Evaluation of Four Inoculation Techniques for Infecting Corn Ears with *Stenocarpella maydis*

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ABSTRACT

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Four techniques for inoculating corn (*Zea mays*) ears with *Stenocarpella maydis* were evaluated on six inbreds and four hybrids. Depositing a spore suspension into the sheath cavity or placing infected popcorn into the whorl resulted in low levels of infection. Spraying a conidial suspension on the silks resulted in an intermediate level of infection, and injecting a conidial suspension directly into the ear resulted in very high levels of infection. The silk spray method was selected as the best technique because of the level of infection, the ability to distinguish between resistant and susceptible inbred lines, and ease of application.

Diplodia ear rot of corn (*Zea mays* L.), caused by *Stenocarpella maydis* (Berk.) Sutton (= *Diplodia maydis* (Berk.) Sutton), was recognized in 1934 as the most serious ear rot of corn in the central United States (7). In 1943, *S. maydis* was found to be widespread, occurring in all but three states east of the Rocky Mountains and in California (4). Although the ear rot is widely distributed over the corn belt, epidemics are limited in extent, occurring in areas in close proximity to the inoculum source (9). In 1983, Latterell and Rossi reported an increased incidence in the disease in the mid-Atlantic states, especially under stress conditions, and suggested that the use of susceptible germ plasm and no-till farming were contributing factors (4).

Of the control methods available, genetic resistance may offer the most promise in avoiding losses to the disease. Screening for resistant lines requires a reliable method of inoculation that will distinguish between resistant and susceptible genotypes, yield consistent results between years and within lines, and simulate the natural mode of infection as much as possible (8). Finally, the technique must have practical application in a breeding program, allowing for large numbers of plants to be screened in a limited amount of time.

Numerous techniques have been used to inoculate corn plants with *S. maydis*. Inoculation of the whorl has resulted in infection when a conidial suspension was placed in the whorl approximately 10 days before tasseling and has been used in inbred evaluation (12). Spraying the ear with a conidial suspension was used by Ullstrup (9) but rejected by Villena (10). Inoculation of the sheath has resulted in infection when infected grains or conidial suspensions were placed in the sheath cavity behind the ear (4). Several methods of direct introduction of *S. maydis* into the ear have been successful in causing infection. These include inserting infected toothpicks into the middle (1,10) or butt of the ear (10); drilling holes in the ear and inserting infected grain (3) or spores (5); and injecting a conidial suspension with a hypodermic syringe into the middle, tip, or butt of the ear (10) or both the tip and butt (4). Other techniques evaluated and rejected by Villena include brushing the ear or silks with an agar-spore slurry and dropping a spore suspension among the silks with a medicine dropper (10).

Before beginning studies to evaluate genetic resistance in maize to *S. maydis*, we wished to evaluate recommended inoculation techniques under local environmental conditions. Preliminary studies in 1987 indicated that different methods of inoculating plants would result in very different levels of infection (J. C. Klapproth and J. A. Hawk, *unpublished*). The objective of this study was to evaluate four inoculation techniques: 1) dropping infected grain into the whorl, 2) spraying the silks with a conidial suspension, 3) applying a conidial suspension into the sheath cavity, and 4) injecting a conidial suspension directly into the ear.

MATERIALS AND METHODS

Two experiments were done in 1988 and repeated in 1989. The first was an evaluation of inbred lines and the second an evaluation of hybrids. The experimental design was a split plot with four replications, with genotypes as the main plot and inoculation treatments as the subplot. Each subplot consisted of one 5.3-m row, with 20–24 plants per row. All plots were located on the University of Delaware farm in Newark. Fertilizer and herbicide were applied based on University of Delaware recommendations. In 1988, insects were controlled with granular BT in the whorl; in 1989, 10 kg/ha of carbofuran 15G was applied at planting in addition to the BT in the whorl. Rainfall of 61 cm in 1988 and 74 cm in 1989 was recorded. Plots received overhead sprinkler irrigation when required.

Genotypes evaluated. In the first experiment, six inbreds were chosen to represent a range of resistant, intermediate, and susceptible lines. Based on earlier observations, MBS613 was expected to exhibit resistance to *S. maydis*, VA26Ht and VA35 were expected to be moderately resistant, and B73Ht was expected to be susceptible. H111 was suggested as a line with possible resistance (11), and LH51 was tested because of its widespread use in commercial breeding programs.

In the second experiment, four hybrids were evaluated. Hybrids expected to exhibit some resistance included Pioneer 3192 (K. Byrnes, *personal communication*) and MBS613×VA26Ht. Susceptible hybrids included Pioneer Experimental XC (K. Byrnes, *personal communication*) and LH132×LH51.

Inoculum preparation. *S. maydis* was isolated from corn ears collected at Middletown and Newark, DE (three isolates), and Lancaster County, PA, in 1986. In addition, H. Warren provided two isolates from West Lafayette, IN. Isolates were grown on corn leaf water agar (autoclaved corn leaves placed on 2% water agar) at approximately 25 C with a 12-hr photoperiod. After 3 wk, the plates were flooded with sterile distilled water and scraped with a rubber policeman. Spore concentration was determined by making two counts with a hemacytometer. The spore suspension was adjusted to 250,000 spores per milliliter by adding sterile distilled water.

For techniques that used infested grain, 150 g of popcorn seed and 100 ml of distilled water were autoclaved for 40 min on two consecutive days in a 375-ml plastic box, then inoculated with a 10-ml conidial suspension of equal amounts of all isolates. The boxes were incubated at 25 C, 12-hr photoperiod,

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for 6–8 wk.

Inoculation techniques. In both experiments, four inoculation techniques plus an untreated control were evaluated. The four techniques included: 1) whorl method—approximately five grains of infected popcorn were placed into the whorl at the V12 growth stage (6); 2) silk method—at full silk, silks were sprayed with approximately 15 ml of a spore suspension using a 1-L Polysprayer 2 compressed air sprayer (Model 94-2P) until the spore suspension ran off, and ears were then covered with a shoot bag (#217 Glassine water repellent bag); 3) sheath method—1 ml of the spore suspension was squirted between the base of the ear and the ear leaf at mid-silk with an Ideal 50-ml pistol grip syringe (Model 76L-1959); 4) injection method—1 ml of the spore suspension was injected into the kernels in the middle of the ear approximately 20 days after mid-silk using a 50-ml pistol grip syringe with an insulin needle; and 5) control plots were not inoculated.

Disease rating. Inbred and hybrid ears were harvested 40 and 60 days after mid-silk, respectively. The incidence of infected ears was calculated by dividing the number of infected ears by the total number of ears present in the plot and multiplying by 100.

Disease severity of individual ears was determined in the field on a 0–5 rating scale (kernels were not removed from the cob) where 0 = no visible infection, 1 = 1–25% infected kernels, 2 = 26–50% infected kernels, 3 = 51–75% infected kernels, 4 = 76–100% infected kernels, and 5 = all kernels and cob completely rotted. The disease severity for the plot was calculated as a mean of individual values.

Data analysis. Data from individual years were analyzed as a split-plot design. Data for both years were then combined and analyzed with year being treated as a random factor. For the combined data analysis, the line effects were tested using the mean square for year × line as the error term and the treatment and line × treatment effects were tested using the mean square for year × line × treatment as the error term. Least significant differences for treatment combinations were computed using the approach in Cochran and Cox (2) for split-plot designs.

RESULTS

Inbreds. Line, treatment, and the interaction of line and treatment were significant for both the incidence of infected ears and the disease rating in 1988, 1989, and in both years combined.

Of the four inoculation techniques, the injection method yielded the highest incidence of disease, with an average of 94% of the ears infected over both years (Table 1). Disease ratings for inbred lines ranged from 2.7 to 4.1 in 1988 and from 1.1 to 3.6 in 1989, with significant differences between lines (Table 2). MBS613 exhibited the lowest disease severity in both years. Of the four lines with high disease ratings in 1988, three (B73Ht, VA35, and H111) also exhibited high disease ratings in 1989.

The silk spray technique yielded a lower, although still acceptable, incidence of infected ears, with an average of 50% of ears infected over both years (Table 1). The incidence of infected ears varied considerably between lines, ranging from an average of 35% for MBS613 to 80% for B73Ht. The overall incidence of infected ears was lower in 1989 than 1988. Disease ratings ranged from 1.0 to 3.7 in 1988 and 0.5 to 2.4 in 1989, with significant differences between lines in both years (Table 2). Inbreds MBS613, Va26Ht, Va35, and H111 had significantly lower disease incidence and ratings than B73Ht and LH51 in the combined years analysis.

The whorl method of inoculation resulted in an average of 30% infected ears over both years (Table 1). Like the silk spray method, the incidence of infected ears varied considerably between lines, ranging from 6% for H111 to 58% for B73Ht. Disease ratings ranged from 0.2 to 1.8 in 1988 and 0.2 to 2.6 in 1989, significantly lower than values for either the silk spray or injection methods (Table 2). In both years, MBS613 and H111 exhibited the lowest disease incidence, whereas B73Ht had the highest values for both disease incidence and rating.

Table 1. Mean incidence of infected ears for six maize inbreds inoculated with *Stenocarpella maydis* using five inoculation techniques in 1988 and 1989

	Injection			Silk			Whorl			Sheath			Control		
Inbred	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined
MBS613	95[a,b]	76	86	47	23	35	11	9	10	18	4	11	6	9	7
VA26Ht	83	94	89	34	39	37	37	26	32	8	2	5	8	13	10
VA35	100	100	100	60	18	39	44	36	40	43	8	26	1	0	1
H111	96	100	98	37	43	40	6	7	6	2	2	2	0	3	2
B73Ht	98	88	93	90	71	80	52	63	58	45	17	31	1	11	6
LH51	100	96	98	85	58	71	30	41	35	57	8	33	2	1	2

[a]LSD ($P = 0.05$) between treatments within a line = 17 (1988), 15 (1989), and 23 (years combined); and between lines within a treatment = 18 (1988), 16 (1989), and 25 (years combined).

[b]Disease incidence calculated by dividing the total number of infected ears by the total number of ears and multiplying by 100.

Table 2. Mean disease rating for six maize inbreds inoculated with *Stenocarpella maydis* using five inoculation techniques in 1988 and 1989

	Injection			Silk			Whorl			Sheath			Control		
Inbred	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined
MBS613	2.7[a,b]	1.1	1.9	1.4	0.6	1.0	0.2	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.2
VA26Ht	3.1	2.8	2.9	1.0	1.4	1.2	0.6	1.0	0.8	0.1	0.1	0.1	0.1	0.3	0.2
VA35	4.1	3.1	3.6	1.6	0.5	1.1	1.0	1.0	1.0	0.9	0.2	0.6	0.1	0.0	0.0
H111	3.8	3.6	3.7	1.4	1.4	1.4	0.2	0.2	0.2	0.1	0.0	0.0	0.0	0.0	0.0
B73Ht	3.8	3.1	3.5	3.7	2.4	3.0	1.8	2.6	2.2	1.1	0.5	0.8	0.0	0.3	0.2
LH51	4.0	2.8	3.4	3.3	1.8	2.6	0.8	1.1	0.9	1.3	0.2	0.8	0.1	0.0	0.0

[a]LSD ($P = 0.05$) between treatments within a line = 0.6 (1988), 0.6 (1989), and 0.9 (years combined); and between lines within a treatment = 0.7 (1988), 0.6 (1989), and 1.0 (years combined).

[b]Disease rating on a 0–5 scale where 0 = no visible infection, 1 = 1–25% infected kernels, 2 = 26–50% infected kernels, 3 = 51–75% infected kernels, 4 = 76–100% infected kernels, and 5 = all kernels and cob completely rotted.

The sheath inoculation technique yielded low numbers of infected ears, with an average of 18% infection over both years (Table 1). This method was not significantly different from the control in 1989. Differences between lines were detected in 1988 for both disease incidence and ratings; however, disease ratings for all lines were low, ranging from 0.1 to 1.3 (Table 2).

Hybrids. Among all hybrids and inoculation techniques evaluated, only treatments were significant for both the incidence of infected ears and the disease rating; line (hybrid) and the line by treatment interaction were not significant except for a significant difference between lines (hybrids) for disease rating in 1989.

Of the four inoculation techniques, the injection method yielded the highest incidence of infected ears among hybrids, with an average of 96% infected ears over both years (Table 3). However, there were no significant differences between hybrids in either the incidence of infected ears or disease rating (Table 4).

The silk spray technique yielded an average incidence of 63% infected ears over both years (Table 3). There were significant differences between hybrids for disease incidence in 1988 but not in 1989. There were no significant differences between hybrids for disease rating either year (Table 4).

The average incidence of infected ears and disease rating for the sheath and whorl methods did not differ significantly from the controls in either year (Tables 3 and 4).

DISCUSSION

The injection technique yielded the highest incidence of infected ears in both inbreds and hybrids. Among inbred lines, the technique identified one line (MBS613) with significantly lower disease ratings in both years and lower incidence in 1989. Among the other inbred lines, disease ratings were very high and many ears were completely rotted. Among the hybrids tested, the injection method did not identify any significant differences in either incidence or disease ratings. Injection of spores directly into the ear is an artificial method of inoculation that may bypass or overcome some types of resistance (8) but may measure the ability of the ear to contain the fungus once infected (5). The technique is relatively time-consuming but results in consistent infection under variable environmental conditions.

The silk spray technique yielded an intermediate incidence of infected ears in both inbreds and hybrids. The technique was successful in identifying resistant and susceptible inbred lines, with B73Ht and LH51 having significantly higher disease incidence and severity than other inbreds. The silk spray may more closely simulate the natural infection process and may provide a more accurate measure of disease resistance. In addition, the technique is fairly easy to apply.

The whorl inoculation technique yielded significantly lower incidence and disease ratings in both inbreds and hybrids than either the silk spray or injection techniques. In a previous study, infection levels resulting from whorl inoculations were not significantly different from the controls in the absence of overhead irrigation (J. C. Klapproth and J. A. Hawk, *unpublished*). Among inbred lines, the whorl technique did identify significant differences between lines in both incidence and disease ratings, with MBS613 and H111 having the lowest incidence and disease ratings, whereas B73Ht had the highest. The whorl method has the advantage that the grain is very easy to apply and does not injure the plant.

The sheath inoculation technique resulted in very low levels of disease incidence and ratings in both inbreds and

Table 3. Mean incidence of infected ears for four maize hybrids inoculated with *Stenocarpella maydis* using five inoculation techniques in 1988 and 1989

	Injection			Silk			Whorl			Sheath			Control			Mean		
Hybrid	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined
LH132 × LH51	100[a,b]	74	87	73	61	67	17	22	20	16	14	15	1	26	14	42	39	40
Pioneer 3192	99	100	99	48	57	53	1	6	4	30	4	17	0	0	0	36	34	35
Pioneer XC	100	99	100	68	69	69	4	32	18	13	11	12	0	4	2	37	43	40
MBS613 × VA26Ht	98	96	97	61	64	62	19	39	29	15	17	16	10	15	12	41	46	43
Mean	99	92	96	63	63	63	11	25	18	18	12	15	3	11	7	39	40	40

[a]LSD ($P = 0.05$) between lines averaged over treatments = 10 (1988), 9 (1989), and 10 (years combined); between treatments averaged over lines = 9 (1988), 14 (1989), and 11 (years combined); between treatments within a line = 19 (1988), 28 (1989), and 21 (years combined); and between lines within a treatment = 19 (1988), 27 (1989), and 21 (years combined).

[b]Disease incidence calculated by dividing the total number of infected ears by the total number of ears and multiplying by 100.

Table 4. Mean disease rating for four maize hybrids inoculated with *Stenocarpella maydis* using five inoculation techniques in 1988 and 1989

	Injection			Silk			Whorl			Sheath			Control			Mean		
Hybrid	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined	1988	1989	Years combined
LH132 × LH51	4.0[a,b]	2.9	3.5	2.6	2.0	2.3	0.4	0.4	0.4	0.2	0.2	0.2	0.0	1.0	0.5	1.5	1.3	1.4
Pioneer 3192	3.9	4.0	4.0	1.8	2.2	2.0	0.1	0.1	0.1	1.1	0.1	0.6	0.0	0.0	0.0	1.4	1.3	1.3
Pioneer XC	4.0	4.0	4.0	2.5	2.6	2.5	0.1	1.0	0.5	0.3	0.3	0.3	0.0	0.1	0.0	1.4	1.6	1.5
MBS613 × VA26Ht	3.9	3.8	3.9	2.1	2.2	2.2	0.3	0.8	0.6	0.2	0.3	0.2	0.2	0.3	0.3	1.4	1.5	1.4
Mean	4.0	3.7	3.8	2.3	2.3	2.3	0.2	0.6	0.4	0.4	0.2	0.3	0.1	0.3	0.2	1.4	1.4	1.4

[a]LSD ($P = 0.05$) between lines averaged over treatments = 0.4 (1988), 0.2 (1989), and 0.4 (years combined); between treatments averaged over lines = 0.4 (1988), 0.6 (1989), and 0.4 (years combined); between treatments within a line = 0.7 (1988), 1.1 (1989), and 0.8 (years combined); and between lines within a treatment = 0.8 (1988), 1.0 (1989), and 0.8 (years combined).

[b]Disease rating on a 0–5 scale where 0 = no visible infection, 1 = 1–25% infected kernels, 2 = 26–50% infected kernels, 3 = 51–75% infected kernels, 4 = 76–100% infected kernels, and 5 = all kernels and cob completely rotted.

hybrids in both years. The sheath technique is fairly time-consuming and more damaging to the plant than either the silk spray or whorl methods.

Based on these results, the silk spray technique has been selected as the best technique for screening for resistance to Diplodia ear rot. This technique results in reasonable levels of infection, may be more similar to natural infection (8), is less damaging to the plant, can identify significant differences between inbred lines, and is reasonably easy to apply.

Results from this study support earlier observations that inbreds MBS613 and H111 may exhibit some resistance to Diplodia ear rot, whereas inbred B73Ht is more susceptible. The susceptibility of the elite inbred B73Ht supports the suggestion that susceptible germ plasm may be an important factor in the increased occurrence of Diplodia ear rot (4).

Little or no significant differences were observed between hybrids with any of the inoculation techniques evaluated. This may be attributable to a lack of resistance in the hybrids selected; evaluation of a larger number of hybrids might have shown differences. Although there were no significant differences detected, Pioneer 3192 had the lowest disease incidence and ratings in both years for the whorl and silk spray techniques, which supports earlier observations (K. Byrnes, *personal communication*).

Results indicate that the performance of the inbred lines was related to the method by which the plants were inoculated, resulting in a significant line by treatment interaction. This interaction of genotype and inoculation technique should be considered when choosing an inoculation method and when comparing results from different studies.

Finally, a better understanding of how the ear becomes infected, the time of infection, the role of insects in spreading the disease, and identification of traits that may contribute to disease resistance will be necessary in choosing a technique that most accurately measures plant resistance to *S. maydis*.

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