Evaluation of Methods for Identification of Corn Genotypes with Stalk Rot and Lodging Resistance

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ABSTRACT

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Methods of evaluating corn (*Zea mays*) stalk quality at or near anthesis and stalk rot susceptibility following inoculation were examined for 3 yr to identify those most effective in predicting premature death of plants and stalk lodging in multiple environments. Stalk quality measurements, including rind puncture, rind thickness, stalk push test, pith density or pith moisture, and susceptibility to the stalk rotting fungi *Stenocarpella maydis* and *Colletotrichum graminicola*, were determined on eight single-cross hybrids grown in Urbana, Illinois. Data on premature death of plants and lodging were collected from the same hybrids grown near Urbana in 1984 and at 16 locations throughout the midwestern United States in 1985 and 1986. Hybrid rank for rind puncture at anthesis was more consistently correlated with hybrid rank for lodging and premature death of plants than other stalk quality measurements or rating of stalk rot susceptibility following inoculation. Rind puncture was the most desirable method for evaluating stalk quality because it was simple to use and nondestructive to plants. This is the first study to demonstrate that measurements taken at anthesis or prior to anthesis can predict premature death of plants and lodging of corn hybrids in multiple locations.

Additional keywords: rind penetrometer

Stalk rot and lodging are major problems of corn (*Zea mays* L.), with annual losses estimated at 5–20% (4). Several species of fungi and bacteria cause stalk rot either alone or in combination. These organisms decay pith tissue in lower stalk internodes, which often results in premature death of the plant. As the rotting pith tissue pulls away from the stalk rind, the structural integrity of the stalk changes and the plant may lodge unless the stalk rind is strong enough to support the weight of the upper plant. Stalk rot and lodging vary in severity among years and locations, depending on environmental conditions and susceptibility of corn hybrids (4).

Yield loss due to stalk rot and lodging has directed research toward developing corn hybrids with improved stalk quality. Improvements have been achieved by selecting for both stalk rot and lodging resistance (22). Resistance to stalk rot is related to amount of pith decay following infection by stalk rotting organisms. Lodging resistance is related to the structure and strength of stalk tissue in addition to the susceptibility to stalk rotting organisms.

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Current methods to identify genotypes with lodging and stalk rot resistance are cumbersome. Selection for stalk rot resistance requires inoculation and evaluation of subsequent disease development, both of which are laborious. Selection for lodging resistance and naturally occurring stalk rot requires evaluation of premature death and lodging at harvest, which is best done at multiple locations, may be expensive, and usually is confounded with patterns of natural occurrence. Environmental conditions, which affect the amount of stalk rot and lodging, vary from year to year, further complicating the selection of resistant genotypes. With the widely used methods of selection, superior genotypes cannot be identified until after anthesis, which prohibits more efficient breeding procedures.

Objectives of this study were: 1) to determine the correlation among several methods of measuring stalk quality and susceptibility to stalk rot following inoculation with premature death of plants and lodging from plots throughout the Midwest and 2) to determine if measurements taken at or near anthesis could identify superior genotypes.

MATERIALS AND METHODS

Stalk quality measurements and susceptibility to stalk rot. Stalk quality and stalk rot susceptibility were evaluated on eight single-cross hybrids (Table 1) grown at the Agronomy/Plant Pathology South Farm, Urbana, Illinois. Hybrids were selected on the basis of popularity among commercial growers and a range of stalk rot and lodging resistance. The treatment design was a factorial arrangement of four dates of evaluation and eight hybrids. The experimental design was a split plot of a randomized complete block with dates of evaluation in main plots and hybrids in subplots with four replications. Each experiment unit was eight 5.3-m rows of a hybrid with rows spaced 0.76 m apart and a plant population of 62,500/ha. The two outer rows of each eight-row subplot were used as borders. Planting dates were 8 May 1984, 23 April 1985, and 29 April 1986. Stalk quality measurements were done at 2-wk intervals from 2 wk prior to anthesis until 4 wk after anthesis. The date of each measurement varied among hybrids on the basis of maturity.

Stalk quality measurements included rind puncture, rind thickness, pith moisture, pith density, and the push test. All five measurements were made in each year except for the push test, which was not done in 1984. Pith moisture and pith density were measured on the same plants in a single row. Rind thickness, rind puncture, and the push test were done in single rows. Susceptibility to anthracnose stalk rot caused by *Colletotrichum graminicola* (Ces.) G.W. Wils. and Diplodia stalk rot caused by *Stenocarpella maydis* (Berk.) Sutton (syn. = *Diplodia maydis* (Berk.) Sacc.) was determined in one row each 4 wk after inoculation. Inoculations were done at anthesis and at 2 and 4 wk after anthesis but not at 2 wk prior to anthesis. Twenty, 15, and 10 consecutive plants per row were evaluated in 1984, 1985, and 1986, respectively; three plants at either end of each row were not used.

Rind puncture, rind thickness, and pith moisture and density were measured in the middle of the first elongated internode above the brace roots. Rind puncture (rind strength) was determined with a penetrometer, a modified Chatillon fruit and vegetable tester (Master Gauge Co., Chicago, IL) with a spring-loaded force (kilogram) gauge and a sharp point attached to the shaft. The penetrometer was pressed against stalks to determine the force necessary to puncture the stalk rind. Rind thickness was determined by cutting stalks with a knife, scraping away pith tissue, and measuring the rind thickness with a caliper. The procedures used to determine pith moisture and density were similar to those described by others (15,16). Stalk push tests measured the force (kilograms) necessary to push individual stalks 45° from the vertical with the force gauge, which was modified with a U-shaped attachment on the shaft. The attachment was placed against stalks approximately 0.75 m above the ground. Pressure was applied until stalks bent 45° from the vertical, and the required force was recorded.

Inocula of *C. graminicola* and *S. maydis* were produced from 2-wk-old cultures grown at room temperature with 12 hr of light on oatmeal and potato-dextrose agar, respectively. Conidia were washed from the culture surface with distilled water, and the resulting suspension was adjusted to 2×10^5 conidia per milliliter by dilution with deionized water. Plants were inoculated by injecting 2 ml of the suspension into the first elongated internode with a 50-ml syringe. Disease development was evaluated 4 wk after inoculation by splitting stalks lengthwise and rating pith discoloration. Diplodia stalk rot severity was rated on a scale of 1–5, where 1 = 0–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% of the inoculated internode discolored and 5 = discoloration beyond the inoculated internode (11). Ratings for anthracnose stalk rot were based on the sum of the numbers of internodes with discoloration of 75% or more plus the total number of internodes with discoloration (21). Individual plant ratings for stalk quality measurements and stalk rot susceptibility were averaged for each subplot within each replicate.

Stalk lodging and premature death of plants. In 1984, the eight hybrids were grown at one location near Urbana. In 1985 and 1986, hybrids were grown at 16 locations in Illinois, Indiana, Ohio, and Nebraska (Fig. 1). At each location, hybrids were arranged in a randomized complete block treatment design with four replications. Each replicate contained 20 7.6-m rows spaced 0.76 m apart. Experimental units consisted of two-row plots of each hybrid with two rows of border on each side of a replicate. Replicates were not contiguous but were placed throughout a wide area in each field. All plots were overplanted and thinned to 62,500 plants per hectare.

Stalk lodging, lodging potential, and premature plant death were measured at each location during the last week of September or the first 2 wk of October. Prematurely dead plants were determined by the presence of brown, necrotic stalks that could be crushed between the thumb and forefinger. Stalk lodging was determined by counting the number of stalks that were bent or broken with ears lying on the ground. Lodging potential was estimated by pushing plants 15 cm from the vertical and counting the number of stalks that broke or collapsed. Lodging potential estimated the possibility of lodging if high winds or delayed harvest occurred. All data were converted to percentage of plants per plot.

Data analysis. Analysis of variance was done with SAS software (SAS Institute, Cary, NC). Stalk quality measurements and stalk rot rating following inoculation were analyzed by appropriate procedures for a split-plot arrangement, with dates of sampling as whole plots and hybrids as subplots. Data from the 3 yr were combined after completing homogeneity of variance tests on appropriate error terms.

Stalk lodging, lodging potential, and premature plant death data were analyzed separately for each location using the procedure for a randomized complete block design. Homogeneity of variance was tested for error terms, and data from the locations were combined within and among years.

Spearman's rank correlation coefficients were calculated between hybrid rank for the five stalk quality measurements or the two stalk rot ratings and hybrid rank for percent stalk lodging, lodging potential, and premature death of plants. Correlations were done within years and averaged over years.

RESULTS

Stalk quality measurements and susceptibility to stalk rot. Significant differences in stalk quality measurements and stalk rot ratings were found among hybrids in each year and among hybrids, years, and dates of sampling in the combined analysis. In the combined analysis, the year × hybrid, year × date, and date × hybrid interactions were significant. Individual hybrid stalk quality measurements at anthesis and stalk rot rating following inoculations made 2 wk after anthesis were representative of hybrid ranks at other sampling dates (Table 1). The highest values for rind puncture, rind thickness, and the push test occurred at anthesis (Table 2). Pith moisture and density values were highest 2 wk prior to anthesis and declined at later sampling dates. Stalk rot ratings for anthracnose and Diplodia stalk rot were higher with inoculation at 2 and 4 wk after anthesis than at anthesis.

Stalk lodging and premature death of plants. Stalk lodging, lodging potential, and premature death of plants differed significantly among hybrids each year and among hybrids and years in the combined analysis. In the combined analysis, the year × hybrid interaction was significant. Part of the apparent reason for a significant year × hybrid interaction was the greater severity of stalk lodging and premature death of plants in 1986 than in 1984 or 1985. In general, there was little change in rank of hybrids over years. Premature death of plants among hybrids averaged over all years and locations ranged from 11.6 to 30.4%. Stalk lodging and lodging potential ranged from 1.8 to 10.4% and from 8.2 to 23.7%, respectively (Table 3). The hybrid B73 × C123 had the highest percentage of

Table 1. Hybrid means of five stalk quality measurements and ratings for susceptibility to stalk rot following inoculation with *Colletotrichum graminicola* or *Stenocarpella maydis* at Urbana, Illinois, in 1984, 1985, and 1986

Hybrid	Rind puncture[x] (kg)	Rind thickness[x] (mm)	Push test[y] (kg)	Pith moisture[x] (%)	Pith density[x] (g/cm^3)	Anthracnose stalk rot rating[z]	Diplodia stalk rot rating[z]
B73 × MS71	7.1	0.62	10.4	91.6	0.52	5.0	3.6
B73 × Mo17	7.5	0.64	11.1	91.7	0.64	4.9	3.0
A634 × Mo17	7.4	0.62	10.3	92.6	0.68	5.5	3.2
B73 × LH38	8.2	0.64	10.9	91.2	0.57	5.7	2.9
B73 × LH51	8.3	0.69	13.5	92.0	0.61	5.1	3.2
LH119 × LH51	8.5	0.70	13.0	92.3	0.58	4.9	3.1
FR27 × Mo17	8.3	0.67	11.7	92.4	0.64	4.6	2.8
B73 × C123	6.8	0.64	10.4	92.4	0.66	7.5	2.3
FLSD ($P = 0.05$)	0.6	0.02	1.9	1.1	0.08	0.7	0.5

[x] Means are for a total of 180 plants measured at anthesis from four replicates in 3 yr.
[y] Means are for a total of 100 plants measured at anthesis from four replicates in 2 yr.
[z] Anthracnose stalk rot rating was based on number of discolored internodes plus number >75% discolored (20). Diplodia stalk rot rating was based on percent discoloration of inoculated internode (10). Individual hybrid means are from a total of 180 plants inoculated 2 wk after anthesis from four replicates in 3 yr.

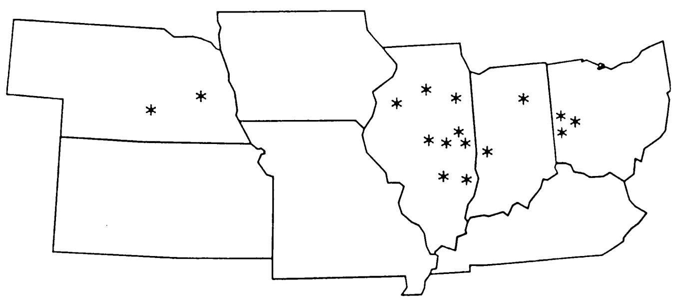

Fig. 1. Sixteen locations where eight corn hybrids were grown in 1985 and 1986 for evaluation of premature plant death, stalk lodging, and potential lodging.

premature death of plants, stalk lodging, and lodging potential throughout the study, whereas LH119 × LH51 had the lowest values for these variables.

Spearman's rank correlation coefficients. Of the five stalk traits measured, rind puncture was most closely associated with stalk lodging, lodging potential, and premature death of plants, with r_s values ranging from −0.37 to −0.97 (Table 4). In general, hybrid ranks for rind puncture at Urbana were correlated with hybrid ranks at other locations each year and combined over years for stalk lodging and lodging potential. Hybrid rank for rind puncture also was significantly correlated with hybrid rank for premature death of plants in 1986 and averaged over all years, but these correlations were not significant in 1984 or 1985. There was some association between hybrid rank for rind thickness at 2 wk prior to anthesis and at anthesis and hybrid rank for stalk lodging and lodging potential, but the relationship was not significant when rind thickness was measured 2 or 4 wk after anthesis. Hybrid rank for the push test was correlated with hybrid rank for stalk lodging, lodging potential, and premature death in 1986 at all four times of measurement; however, the push test did not successfully rank hybrids in 1985. Hybrid rank for pith moisture was significantly correlated with hybrid rank for stalk lodging and lodging potential when taken 4 wk after anthesis in 1984 and 1985; these traits were not correlated in 1986 or averaged over years, however. Hybrid rank for pith density and stalk rot rating following inoculation with either *C. graminicola* or *S. maydis* were not consistently related to hybrid rank for stalk lodging, lodging potential, or premature death of plants.

DISCUSSION

Hybrid rank for rind puncture was more consistently correlated with hybrid rank for stalk lodging, lodging potential, and in some cases premature plant death than the other measures of stalk quality. Hybrid rank for rind thickness or the push test was also correlated with hybrid rank for stalk lodging in some years and dates of measurement. When significant, correlations were negative, indicating that resistance to lodging is associated with thicker and stronger rinds as measured by increased force necessary to puncture or push over stalks, as previously reported (10,12,13,18,23). Evaluation of stalk strength may be an indirect measure of plant health, since strong stalks are associated with healthy, living cells that are more resistant to stalk-rotting fungi (2,7,10). Several advantages make the rind puncture method better than other measurements as concluded by others (1,17,20). First, hybrid rank for rind puncture was fairly consistently correlated with hybrid rank for stalk lodging throughout the study. Second, rind puncture was faster and easier to do, which is extremely important if large numbers of plants are to be evaluated. Third, rind puncture was not destructive to the plants, thus allowing plants to mature and produce seed. Hybrid rank for rind thickness and push tests were also somewhat correlated with hybrid rank for lodging, but these measurements destroyed plants and seed could not be obtained.

Results of this study indicate superior genotypes for stalk lodging may be identified even before anthesis, as collaborated by data from various locations. Hybrid rank based on rind puncture taken at anthesis most consistently identified the hybrids with superior lodging resistance. This agrees with the conclusions of Colbert and Zuber (6), who reported rind puncture readings from hybrids of known stalk quality rather than actual field data. Measuring rind puncture at anthesis would improve a corn breeding program by allowing selection for lodging resistance to be made before pollination. It also could be used to identify the best experimental hybrids for future testing in multiple locations, thus saving time and expense.

The trends in measurements of rind puncture and rind thickness may be related to seasonal trends of stalk strength. Measurements taken at anthesis were generally higher than at other times. After anthesis, the developing ear has priority for carbohydrates produced by the plant even at the expense of other tissue, which begins to senesce (8,9). Senescence of tissue also is responsible for the reduction in rind puncture, rind thickness, pith moisture, and pith density after anthesis. Measurements made at anthesis may be an indication of relative stalk health before it is affected by factors such as kernel number and ear size. The increased variation due to ear size would make it increasingly difficult to accurately identify truly superior genotypes.

To avoid complications from yearly variation in rind puncture measurements, a minimum value could be estab-

Table 2. Means of five stalk quality measurements and ratings for susceptibility to stalk rot combined over eight corn hybrids grown at Urbana, Illinois, in 1984, 1985, and 1986

	Time of rating or inoculation with stalk rot fungi[v]			
Measurement	2 wk Before anthesis	Anthesis	2 wk After anthesis	4 wk After anthesis
Rind puncture (kg)[w]	6.30 b	7.70 a	7.60 a	6.50 b
Rind thickness (mm)[w]	0.62 c	0.66 a	0.65 ab	0.64 bc
Push test (kg)[x]	4.30 b	5.20 a	4.90 ab	4.50 ab
Pith moisture (%)[w]	93.50 a	91.90 b	91.50 bc	91.10 c
Pith density (g/cm^3)[w]	0.62 a	0.61 a	0.59 a	0.49 b
Anthracnose stalk rot[y]	...[z]	4.40 b	5.40 a	5.40 a
Diplodia stalk rot[y]	...[z]	2.70 b	3.0 ab	3.30 a

[v] Means followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's LSD test.
[w] Means are from a total of 1,440 plants measured from four replicates in 3 yr.
[x] Means are from a total of 800 plants measured from four replicates in 2 yr.
[y] Anthracnose stalk rot rating was based on number of discolored internodes plus number >75% discolored (20). Diplodia stalk rot rating was based on percent discoloration of inoculated internode (10). Inoculations were made at anthesis, 2 wk after anthesis, or 4 wk after anthesis and rated 4 wk after inoculation. Sampling period means are from a total of 540 plants inoculated from four replicates in 3 yr.
[z] Inoculations not made.

Table 3. Mean premature death, stalk lodging, and lodging potential of eight corn hybrids grown at Urbana, Illinois, in 1984, 1985, and 1986[w]

Hybrid	Premature death[x]	Stalk lodging[y]	Lodging potential[z]
B73 × MS71	27.7	6.2	21.4
B73 × Mo17	22.1	6.1	17.0
A634 × Mo17	23.9	5.5	18.4
B73 × LH38	11.8	2.5	11.6
B73 × LH51	15.9	2.2	10.1
LH119 × LH51	11.6	1.8	8.2
FR27 × Mo17	14.6	2.8	10.8
B73 × C123	30.4	10.4	23.7
FLSD ($P = 0.05$)	3.5	1.7	3.0

[w] Data are from four replicates of approximately 72 plants from one location in 1984 and from 16 locations in 1985 and in 1986. Data were collected during the last week of September or the first 2 wk of October.
[x] Determined by the presence of brown, necrotic stalks that could be crushed between the thumb and forefinger.
[y] Determined by counting the number of stalks that were bent or broken with ears lying on the ground.
[z] Estimated by pushing plants approximately 15 cm from the vertical and counting the number of stalks that broke or collapsed.

Table 4. Spearman's rank correlations[x] between means of stalk quality measurements and ratings for susceptibility to stalk rot made on four dates in 1984, 1985, and 1986 and premature death of plants on lodging means of eight corn hybrids

		Premature death of plants				Stalk lodging				Lodging potential			
Measurement	**Date[y]**	**1984**	**1985**	**1986**	**Combined**	**1984**	**1985**	**1986**	**Combined**	**1984**	**1985**	**1986**	**Combined**
Rind thickness	A−2wk	−0.40	−0.43	−0.64	−0.55	−0.57	−0.86**	−0.74**	−0.55	−0.59	−0.64	−0.71*	−0.79*
	A	−0.28	−0.16	−0.83**	−0.54	−0.59	−0.79*	−0.86**	−0.73*	−0.60	−0.40	−0.81**	−0.72*
	A+2wk	0.07	−0.11	−0.47	−0.26	−0.33	−0.58	−0.53	−0.52	−0.31	−0.41	−0.49	−0.55
	A+4wk	−0.18	−0.14	−0.16	−0.17	−0.59	−0.71*	−0.18	−0.45	−0.50	−0.43	−0.16	−0.48
Rind puncture	A−2wk	−0.42	−0.37	−0.77*	−0.63	−0.51	−0.87**	−0.83**	−0.67	−0.61	−0.62	−0.79**	−0.83**
	A	−0.67	−0.55	−0.85**	−0.80*	−0.78*	−0.90**	−0.80*	−0.87**	−0.86**	−0.79*	−0.78*	−0.97**
	A+2wk	−0.54	−0.41	−0.81**	−0.73*	−0.63	−0.77*	−0.78*	−0.72*	−0.74*	−0.66	−0.82**	−0.89**
	A+4wk	−0.51	−0.68	−0.78*	−0.79*	−0.67	−0.86*	−0.75*	−0.82**	−0.75*	−0.83**	−0.68	−0.87**
Push test	A−2wk	...[z]	−0.31	−0.79*	−0.36	...	−0.79*	−0.76*	−0.47	...	−0.61	−0.72*	−0.63
	A	...	−0.16	−0.81**	−0.33	...	−0.49	−0.87**	−0.55	...	−0.13	−0.90**	−0.51
	A+2wk	...	0.11	−0.79*	−0.33	...	−0.55	−0.74*	−0.60	...	−0.24	−0.72*	−0.31
	A+4wk	...	0.30	−0.85**	−0.69	...	−0.55	−0.90**	−0.84**	...	−0.59	−0.88**	−0.75*
Pith moisture	A−2wk	−0.12	0.36	0.49	0.36	−0.18	0.28	0.37	0.19	−0.01	0.41	0.29	0.36
	A	0.26	−0.43	0.29	0.61	0.52	−0.19	0.24	0.63	0.40	−0.46	0.15	0.35
	A+2wk	0.03	0.34	−0.10	0.07	−0.17	−0.57	−0.21	−0.38	−0.19	−0.59	−0.24	−0.33
	A+4wk	−0.66	−0.03	−0.52	−0.38	−0.74*	−0.72*	−0.61	−0.52	−0.80*	−0.71*	−0.63	−0.69
Pith density	A−2wk	0.12	0.22	0.26	0.50	0.19	0.45	0.21	0.38	0.10	0.17	0.19	0.12
	A	0.22	−0.14	0.29	0.31	0.32	0.61	0.26	0.29	0.20	0.11	0.21	0.07
	A+2wk	0.05	−0.23	0.21	0.38	0.33	0.50	0.12	0.33	0.19	0.24	0.04	0.19
	A+4wk	0.33	0.08	0.14	−0.07	0.55	0.27	0.24	0.31	0.31	0.25	0.19	0.19
Anthracnose stalk rot	A	0.30	0.61	0.19	0.39	0.17	0.26	0.25	0.28	0.33	0.52	0.26	0.53
	A+2wk	0.60	0.41	0.51	0.60	0.55	0.57	0.40	0.56	0.62	0.56	0.38	0.79*
	A+4wk	0.49	0.55	0.36	0.57	0.44	0.61	0.43	0.43	0.60	0.60	0.45	0.66
Diplodia stalk rot	A	−0.18	0.10	−0.49	−0.37	−0.38	−0.43	−0.46	−0.43	−0.20	−0.25	−0.51	−0.11
	A+2wk	0.37	0.16	−0.22	0.18	0.21	−0.28	−0.27	−0.17	0.31	0.05	−0.17	0.04
	A+4wk	0.62	0.41	−0.38	0.29	0.23	−0.36	−0.45	−0.10	0.39	0.07	−0.51	0.18

[x] * = Significant at $P < 0.05$, ** = significant at $P < 0.01$.

[y] A = anthesis, A−2wk = anthesis minus 2 wk, A+2wk = anthesis plus 2 wk, and A+4wk = anthesis plus 4 wk.

[z] Push test was not done in 1984.

lished each year by measuring standard genotypes whose stalk rot and lodging resistances are unacceptable and acceptable. Plants or genotypes more susceptible than the resistant standard or not different from the susceptible standard could be discarded.

Improvements in stalk strength often are associated with significant changes in other agronomic traits, including grain yield (14,19). Although few studies have been done, selection for increased rind puncture has not been found to adversely affect yield (1,5). If a negative response of yield was found, a selection index including both yield and rind puncture could be used to simultaneously select for both traits.

Stalk rot rating following inoculation with either *C. graminicola* or *S. maydis* was not consistently related to rank of hybrids for stalk lodging, lodging potential, or premature death of plants, although the eight hybrids used in this study have been grown extensively and do not have the range of susceptibility that may be found in hybrid development programs (D. G. White, *personal observation*). With the possible exception of B73 × MS71, all hybrids would be considered acceptable for their level of resistance to *S. maydis*. Only B73 × C123 was extremely susceptible to anthracnose stalk rot, as was indicated by the high rating following inoculation. Surveys have shown *C. graminicola* to be more important than *S. maydis* as a stalk rot pathogen in Illinois (3). *C. graminicola* was likely more prevalent at the various locations, which may account for the high level of premature death of plants and lodging of B73 × C123. Inoculation of hybrids identified B73 × C123 as extremely susceptible to *C. graminicola*. The use of this hybrid in the central corn belt was discontinued because of susceptibility to anthracnose stalk rot. Therefore, the practice of inoculation and rating for stalk rot severity may have some value in identifying extremely susceptible genotypes, but it was not helpful in ranking hybrids in this study, possibly because the narrow range of susceptibility.

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