Evaluation of Iowa Stiff Stalk Synthetic for resistance to gray leaf spot

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Abstract
Gray leaf spot (GLS) of maize (Zea mays), caused by Cercospora zeae-maydis, has become an increasing disease problem in the United States. Resistance to this pathogen is generally higher in inbred lines of Lancaster origin compared to lines derived from Iowa Stiff Stalk Synthetic (BSSS). This study was conducted to determine whether recurrent selection for yield had altered the level of GLS resistance in BSSS and to identify BSSS(R)Cl I S1 lines that combine GLS resistance with high yield. The distribution of GLS ratings for S1 lines derived from BSSSCO and BSSS(R)Cl I were very similar, indicating that selection for yield had not altered GLS resistance levels. Although the mean rating for both cycles was a susceptible 7 (1 = resistant, 9 = susceptible), S1 lines with intermediate levels of resistance (4-6) were identified. The 250 BSSS(R)Cl I S1 lines were crossed to LH51, and the testcrosses were evaluated for yield and agronomic performance. S1 lines were identified which combine intermediate levels of GLS resistance with above-average standability and yield. These S1 lines will be recombined to develop an Iowa Stiff Stalk Synthetic population adapted to eastern maize growing conditions.

Keywords
maize breeding

Disciplines
Agricultural Science | Agronomy and Crop Sciences | Plant Breeding and Genetics | Plant Pathology

Comments

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Evaluation of Iowa Stiff Stalk Synthetic for Resistance to Gray Leaf Spot

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ABSTRACT


Gray leaf spot (GLS) of maize (Zea mays), caused by Cercospora zeae-maydis, has become an increasing disease problem in the United States. Resistance to this pathogen is generally higher in inbred lines of Lancaster origin compared to lines derived from Iowa Stiff Stalk Synthetic (BSSS). This study was conducted to determine whether recurrent selection for yield had altered the level of GLS resistance in BSSS and to identify BSSS(R)C11 S1 lines that combine GLS resistance with high yield. The distribution of GLS ratings for S1 lines derived from BSSSC0 and BSSS(R)C11 were very similar, indicating that selection for yield had not altered GLS resistance levels. Although the mean rating for both cycles was a susceptible 7 (1 = resistant, 9 = susceptible), S1 lines with intermediate levels of resistance (4–6) were identified. The 250 BSSS(R)C11 S1 lines were crossed to LH51, and the testcrosses were evaluated for yield and agronomic performance. S1 lines were identified which combine intermediate levels of GLS resistance with above-average standability and yield. These S1 lines will be recombined to develop an Iowa Stiff Stalk Synthetic population adapted to eastern maize growing conditions.

Additional keywords: maize breeding

Gray leaf spot (GLS) of maize, Zea mays L., caused by Cercospora zeae-maydis, has become an increasing problem in the United States (3,6,10). The increased severity and spread of GLS has been primarily associated with no-till, continuous-maize-production practices which provide an overwintering site for the pathogen and a source of early inoculum the following season (3,8). Early infection may result in substantial yield losses and the predisposition of plants to secondary plant pathogens (9,11). The adverse economical and ecological effects of moldboard tillage and commercial fungicides make genetic resistance the best strategy for GLS control. Studies have been conducted to determine the mode of inheritance of genetic resistance. Manh (7) determined, by generation mean analysis, that inheritance of resistance in the inbred Va14 is predominantly quantitative and possibly additive. Thompson et al (13) indicated that additive effects are of major importance and that inbred evaluation per se should provide a good estimate of hybrid performance for GLS resistance. Similar results have also been reported by Elwinger et al (2), Huff et al (5), and Ulrich et al (14).

Although GLS resistance has been reported in some inbreds, until recently very few commercial hybrids or elite inbreds have shown resistance to this pathogen (1,4,11). GLS resistance is generally higher in inbreds of Lancaster origin than in lines developed from Iowa Stiff Stalk Synthetic (BSSS) (14). For this reason, research was initiated to determine whether 1 cycles of recurrent selection for yield in BSSS had altered the susceptibility of this synthetic to GLS. An additional objective was to identify S1 lines of BSSS(R)C11 that combined GLS resistance with adequate yield, standability, and grain moisture.

MATERIALS AND METHODS

One hundred random, self-pollinated ears were harvested from both BSSSC0 and BSSS(R)C11 at Newark, Delaware, in the summer of 1988. In a separate planting of BSSS(R)C11, an additional 1,000 plants were self-pollinated; and the best 250 S1 ears were selected at maturity based on early pollination date, standability, root lodging, and general plant appearance. Two separate experiments were conducted on the above lines in the summer of 1989.

Experiment 1: S1 line evaluation for GLS resistance. In experiment 1, the 450 S1 lines (100 random C0, 100 random

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C11, and 250 selected C11) were evaluated for GLS resistance. Plot size and test sites were as follows: 2.8-m single-row plots at Millersville and Bainbridge in Pennsylvania, and Marion in North Carolina; and 5.8-m single-row plots at Chambersburg, Pennsylvania. Row spacing was approximately 0.75 m at all locations, with all plots being thinned to 60,000 plants per hectare. All four sites had been in continuous corn production for several years prior to experiment I and had histories of GLS development. Reduced tillage practices were used at all sites except Marion, where conventional tillage was employed. Plants at Marion were inoculated at the midwhorl stage with sorghum grains that were colonized with a local isolate of *C. zeae-maydis*. This inoculum was placed in the whorl to initiate disease development. All plots in experiment I were thinned to 1–9 scale, with 1 being the most resistant.

The experimental design for experiment I was an incomplete block with locations serving as individual replications of the experiment. Each location (replication) had 10 sets (blocks), with each set including 10 random BSSSC0 S1 lines, 10 random BSSS(R)C11 lines, 25 selected BSSS(R)C11 lines, and three inbred checks (LH191, LH51, and B73Ht). The composition of the individual sets was constant across locations. The resistant and susceptible inbred checks were used to monitor disease progression but were not included in the analysis.

To determine the variation within the three BSSS groups, degrees of freedom were partitioned into the variation among S1 lines for the three groups. In addition, degrees of freedom were partitioned into two comparisons to determine whether the mean GLS rating for BSSS0 was significantly different from each of the two BSSS(R)C11 groups.

Experiment 2: Evaluation of BSSS(R) C11 select testcrosses for yield and agronomic performance. Experiment 2 evaluated the yield and agronomic performance of the 250 selected BSSS(R) C11 S1 lines in hybrid combination. Hybrids were formed by planting 12 kernels of each S1 ear in an isolation block in Homestead, Florida. All S1 plants were detasseled, with LH51 used as the male tester. In early January 1989, five ears from each S1 row were harvested, shelled, and bulked to form the 250 BSSS(R)C11 testcrosses.

Entries for experiment 2 were planted in the spring of 1989 at four locations: Georgetown, Little Creek, and Odessa in Delaware; and Queenstown, Maryland. All locations utilized two-row plots 5.8 m in length with 0.75-m spacings between rows. Plots were overplanted and thinned to 59,000 plants per hectare at Queenstown, 64,000 plants per hectare at Little Creek, and 69,000 plants per hectare at the remaining two locations. All sites received fertilizer and herbicide applications based on University of Delaware soil-test results and weed-control recommendations. At Georgetown, a solid-set irrigation system was used to ensure a minimum of 1.3 cm of water per week including rainfall. The remaining three sites were not irrigated. All plots were machine harvested and yield adjusted to 15.5% moisture. Data were collected on final stand counts, grain weight, grain moisture, and the number of plants per plot that stalk lodged, root lodged, and dropped ears.

The experimental design for experiment 2 was a 16 × 16 simple lattice with two replications at each location. Each replication consisted of 250 S1 hybrids plus six commercial checks (Agway 788, Agway 838, Hytest 712, Cargill 7877, Cargill 7993, and Pioneer 3343).

**RESULTS AND DISCUSSION**

**Experiment I: S1 line evaluations for GLS resistance.** Environmental conditions for experiment I were 3.8 cm of water per week available for disease development, i.e., high relative humidity, prolonged morning dews, and abundant rainfall. At Millersville, mature lesions were observed in mid-June. Unfortunately, the plants at this location were severely infected by *Bipolaris zeicola* (G.L. Stout) Shoemaker; therefore, this location was not used in the combined analyses. Disease symptoms were observed in mid-July at both Marion and Bainbridge. Plants at Marion were rated on 15 August and 1 September, and at Bainbridge they were rated on 10 August and 6 September. Disease development at Chambersburg was later, with initial disease symptoms observed in mid-August. Only one rating was taken at this location (7 September).

Because of the limited number of locations evaluated for the early rating, only data from the later ratings are presented.

The frequency distributions of second GLS ratings for the three groups of S1 lines were very similar (Fig. 1). The mean rating for BSSS0 was 7.0, which was similar to the BSSS(R)C11 populations, which rated 7.1. The range of ratings for BSSS0 was from an intermediate 4.5 to a susceptible 9.0. The range for the BSSS(R)C11 groups was similar, from an intermediate 5.0 to a susceptible 9.0. Means of LH51, LH191, and B73Ht were 5.3, 5.7, and 8.7, respectively.

The pooled analysis of variance (Table 1) for the GLS disease rating indicated no significant difference due to location. The location by set interaction was highly significant (*P* = 0.001). This occurred because some sets performed differently across locations. Highly significant variation among S1 lines within each set reflects the heterogeneity expected between S0 plants in the BSSS synthetic. Both comparisons, BSSS0 vs. BSSS(R)C11 random and BSSS0 vs. BSSS(R)C11 select, were not significant, indicating that selection for yield in BSSS had not changed GLS resistance.

An additional objective of experiment I was to identify S1 lines from the BSSS(R)C11 groups that had good GLS ratings. Elite inbreds currently available from BSSS generally have low levels of resistance to GLS (14). Several S1 lines were identified that performed as well as or better than the two resistant checks. The mean GLS rating for this group was an intermediate 5.0 compared to a susceptible rating of 7.1 for all BSSS(R)C11 S1 lines. In addition, general field observations indicated that several of these lines had desirable agronomic characteristics, i.e., good stalk quality and

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**Fig. 1.** Distribution of mean gray leaf spot ratings for 100 random BSSS0, 100 random BSSS(R)C11, and 250 select BSSS(R)C11 S1 lines evaluated at three locations, 1989.
ear development, and stay green.

Experiment 2: Evaluation of BSSS(R) C11 select testcrosses for yield and agronomic performance. Experiment 2 measured the yield and agronomic performance of the 250 BSSS(R)C11 select testcrosses. Significant differences for yield, percentage of stalk lodging, and percentage of grain moisture at harvest existed among locations (Tables 2 and 3). Differences in yield and percentage of stalk lodging were primarily a result of conditions at some locations that were not optimal for maize growth, i.e., high rainfall, below-average temperatures, a limited number of cloud-free days during the grain-filling period, and high levels of European corn borer (Ostrinia nubilalis Hübner).

Significant testcross differences were observed for yield, percentage of grain moisture at harvest, and percentage of stalk lodging (Table 3). Significant variation among testcrosses was expected, because each S1 line was derived from a different S0 plant in the heterogeneous BSSS(R)C11 population. The lack of significant testcross differences for dropped ears and percentage of root lodging was because of the low occurrence of these traits during the 1989 growing season. For all traits, the treatment by location interaction was not significant, indicating similar ranking of testcrosses across locations.

Testcross differences resulted in a wide distribution of measurements for yield, percentage of stalk lodging, and percentage of grain moisture at harvest (Table 2). The mean yield of all BSSS(R)C11 hybrids was 7,738 kg/ha, with a range from 6,122 to 9,324 kg/ha. The mean yield of the commercial checks (9,618 kg/ha) was 1,880 kg/ha greater than the BSSS(R)C11 testcross mean. None of the BSSS(R)C11 testcrosses outyielded the commercial checks.

The percentage of stalk lodging for BSSS(R)C11 testcrosses across locations ranged from 10 to 42.5% with a mean of 26.3%. The percentage of stalk lodging for the commercial checks ranged from 6.9 to 15.6% with a mean of 9.9%. The choice of a different inbred tester may improve the stalk-lodging performance of the testcrosses. Although LH51 is a commonly used inbred in hybrid development, other inbred testers may decrease the amount of stalk lodging.

The best 10 S1 (elite group) lines were selected based on independent culling for above-average yield, standability, and GLS resistance obtained from data in experiments 1 and 2. The mean yield for the elite group was 8,439 kg/ha. This was 701 kg/ha greater than the mean yield of all BSSS(R)C11 hybrids, but it was 1,179 kg/ha less than the mean of the six commercial hybrids. The mean percentage of stalk lodging of the elite group was 19.3%, compared to 26.3% for all BSSS(R)C11 testcrosses and 9.9% for the commercial checks. The mean GLS rating for the elite S1 testcrosses was 5.7, with a range from 5.3 to 6.7. The mean for all BSSS(R)C11 S1 testcrosses was 1.4 rating units higher, at 7.1.

These results indicate that variation exists in BSSS(R)C11 for GLS resistance and important agronomic traits. S1 lines which combine intermediate levels of GLS resistance and desirable agronomic traits will be used to initiate a recurrent selection program to adapt the BSSS synthetic to eastern growing conditions.

**Table 1.** Pooled analysis of variance for gray leaf spot ratings at Marion, North Carolina, and Bainbridge and Chambersburg, Pennsylvania, 1989.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>Sets</td>
<td>9</td>
<td>4.63***</td>
</tr>
<tr>
<td>Location x set</td>
<td>18</td>
<td>2.14***</td>
</tr>
<tr>
<td>Entries/set</td>
<td>440</td>
<td>1.70***</td>
</tr>
<tr>
<td>Among BSSS0</td>
<td>90</td>
<td>1.80***</td>
</tr>
<tr>
<td>Among BSSS(R)C11 random</td>
<td>90</td>
<td>1.58***</td>
</tr>
<tr>
<td>Among BSSS(R)C11 select</td>
<td>240</td>
<td>1.58***</td>
</tr>
<tr>
<td>BSSS0 vs. BSSS(R)C11 random</td>
<td>1</td>
<td>0.34</td>
</tr>
<tr>
<td>BSSS0 vs. BSSS(R)C11 select</td>
<td>1</td>
<td>1.82</td>
</tr>
<tr>
<td>Location x entry/set</td>
<td>859</td>
<td>0.65</td>
</tr>
<tr>
<td>Total</td>
<td>1,328</td>
<td></td>
</tr>
</tbody>
</table>

*** = Significant at the 0.001 level.

**Table 2.** Mean yield, percentages of stalk lodging, grain moisture at harvest, and root lodging, and number of dropped ears for 250 BSSS(R)C11 select testcrosses and six commercial checks grown at four locations in 1989.

<table>
<thead>
<tr>
<th>Location</th>
<th>Yield (kg/ha)</th>
<th>Stalk lodging (%)</th>
<th>Grain moisture (%)</th>
<th>Root lodging (%)</th>
<th>Dropped ears (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odessa, DE</td>
<td>6,799</td>
<td>19.2</td>
<td>19.2</td>
<td>2.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Georgetown, DE</td>
<td>7,794</td>
<td>20.3</td>
<td>21.5</td>
<td>6.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Little Creek, DE</td>
<td>7,903</td>
<td>32.9</td>
<td>29.2</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Queenstown, MD</td>
<td>8,630</td>
<td>31.1</td>
<td>23.0</td>
<td>0.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Mean</td>
<td>7,782</td>
<td>25.9</td>
<td>23.2</td>
<td>2.7</td>
<td>0.7</td>
</tr>
<tr>
<td>CV</td>
<td>11</td>
<td>34.9</td>
<td>6.0</td>
<td>137.7</td>
<td>209.3</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>1,028</td>
<td>9.6</td>
<td>1.7</td>
<td>NS*</td>
<td>NS*</td>
</tr>
</tbody>
</table>

*Not significant.

**Table 3.** Pooled analysis of variance across locations for yield, percentages of stalk lodging, grain moisture at harvest, and root lodging, and number of dropped ears for 250 BSSS(R)C11 select testcrosses and six commercial checks.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locations</td>
<td>3</td>
<td>289,973,760***</td>
</tr>
<tr>
<td>Replicates/loc</td>
<td>24</td>
<td>3,899,520***</td>
</tr>
<tr>
<td>Treatments</td>
<td>255</td>
<td>2,903,423***</td>
</tr>
<tr>
<td>Trt. X location</td>
<td>765</td>
<td>1,100,864</td>
</tr>
<tr>
<td>Pooled error</td>
<td>900</td>
<td>852,113</td>
</tr>
<tr>
<td>Total</td>
<td>2,047</td>
<td></td>
</tr>
</tbody>
</table>

*** = Significant at the 0.001 level; ** = significant at the 0.01 level.

**LITERATURE CITED**

Susceptibility of Apple Fruit to *Botryosphaeria dothidea* and Isolate Variation

K. C. PARKER, Former Graduate Research Assistant, and T. B. SUTTON, Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616

**ABSTRACT**


The susceptibility of apple fruit, cultivar Golden Delicious, to *Botryosphaeria dothidea* was investigated by inoculating fruit in the orchard three times during the growing seasons of 2 successive yr. Immature and mature fruit were equally susceptible to *B. dothidea*; both required a 1- to 1.5-mo incubation period before symptom development. The time of infection of apple fruit in the orchard by *B. dothidea* was investigated by sampling apples at three locations throughout the season and monitoring disease development in the laboratory. This test demonstrated that fruit can become infected within 7 wk of petal fall, even though macroscopic symptoms do not occur until later. Variation in isolate aggressiveness was investigated with five isolates of *B. dothidea* collected from infected fruit and limb cankers of trees in four locations in North Carolina. Detached apple fruit were inoculated with the five isolates, and fruit sections from the inoculated regions were removed and examined for disease development over 1 mo. All isolates were pathogenic but varied in aggressiveness.

Infection by *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not. results in fruit rot (white rot or bot rot) and limb cankers on apples (*Malus X domestica* Borkh.) in warm growing regions of the world. The disease accounts for up to 50% fruit loss (11) and extensive limb loss in the southeastern United States. Recently, *B. dothidea* has been associated with limb cankers in the mid-Atlantic apple-growing region of the United States (J. Ryutter, personal communication).

Although fruit rot symptoms often are not noticed until 6-8 wk before harvest (6), results from previous studies differ as to the time of initial infection. Eid (3) presented evidence that initial infections occur soon after the fruit are formed. He determined that fruit infection occurs before late May and that fruit are susceptible throughout the season. Other investigators found that infections are uncommon until the soluble-solids levels reach 10.5% (6). Several mechanisms of resistance of young fruit to rot have been suggested. Sitterly and Shay (9) proposed that low soluble-sugar concentrations provide resistance in immature fruit, and Hwang (5) suggested that concentrations of phenols, reducing sugars, fruit acids, and amino acids, or unspecified physiological or morphological alterations resulting from the interaction of these components, may be involved in the resistance of young fruit. Soluble solids are monitored by growers to determine fruit maturity, and a preventative spray program, based on application of benomyl or thiophanatemethyl, is initiated when the soluble-solids level reaches 10% (1,6).

The morphological and physiological characteristics of individual isolates of *B. dothidea* vary greatly on potato-dextrose agar: mycelium color ranges from white to yellow, gray, or olive (4,5); aerial mycelia may be sparse or abundant; and rates of mycelial growth and sporulation are different. These in vitro differences do not necessarily indicate variation in aggressiveness. If variation is present, however, it could influence the results of research, since controlled studies typically employ a limited number of isolates.

Differences in pathogenicity and virulence among isolates of *B. dothidea* have been reported on apple, but most of the work has been done on the development of stem and trunk cankers rather than on fruit rot (2,3). Latorre (7) studied differences in pathogenicity and virulence (expressed as the ability to rot fruit) among five isolates of *B. dothidea*. All were pathogenic but varied in virulence. Sutton (12) also found that isolates varied in their ability to infect fruit (termed pathogenicity); however, isolates recovered from cankers were generally as pathogenic as those from apple fruit. No significant differences were found among collection locations; differences in disease severity among locations were attributed to environmental conditions rather than isolate variation. In both studies, lesion size was the only measure of variation among isolates.

The purpose of our study was to determine the time of fruit infection by *B. dothidea* and to compare the pathogenicity and aggressiveness of the isolates of *B. dothidea* used in our study.

**MATERIALS AND METHODS**

**Orchard inoculations.** Apple fruit were inoculated in the orchard to determine when the fruit became susceptible. The experiment was initiated each year at the conclusion of the June drop. In 1990, the experiment was conducted at the Mountain Horticultural Crops Research Station (MHCRS), Fletcher, North Carolina. On 18 June, 16 July, and 14 August, 25 fruit on each of six non-sprayed Golden Delicious trees were inoculated. Fruit on three of the trees were not wounded before inoculation; fruit on the other three trees were wounded. The wounds, 1-2 mm deep, were made with a tool made of five no. 1 insect pins pushed through a cork stopper.

Inoculum was prepared from 2-3-wk-old cultures of isolates 1501, 1502, CB1, CB2, SH4, SH6, and RFC of *B. dothidea* that had been grown on potato-dextrose agar under continuous fluorescent light and at room temperature (22 C). Deionized water was poured into each petri dish, and the surface of the mycelial mat was scraped to remove the conidia. The resulting suspensions were mixed and blended for 15 sec, filtered through a double layer of cheesecloth, and the inoculum standardized to 1 X 10^6 conidia per milliliter. The area to be inoculated on each fruit was marked with a wax pencil. Fruit were inoculated by placing in each marked spot a 2.5 X 2.5-cm piece of four-ply laboratory towel that had...