Effect of Macroconidial Suspension Volume and Concentration on Expression of Resistance to *Fusarium graminearum* in Maize

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ABSTRACT

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The effects of volume and concentration of macroconidial suspensions applied to maize silks on resistance to *Fusarium graminearum* infection were investigated on three maize hybrids (Pride K127, Pioneer 3953, and Dekalb DK-415). Plants were inoculated in the field by injecting a spore suspension into the silk channel of individual ears. Disease severity and deoxynivalenol (DON) concentration in grain were determined at harvest. In three experiments, plants were inoculated with 1) six conidial suspension volumes ranging from 0.5 to 10 ml in 1991 and 1992; 2) seven spore concentrations ranging from 0 to 2×10^6 spores per milliliter in 1990, 1991, and 1992; and 3) three spore concentrations (2×10^5, 5×10^5, and 2×10^6 spores per milliliter) inoculated at six silk ages (1, 3, 5, 7, 12, and 18 days after silk emergence) in 1991 and 1992. On average, Pride K127 was most resistant, followed by Pioneer 3953 and Dekalb DK-415. Disease severity, and in most cases DON, increased with increased conidial suspension volume and concentration, with greater effects in susceptible hybrids. Disease severity decreased with silk age over all spore concentrations. When screening for resistance to infection via the silk, 2 ml of a 5×10^5 spores per milliliter suspension injected into the silk channel at approximately 6 days after silk emergence should give adequate differentiation of genotypes.

Fusarium spp. are the causal agents of some of the most important ear molds of maize (*Zea mays* L.) in Canada, the United States, Europe, and other countries (10). *Fusarium graminearum* Schwabe (sexual state: *Gibberella zeae* (Schwein.) Petch) is of considerable economic importance due to the production of mycotoxins such as deoxynivalenol (DON, vomitoxin), which contaminate infected grain. When infected grain is fed to livestock such as swine, deoxynivalenol induces emesis characterized by vomiting, feed refusal, and decreased weight gain (12).

F. graminearum ear rot is characterized by growth of pinkish mold on silks, kernels, and husks. Inoculum is dispersed via wind, rain, insects, and birds. Entry into the maize ear can occur through wounds or by growth of the mycelium down the silks to the kernels and cob from spores germinating on the silks (3,4,10).

Since most maize hybrids are susceptible to this pathogen, development of resistance is an important plant breeding goal. The occurrence of *F. graminearum* infections is sporadic, so relying on natural infection to screen germ plasm for resistance is only useful in years when the environment favors infection. Therefore, inoculation techniques are required.

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Various techniques have been proposed to screen for resistance to the two modes of entry (i.e., wounding vs. silk), but none of these techniques have undergone rigorous testing for routine use in breeding programs.

Since 1986, we have used a screening technique for maize resistance to infection via the silk. The technique involves the injection of a conidial suspension into the silk channel (region between the cob–kernel tip and the husk tip where the silks emerge) of individual ears, followed by evaluation of disease severity at grain harvest in mid-October. Using this technique, we identified inbred lines with high resistance to infection via the silk (6). To standardize this technique for routine use, we investigated various parameters associated with its application, including time of inoculation or silk age (5) and isolate effects (7).

The objectives of this study were to investigate two additional parameters involved in the silk channel inoculation technique: conidial suspension volume and spore concentration. Our specific objectives were to 1) determine the effect of volume of suspension injected into the silk channel on disease severity ratings and DON concentration, 2) determine the effect of spore concentration in the suspension on disease severity ratings and DON concentration, and 3) investigate the interaction between spore concentration and time of inoculation.

MATERIALS AND METHODS

A macroconidial suspension of *F. graminearum* was prepared as previously described (6) using a mixture of three isolates (DAOM194276, DAOM180378, and DAOM212678) obtained from the Canadian Collection of Fungus Cultures, Agriculture Canada, Ottawa, all originating from naturally infected maize ears. Individual plants were inoculated by injecting suspensions into the silk channel of primary ears using a graduated, 10 cm^3, self-refilling automatic vaccinator attached to a 2.5-L backpack container (Nasco Co., Fort Atkinson, WI). In the experiments to evaluate the effects of conidial suspension volume and concentration, inoculations were conducted when silks were elongated, pollinated, and had some tip browning (approximately 5–7 days after silk emergence). After inoculation, humid conditions were maintained by overhead sprinkler irrigation with 2–5 mm of water twice daily (early morning and late afternoon) for 4 wk.

Three maize hybrids differing in susceptibility, Pride K127 (resistant), Pioneer 3953 (susceptible), and Dekalb DK-415 (susceptible), were used in all experiments. Plants were grown at the Central Experimental Farm, Agriculture Canada, Ottawa, Ontario, in 3.8-m rows of 12 plants, with 76 cm between rows. The center 10 plants of each row were inoculated. The severity of ear rot symptoms was evaluated in mid-October at normal grain harvest using a 7-class rating scale, where 1 = no symptoms, 2 = 1–3%, 3 = 4–10%, 4 = 11–25%, 5 = 26–50%, 6 = 51–75%, and 7 = more than 75% of the kernels visibly moldy. Ears were rated individually for disease severity, and a mean rating was calculated for each row.

Analyses of DON concentrations were conducted in 1991 and 1992 for Pride K127 and Dekalb DK-415 in the conidial suspension volume and concentration studies. Harvested ears were bulked within each row, bagged in mesh sacks, and air-dried for 2 wk. Each bag of 10 ears was then hand-shelled, and the cob was discarded. Each bulked kernel sample was mixed thoroughly to ensure random distribution of kernels, and a 50-g subsample was ground to a fine powder in a Retsch Ultra Centrifugal Mill Type ZM1 (Brinkman Instruments, Rexdale, Ont.) with a 0.75-mm mesh. All subsamples were stored at −20 C until DON analysis.

For DON analysis, each sample was analyzed in duplicate at the Plant

Research Centre's mycotoxin lab. DON concentration was reported as the mean of the two analyses. Recoveries were 95.6 and 93.6% on two separate occasions, with coefficients of variance less than 1% for each. A DON standard curve was linear up to 1.5 ng injected on column, and detection limit was typically in the order of 0.1 μg of DON per gram of sample.

For DON extraction, 1 g of ground kernels was extracted with methanol: water (1:9, 5 ml) for 1 h on an end-over-end mixer. The extracts were centrifuged (2,000 rpm, 5 min), and aliquots of the supernatant (1 ml) were added to Varian Chem Elute 1001 solid-phase extraction columns. Each column was washed with ethyl acetate (7 × 1 ml), and the washing from each sample was evaporated to dryness using a stream of nitrogen gas.

Extracts were cleaned on silica gel columns prepared using Kieselgel 60 (0.5 g, 70–230 mesh) dry packed in a disposable glass pipette (145 mm) plugged with glass wool, and prewashed with toluene: acetone (95:5, 1 ml). Sample residues were taken up in dichloromethane (500 μl) and placed on the silica columns with additional dichloromethane rinses (2 × 100 μl). Columns were washed with toluene:acetone (95:5, 5 × 1 ml), and these washings were discarded. DON was eluted off the columns with dichloromethane:methanol (95:5, 6 × 1 ml), and the eluants were dried under a stream of nitrogen gas.

To derivatize DON, each sample was taken up in toluene:acetonitrile (95:5, 500 μl, dried overnight using molecular sieve type 3A) and derivatized with heptaflorobutyrylimidazole (HFBI, 50 μl). Samples were mixed (2 min) in a vortex mixer and heated in a water bath (65 C, 1 h). After cooling, saturated aqueous $NaHCO_3$ solution (1 ml) was added to quench the reaction. Fifty micrometers of the organic layer was made up to 1 ml with hexane (dried over molecular sieve type 3A) for chromatographic analysis.

Samples were analyzed on a Varian Vista 6000 Gas Chromatograph equipped with an electron capture detector, coupled to a Varian Model 8000 Auto Sampler and a Varian GC-Star data handling system. One microliter of hexane solution was injected onto a DB-5 column (15 m, 0.55 mm ID) with a helium carrier gas (12 ml/min) and a makeup gas of argon:methane (95:5, 14 ml/min). Injector temperature was isothermal at 230 C. Detector temperature was set at 300 C. Oven temperature was programmed with a 5-min hold at 200 C, ramped to 275 C at 6 C/min, and held at 275 C for 1.5 min. Quantification was obtained by comparison with authentic standards.

Conidial suspension volume study. In 1991 and 1992, a 3 × 6 factorial experiment arranged in a split plot design with four replicates was conducted. The three hybrids were randomized among the main plot units so that each consisted of six single-row subplot units. The inoculation treatments were randomized among the subplot units consisting of six different conidial suspension volumes (0.5, 1.0, 2.0, 4.0, 6.0, and 10.0 ml) all at a concentration of 5×10^5 spores per milliliter.

Spore concentration study. In 1990, 1991, and 1992, a 3 × 7 factorial experiment was conducted similar to the volume study described above; however, the inoculation treatments were randomized among the seven subplot units consisting of the injection of 2 ml of conidial suspension at seven concentrations: 0 (sterile water), 10^4, 10^5, 2×10^5, 5×10^5, 10^6, and 2×10^6 spores per milliliter.

Time of inoculation (silk age) and spore concentration study. In 1991 and 1992, a 3 × 6 × 3 factorial experiment was conducted. The three hybrids were randomized to the main plot units. Six inoculation times (1, 3, 5, 7, 12, and 18 days after silk emergence) were randomized to the subplot units, each of which consisted of three single-row sub-subplot units among which three spore concentrations (2×10^5, 5×10^5, and 2×10^6 spores per milliliter) were randomized. Suspension volume was kept standard at 2 ml.

Statistical analyses. Residual error terms were tested for normal distribution using the Kolmogorov D statistic (8). Using SAS statistical programs (8), mean disease severity ratings and DON concentrations were analyzed separately by year for each experiment using standard analysis of variance procedures, and error mean squares were tested for homogeneity to determine if data could be pooled over years (9).

Table 1. Analysis of variance for the effect of conidial suspension volume on disease severity and deoxynivalenol concentration using a silk channel injection technique to assess resistance of maize to *Fusarium graminearum*

		Disease severity			Deoxynivalenol concentration	
		Mean squares			Mean squares	
Source	df	1991	1992	df	1991	1992
Replicate	3	0.90	3.21	3	15.54	5,843.34
Genotype	2	10.23*[a]	53.82**	1	785.45*	40,233.55*
Error a	6	1.15	3.4	3	73.34	3,632.52
Volume	5	4.64**	17.44**	5	31.82	8,763.27**
Genotype × volume	10	0.59	1.64*	5	2.80	2,233.09
Error b	45	0.37	0.69	30	88.64	1,200.30

[a] * = Significant at the 0.05 probability level; ** = significant at the 0.01 probability level.

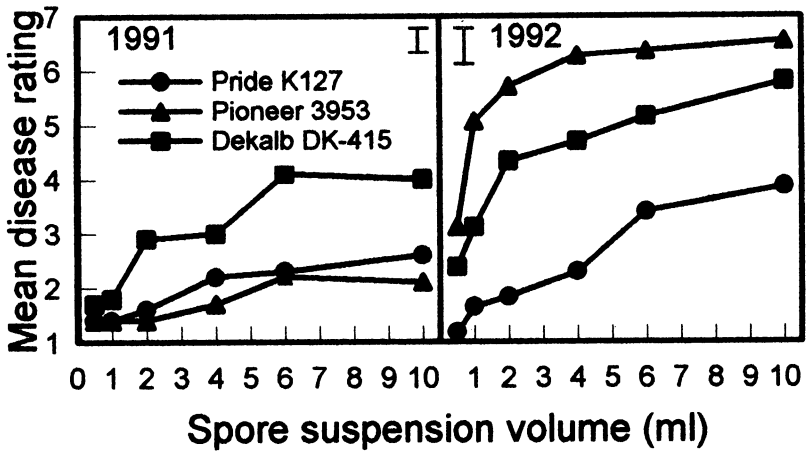

Fig. 1. The effect of conidial suspension volume on disease severity of three maize hybrids inoculated by injecting a conidial suspension of *Fusarium graminearum* into the silk channel in 1991 and 1992. Disease severity ratings were on a 1–7 scale, where 1 = no infection and 7 = more than 75% of the kernels visually moldy. Bar indicates standard error of the difference = 0.4 (1991) and 0.6 (1992).

RESULTS

In all experiments, residual error terms were found to be normally distributed ($P > 0.05$), as tested by the Kolmogorov D statistic. Error mean squares were homogeneous for disease severity ratings in the spore concentration study, tested using Bartlett's test (9); however, since significant year × genotype interactions were obtained in the pooled analysis of variance, data were analyzed separately for each year. In the other two studies, error mean squares were heterogeneous ($P < 0.05$) for both disease severity and DON concentration, so data were analyzed separately for each year.

Conidial suspension volume. For disease severity ratings, genotype and suspension volume effects were significant

in both years (Table 1). Genotype × volume interactions were significant in 1992, but this source of variation represented only a small proportion of the total sum of squares.

Disease severity ratings were on average higher in 1992 than in 1991 (Fig. 1). The most consistently resistant hybrid was Pride K127. Pioneer 3953 appeared to be resistant in 1991 but was highly susceptible in 1992. Dekalb DK-415 was susceptible in both years.

In 1991, increasing the suspension volume from 0.5 to 10 ml had no significant effect on disease severity ratings for Pride K127 or Pioneer 3953 (Fig. 1). However, increased volume did increase disease severity in the more susceptible hybrid Dekalb DK-415. In 1992, when disease severity was higher for all hybrids, the effect of volume was more significant. In both years, the most resistant hybrid, Pride K127, was classified as susceptible (infection reached the kernels, ratings greater than 2) when more than 4 ml was injected into the silk channel.

For DON concentrations, genotype effects were significant in both years, but volume effects were significant only in 1992 (Table 1). Genotype × volume interactions were not significant either year. DON concentrations in 1991 ranged from 1.38 μg/g (Pride K127) to 14.21 μg/g (Dekalb DK-415) (Fig. 2). In 1992, DON concentrations were higher, ranging from 3.5 μg/g (Pride K127) to 127.2 μg/g (Dekalb DK-415).

No correlation between disease severity ratings and DON concentrations was found in 1991; however, in 1992 significant ($P < 0.01$) positive correlations were found for both hybrids ($r = 0.948$, Pride K127; $r = 0.930$, Dekalb DK-415).

Spore concentration. For disease severity ratings, genotype and spore concentration main effects were significant at the 0.01 probability level in all 3 yr (Table 2). Genotype × concentration interactions were significant in two (1991 and 1992) of the 3 yr; however, the sum of squares was small compared to the sum of squares for genotypes.

In all 3 yr, inoculation with sterile water resulted in almost no infection; all hybrids had mean disease ratings of 1.0–1.1. Increasing the spore concentration from 10^4 to 2×10^6 spores per milliliter increased disease severity in all hybrids each year, with greater severity expressed by more susceptible hybrids (Fig. 3).

As with the suspension volume experiment, Pride K127 was consistently the most resistant hybrid and only showed susceptible levels of infection (ratings greater than 2) in 1990 after inoculation with 5×10^5 spores per milliliter and in 1991 after inoculation with 10^6 spores per milliliter. The highest rating for Pride K127 was only 3.1 (1990, 2×10^6 spores per milliliter). Dekalb DK-415 was susceptible in all 3 yr, with average ratings greater than 2 in 1990 and 1992 with 10^4 spores per milliliter and in 1991 with 10^5 spores per milliliter. Pioneer 3953 was less consistent among years, appearing susceptible (ratings greater than 2) in 1990 after inoculation with 10^5 spores per milliliter, resistant in 1991, and highly susceptible (ratings greater than 4) in 1992 with 10^3 spores per milliliter. This was also observed with Pioneer 3953 in the conidial suspension volume study.

Genotype and spore concentration effects were significant for DON concentration in both 1991 and 1992 (Table 2). Genotype × concentration interaction effects were significant in 1991, but not in 1992. DON was detected in some of the ears inoculated with sterile water, indicating that natural infection did take place. In these water inoculations, Pride K127 had average DON concentrations in 1991 and 1992 of 0.6 and 0.2 μg/g, respectively, while Dekalb DK-415 had 1.1 and 4.5 μg/g, respectively.

In contrast to the volume study, high concentrations of DON were found for Dekalb DK-415 in 1991 (Fig. 4). In 1991, there was a sharp increase in DON concentration for this hybrid above a concentration of 5×10^5 spores per milliliter. However, in 1991, spore concentration had little effect on DON levels for Pride K127. DON concentration was significantly correlated with disease severity ratings in 1991: $r = 0.972$, $P < 0.01$ (Pride K127); $r = 0.857$, $P < 0.05$ (Dekalb DK-415). In 1992, DON concentrations only ranged from 0.8–5.5 μg/g for Pride K127. Concentrations for Dekalb DK-415 were higher in 1992 for the 10^4 and 10^6 concentrations, but the sharp increase for 2×10^6 spores per milliliter was not observed as it was in 1991. In 1992, DON concentrations were not correlated to disease severity ratings for Pride K127 (due to the small range in DON values); however, a significant ($P < 0.05$) positive correlation ($r = 0.875$) was found for Dekalb DK-415.

Time of inoculation (silk age) and spore concentration study. In both 1991 and 1992, genotype, time of inoculation, genotype × time interaction, spore con-

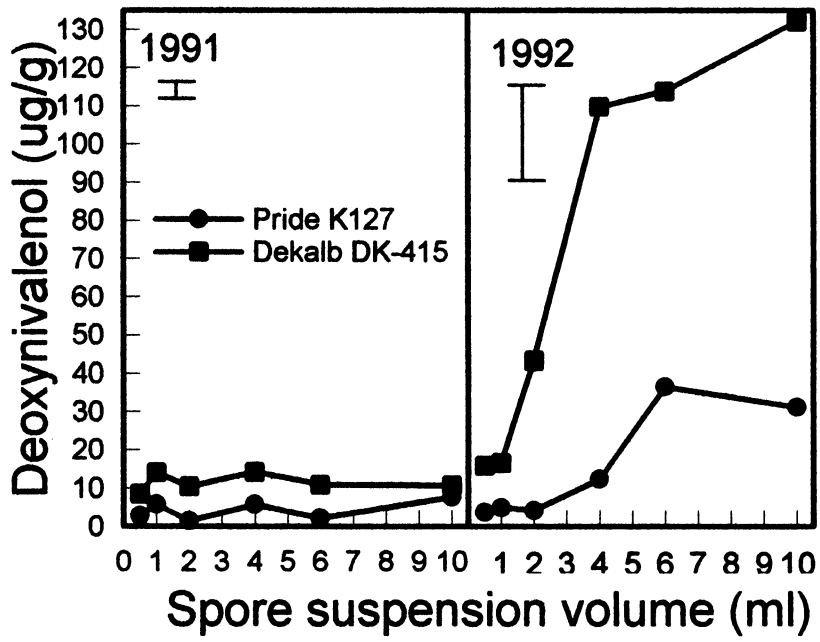

Fig. 2. The effect of conidial suspension volume on DON concentration of two maize hybrids inoculated by injecting a conidial suspension of *Fusarium graminearum* into the silk channel in 1991 and 1992. Bar indicates standard error of the difference = 6.7 (1991) and 24.5 (1992).

Table 2. Analysis of variance for the effect of spore concentration on disease severity and deoxynivalenol concentration using a silk channel injection technique to assess resistance of maize to *Fusarium graminearum*

	Disease severity				Deoxynivalenol concentration		
		Mean squares				Mean squares	
Source	df	1990	1991	1992	df	1991	1992
Replicate	3	1.09	0.37	0.63	3	355.92	1,092.89
Genotype	2	10.51**[a]	23.52**	73.73**	1	9,694.71*	17,428.67*
Error a	6	0.67	0.44	1.61	3	598.73	1,383.07
Concentration	6	10.73**	6.56**	12.63**	6	2,095.57**	1,158.87*
Genotype × concentration	12	0.47	1.97**	2.38**	6	1,559.31**	831.25
Error b	54	0.31	0.21	0.32	36	249.23	421.68

[a] * = Significant at the 0.05 probability level; ** = significant at the 0.01 probability level.

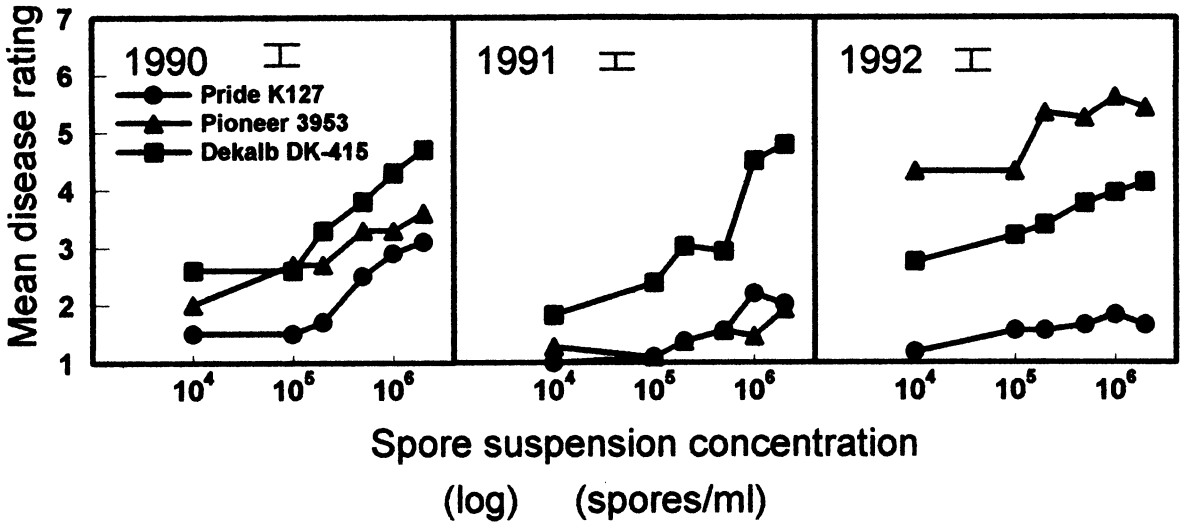

Fig. 3. The effect of spore concentration on disease severity of three maize hybrids inoculated by injecting a conidial suspension of *Fusarium graminearum* into the silk channel in 1990, 1991, and 1992. Disease severity ratings were on a 1–7 scale, where 1 = no infection and 7 = more than 75% of the kernels visually moldy. Bar indicates standard error of the difference = 0.4 (1990), 0.3 (1991), and 0.4 (1992).

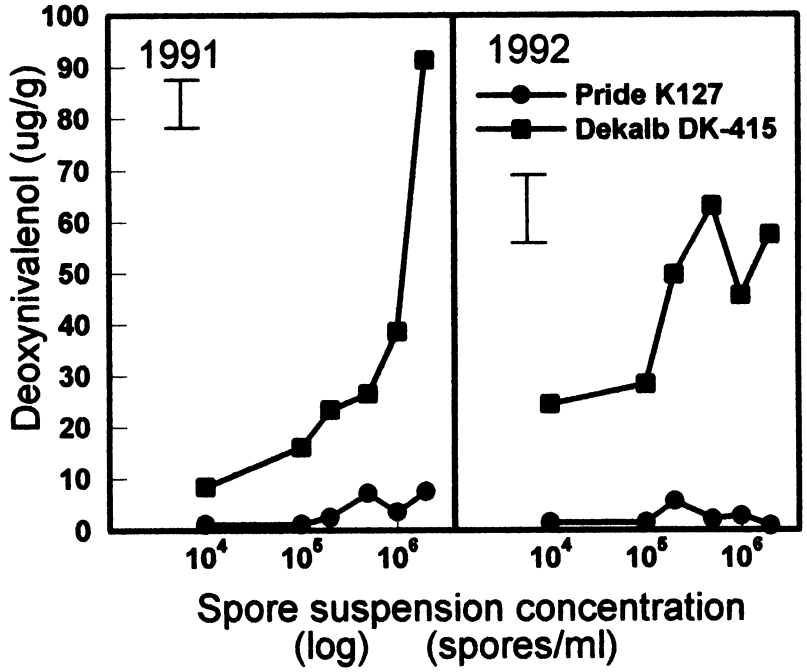

Fig. 4. The effect of spore concentration on DON concentration of two maize hybrids inoculated by injecting a conidial suspension of *Fusarium graminearum* into the silk channel in 1991 and 1992. Bar indicates standard error of the difference = 11.2 (1991) and 14.5 (1992).

Table 3. Analysis of variance for the effect of spore concentration and time of inoculation on disease severity using a silk channel injection technique to assess resistance of maize to *Fusarium graminearum*

Source	df	Mean squares 1991	Mean squares 1992
Replicate	3	1.64	0.84
Genotype	2	17.99**[a]	88.80**
Error a	6	1.47	0.63
Time	5	76.65**	48.50**
Genotype × time	10	3.34**	15.40**
Error b	45	0.99	0.63
Concentration	2	9.78**	4.73**
Genotype × concentration	4	1.88**	0.32
Time × concentration	10	2.24**	1.00**
Genotype × time × concentration	20	1.00*	0.24
Error c	108	0.53	0.27

[a] * = Significant at the 0.05 probability level; ** = significant at the 0.01 probability level.

centration, and time × concentration interaction effects were significant at the 0.01 probability level (Table 3). Genotype × concentration interactions were significant in 1991 ($P < 0.01$) but not in 1992. The three-way interaction of genotype × time × concentration was also significant in 1991 ($P < 0.05$) but not in 1992.

As with the volume and concentration experiments, disease severity levels were higher in 1992 than in 1991, except for the more resistant hybrid Pride K127 (Fig. 5). Generally, increasing the spore concentration from 2×10^5 to 2×10^6 spores per milliliter did not significantly increase disease severity levels. For all treatment combinations, disease severity levels decreased with increasing silk age after 4 days after silk emergence (time of inoculation). Inoculations at 12 or 18 days after silk emergence produced little or no infection, even for the most susceptible hybrid Dekalb DK-415 inoculated with the highest inoculum concentration (2×10^6 spores per milliliter). Pride K127 was again the most resistant hybrid, and Pioneer 3953 and Dekalb DK-415 were susceptible. As with the volume and concentration studies, Pioneer 3953 was more moldy in 1992 than in 1991.

DISCUSSION

Increasing the volume and concentration of conidial suspension injected into the silk channel of maize ears generally increased the severity of disease symptoms and DON concentration at harvest. This effect was more pronounced in susceptible than in resistant hybrids and in years with overall higher levels of infection. Significant differences in DON concentration were found among hybrids. DON concentration was highly correlated with visual ratings of disease severity.

An increase in conidial suspension

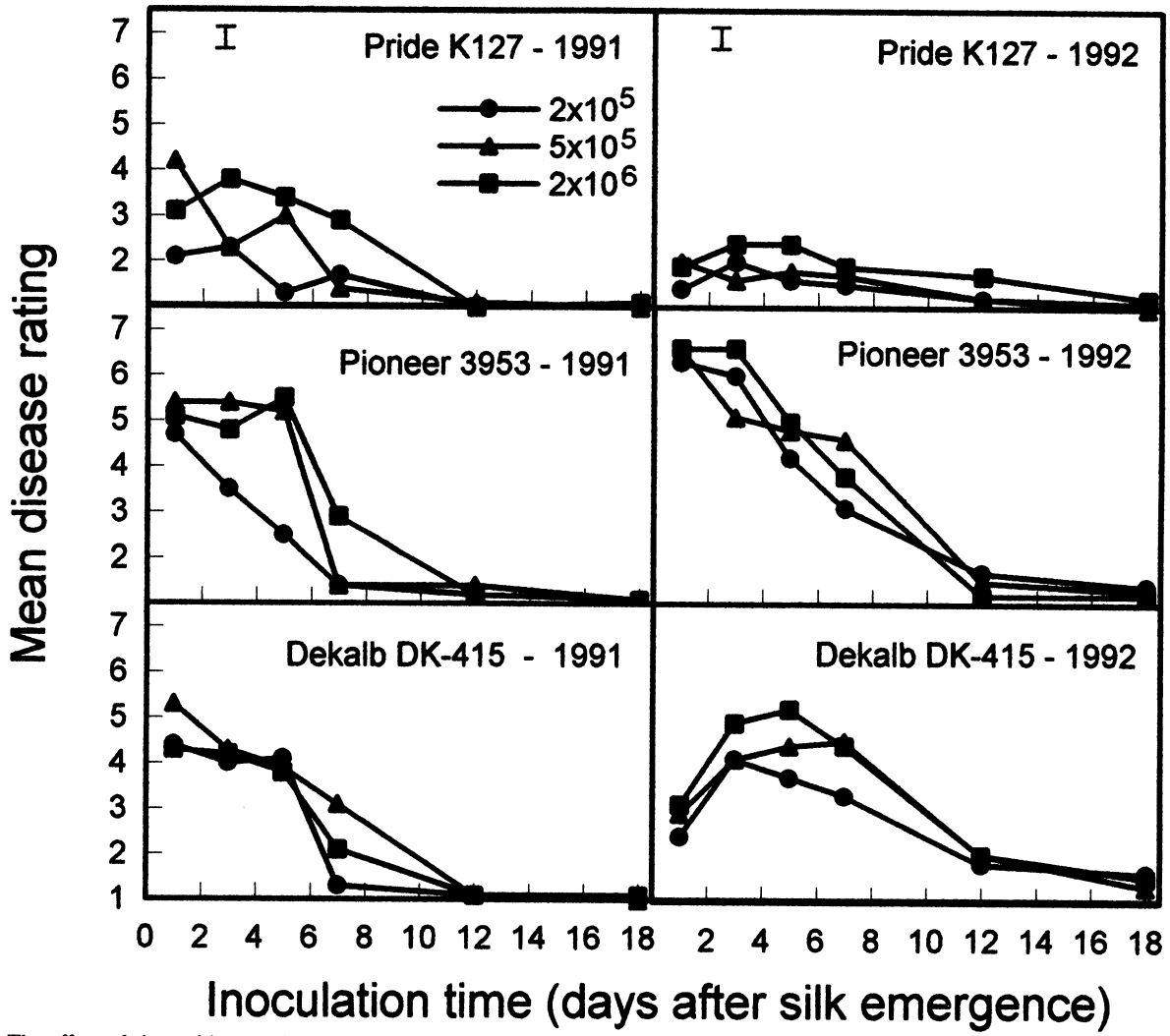

Fig. 5. The effect of time of inoculation on disease severity of three maize hybrids inoculated by injecting a conidial suspension of *Fusarium graminearum* into the silk channel at three different spore concentrations in 1991 and 1992. Disease severity ratings were on a 1–7 scale, where 1 = no infection and 7 = more than 75% of the kernels visually moldy. Bar indicates standard error of the difference = 0.4 (1991) and 0.4 (1992).

volume may increase the probability that inoculum will be forced down the silk channel and onto the cob tip and kernels, thus overcoming the natural barrier of the silk and any silk resistance. Thus, genotypes with useful silk resistance will not be selected, as they will appear susceptible. The ability to differentiate among genotypes will be reduced, and selection will be ineffective. In screening for resistance to silk infection, lower volumes of conidial suspension must be injected. We have routinely used a volume of 2 ml. The data in this study support the use of this volume; neither 0.5 nor 1.0 ml differentiated among the genotypes, and 4.0 ml resulted in high levels of susceptibility (greater than 2) in the resistant hybrid Pride K127 both years. This would indicate that the silk resistance barrier was overcome. Almost all maize genotypes, inbreds and hybrids, can absorb a 2-ml volume of liquid in their silk channels. We have tested various volumes (0.5–10.0 ml) of dye injected into the silk channel of numerous genotypes and have found that 2 ml always stays within the silk channel and does not flood the ear (*unpublished*). Previous studies used 1 ml (2) to 5 ml (1,10). Ullstrup (11) used 5 ml of a 10^5 spores per milliliter suspension sprayed onto exposed silks in one treatment and injected into the silk channel in a second treatment. He compared these two inoculation techniques to a third, the insertion of a colonized toothpick into the center of the ear (wounding technique). He achieved the best genotype differentiation with the silk spray method and the least with the injection of the conidial suspension into the silk channel, which gave too severe disease ratings. Ullstrup's silk channel ratings were more severe than the toothpick technique, which is usually expected to be the most severe technique due to the wounding. The results of our study suggest that the severity of disease ratings with the injection technique of Ullstrup may have been due to the large volume (5 ml) of conidial suspension injected.

We also have noted that in addition to conidial suspension volume, the position of the needle relative to the silk channel is important. The needle must be at right angles to the silk channel; otherwise inoculum will be injected down the silk channel to the kernels. If this happens, disease severity ratings will be high, and a measurement of resistance to infection via the silk will not be obtained. This problem would be compounded and lead to even higher disease ratings and less differentiation if inoculum volumes are high.

Increasing the spore concentration in the suspension increased disease severity. However, the resistant hybrid Pride K127 still had comparatively low levels of disease severity, even at the highest concentrations. Similarly, Drepper and Renfro (1), when wound-inoculating ears with *F. moniliforme* J. Sheld., found no significant effect on disease severity for inoculum concentrations of 10^6 and 10^7 spores per milliliter. Ullstrup (11) found a positive correlation between disease severity and spore concentration when ears were inoculated with *Diplodia maydis* (Berk.) Sacc. by spraying a conidial suspension on the silks.

Even our lowest concentration (10^4 spores per milliliter) differentiated between susceptible and resistant hybrids. In other field experiments, we have achieved infection in susceptible hybrids with concentrations as low as 100 spores per milliliter (*unpublished*).

Studies on inoculation techniques have used conidial suspension concentrations ranging from 450 spores per milliliter (11) to 10^8 spores per milliliter (1), while some do not mention the concentration used (2). No one standard concentration has been used with a given technique. We have chosen 5×10^5 spores per milliliter as the concentration to routinely use in screening for silk resistance. This concentration differentiates among genotypes and is easy to obtain with our inoculum culture method. In this study, at 5×10^5 spores per milliliter, the susceptible hybrid Dekalb DK-415 had ratings greater than 3, and Pioneer 3953 had ratings greater than 3 for all years except 1991. Concentrations lower than 5×10^5 spores per milliliter can be used, as indicated by our data; however, less differentiation among genotypes may occur in some years.

In previous studies, we found a significant effect of silk age on disease severity (5). In the present study on the interaction between spore concentration and time of inoculation, this effect was again observed. In all treatment combinations, disease severity decreased with increasing time of inoculation or silk age. We had hypothesized that although spore concentration did not appear to have a major effect on disease severity in more resistant genotypes when inoculated at approximately 6 days after silk emergence, a more significant effect may occur when higher spore concentrations are used at different silk ages. The results of this study suggest that this is not the case. In general, it did not matter what time the inoculation was conducted; there was little or no significant difference in disease severity among spore concentrations ranging from 2×10^5 to 2×10^6 spores per milliliter. Thus, there appears to be no silk age that results in a more susceptible reaction at higher spore loads. Even a small amount of inoculum can result in high levels of infection if inoculation occurs when the plant is receptive.

The results of these studies also indicated that for some hybrids, such as Pioneer 3953, a significant amount of year-to-year variation can occur. Unlike Pride K127 and Dekalb DK-415, which were consistently rated resistant and susceptible, respectively, Pioneer 3953 was rated susceptible in 1990 and 1992 but resistant in 1991. Across the experiments, there was little variation. For example, using an inoculation of 2 ml of 5×10^5 spores per milliliter 7 days after silk emergence, Pride K127 was rated 2.3 in 1990, 1.4–1.6 in 1991, and 1.7–1.9 in 1992; Dekalb DK-415 was rated 3.8 in 1990, 2.9–3.1 in 1991, and 4.0–4.6 in 1992; and Pioneer 3953 was rated 3.3 in 1990, only 1.4–1.6 in 1991, and 4.6–6.1 in 1992. Thus, infection levels were highest for Pride K127 in 1990 and highest for the two other, more susceptible, hybrids in 1992. We cannot explain the year-to-year variation in Pioneer 3953. During inoculation and fungal growth in July and August, temperatures ranged from a maximum and minimum of 14.8–27.1 C in 1990, 16.5–27.9 C in 1991, and only 13.2–23.1 C in 1992. Precipitation for the 2 mo totaled 252 mm in 1990, 172.8 mm in 1991, and 264.4 mm in 1992. Thus, in a year with high rainfall and high temperatures (1990), resistance appeared to be overcome in Pride K127, as evidenced by the slightly higher disease ratings, whereas for the two more susceptible hybrids, more infection was obtained in a year with high precipitation but low temperatures (1992). A dry year like 1991, with below-average precipitation and high temperatures, resulted in the lowest infection levels for all hybrids. These trends were also observed in our screening nursery.

In conclusion, when a conidial suspension of *F. graminearum* was injected into maize ears to screen for resistance to infection via the silk, increasing the volume or concentration of inoculum injected increased disease severity and DON concentrations. This effect was more pronounced in susceptible genotypes and in years with higher overall infection levels. Disease severity decreased with silk age for all spore concentrations. We suggest that a volume of 2 ml and a concentration of 5×10^5 spores per milliliter be injected into the silk channel approximately 6 days after silk emergence when using the silk channel inoculation screening technique.

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