Effect of Anthracnose Leaf Blight on Stalk Rind Strength and Yield in F_1 Single Crosses in Maize

M. S. ZUBER, Professor of Agronomy, University of Missouri, Columbia 65211; T. C. AINSWORTH, Plant Breeder, Ainsworth Seed Company, Mason City, IL 62664; M. H. BLANCO, Plant Pathologist, O's Gold Seed Company, Farmer City, IL 61842; and L. L. DARRAH, Research Geneticist, AR, SEA, USDA, Columbia

ABSTRACT

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Fifteen F_1 crosses among six maize (*Zea mays*) inbred lines were planted in a replicated split-plot design. Plants in one row of the split plot were inoculated with conidia of *Colletotrichum graminicola* at the six- to eight-leaf stage of development. Control plants in the second row of the split plot were not inoculated. The 15 inoculated F_1 crosses yielded significantly less grain and had more stalk lodging, lower rind puncture values, higher leaf blight ratings, and less grain moisture. Some inbred lines as parents of hybrids contributed high levels of resistance. When inoculated and uninoculated treatments were compared, hybrids from these lines had smaller differences in grain yield than did other hybrids. Several F_1 crosses were highly resistant to the leaf blight phase but had large yield reductions and high percentages of stalk lodging. This result suggests that resistance to leaf blight is affected by a different genetic mechanism than that affecting stalk quality. Rind puncture was a useful technique for identifying genotypes with resistance to the stalk rot phase of the disease, as reflected by differences in rind strength.

Additional key words: rind component

During the past 5 years, anthracnose of maize (*Zea mays* L.) caused by *Colletotrichum graminicola* (Ces.) G. W. Wils. has caused increased damage in the eastern part of the central to southern corn belt (3,8). The pathogen survives from season to season on maize plant residue. Disease development is favored by warm, humid weather and cloudy days, especially during the latter part of the growing season (3,10). Spores are disseminated by splashing rain and wind (3).

Resistant hybrids have been difficult to use because the maize genotypes respond differently to the pathogen when it occurs as leaf blight than when it occurs as stalk rot. The reactions have no apparent direct relationship, which suggests that different mechanisms of resistance are involved for leaf blight and stalk rot (4).

Several researchers have reported different disease reactions and yields among F_1 single crosses. Using artificial inoculation, Smith (8) found significant yield losses that closely parallel the intensity of visible leaf blight. Perkins and Hooker (7) reported dry grain weight losses averaging 9.5% among 36 dent maize hybrids under natural levels of anthracnose stalk rot infection.

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Studies with *Diplodia maydis* (Berk.) Sacc. in Missouri have linked stalk infection with increased lodging and decreased crushing strength values but not with rind thickness and weight of stalk section (1,5). In a later report, which introduced a nondestructive rind puncture method, crushing strength and rind puncture values were closely associated (2).

This study was designed to evaluate rind puncture as a tool for assessing stalk strength among single crosses inoculated with *C. graminicola* and to examine grain loss in relation to stalk rot and stalk rind strength.

MATERIALS AND METHODS

We conducted an experiment on the Ainsworth Seed Farm near Mason City, IL, in 1978. The 15 possible single crosses among six inbred lines were studied. The split-plot design, replicated four times, consisted of adjacent, paired rows of each hybrid; one row was inoculated and the other not inoculated. This experimental design was selected because our major interest was such highly variable traits as yield, stalk strength, and stalk lodging. Treatments were compared within similar microenvironments (split plots) to allow greater precision in tests of statistical significance of subplot effects (9).

A plot consisted of a single row 5.2 m long with 0.76 m between rows. The experiment was machine planted on 1 June. Each plot was overplanted, then thinned to 24 plants for a final population of about 61,500 plants per hectare.

Two isolates of *C. graminicola* (originally recovered from infected stalk tissue in central Illinois in 1977) were used for inoculation. Both isolates were previously tested for pathogenicity and used successfully in stalk rot inoculations. Inoculum was produced by culturing the isolates separately in Erlenmeyer flasks on autoclaved sorghum grains. Each flask was started by transferring an agar block (cultured on oatmeal agar for 2 wk) to the sterile grains. About 21 days later, infected grain was removed from the flasks to dry, and a mixed composite was made from kernels infected with each isolate. Fifteen to 20 infected sorghum grains were placed in the whorl of each plant on 30 June. Rain fell on two successive nights following inoculation.

Table 1. Analysis of variance for F_1 crosses inoculated and not inoculated with *Colletotrichum graminicola*[a]

Source of variation	d.f.	Stand (%)	Lodging (%) Root	Lodging (%) Stalk	Dropped ears (%)	Leaf blight ratings[b]	Rind puncture (kg)	Yield (q/ha)	Grain moisture (%)
Replication	2	0.77	352	40	0.25	0.43	1.96	17	4.28[c]
Hybrids (H)	14	10.53	232	645[d]	16.95[d]	6.33[d]	1.88[d]	593[d]	28.49[d]
Whole-plot error	28	8.21	204	94	5.04	0.27	0.43	91	1.04
Inoculated vs. uninoculated (I)	1	12.35	133	592[c]	15.17	20.54[d]	10.98[d]	2,104[d]	25.06[d]
H × I	14	23.92	73	81	7.19	0.33	0.25	145	0.69[c]
Split-plot error	30	23.15	122	91	4.72	0.21	0.20	123	0.33
CV (%)		5	115	62	128	15	14	15	3

[a] Data for characteristics given as mean squares.
[b] On a five-point scale of increasing severity.
[c] Significant at $P = 0.05$.
[d] Significant at $P = 0.01$.

The humid and somewhat cloudy weather favored disease development.

We recorded a plant as being stalk lodged when the stalk was broken below the ear. Root-lodged plants were those that leaned 30° or more from the vertical. We rated leaf disease subjectively on the basis of extent of necrotic host tissue. Symptoms ranged from small necrotic flecks (rating of 1) to larger, coalesced lesions killing about 20% of the infected leaf area (rating of 5). Individual plants within each plot were rated, and a plot mean was used for the analysis of variance.

We computed averages of F_1 crosses with common parents (designated line means) to determine the contributions of specific inbred lines. For example, the yield line mean of the parent Mo17Ht uninoculated is 83.1 q/ha, which is the average of B73×Mo17Ht, or 91.5; AH30 × Mo17Ht, 87.2; B79 × Mo17Ht, 92.8; AH17 × Mo17Ht, 60.3; and Mo12 ×

Table 2. Effects of inoculation with *Colletotrichum graminicola* on F_1 single crosses of maize

Pedigree	Treatment	Lodging (%) Root	Lodging (%) Stalk	Dropped ears (%)	Leaf blight rating[a]	Rind puncture (kg)	Yield (q/ha)	Grain moisture (%)
B73Ht × Mo17Ht	Uninoculated	6.9	5.6	0.0	3.3	4.0	91.5	18.9
	Inoculated	13.9	9.7	0.0	4.9	3.2	87.1	18.1
	Difference	+7.0	+4.1	0.0	+0.7	−0.8[b]	−4.4	−0.8[b]
AH30[c] × Mo17Ht	Uninoculated	12.4	4.2	1.4	3.3	3.0	87.2	17.8
	Inoculated	8.2	9.7	2.7	4.3	2.6	76.0	16.8
	Difference	−4.2	+5.5	+1.3	+1.0[b]	−0.4[b]	−11.2[b]	−1.0[b]
B79 × Mo17Ht	Uninoculated	1.4	37.5	0.0	3.7	2.9	92.8	17.7
	Inoculated	4.2	35.3	0.0	5.0	2.5	76.1	16.3
	Difference	+2.8	−2.2	0.0	+1.3[b]	−0.4[b]	−16.7[b]	−1.4[b]
AH17[c] × Mo17Ht[d]	Uninoculated	18.1	8.3	6.9	3.0	4.3	60.3	18.3
	Inoculated	13.8	12.4	5.4	3.7	3.6	54.6	16.9
	Difference	−4.3	+4.1	−1.5	+0.7[b]	−0.7[b]	−5.7	−1.4[b]
Mo12 × Mo17Ht	Uninoculated	13.5	16.1	6.5	2.0	4.2	83.9	20.8
	Inoculated	21.8	16.2	0.0	2.0	3.6	66.7	21.6
	Difference	+8.3	+0.1	−6.5[b]	0.0	−0.6[b]	−17.2[b]	+0.8[b]
B73Ht × AH17[c]	Uninoculated	5.6	2.8	1.4	2.7	4.0	95.6	20.5
	Inoculated	1.4	5.7	2.8	3.3	3.4	94.7	18.8
	Difference	−4.2	+2.9	+1.4	+0.6[b]	−0.6[b]	−0.9	−1.7[b]
AH30[c] × AH17[c]	Uninoculated	11.1	5.6	4.2	2.7	4.1	84.8	18.8
	Inoculated	11.2	9.8	1.4	4.0	3.9	76.2	17.9
	Difference	+0.1	+4.2	−2.8[b]	+1.3[b]	−0.2	−8.6	−0.9[b]
B79 × AH17[c]	Uninoculated	4.2	25.0	2.8	3.0	3.5	80.6	20.4
	Inoculated	8.6	39.4	1.4	3.7	2.3	74.2	19.3
	Difference	+4.4	+14.4[b]	−1.4	+0.7[b]	−1.2[b]	−6.4	−1.1[b]
Mo12 × AH17[c]	Uninoculated	17.2	7.0	4.2	1.0	4.5	73.8	22.7
	Inoculated	22.8	12.7	1.4	1.3	4.0	85.2	22.3
	Difference	+5.6	+5.7	−2.8[b]	+0.3	−0.5[b]	+11.4[b]	−0.4
B73Ht × AH30[c,d]	Uninoculated	0.0	4.2	0.0	4.3	3.2	71.7	17.5
	Inoculated	0.0	16.7	0.0	5.0	2.8	52.4	16.3
	Differences	0.0	+12.5[b]	0.0	+0.7[b]	−0.4[b]	−19.3[b]	−1.2[b]
B73Ht × B79	Uninoculated	0.0	19.2	0.0	3.3	2.7	82.0	18.9
	Inoculated	4.1	14.8	0.0	4.7	2.5	78.7	17.7
	Difference	+4.1	−4.4	0.0	+1.4[b]	−0.2	−3.3	−1.2[b]
B73Ht × Mo12	Uninoculated	16.7	2.8	0.0	2.3	4.0	87.1	20.2
	Inoculated	5.6	14.6	1.3	4.0	2.6	60.4	18.3
	Difference	−11.1	+11.8[b]	+1.3	+1.7[b]	−1.4[b]	−26.7[b]	−1.9[b]
AH30[c] × B79	Uninoculated	2.7	21.9	2.7	3.3	3.1	64.7	18.9
	Inoculated	5.3	13.4	0.0	4.7	2.6	65.3	16.9
	Difference	+2.6	−8.5[b]	−2.7	+1.4[b]	−0.5[b]	−1.4	−2.0[b]
B79 × Mo12	Uninoculated	8.3	23.6	1.4	1.0	3.6	83.6	21.1
	Inoculated	15.3	43.1	1.4	2.3	2.2	60.7	20.3
	Difference	+7.0	+19.5[b]	0.0	+1.3[b]	−1.4[b]	−22.9[b]	−0.8[b]
AH30[c] × Mo12	Uninoculated	6.9	8.5	0.0	1.3	4.4	76.7	25.1
	Inoculated	25.4	15.4	1.3	2.7	3.3	64.9	24.1
	Difference	+18.5	+6.9	+1.3	+1.4[b]	−1.1[b]	−11.8[b]	−1.0[b]
	LSD	NS[e]	7.8	1.8	0.4	0.4	9.1	0.5

[a] On a five-point scale of increasing severity.
[b] Significant at $P = 0.05$.
[c] Private line.
[d] Hybrids AH17 × Mo17Ht and B73Ht × AH30 are related.
[e] Differences among means not significant.

Mo17Ht, 83.9. All possible correlation coefficients were calculated to determine the interrelationships among the various characteristics observed.

RESULTS AND DISCUSSION

Analyses of variance (Table 1) showed highly significant differences among F_1 crosses for stalk lodging, dropped ears, leaf blight, rind puncture, grain yield, and grain moisture. With the exception of dropped ears, a significant difference was also found between uninoculated and inoculated treatment means for the same attributes. The only significant difference was found for grain moisture in uninoculated vs. inoculated F_1 hybrids.

Five of the 15 F_1 crosses had significantly more stalk lodging when inoculated than when uninoculated (Table 2). This result indicates that the inoculation that caused leaf blight ultimately increased stalk lodging. We were not able to determine how much of the stalk rot was caused by direct infection by the pathogen and how much by the predisposing influence of leaf tissue necrosis. However, we suspect that stalk rot was enhanced by the combination of direct infection and predisposition by leaf disease.

Root lodging was not significantly affected by either hybrids or treatments. Dropped ear means were significantly different among hybrids but not between treatments. About half of the 15 hybrids had more dropped ears when inoculated than when uninoculated, so we conclude that inoculation with the pathogen did not affect this character.

Thirteen F_1 crosses had significantly higher leaf blight ratings when inoculated than when uninoculated. The two crosses that were not significantly different had low mean ratings for both the uninoculated and inoculated treatments, which indicates high levels of resistance. These crosses were Mo12×Mo17Ht and Mo12×AH17.

Each of the 15 F_1 single crosses had lower rind puncture values when inoculated than uninoculated, and differences were significant for 13. Although stalks were not routinely examined, the most susceptible plants had soft stalks that were often discolored with black, shiny lesions on the rind surface. These symptoms are typical of anthracnose stalk rot in dent maize (3). Several isolations made from infected stalks confirmed colonization by *C. graminicola*, but no attempt was made to identify further or quantify additional stalk rotting organisms.

Grain yield was significantly reduced in seven of the inoculated 15 F_1 crosses. Reductions ranged from a low of 0.9 q/ha for B73Ht × AH17 to a high of 26.7 q/ha for B73Ht × Mo12. Grain moisture levels were significantly reduced in 14 of the 15 crosses by inoculation with *C. graminicola*.

When uninoculated and inoculated treatments are averaged for all 15 F_1 crosses (Table 3), we see that inoculation with *C. graminicola* significantly increased lodging and leaf blight and decreased rind strength, grain yield, and grain moisture.

We also computed the parental line means for each of the six parents by averaging the five F_1 crosses with a common parent. This information is useful in determining the level of resistance conferred by a parental line to its progeny. Hybrids with the inbred line AH17 as the common parent had the lowest yield difference (2.0 q/ha, not significant) between the uninoculated and inoculated treatments, whereas Mo12 had the largest difference (13.4 q/ha) (Table 4). The remaining four parental line means had similar differences, ranging around 10 q/ha.

Hybrids from Mo17Ht had the smallest difference in stalk lodging between uninoculated and inoculated treatments (2.4%, not significant), and those from Mo12 had the greatest difference (8.8%). All six parental line means had more stalk lodging when inoculated than when uninoculated, but only three of the six differences were statistically significant.

The rind puncture value was significantly lower for all means of the inoculated treatments. Hybrids with the common parent AH 30 had the least reduction and those from Mo12 had the greatest.

Leaf blight ratings were the least different in crosses involving AH17 (0.7) and the most different in the inbred line B79 (1.2). In each of the six parental line means, the leaf blight rating was higher and significantly different for the inoculated treatment.

All possible correlation coefficients among the characteristics are presented in Table 5. Although the coefficients were not large enough to be used for prediction, they may serve as indicators if a relationship exists between different characteristics when hybrids are subjected to uninoculated and inoculated treatments. Only the r values that were statistically significant are considered.

Root lodging was positively correlated with dropped ears and rind puncture for the uninoculated treatment and with grain moisture for the inoculated treatment. Root lodging was negatively correlated with leaf blight ratings for

Table 3. Mean comparison of the effects of inoculation with *Colletotrichum graminicola* on maize plants

Treatment	Lodging (%) Root	Lodging (%) Stalk	Dropped ears (%)	Leaf blight rating[a]	Rind puncture (kg)	Yield (q/ha)	Grain moisture (%)
Uninoculated	8.3	12.8	2.1	2.7	3.7	81.2	19.8
Inoculated	10.8	17.9	1.3	3.6	3.0	71.5	18.7
LSD ($P = 0.05$)	NS[b]	4.0	NS	0.2	0.2	4.7	0.2

[a] On a five-point scale of increasing severity.
[b] Not significant.

Table 4. Response of parental lines to inoculation with *Colletotrichum graminicola*

Inbred line	Treatment	Yield (q/ha)	Stalk lodging (%)	Rind puncture (kg)	Leaf blight rating[a]
Mo17Ht	Uninoculated	83.1	14.3	3.68	3.06
	Inoculated	72.1	16.7	3.11	3.78
	Difference	−11.0[b]	+2.4	−0.57[b]	+0.72[b]
B73	Uninoculated	85.6	6.9	3.60	3.18
	Inoculated	74.7	12.3	2.92	4.20
	Difference	−10.9[b]	+5.4[b]	−0.68[b]	+1.02[b]
AH30[c]	Uninoculated	77.4	8.9	3.54	2.98
	Inoculated	67.0	13.0	3.04	4.14
	Difference	−10.4[b]	+4.1	−0.50[b]	+1.16[b]
B79	Uninoculated	81.0	25.4	3.16	2.86
	Inoculated	71.0	29.2	2.43	4.08
	Difference	−10.0[b]	+3.8	−0.73[b]	+1.22[b]
AH17[c]	Uninoculated	79.0	9.7	4.10	2.48
	Inoculated	77.0	15.6	3.44	3.18
	Difference	−2.0	+5.9[b]	−0.66[b]	+0.70[b]
Mo12	Uninoculated	81.0	11.6	4.17	1.52
	Inoculated	67.6	20.4	3.14	2.46
	Difference	−13.4[b]	+ 8.8[b]	−1.03[b]	+0.94[b]

[a] On a five-point scale of increasing severity.
[b] Significant at $P = 0.05$.
[c] Private lines.

Table 5. Correlation coefficients for various characteristics of maize plants inoculated and not inoculated with *Colletotrichum graminicola*

	Root lodging		Stalk lodging		Dropped ears		Leaf blight rating		Rind puncture		Grain yield		Grain moisture	
	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.	Uninoc.	Inoc.
Root lodging	···	···	−0.18	0.09	0.35[b]	0.26	−0.29[a]	−0.41[b]	0.39[b]	0.15	−0.36[b]	−0.02	0.10	0.46[b]
Stalk lodging	···	···	···	···	0.06	0.01	0.08	−0.09	−0.39[b]	−0.37[b]	−0.04	−0.34[a]	−0.05	0.00
Dropped ears	···	···	···	···	···	···	−0.17	−0.08	0.19	0.08	−0.37[b]	−0.25	−0.01	−0.05
Leaf blight rating	···	···	···	···	···	···	···	···	−0.57[b]	−0.39[b]	−0.05	−0.08	−0.80[b]	−0.80[b]
Rind puncture	···	···	···	···	···	···	···	···	···	···	0.04	0.31[a]	0.50[b]	0.27
Grain yield	···	···	···	···	···	···	···	···	···	···	···	···	0.03	0.14
Grain moisture	···	···	···	···	···	···	···	···	···	···	···	···	···	···

[a] Significant at $P = 0.05$.
[b] Significant at $P = 0.01$.

both uninoculated and inoculated treatments. These results suggest that inoculation with *C. graminicola* may indirectly affect root lodging, but the relationship is not strong. Stalk lodging was negatively correlated with rind puncture for both the uninoculated and inoculated treatments. The *r* values were not large, probably because of the rather low incidence of natural stalk breakage. The correlation coefficient was significant and negative between stalk lodging and yield only for the inoculated treatment, suggesting that inoculation had a negative effect on yield through reduced stalk quality. Dropped ears were not directly affected by inoculation. Leaf blight was significantly and negatively correlated with rind puncture values and grain moisture. Rind puncture values were positively correlated with yield under the inoculated treatment and with grain moisture under the uninoculated.

Although clear differences were found between uninoculated and inoculated F_1 crosses in yield, stalk lodging, leaf blight rating, rind puncture, and grain moisture, the differences were probably smaller than might be expected because of natural infection and possible secondary spread of the pathogen from inoculated rows. Differences between uninoculated and inoculated treatments among some F_1 crosses were smaller than others, indicating inherited resistance to the pathogen.

F_1 crosses with the common parent Mo12 generally had low leaf blight ratings, but yield was reduced and stalk lodging increased when inoculated with *C. graminicola*. These results support the contention that leaf blight and reduced stalk quality caused by the pathogen are separate problems and may have different genetic mechanisms (3,4,6).

The significant relationship between leaf blight ratings and rind puncture strength suggests that selection for resistance to the leaf blight phase of anthracnose should enhance rind strength. Research by others has found that the relative destruction of leaf tissue by a pathogen influences, both qualitatively and quantitatively, the synthesis of various enzymes that degrade pith cell walls (6). The anthracnose pathogen has been regarded as unique among the maize stalk rot fungi in its ability to parasitize living tissue (6).

The rind penetrometer has recently been demonstrated as a valuable tool for evaluating inbred lines and hybrids for stalk strength (2). Our results showed that rind puncture values obtained with this instrument should be useful in identifying genotypes that are resistant to the stalk rotting phase of the pathogen. We still do not know whether the rind tissue is directly weakened by the pathogen or indirectly weakened when loss of leaf tissue reduces photosynthate.

Further research is needed into the genetic, biochemical, and parasitic aspects of the anthracnose pathogen as they affect stalk quality in maize.

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