Evaluation of a Silk-Inoculation Technique to Differentiate Reactions of Sweet Corn Hybrids to Common Smut

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We thank J. Gantz, P. Fallah Moghaddam, L. Adams, A. Hightshoe, J. Manuel, Q. Pike, B. Yates, and S. Zimmerman for technical assistance.
Accepted for publication 11 August 1995.

ABSTRACT

Pataky, J. K., Nankam, C., and Kerns, M. R. 1995. Evaluation of a silk-inoculation technique to differentiate reactions of sweet corn hybrids to common smut. Phytopathology 85:1323-1328.

Reactions of 750 sweet corn hybrids to common smut, caused by *Ustilago maydis*, were evaluated in field trials using a silk-channel inoculation technique and natural infection. Hybrids were classified annually from resistant to susceptible based on incidence of galls on primary ears. Means and standard deviations of incidence of ear galls from plots inoculated with *U. maydis* were 34.2 ± 20.9, 20 ± 12.9, and 32.7 ± 17.4% for the 1992, 1993, and 1994 trials, respectively. Means and standard deviations of incidence of ear galls from naturally infected plots were 1 ± 2.4, 4.8 ± 7.1, and 4.5 ± 6.4% for the 1992, 1993, and 1994 trials, respectively. Hybrid means ranged from 0 to 96, 0 to 80, and 0 to 91% for inoculated plots and from 0 to 19, 0 to 47 and 0 to 58% for naturally infected plots in 1992, 1993, and 1994, respectively. Mean incidence of ear galls was greatest for the group of white *sh2* hybrids and least for the white *se* hybrids. Average smut incidence also was higher for groups of early-maturing hybrids than for all other hybrids. The consistency of classifications was compared for hybrids common to trials in different years. The null hypothesis was retained for Chi-square tests of independence of hybrid classifications between two trials, indicating a lack of relationship between trials. Approximately 24% (91 of 383) of all hybrids common to two trials were placed in the same resistance category in both trials. Forty-two percent of the hybrids differed by one category. Inconsistent classifications, differences of two or three categories, occurred for about 34% (131 of 383) of the hybrids common to two trials. Although the silk-channel inoculation method resulted in a significantly higher incidence of ear galls than did natural infection, this technique and our categorization procedure resulted in greater inconsistency between trials than is acceptable. These procedures must be modified to accurately assess the response of hybrids to common smut and to breed for increased levels of smut resistance.

Common smut, caused by *Ustilago maydis* (DC.) Corda (synamorph = *U. zeae* (Beckm.) Unger), causes economic damage on sweet corn (*Zea mays* L.) by reducing the number of marketable ears and by adding to harvest and processing costs. Host resistance is the most efficient way to control smut, but resistance is not well understood, partly because of the lack of a reliable, repeatable, labor-efficient inoculation method.

Response to natural infection by *U. maydis* is the primary basis by which sweet corn hybrids are classified as resistant or susceptible to smut. Natural infection also is the primary selection criterion used to eliminate smut-susceptible lines in breeding programs. Trials based on natural infection have been somewhat unreliable because of the fortuitous occurrence of environmental conditions conducive to smut infection and an association between host growth stage during infection periods and the plant tissues on which galls form (4). For example, incidence of ear galls was 41 and 0% in two plots of a susceptible sweet corn hybrid, Candy Bar, planted 12 days apart in Urbana, IL, in 1989, whereas incidence of tassel galls in the two plantings was 9 and 24%, respectively (J. K. Pataky, *unpublished data*). Similar associations between host growth stages at infection and formation of galls on different plant tissues were observed from 1987 to 1990 in the NE-124 disease and insect nurseries in Indiana, Minnesota, and New York (2; H. L. Warren, S. L. Grier, and M. E. Smith, *personal communications*).

An efficient, reliable inoculation method could improve evaluations of smut resistance. In a review of inoculation techniques used in the early part of this century, Walter (16) concluded that applications of large amounts of inoculum in water did not increase the severity of smut but injury to young, rapidly growing plants did. Introducing sporidia or teliospores into plant wounds created by sand-blasting did not increase smut incidence in recent field trials (4). Christensen (1) noted that spraying sporidia or teliospores into leaf whorls or on seed did not increase the incidence of galls, but injecting sporidial suspensions into whorls of seedlings often caused leaf galls, distortion of the growing point, and death of seedlings. Injection of sporidia between the leaf sheath and stalk resulted in 100% incidence of tassel galls and about 50% incidence of ear galls in greenhouse trials, but only a few smut galls developed on ears when this technique was used in a field trial in North Carolina (14). When similar techniques were tested in field trials in Illinois, incidence of galls on stalks, tassels, and leaf sheaths was substantially higher than noninoculated controls, but incidence of ear galls was only slightly greater than controls (4).

Ear galls are induced effectively by injecting sporidial suspensions into silk channels or ears soon after silks emerge (12,13, 15,17). Incidence of ear galls was nearly 97% in field trials in Georgia when 3 ml of compatible sporidia at a concentration of 10^6 cells/ml was injected in ears when silks had emerged 5 to 10 cm (12). In trials in Illinois, about 50% incidence of ear galls resulted from injections of sporidial suspensions into the silk channel when silks had emerged about 3 to 6 cm (17). The silk-channel inoculation technique was used to evaluate the yield and quality of huitlacoche, edible common smut, in trials in which galls formed on about 40% of the inoculated ears (15). Injection of sporidial suspensions through husk leaves as soon as silks were visible was a reliable method used in a greenhouse study to produce stigma (i.e., silk) infections from which ear galls formed when kernels were allowed to mature (13).

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In this article, we evaluate silk-channel inoculation as a method of differentiating reactions of sweet corn hybrids to common smut.

MATERIALS AND METHODS

A total of 750 commercial sweet corn hybrids were evaluated for their reactions to common smut. Trials in 1992, 1993, and 1994 included 345, 372, and 367 hybrids, respectively. Hybrids common between trials numbered 161, 152, and 70 in 1992 to 1993, 1993 to 1994, and 1992 to 1994, respectively; 49 hybrids were common to all three trials. Each trial included three replicates of hybrids grouped by endosperm mutation and arranged in a randomized complete block design. Blocks of 50 or 60 hybrids were grouped by endosperm mutation, sugary-1 (*su*), sugary enhancer (*se*), and shrunken-2 (*sh2*), to minimize cross-pollination between types of sweet corn. Experimental units consisted of five rows in 1992 and 1993 and three rows in 1994 with 10 to 15 plants per 3.2 m of row and 76 cm spacing between rows. Planting dates were 7 May 1992, 11 May 1993, and 17 May 1994.

TABLE 1. Incidence (percentage) of ear galls of common smut on groups of sweet corn hybrids reaching the full silk growth stage at various dates and inoculated with or infected naturally by *Ustilago maydis*

Year and inoculation date[a]	n[b]	Inoculation Mean (%)	Inoculation Range (%)	Natural infection Mean (%)	Natural infection Range (%)
1992	345	34.2	0–96	1.0	0–20
July 2	6	41.7[c]	2–96	1.9	0–10
July 6	18	45.0[c]	10–80	7.0[c]	0–20
July 9	18	26.2[d]	0–71	0.8	0–4
July 13	54	37.4	0–81	0.9	0–8
July 16	89	30.0	0–90	0.8	0–7
July 20	98	28.4	0–81	0.6	0–6
July 23	56	45.6[c]	2–85	0.5	0–4
July 26	6	37.4	15–65	0.2	0–1
1993	372	20.0	0–80	4.8	0–60
July 8	10	34.5[c]	12–80	11.2[c]	0–34
July 10	26	28.9[c]	4–69	9.4[c]	0–52
July 13	28	28.5[c]	3–63	7.8[c]	0–41
July 16	62	20.4	6–43	4.5	0–25
July 19	99	15.1[d]	3–42	4.0	0–40
July 21	58	21.2	1–52	3.2	0–20
July 23	57	19.1	3–47	6.0	0–60
July 26	32	11.7[d]	0–34	4.0	0–16
1994	367	32.7	0–91	4.5	0–58
July 6	14	27.3	0–67	4.1	0–10
July 9	7	49.6[c]	30–73	18.2[c]	0–58
July 11	41	49.4[c]	12–91	8.0[c]	0–35
July 13	29	36.4	5–62	4.9	0–19
July 15	69	26.5[d]	0–65	4.8	0–20
July 17	25	25.8[d]	4–58	5.6	0–29
July 19	132	35.6	7–82	3.0	0–23
July 21	33	26.3[d]	3–48	4.1	0–28
July 23	17	16.1[d]	0–67	1.8[d]	0–11

[a] Inoculation date = approximate date when hybrids in each group reached the full silk growth stage.
[b] *n* = number of hybrids inoculated.
[c] Mean significantly greater than the grand mean of hybrids inoculated at all other dates as compared by *t* tests.
[d] Mean significantly lower than the grand mean of hybrids inoculated at all other dates as compared by *t* tests.

Two monosporidial lines of *U. maydis*, originally isolated and designated as 2 and 11 by K. J. Leonard (USDA-ARS, University of Minnesota, St. Paul), were obtained from M. L. Carson (USDA-ARS, North Carolina State University, Raleigh) and maintained at −80°C in a 15% glycerol solution. The isolates were identified as different mating types in previous studies (4,14,15). Isolates were transferred from cold storage about 4 weeks prior to the first inoculation and tested for virulence. Inocula of each isolate were produced from 2- to 4-day-old potato-dextrose agar cultures and potato-dextrose broth shake cultures incubated at room temperature. Broth and agar cultures were mixed with water and a few drops of Tween 80 to produce a suspension of about 5,000 sporidia per ml.

Hybrids were evaluated daily from late-June until late-July for the midsilk growth stage. Plants were inoculated once when silks had emerged from husk leaves about 1 to 6 cm, 2 to 4 days after the midsilk stage in one replicate in 1992 and in two replicates in 1993 and 1994. A sporidial suspension (8 ml) was injected directly in the silk channel of the primary ear using a hand-held spray gun (Meterjet, model 23623; Spraying Systems Co., Wheaton, IL) attached to a backpack sprayer (Solo, model 425, Grower Equipment Supply, Hainesville, IL) containing the inoculum (15). Inoculum was mixed during inoculation by shaking backpack sprayers. Because of differences in maturity of hybrids, there were eight inoculation dates in 1992 and 1993 and nine in 1994 (Table 1). Inoculation of each trial required approximately 45, 80, and 65 work-hours in 1992, 1993, and 1994, respectively.

Incidence (percentage) of ears with galls was rated 3 to 7 weeks after inoculation by counting the number of primary ears with and without galls. Incidence of smutted ears also was assessed from noninoculated replicates: two in 1992 and one in 1993 and 1994. Data from replicates inoculated in 1993 and 1994 were subjected to analysis of variance after arcsine square-root transformations to create homogeneous variances. Hybrids were compared by a mean separation procedure (Bayesian LSD [BLSD], $k = 100$). Correlations of smut incidence resulting from inoculation and natural infection were done from hybrid means within years. Smut incidence for hybrids grouped by endosperm mutation (*su*, *se*, and *sh2*) and kernel color (yellow, white, and bicolor) were compared in all

TABLE 2. Criteria used to classify hybrid sweet corn reactions to common smut based on incidence (percentage) of ear galls after silk channel inoculation or natural infection in 1992, 1993, and 1994

Year and incidence (%) from natural infection	Incidence (%) from inoculation and hybrid reaction[a]					
1992	10		10–20	20–45	45–55	>55
0–19	R		MR	M	MS	S
1993	<7	7–11	11–20	20–33	33–40	>40
0	R	R-MR	MR	M(?)	M(?)	MS(?)
0–2.5	R-MR	MR	MR-M	M(?)	M(?)	MS(?)
2.5–5	MR	M(?)	M(?)	M-MS	MS	MS-S
5–10	M(?)	M(?)	M(?)	MS	MS-S	S-MS
>10	M(?)	M(?)	M(?)	MS-S	S	S
1994	<10	10–15	15–28	28–33	38–50	>50
0	R	R-MR	MR	M	MS(?)	S(?)
0–3	R-(?)	R-MR(?)	MR	M	MS	S
3–6	R-M	MR-M	MR-M	M	MS	S
>6	M(?)	M(?)	M(?)	M-S	MS-S	S

[a] Hybrid reactions: R = resistant, MR = moderately resistant, M = moderate, MS = moderately susceptible, and S = susceptible.

possible combinations by Student's t tests. Student's t tests also were used to compare smut incidence for hybrids grouped by maturity (i.e., inoculation date) to that of all other hybrids. All analyses were done using the appropriate procedures of SAS (SAS Institute, Cary, NC).

Trial means and standard deviations, calculated from hybrid means, were used to calculate standardized z scores of smut incidence from inoculation and natural infection for each hybrid. BLSD separations and z scores were used to subjectively determine ranges of smut incidence used to define resistance categories: resistant (R), moderately resistant (MR), moderate (M), moderately susceptible (MS), and susceptible (S). Categories were established initially based on responses to inoculation. R and S categories usually were set at about 1 SD below or above the trial mean, respectively, with some adjustment based on where BLSD values separated the least and most severely infected hybrids from those with intermediate responses. Likewise, MR and MS categories were established at about 0.3 SD below and above the trial means, respectively, with BLSD separations also considered. Classifications then were modified based on response to natural infection (Table 2). The consistency of classifications was compared for hybrids that were common to trials in different years. Chi-square contingency tests were used to determine whether classifications of hybrids in each year were independent. The expected number of hybrids in each cell was calculated as $E_{ij} = [P_{i.} \times P_{.j}] \times n_{ij}$, where E_{ij} = the expected number of individuals in the ij class, i = first-year class (from R to S), and j = second-year class (from R to S), $P_{i.}$ = frequency of individuals in the ith class in year 1, $P_{.j}$ = frequency of individuals in the jth class in year 2, and n_{ij} = total individuals common to the two trials. Observed and expected distributions of E_{ij} were compared by Chi-square tests of independence. Contributions of individual cells to Chi-square values were examined.

RESULTS

Means and standard deviations of incidence of ear galls from plots inoculated with *U. maydis* were 34.2 ± 20.9, 20 ± 12.9, and 32.7 ± 17.4% for the 1992, 1993, and 1994 trials, respectively (Table 2). Means and standard deviations of incidence of ear galls from naturally infected plots were 1 ± 2.4, 4.8 ± 7.1, and 4.5 ± 6.4% for the 1992, 1993, and 1994 trials, respectively (Table 3). Among hybrids, mean incidence ranged from 0 to 96, 0 to 80, and 0 to 91% for inoculated plots and from 0 to 19, 0 to 47 and 0 to 58% for naturally infected plots in 1992, 1993, and 1994, respectively. Correlation coefficients for incidence of ear galls from inoculation and natural infections were 0.10, 0.35, and 0.18 in 1992, 1993, and 1994, respectively. Responses of individual hybrids in each trial and a summary of hybrid responses over several years of trials, including noninoculated trials prior to 1992, are available in detail from J. K. Pataky and in a summarized form in extension reports (5,8,10,11). Classification of hybrids into resistance categories based on incidence of ear galls was somewhat more subjective than similar classifications for common rust, northern leaf blight, and Stewart's bacterial wilt (5,8,10,11)

Incidence of ear galls differed among hybrids grouped by endosperm mutation and kernel color (Tables 3 and 4). Mean incidence of ear galls from inoculation or natural infection usually was greatest for the white *sh2* hybrids and least for the white *se* hybrids, although the range of smut incidence overlapped for all groups of hybrids.

When groups of hybrids with different endosperm types were compared within groups of hybrids with similar kernel color, white and bicolor *sh2* hybrids differed from white and bicolor *se* hybrids (Tables 3 and 4). Mean incidence of ear galls was significantly greater for white *sh2* hybrids than for white *se* hybrids in all six comparisons (Table 4). Mean incidence of ear galls was

TABLE 3. Incidence (percentage) of ear galls of common smut on groups of sweet corn hybrids inoculated with or infected naturally by *Ustilago maydis* in 1992, 1993, and 1994

Year and endosperm type	Kernel color	n[a]	Inoculated Mean (%)	Inoculated SD[b] (%)	Inoculated Range (%)	Natural infection Mean (%)	Natural infection SD[b] (%)	Natural infection Range (%)
1992		345	34.2	20.9	0–96	1.0	2.4	0–33
Shrunken-2	Yellow	105	33.3	21.6	0–85	0.9	1.1	0–11
	Bicolor	56	39.3	21.2	0–85	0.6	1.8	0–4
	White	19	36.4	19.1	0–67	1.3	1.6	0–6
Sugary enhancer	Yellow	47	32.6	20.6	0–79	1.6	3.4	0–15
	Bicolor	37	28.6	17.8	0–58	1.1	2.3	0–12
	White	20	17.4	11.2	2–44	0.5	1.1	0–4
Sugary 1	Yellow	52	39.2	21.6	4–96	1.4	3.6	0–20
	Bicolor	4	49.6	17.0	28–69	0.5	1.1	0–33
	White	5	47.6	18.4	25–71	1.2	1.8	0–4
1993		372	20.0	12.9	0–80	4.8	7.1	0–41
Shrunken-2	Yellow	118	17.9	10.8	0–52	4.5	6.7	0–40
	Bicolor	81	21.9	13.0	4–64	5.4	6.0	0–29
	White	21	26.2	14.3	6–52	7.8	6.9	0–26
Sugary enhancer	Yellow	55	18.9	11.7	3–60	3.9	7.2	0–35
	Bicolor	39	22.3	16.8	4–80	3.6	4.9	0–25
	White	20	14.7	9.8	1–33	4.6	5.4	0–18
Sugary 1	Yellow	32	18.0	10.1	2–44	3.8	8.6	0–41
	Bicolor	5	33.1	31.7	8–70	7.1	14.7	0–33
	White	1	44.0	...	...	46.6	...	...
1994		367	32.7	17.4	0–91	4.5	6.4	0–58
Shrunken-2	Yellow	129	31.5	16.3	0–83	4.7	5.9	0–35
	Bicolor	94	37.8	18.3	4–84	6.6	8.5	0–58
	White	24	39.5	21.3	7–91	6.7	5.6	0–35
Sugary enhancer	Yellow	39	29.3	16.6	0–67	2.2	3.6	0–13
	Bicolor	28	27.1	14.4	2–63	1.3	2.2	0–8
	White	11	24.0	22.5	0–67	1.0	1.9	0–5
Sugary 1	Yellow	38	30.7	14.6	3–62	2.2	3.9	0–19
	Bicolor	3	20.3	14.4	11–37	0	0	0
	White	1	36.7	...	...	2.4	...	...

[a] n = number of hybrids.

[b] SD = standard deviation.

significantly greater for bicolor *sh2* hybrids than for bicolor *se* hybrids in four of six comparisons (Table 4). There were no differences among yellow hybrids, except in 1994 when the mean incidence of ear galls from natural infection for *sh2* hybrids (4.7%) was slightly higher than that for *se* or *su* hybrids (2.2%). Groups of white and bicolor *su* hybrids were not compared in these analyses due to the small sample size of five or fewer hybrids per trial.

When groups of hybrids with different kernel colors were compared within groups of hybrids with similar endosperm mutations, mean incidence of ear galls often was higher for the groups of white and bicolor *sh2* hybrids than for the groups of yellow *sh2* hybrids (Tables 3 and 4). Smut incidence from inoculation averaged about 36, 26, and 40% for white *sh2* hybrids; 39, 22 and 38% for bicolor *sh2* hybrids; and 33, 18, and 32% for yellow *sh2* hybrids in 1992, 1993, and 1994 trials, respectively. Mean incidence of ear galls occasionally was lower for the white and bicolor *se* hybrids than for the yellow *se* hybrids (Tables 3 and 4). Smut incidence from inoculation averaged 17, 15, and 24% for white *se* hybrids; 29, 22, and 27% for bicolor *se* hybrids; and 33, 19, and 29% for yellow *se* hybrids in 1992, 1993, and 1994, respectively.

Incidence of ear galls also differed among hybrids grouped by maturity. Mean incidence was greater for early-maturing hybrids, those at the full-silk stage on one of the first three inoculation dates, than for all other hybrids for seven of nine comparisons from inoculated plots and for six of nine comparisons from naturally infected plots (Table 1). For some groups of mid- and full-season hybrids, smut incidence was lower than that of all other hybrids, especially in 1994. Ranges of smut incidence for hybrids grouped by maturity usually overlapped similarly to overlapping ranges of hybrids grouped by endosperm mutation and kernel color (Table 4).

Criteria used to classify hybrid reactions to smut differed slightly

TABLE 4. Significance of *t* statistics for comparing incidence of ear galls among groups of yellow, bicolor, and white sweet corn hybrids with the sugary 1, sugary enhancer, and shrunken-2 endosperm mutations inoculated with or infected naturally by *Ustilago maydis* in 1992, 1993, and 1994

	Inoculated			Natural infection		
Endosperm type and kernel color	1992	1993	1994	1992	1993	1994
Yellow						
Shrunken-2 vs. sugary enhancer	ns[a]	ns	ns	ns	ns	.02
Shrunken-2 vs. sugary 1	ns	ns	ns	ns	ns	.02
Sugary enhancer vs. sugary 1	ns	ns	ns	ns	ns	ns
Bicolor						
Shrunken-2 vs. sugary enhancer	.01	ns	.002	ns	.08	.002
White						
Shrunken-2 vs. sugary enhancer	<.001	.005	.07	.05	.10	<.001
Shrunken-2						
Yellow vs. bicolor	.10	.02	.007	ns	ns	.05
Yellow vs. white	ns	.01	.07	ns	.05	.05
Bicolor vs. white	ns	ns	ns	.03	ns	ns
Sugary enhancer						
Yellow vs. bicolor	ns	ns	ns	ns	ns	ns
Yellow vs. white	.003	ns	ns	ns	ns	ns
Bicolor vs. white	.01	.05	ns	ns	ns	ns

[a] ns = not significant.

TABLE 5. Classification of reactions[a] to common smut for sweet corn hybrids common to 2 years of trials

	Reaction in first year										
	Observed					Expected[b]					
Years in trial and reaction in second year	R	MR	M	MS	S	R	MR	M	MS	S	Σ
1992–1993											
R	7	6	10	8	1	4.8	4.6	12.1	6.9	3.6	32
MR	4	6	15	5	7	5.5	5.3	14.0	8.0	4.1	37
M	11	6	21	14	6	8.6	8.3	22.0	12.6	6.5	58
MS	2	5	10	5	3	3.7	3.6	9.5	5.4	2.8	25
S	0	0	5	3	1	1.3	1.3	3.4	2.0	1.0	9
Σ	24	23	61	35	18	24	23	61	35	18	161
1993–1994											
R	6	7	4	5	2	3.0	5.3	6.8	4.4	4.4	24
MR	3	10	14	5	6	4.8	8.5	10.8	7.0	7.0	38
M	7	14	18	11	10	7.5	13.4	17.0	11.0	11.0	60
MS	3	2	7	6	9	3.4	6.1	7.7	5.0	5.0	27
S	0	1	0	1	1	0.4	0.7	0.9	0.6	0.6	3
Σ	19	34	43	28	28	19	34	43	28	28	152
1992–1994											
R	2	0	6	2	1	0.8	1.4	5.0	1.9	1.9	11
MR	2	1	8	1	1	0.9	1.7	6.0	2.2	2.2	13
M	1	4	4	6	5	1.4	2.6	9.1	3.4	3.4	20
MS	0	1	9	2	4	1.1	2.1	7.3	2.7	2.7	16
S	0	3	5	1	1	0.7	1.3	4.6	1.7	1.7	10
Σ	5	9	32	12	12	5	9	32	12	12	70

[a] Reactions (R = resistant, MR = moderately resistant, M = moderate, MS = moderately susceptible, and S = susceptible) include more than one category: R = R, R-MR; MR = MR, MR-R, MR-M; M = M, M-MR, M-MS; MS = MS, MS-M, MS-S; and S = S, S-MS.
[b] Expected number of individuals in each category if hybrids were distributed randomly, based on frequencies of individuals in each category in years 1 and 2.

among years (Table 2). In 1992, resistance classifications were based solely on incidence of galls from inoculation because ear galls did not result from natural infection for 64% of the hybrids evaluated, and the range of incidence from natural infection was only 0 to 19%. In 1993 and 1994, resistance classifications based on inoculated hybrids were modified slightly according to responses to natural infection. Ear galls from natural infection were observed for about 60% of the hybrids evaluated in 1993 and 1994, and incidence ranged from 0 to 47% in 1993 and from 0 to 58% in 1994. In each year, smut incidence from inoculation was at least 1 SD below or above the trial mean (i.e., z scores less than –1 or greater than 1) for hybrids classified as R or S, respectively. Smut incidence from inoculation was below or above the trial mean for hybrids in the MR and MS categories. Hybrids in the M category had z scores near 0 or had an incidence of ear galls from natural infection that was greater than expected based on their response to inoculation.

Hybrids common to two trials numbered 161, 152, and 70 in 1992 to 1993, 1993 to 1994, and 1992 to 1994, respectively (Table 5). Approximately 24% (91 of 383) of all hybrids common to two trials were placed in the same resistance category in both trials (Tables 5 and 6). Forty-two percent of the hybrids differed by only one category (e.g., MR in one trial and R or M in the other). Inconsistency between trials, differences of two or three categories, occurred for about 34% (131 of 383) of the hybrids common to two trials (Table 6). There were 28, 24, and 9 hybrids common to the 1992 to 1993, 1993 to 1994, and 1992 to 1994 trials, respectively, that were classified as R or MR in one trial and S or MS in the other trial.

Of the 49 hybrids common to three trials, one S and two R hybrids were classified the same in all trials. There were differences of one, two, three, and four categories for 17, 16, 11, and 2 hybrids, respectively. The responses of 72% of these 49 hybrids could be summarized with a relative degree of confidence (e.g., a hybrid classified M, S-MS, MS(?) would be summarized as MS). There were 8 of the 49 hybrids for which reactions to smut could not be summarized confidently based on 3 years of data.

Chi-square values for the tests of independence were 14.24, 15.39, and 21.19 for the 1992 to 1993, 1993 to 1994, and 1992 to 1994 comparisons, respectively. The hypothesis of independence was retained ($\chi^2_{0.05(16)} = 26.3$) in each case, hence there was little or no relationship between classifications between years. When individual cells of the contingency table were examined, 8, 7, and 10 of the 25 cells accounted for 72, 76, and 75% of the Chi-square values in the 1992 to 1993, 1993 to 1994, and 1992 to 1994 comparisons, respectively. Of the 25 cells that contributed most to the Chi-square values, 12 had higher and 13 had lower observed values than expected. Seven of the twelve cells with observed values that were higher than expected were R or S reactions (i.e., R-R, R-MR, S-S, or S-MS). Nine of the thirteen cells with observed values that were lower than expected were mixed reactions (i.e., R-S, R-MS, MR-S, MR-MS, MS-MR, or S-R).

DISCUSSION

Evaluation of sweet corn hybrid reactions to common smut after inoculation of silk channels with *U. maydis* allowed for separation of hybrid reactions in individual trials, but reactions of individual hybrids were relatively inconsistent (i.e., independent) over trials. Approximately 66% of the hybrids common to two trials were placed in resistance categories that were within one category of each other; however, about 34% of the hybrids were classified inconsistently. Examination of the contribution of individual cells of contingency tables to Chi-square values indicates that highly resistant or susceptible genotypes were somewhat more consistent in their classification than other genotypes. Thus, the silk-inoculation procedures we used were an improvement over natural infection, because incidence of ear galls increased and was less subject to environmental influence; however, these procedures resulted in greater inconsistency between trials than was desired or acceptable for hybrids that were not at either extreme, highly resistant or susceptible. These procedures may be an improvement over previous evaluations of hybrid responses to natural infection (6,7, 9), but greater consistency is needed to accurately assess the response of individual hybrids to smut when the silk-channel inoculation method is used.

Certain groups of hybrids were more susceptible or resistant to *U. maydis* than the entire population of sweet corn hybrids we evaluated. These responses were consistent between trials. Smut incidence was highest for groups of white *sh2* hybrids and least for groups of white *se* hybrids. This concurs with observations in the mid-Atlantic states, where in the past few years smut has been particularly troublesome on white *sh2* hybrids (E. Kee, University of Delaware, *personal communication*). Groups of early-maturing hybrids also appeared to be more susceptible to smut than other sweet corn hybrids. Differences among these groups must be interpreted cautiously.

Our results should not be interpreted as indicating that the *sh2* endosperm mutation or white kernel color are related to reactions to smut. Distinctly different reactions between groups of white *se* hybrids and white *sh2* hybrids and the lack of differences in smut incidence among groups of *sh2*, *se*, and *su* yellow hybrids are evidence that kernel color and endosperm mutation are not related to reactions to smut. The susceptibility of white *sh2* hybrids probably results from a relatively small number of smut-susceptible white *sh2* inbreds that are used extensively in hybrid combination and share a relatively common genetic background. Crosses between yellow *sh2* inbreds and these widely used, genetically similar, smut-susceptible white *sh2* inbreds also would explain the higher incidence of ear galls observed on bicolor *sh2* hybrids in our trials. The lower incidence of ear galls on white *se* hybrids and, to a lesser extent, on bicolor *se* hybrids can be explained by a few widely used, genetically similar, smut-resistant white *se* inbreds. Converting smut-resistant white *se* inbreds to *sh2* would be one way to improve smut resistance among white *sh2* inbreds. The wide range of reactions to smut among yellow hybrids in our trials and the similar mean response among endosperm mutants for yellow hybrids can be explained by a larger number of commercially used yellow inbreds with greater genetic diversity for smut reactions than that for commercially used white inbreds.

The higher incidence of smut in inoculated plots of early-maturing hybrids is confounded with different inoculation dates. It is impossible to separate the susceptible reactions of inoculated, early-maturing hybrids from the effects of different levels of inoculum among dates or from environmental conditions during early July that may have been more conducive to infection than later conditions. Since smut incidence among groups of early-maturing hybrids also was greater in naturally infected plots, which would not have resulted from differences in inoculum, and weather condi-

TABLE 6. Consistency of classification of sweet corn hybrid reactions to common smut trials in 1992, 1993, and 1994

	Comparison of trials			
Degree of consistency[a]	1992–1993	1993–1994	1992–1994	Total
Consistent	40 (25)[b]	41 (27)	10 (14)	91 (24)
Relatively consistent	61 (38)	66 (43)	34 (49)	161 (42)
Inconsistent	42 (26)	28 (18)	19 (27)	89 (23)
Highly inconsistent	18 (11)	17 (11)	7 (10)	42 (11)
Total	161	152	70	383

[a] Hybrids were classified as follows: consistent = same in both trials; relatively consistent = same within one category between trials; inconsistent = two-category difference between trials; and highly inconsistent = three-category difference between trials.

[b] Numbers in parentheses represent the percentage of the total for each comparison.

tions during the early part of July were considerably different among 1992, 1993, and 1994, we believe that the best explanation for these results is increased smut susceptibility of many early-maturing inbreds, similar to that of the white *sh2* inbreds.

For all comparisons of groups of hybrids, ranges of reactions overlapped considerably. Although a particular group of sweet corn hybrids may be more susceptible or resistant than other groups, an individual hybrid may be very different from the general response of its group. Our comparisons identify groups of sweet corn germ plasm that need higher levels of smut resistance, namely the white *sh2* and early-maturing materials, but reactions of individual hybrids still must be known for growers to make informed decisions about using resistance to control smut. Modification of the silk-channel inoculation method may produce a technique that is superior to natural infection for evaluating responses of sweet corn hybrids to common smut. Nevertheless, this technique must be adapted to produce less variable results if we wish to improve our understanding of resistance to *U. maydis* and our ability to control this disease.

Certain aspects of our procedures should be modified in future trials. Pope and McCarter (12) obtained nearly 97% incidence of ear galls using techniques similar to ours and inoculum concentrations 200 times greater than ours. Snetselaar and Mims (13) observed that sporidia grown on solid medium or for long periods of time in liquid cultures may need to undergo mitotic division before mating, whereas log-phase sporidia from 12- to 24-h-old shake cultures mate immediately. Although the concentrations used by Pope and McCarter (12) might preclude our ability to separate differences among sweet corn genotypes, higher concentrations of log-phase sporidia from shake cultures should improve our evaluations.

Efficient use of labor also must be considered. Our smut evaluations required 40 to 80 work-hours of labor solely to inoculate plants during pollination, our busiest period of the year. An inoculation procedure that could be completed and that would allow for selection prior to pollination would be useful to most sweet corn breeding and pathology programs. In 1994, we reduced the number of rows in each experimental unit to inoculate plants with greater precision, decreasing the number of plants inoculated per experimental unit from about 50 to 70 to 30 to 45. We were more comfortable with assessments of incidence when 50 or more plants were evaluated in each experimental unit. Possibly, severity of infection could be evaluated in addition to incidence when sample size is less than 50 per plot and improved inoculation procedures produce more uniform infection.

The silk-channel inoculation method may mask some morphological forms of resistance. Kyle (3) associated smut resistance with protection of ears by thick, tight husk leaves. Our procedure would not have detected hybrids with this type of resistance. However, comparisons of reactions to silk-channel inoculation and natural infection might be used to test the value of morphological resistance to *U. maydis*, especially among sweet corn populations that have undergone mass selection for husk protection from ear worms.

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