Cargill 3777). However, the final outcome for these hybrids was one of susceptibility. Less than 10% of the large number of hybrids tested had significantly stable slopes (slopes of less than 1.0). The stability analysis demonstrates the difficulty in accurately predicting how commercial hybrids would respond during *F. graminearum* outbreaks, as changing environmental conditions (location and year) have a pronounced effect upon hybrid performance. Because the hybrids selected for testing represent over 90% of the market

Table 3. Overall rankings of 61 commercial hybrids to *F. graminearum* susceptibility, and a comparison of the stability of the results over different environments (years). Hybrids grown at Ridgetown, Ontario in 1994 and 1995 and grown at Ottawa, Ontario in 1995

Hybrid		Final Rank		Stability Analysis[2]	
Name	CHU[1]	SILK	PIN	SILK Slope	PIN Slope
PICKSEED 4990	2850	MR	MR	0.31	0.65
PIONEER 3527	3360	MR	MR	0.15*	0.88
CIBA G-4106	2880	MR	S	0.17	0.91
PIONEER 3790	2850	S	MR	1.36	1.59*
PIONEER 3921	2580	S	S-MR	2.33	1.12
CARGILL 1927	2670	S	S-MR	0.29	0.55
PRIDE K154	2680	S	S-MR	0.77	0.04
CARGILL 3577	3010	S	S-MR	0.12	0.80
DEKALB DK 485	3040	S	S-MR	1.28	1.09
HYLAND 2241	2500	S	S	2.11**	0.64
CIBA G-4030	2570	S	S	0.81	1.00
PIONEER 3907	2600	S	S	2.49	0.93*
CIBA G-4043	2600	S	S	2.02*	1.05*
HYLAND 2272	2600	S	S	1.62	1.36
PICKSEED 2620	2620	S	S	1.07*	0.42
PICKSEED 2621	2630	S	S	0.32	0.81
DEKALB DK352	2650	S	S	1.51*	0.65
PRIDE 6352	2660	S	S	1.21	0.48
NK N2555	2660	S	S	0.54	0.97
CARGILL 3427	2680	S	S	0.27	0.65
NK N2409	2690	S	S	0.72	1.71
PIONEER 3876	2700	S	S	2.29	1.00
AGRI 280	2700	S	S	1.16	0.88
PRIDE 6355	2720	S	S	1.02	0.04
CIBA G-4120	2730	S	S	0.66	1.51*
CIBA G-4023	2750	S	S	1.21	1.53**
FERG TFSX1194A	2750	S	S	0.86	0.64
PRIDE K213	2750	S	S	1.7	–0.06
CARGILL 2927	2760	S	S	1.56	1.36
DEKALB DK401	2760	S	S	1.20	1.72*
NK N2879	2760	S	S	0.43	1.66
CIBA G-4070	2790	S	S	0.65	0.82
NK N2933	2800	S	S	0.54	1.20**
GREAT LAKES GL420	2800	S	S	1.53*	1.47
PIONEER 3861	2810	S	S	2.76	1.59
NK N3030	2810	S	S	0.37	1.21*
MYCOGEN 4120	2830	S	S	0.60	1.06
DEKLAB DK421	2830	S	S	1.14*	0.97
AGRI 350	2850	S	S	1.67	0.92
PICKSEED 5545	2880	S	S	0.81	1.22
CIBA G-4148	2900	S	S	0.96	1.24
PIONEER 3769	2950	S	S	2.26	0.96
MYCOGEN 4670	2950	S	S	0.69*	1.09
PIONEER 3751	3000	S	S	0.98	0.33
RENK RK 616	3020	S	S	1.67	1.40*
FIRST LINE HADES	3030	S	S	0.45	1.01*
HYLAND 2527	3040	S	S	0.62	1.06
PIONEER 3733	3050	S	S	1.11	1.44
CARGILL 3777	3070	S	S	0.47	0.41*
CIBA G-4273	3100	S	S	1.26	1.73**
PRIDE K423	3100	S	S	0.67	0.87*
PRIDE K463	3200	S	S	0.14	0.21
PICKSEED 8855	3200	S	S	–0.11	0.61
RENK RK657	3200	S	S	1.37*	1.12
FIRST LINE TEKILA	3200	S	S	0.39	1.24*
PIONEER 3573	3220	S	S	0.72	1.72*
GREAT LAKES GL536	3280	S	S	1.56	0.94*
PRIDE K470	3300	S	S	0.59	1.27*
PRIDE 6372	2950	S	S-HS	0.34	–0.17
PIONEER 3787	2900	HS	S	0.93	1.62*
PIONEER 3737	3000	HS	HS	1.56	2.02*

[1] Crop Heat Units, 2500 to 2765, 2780 to 2941 and 2950 to 3300 CHU is approximately equal to the FAO's (Food and Agricultural Association) 200–325 RM, 350–410 RM, and 410–480 RM respectively.

[2] Slopes obtained from a regression against an environmental mean (the average mold severity of all maize hybrids). As slopes approach 0 they are said to have greater stability. Slopes increasing or decreasing away from 0 are said to become increasingly unstable. Slopes marked with a * are significant at $P<0.05$, and ** are significant at $P<0.01$.

share in Ontario, the maize feed industry continues to be vulnerable to *F. graminearum* epidemics.

Pick 4990 and Pioneer 3527 both exhibited consistent mean mold severity ratings across the years for SILK inoculations and had slopes that approached zero, with the P value of Pioneer 3527 being <0.05 andthat of Pick 4990 being close to significant (0.08). Both Pioneer 3527 and Pick 4990 had mean ratings below the MR check (and therefore ranked as MR) and had relatively low interaction with the environment (stable results across the years). Pioneer 3527 was somewhat more tolerant of *F. graminearum* infection than Pick 4990 as it had lower mean mold severity scores in 6 of the 7 tests (3 years of SILK inoculations and 4 years of PIN inoculations). These two hybrids were ranked as the best out of the 61 tested for *F. graminearum* resistance. However, the best hybrids

Table 4. Analysis of variance for the effect of mist irrigation and hybrid on mean mold severity ratings within each hybrid, 1995

Inoculation Method	Source	*df*	Mean squares	F-ratio	*P*
SILK	Misting	1	11.77	39.20	0.0001
	Hybrid	1	3.0	9.98	0.005
	Misting* Hybrid	1	1.23	4.10	0.056
	Error	20	0.30		
PIN	Misting	1	19.19	64.65	0.0001
	Hybrid	1	11.54	38.88	0.0001
	Misting* Hybrid	1	8.98	30.25	0.0001
	Error	115	0.30		

Table 5. Effect of overhead misting on the separation of check hybrids, susceptible and resistant to gibberella ear rot, using silk or pin inoculations at Ridgetown, Ontario

Inoculation regime			Mean mold severity score (1–7) of hybrids[1]						
Method	Year	Misting	3737	3790	G-4106	G-4148	G-4030	3953	CV (%)
SILK	1994	No mist	3.9 a[2]	2.2 b	1.4 b	1.6 b	–	–	18.8
		Mist	3.6 a	2.4 bc	2.0 c	2.5 b	–	–	12.0
	1995	No mist	1.5 a	1.3 a	1.6 a	–	1.8 a	–	12.0
		Mist	3.2 b	4.0 ab	3.8 b	–	5.3 a	–	7.9
PIN	1994	No mist	2.3 a	2.4 a	2.1 a	2.0 a	–	–	7.5
		Mist	3.0 a	2.8 a	2.3 b	2.3 b	–	–	9.3
	1995	No mist	3.0 a	3.0 a	3.0 a	–	3.0 a	3.0 a	5.6
		Mist	4.6 a	2.9 c	3.0 c	–	4.7 a	3.6 b	4.3

[1] 3737 = Pioneer 3737, 3790 = Pioneer 3790, G-4106 = Ciba G-4106, G-4148 = Ciba G-4148, G-4030 = Ciba 4030, 3953 = Pioneer 3953.
[2] Values followed by the same letter within rows are not significantly different, Tukey's test using PROC GLM, in SAS (1991).

have less than ideal resistance as the observed disease ratings would still translate into economic loss.

SILK inoculations studies

There were no significant differences in mold severity over all hybrids when either of the three schemes of timing inoculations were used in 1994 and 1995. A trend was seen in both years, where the highest ratings were obtained at silk browning, followed by 7 days post silk and lastly at 195 CHU. These ratings ranged from 3.0 to 3.7 in 1994, and 3.1 to 3.5 for 1995. Timing using silk browning as a guide is recommended for silk channel inoculations because it is easier and is less labour intensive than the other timing methods.

Table 4 represents the ANOVA completed on the effect of mist and hybrid on mold severity within each hybrid (i.e. it is not the accompanying ANOVA for Table 5). Mist irrigation and hybrid both had a significant effect upon the dependent variable, with mist irrigation causing higher mold severity. Misting was needed in one of the years (1995) to obtain a separation in the average response of hybrids to *F. graminearum* inoculation (Table 5). This is probably due to the lower amount of precipitation received at Ridgetown in 1995.

Miller (1994) demonstrated that August and September rainfall was higher than normal during natural *F. graminearum* outbreaks in Ontario in 1986 and 1987. Precipitation in these months influences the severity of the fungal infection (Sutton, 1982). In 1995, a relatively dry August and a September where precipitation was only 35.7% of the average monthly amount, was the likely reason that irrigation was needed to obtain a separation of hybrid response. In 1994, significant hybrid differences were obtained under both mist and non-mist conditions, although the data were stronger under the misting regime (P=0.0001 versus P=0.0113 for no mist, not shown). Irrigation in this year also

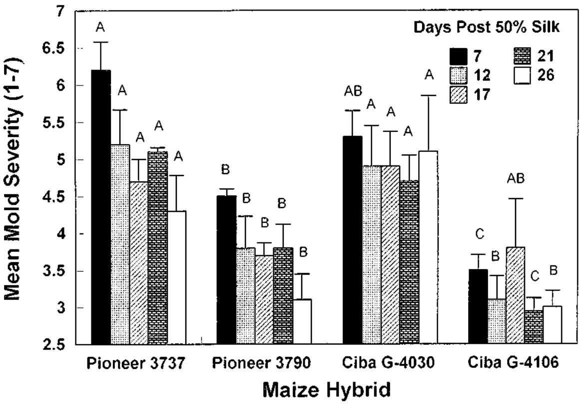

Figure 1. Time course of mean mold severity scores for maize hybrids inoculated with the PIN method, 1994. Means are ± standard error. Bars marked by the same letter are not significantly different, Tukey's test using PROC GLM, in SAS (1991). Means are compared across hybrids at each day only, not within hybrids, (i.e. all hybrids are compared at day 7, then at each following day).

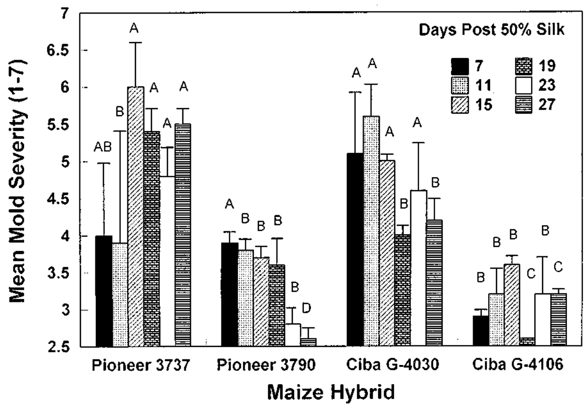

Figure 2. Time course of mean mold severity scores for maize hybrids inoculated with the PIN method, 1995. Means are ± standard error. Bars marked by the same letter are not significantly different, Tukey's test using PROC GLM, in SAS (1991). Means are compared across hybrids at each day only, not within hybrids, (i.e. all hybrids are compared at day 7, then at each following day).

resulted in a better separation of the mold severity ratings. The highest mean rating of the susceptible hybrid Pioneer 3737 was significantly different from the resistant hybrids without misting, whereas Pioneer 3737 was different from both resistant hybrids, and the other susceptible hybrid (Ciba G-4148) was different from one resistant hybrid (Ciba Geigy G-4106) under misting. In 1995, one of the susceptible check hybrids had mold severity ratings higher than the tolerant checks under mist, whereas no hybrid distinctions could be made under the no mist regime (Table 5). The silk channel results are in agreement with Reid and Hamilton (1996) who found that irrigation generally resulted in higher disease ratings for silk channel inoculated hybrids and that for one out of the two years tested, irrigation affected classification of hybrid susceptibility.

PIN inoculations studies

Hybrid scores were compared to days after silking to identify the time in the season that was conducive to high mold ratings (Figures 1 and 2). In 1995, the mold severity generally peaked between 11 to 15 days after 50% silking. This includes the two extra hybrids tested in this year, Pioneer 3953, which peaked at day 15, and Ciba G-4148, which peaked at day 11. For 1994, mean values between days 12 to 17 were consistent with the peak values seen in 1995 at approximately the same time (Figures 1 and 2). Scores at 7 days are higher than those obtained in 1995, but this is not significant. Hybrid, time of inoculation and the hybrid by time interaction all had a significant effect upon the dependent variable (ANOVA not shown).

Time of inoculation was significant when hybrids were compared to one another at each date (Figures 1 and 2). No discrimination of the hybrids was possible at day 7 in 1995 and discrimination was generally better at the mid to late dates after 50% silking in both years. Consistent and high scores for all hybrids over both years were obtained when ears were inoculated at approximately 15 days after 50% silk emergence and therefore this seems to be the best time to complete PIN inoculations. These results also demonstrate the significant interaction of time and hybrid. For example, in 1995 the mold scores for Pioneer 3790 steadily declined for each observation date, whereas other hybrids (e.g. Ciba G-4106) increased to peak scores at day 15 and then decreased (Figure 2). Because of the interaction between hybrid and timing, all hybrids may not completely perform as expected at inoculation dates outside of the window of differentiation identified here. The other benefit of doing kernel inoculations at this time is that there is a reduced chance of puncturing the rachis. This could affect results by concentrating fungal infection symptoms in the cob instead of in the kernels where the visual ratings take place.

As with SILK inoculations, misting in the PIN inoculation material had a significant effect on mean mold severity scores (Table 4). Not all hybrids responded to the same degree to irrigation as there was also a significant interaction between hybrid and misting, but generally misting created higher mold severity scores. No discrimination between hybrids was possible without mist (Table 5) in either 1994 or 1995. When discrimination was possible, all of the check hybrids fell accordingly into their ratings of highly susceptible to moderately resistant for 1995.

Irrigation in the form of misting was beneficial for both of the inoculation methods. Misting resulted in significantly higher mold scores, and was needed to obtain hybrid discrimination in one year for the SILK method and both years for the PIN method. Reid and Hamilton (1996) found that irrigation did not increase severity ratings under PIN inoculations to the extent seen here. This could be due to the different hybrids used in the two studies, or could be due to the actual irrigation system. Reid and Hamilton (1996) did not use a misting system, and they speculated that their irrigation system may wash conidia out of the wound. The mist irrigation system is useful in obtaining more consistent severity scores and is needed to obtain a proper separation of hybrid response to *F. graminearum* infection. Irrigation may be more important in warmer or drier growing regions.

In summary, the great majority of commercial hybrids tested in this study are susceptible to disease caused by *F. graminearum*. During times of natural epidemics, this would result in economic loss through reductions in crop yield and quality. The goal now is to identify genotypes with greater resistance to this fungus. These results confirm the ability to screen large numbers of commercial hybrids and will help to consistently identify resistant genotypes. Information on commercial hybrids aid pork producers in selecting hybrids that may lower the risk of mycotoxicoses in swine.

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