# Aggressiveness of *Gibberella fujikuroi* (*Fusarium moniliforme*) Isolates to Grain Sorghum Under Greenhouse Conditions

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**ABSTRACT**

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Eleven strains of *Gibberella fujikuroi* mating population F and two strains of mating population A were tested for aggressiveness to grain sorghum plants grown under greenhouse conditions. All strains were tested against the cultivar Wheatland, and four strains were tested against Wheatland, SC599, B1778, and BKS45. No significant ($P = 0.05$) differences in aggressiveness were detected. Two cultivars previously described as resistant exhibited shorter main lesions than the susceptible cultivars, but this correlation was not significant when total lesion length was used to measure aggressiveness. *Fusarium* spp. could also be recovered from uninoculated control plants, but fewer than 10% of the isolates recovered from these plants were the same as the isolates used in this study, as measured by identity of vegetative compatibility groups.

Additional keywords: grain mold, *Sorghum bicolor*, stalk rot

Stalk rot of grain sorghum (*Sorghum bicolor* (L.) Moench) is a late-season disease of maturing plants and may be caused by several different fungi, including *Fusarium moniliforme* J. Sheld. (perfect stage *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura), *F. graminearum* Schwabe (perfect stage *G. zeae* (Schwein.) Petch), and *Macrophomina phaseolina* Tassi (Goidanich) (27,28), but during growth in the field plants may be colonized by numerous pathogenic and nonpathogenic fungi, including at least eight different *Fusarium* spp. (18). Stalk rot disease symptoms are seldom manifested during vegetative growth of the host, and the fungi responsible for the disease are often assumed to be latent. Average annual losses attributable to stalk rots in Kansas are estimated at 4%, but losses may approach 50% in some areas with stalk rot incidence of 90–100% in individual fields (7).

*F. moniliforme* is the most common of the three fungi primarily associated with sorghum stalk rot in Kansas. This fungus can be found in maize and sorghum roots, stalks, and seeds throughout the life of the plant and is endemic in cultivated fields worldwide (16,18,23,25,31). Some strains of this fungus produce significant quantities of mycotoxins, such as moniliformin (20), fusarin C (29), fusaric acid (20), and fumonisins (6,19,22), which may play an important role in sorghum grain mold (30) and may reduce sorghum grain quality.

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*F. moniliforme* can be subdivided into at least two different biological species, or *G. fujikuroi* mating populations (15,17). These biological species are separable on the basis of their ability to cross with standard tester strains but not necessarily on their vegetative morphological characters. They also differ in their host preference, with *G. fujikuroi* mating population A predominating on maize and *G. fujikuroi* mating population F predominating on sorghum (19). Our objectives in this study were to determine if there were differences in aggressiveness in the greenhouse among strains of this fungus that represent the predominant vegetative compatibility groups (VCGs) found in Kansas, and to determine if field-identified "susceptible" and "resistant" sorghum cultivars differed in their response to this fungus. Preliminary reports of this work have been previously published (8,9).

## MATERIALS AND METHODS

**Strains.** Isolates of *G. fujikuroi* used in this study are listed in Table 1. These isolates were selected so that each of the predominant VCGs in *G. fujikuroi* mating population F from Kansas (12) was represented. In addition, two isolates of *G. fujikuroi* mating population A (10,17,24) were also used. All isolates were heterokaryon self-compatible (4).

**Toothpick inoculum preparation.** Isolates of *G. fujikuroi* were grown on minimal medium (3) slants for 7 days. Round wooden toothpicks about 6 cm long were boiled in distilled water for 1 hr to remove any toxic substances that might inhibit fungal growth. The water was decanted and discarded, and the procedure was repeated three more times. Dry toothpicks were packed into glass jars (25 × 60 mm), and 5 ml of potato-dextrose broth (Difco) were added to thoroughly moisten the toothpicks. The jars were autoclaved, cooled, inoculated with pieces of agar cultures, and incubated at 20 C for 10 days under 12-hr periods of alternating light and dark.

**Pathogenicity tests with mature plants.** Grain sorghum seeds were treated with hot water according to the method developed by Daniels (5) for maize to remove any naturally occurring *Fusarium* that may have been in or on the seed. Absence of *Fusarium* spp. propagules in treated seeds was verified by plating a representative sample of each seed lot on potato-dextrose agar (Difco). Plants were grown in 25.4-cm plastic pots (one plant per pot) containing Sunshine Mix No. 1 (Fisons Horticulture Inc., Vancouver, BC). A 20-20-20 (NPK) soluble fertilizer was applied biweekly. Plants were inoculated approximately 2 wk after full bloom by using an ice pick to create a hole in the peduncle midway between the base of the inflorescence and the flag leaf. A toothpick was then inserted into the hole.

**Table 1.** Strains of *Gibberella fujikuroi* (*Fusarium moniliforme*) tested for aggressiveness to grain sorghum plants grown under greenhouse conditions

| Isolate VCG[y] | Location | Host material[z] |
|---|---|---|
| A-00102 A-1 | San Joaquin Co., CA | S |
| A-00149 A-7 | Visalia, CA | MSt |
| F-00728 F-11 | Powhattan, KS | SSt |
| F-00921 F-6 | Chase, KS | SSt |
| F-01046 F-5 | Wabaunsee, KS | MSt |
| F-01154 F-8 | Atlanta, KS | SSt |
| F-01155 F-9 | Atlanta, KS | SSt |
| F-01163 F-7 | Latimer, KS | SSt |
| F-01183 F-2 | Moundridge, KS | SSt |
| F-01244 F-10 | Green, KS | SSe |
| F-01292 F-1 | Linn, KS | SSe |
| F-01321 F-3 | Linn, KS | SSt |
| F-01383 F-4 | Wakeeney, KS | SSe |

[y] Vegetative compatibility group.
[z] S = sorghum, MSt = maize stalk, SSt = sorghum stalk, SSe = sorghum seed.

Inoculated plants were arranged on a greenhouse bench in a completely randomized design using five replications per treatment. Incubation conditions in the greenhouse varied somewhat depending upon season. Supplemental lighting was used to provide a 14-hr light and 10-hr dark cycle. Temperature control followed the same cycle and alternated between approximately 30 C light and 18 C dark.

Eighteen days after inoculation, the peduncle was split longitudinally, and stem decay was measured. Two measurements, main and total lesion length, were taken. Main lesion length was the distance on either side of the inoculation point where the discoloration extended across the entire width of the pith tissue. Total lesion length was the distance on either side of the inoculation point at which discoloration could be observed. Analysis of variance for main and total lesion lengths was performed after a square root transformation of the data. The experiment was conducted twice.

Following analysis of the first two experiments, two each of the *G. fujikuroi* mating population F isolates that demonstrated the greatest and least aggressiveness were selected for use in evaluating cultivar resistance. Inoculum and plant preparation, and inoculation procedures for testing cultivar response to these four isolates were the same as in previous experiments. Cultivars of grain sorghum used were SC599 and B1778 (resistant to *F. moniliforme*), and Wheatland and BKS45 (susceptible to *F. moniliforme*) (2). Inoculated plants for each experiment were placed on greenhouse benches in a completely randomized design with five replications per treatment. Greenhouse conditions were the same as in the previous experiment. Stalks were split and lesions measured 18 days after inoculation as in the previous experiments. Analysis of variance for a factorial design was performed after a square root transformation of main and total lesion lengths. Sorghum cultivar and fungal isolate were used as variables. The experiment was conducted twice. All analyses were done with the MSTAT-C (Michigan State University, E. Lansing) statistical program.

**Recovery of *Fusarium* isolates from infested stalks.** Tissue samples, 2 $mm^3$, were taken from the pith of each split stalk in one test each of isolate aggressiveness and cultivar resistance. Samples were taken from the end of each lesion, as denoted by red discoloration of the tissue, and from each additional internode if the discolored area spanned more than two nodes. If only two samples were taken, then they were taken as far from the site of the toothpick inoculation as possible. Tissue blocks were placed on a peptone-pentachloronitrobenzene (PCNB) medium (21) and incubated for 7–10 days at 25 C. Only one fungal isolate was taken per tissue sample, unless sectors with different morphologies were observed, in which case a sample from each sector was taken. Small mass transfers, 1-$mm^3$ agar blocks, were made from the peptone-PCNB medium to a complete medium (3) and maintained on these slants at 4 C until the analyses of the derivative nitrate-nonutilizing (*nit*) sectors (see below) were complete.

***Nit* mutants and tests for vegetative compatibility.** For each of the 13 strains, two complementary NitM mutants were generated on minimal medium + 1.5% $KClO_3$ to serve as testers (3,11). These "standard" NitM testers were purified by isolating a uninucleate microconidium via micromanipulation (11). *Nit* mutants in isolates from stalk tissue were derived by culturing these isolates on potato-dextrose agar + 1.5% $KClO_3$, a procedure that is known to give an excess of *nit1* mutants (3). Chlorate-resistant nitrate-utilizing (*crn*) sectors (14) resulting from this process were discarded, but no *nit* mutants obtained on this medium were phenotyped further.

For vegetative compatibility tests, the "standard" NitM mutants from the strain inoculated into the plant were paired with *nit* mutants from all isolates derived from that plant using the multiwell plate technique of Klittich and Leslie (13). In cases where both pairings with both of the standard testers were negative, the pairing test was repeated once.

## RESULTS

**Isolate aggressiveness.** Results of the two experiments measuring isolate aggressiveness were similar but not identical (Table 2). Those isolates considered to be most aggressive in the first run of the experiment tended to remain in that group (F-01046 and F-01163) and likewise for those considered to be least aggressive (A-00149, F-00728, and F-01183). The greatest variability occurred with isolates that fell toward the middle. Significant differences in total lesion length were observed at $P = 0.10$, but not at $P = 0.05$. Extensive variability was observed for both main and total lesion lengths. Main and total lesion lengths were significantly correlated ($P = 0.01$), with $r = 0.84$ for experiment 1 and $r = 0.60$ for experiment 2.

**Cultivar resistance.** As a result of the previous experiments, the two sorghum strains that were considered the most aggressive (F-01155 and F-01163) and two that were the least aggressive (F-00728 and F-01183) were selected to screen four lines of inbred sorghum. In this screening (Tables 3 and 4), there was no significant interaction between isolates and cultivars. As with the experiments on isolate aggressiveness, extensive variability was observed for both main and total lesion lengths, but they were significantly ($P = 0.01$) correlated with $r = 0.71$ for experiment 1 and $r = 0.45$ for experiment 2. There were no significant ($P = 0.05$) differences between fungal isolates in aggressiveness in either main or total lesion length, but all caused significantly longer lesions than observed in the uninoculated control (Table 3).

Among cultivars, patterns were more difficult to discern. Two cultivars—SC599 and B1778—were previously described as resistant to *F. moniliforme*, and two other cultivars—Wheatland and BKS45—had been described as susceptible to the pathogen (2). If the main lesion was used as the measure of resistance, then resistant and susceptible cultivars could be separated whenever the mean measurement was used. Using this criterion in experiment 1, however, B1778 would be scored as susceptible. If total lesion length is used as the measure of aggressiveness, then B1778 and possibly Wheatland would be scored as resistant, and BKS45 and SC599 would be scored as susceptible. These differences reflect primarily the differences observed in experiment 2, since there was no significant difference between cultivars in experiment 1. Thus, B1778 was consistently resistant and BKS45 was consistently susceptible by

**Table 2.** Greenhouse test of isolate aggressiveness of 13 strains of *Gibberella fujikuroi* (*Fusarium moniliforme*) to *Sorghum bicolor* cv. Wheatland

| | Main lesion length (cm) | | | Total lesion length (cm) | | |
|---|---|---|---|---|---|---|
| Isolate | Experiment 1[y] | Experiment 2 | Mean | Experiment 1 | Experiment 2 | Mean |
| No fungus | 8.4 c[z] | 0.3 d | 4.4 b | 18.6 a | 1.2 d | 9.9 b |
| A-00102 | 30.7 abc | 4.4 c | 17.6 a | 38.1 a | 8.2 c | 23.1 a |
| A-00149 | 7.7 c | 5.0 bc | 6.3 ab | 24.7 a | 9.4 bc | 17.0 a |
| F-00728 | 8.6 c | 7.3 a | 8.0 a | 18.2 a | 13.7 abc | 15.9 a |
| F-00921 | 26.7 abc | 7.2 ab | 16.9 a | 40.6 a | 11.8 abc | 26.2 a |
| F-01046 | 35.4 ab | 6.9 ab | 21.2 a | 40.6 a | 18.1 a | 29.4 a |
| F-01154 | 28.4 abc | 6.9 ab | 17.6 a | 30.4 a | 10.3 abc | 20.3 a |
| F-01155 | 27.3 abc | 8.0 a | 17.7 a | 38.4 a | 11.6 abc | 25.0 a |
| F-01163 | 41.7 a | 7.0 ab | 24.3 a | 53.9 a | 9.6 bc | 31.7 a |
| F-01183 | 10.6 bc | 5.3 abc | 8.0 a | 27.6 a | 13.3 abc | 20.4 a |
| F-01244 | 23.1 abc | 6.2 abc | 14.7 a | 39.6 a | 12.0 abc | 25.8 a |
| F-01292 | 22.6 abc | 7.1 a | 14.8 a | 35.8 a | 15.3 ab | 25.5 a |
| F-01321 | 25.2 abc | 7.1 ab | 16.1 a | 38.9 a | 12.5 abc | 25.7 a |
| F-01383 | 20.5 abc | 7.8 a | 14.2 a | 29.2 a | 15.1 ab | 22.2 a |

[y] One plant per replicate, with five replications in each experiment.
[z] Values followed by a common letter are not significantly different ($P = 0.05$).

both measures in our screening, but the resistant–susceptible status of the SC599 and Wheatland cultivars is not clear.

**Recovery of infesting isolate.** In one of each of the two replicate experiments, we reisolated *Fusarium* spp. from infested tissue to determine if the isolates recovered from the discolored area were identical to the strain we used to infest the tissue. We recovered *Fusarium* spp. from all plants in these experiments, including the uninoculated controls. We found recovery rates of inoculated isolates ranging from 10 to 100% (Table 5). In only three instances (F-00728/Wheatland II, F-01163/SC599, and F-01244/Wheatland I) were fewer than 50% of the recovered isolates identical to the inoculated isolate, whereas in 16 of 29 cases, more than 80% of the recovered isolates were the same as the inoculated isolate. Of the 45 isolates recovered from uninoculated control plants, three isolates from different plants were F-00728, but none of the remaining 42 isolates was the same as any other isolate used in this test.

## DISCUSSION

Breeding for resistance to Fusarium stalk rot has been a relatively slow and difficult process (1,2). Part of the problem may be attributed to the relatively recent recognition that there are two biological species within *F. moniliforme* (12,15,17). In all previous breeding work, it is now unknown whether maize isolates (*G. fujikuroi* mating population A), have been used to screen maize and, likewise, whether sorghum isolates (*G. fujikuroi* mating population F) have been used to screen sorghum for resistance. The differential distribution of the members of these two mating populations on maize and sorghum (19) suggests that there may indeed be significant differences in at least host preference. Within the *G. fujikuroi* mating population F isolates from Kansas, 11 VCGs account for approximately 75% of the examined isolates (12). We wanted to determine whether there were differences in aggressiveness among isolates from different VCGs. If differences exist, then selection of the proper strain in an artificial inoculation situation is mandatory to accurately measure resistance.

Our results revealed no detectable differences in aggressiveness among the common VCGs of *G. fujikuroi* mating population F from Kansas toward the sorghum cultivars that we examined. At first this finding was surprising; however, in retrospect, the VCGs identified may be the most common because they are the most aggressive. To find differences in aggressiveness, it may be necessary to examine and compare isolates from less frequent VCGs with their more frequently occurring counterparts or examine a more genetically diverse set of sorghum cultivars. On the other hand, the relatively low amount of genetic vari-

**Table 3.** Greenhouse test of isolate aggressiveness of four strains of *Gibberella fujikuroi* (*Fusarium moniliforme*) to four cultivars[x] of *Sorghum bicolor*

| | Main lesion length (cm) | | | Total lesion length (cm) | | |
|---|---|---|---|---|---|---|
| Isolate | Experiment 1[y] | Experiment 2 | Mean | Experiment 1 | Experiment 2 | Mean |
| No fungus | 1.5 b[z] | 3.6 b | 2.6 b | 3.7 b | 6.4 b | 5.1 b |
| F-00728 | 6.9 a | 7.3 a | 7.1 a | 13.3 a | 27.2 a | 20.3 a |
| F-01155 | 5.9 a | 6.4 a | 6.2 a | 14.6 a | 28.0 a | 21.3 a |
| F-01163 | 7.0 a | 6.1 a | 6.6 a | 17.7 a | 27.5 a | 22.6 a |
| F-01183 | 7.0 a | 6.6 a | 6.8 a | 12.9 a | 25.1 a | 19.0 a |

[x] Wheatland and BKS45 (susceptible to *F. moniliforme*), and SC599 and B1778 (resistant to *F. moniliforme*) (2).
[y] One plant per replicate, with five replications per cultivar in each experiment.
[z] Values followed by a common letter are not significantly different ($P = 0.05$).

**Table 4.** Greenhouse test of cultivar resistance of four cultivars of *Sorghum bicolor* to four strains[v] of *Gibberella fujikuroi* (*Fusarium moniliforme*)

| | Main lesion length (cm) | | | Total lesion length (cm) | | |
|---|---|---|---|---|---|---|
| Cultivar | Experiment 1[w] | Experiment 2 | Mean | Experiment 1 | Experiment 2 | Mean |
| Wheatland[x] | 5.8 a[y] | 7.6 a | 6.7 a | 12.6 a | 19.4 b | 16.1 ab |
| BKS45[x] | 6.2 a | 8.0 a | 7.1 a | 12.7 a | 28.6 a | 20.7 a |
| B1778[z] | 6.4 a | 3.5 b | 5.0 b | 12.0 a | 16.7 b | 14.5 b |
| SC599[z] | 4.2 b | 4.9 b | 4.6 b | 12.4 a | 26.6 a | 19.5 a |

[v] F-00728, F-0115, F-01163, and F-01183.
[w] One plant per replicate, with five replications per cultivar in each experiment.
[x] Susceptible to Fusarium stalk rot (2).
[y] Values followed by a common letter are not significantly different ($P = 0.05$).
[z] Resistant to Fusarium stalk rot (2).

**Table 5.** Recovery of *Gibberella fujikuroi* strains from infected sorghum cultivars in the greenhouse

| Inoculated strain | Cultivar | Number of plants | Number of fungal isolates | Isolates identical to inoculated isolate (%) |
|---|---|---|---|---|
| No fungus | Wheatland I | 5 | 10 | —[x] |
| | Wheatland II | 5 | 10 | —[x] |
| | SC599 | 5 | 6 | —[y] |
| | BKS45 | 5 | 10 | —[x] |
| | B1778 | 5 | 9 | —[z] |
| A-00102 | Wheatland I | 5 | 13 | 69 |
| A-00149 | Wheatland I | 5 | 11 | 91 |
| F-00728 | Wheatland I | 5 | 11 | 82 |
| | Wheatland II | 5 | 17 | 47 |
| | SC599 | 5 | 13 | 77 |
| | BKS45 | 5 | 14 | 64 |
| | B1778 | 5 | 10 | 90 |
| F-00921 | Wheatland I | 5 | 10 | 90 |
| F-01046 | Wheatland I | 5 | 11 | 91 |
| F-01154 | Wheatland I | 5 | 12 | 83 |
| F-01155 | Wheatland I | 5 | 10 | 80 |
| | Wheatland II | 5 | 13 | 69 |
| | SC599 | 5 | 10 | 100 |
| | BKS45 | 5 | 10 | 90 |
| | B1778 | 5 | 12 | 83 |
| F-01163 | Wheatland I | 5 | 11 | 91 |
| | Wheatland II | 5 | 12 | 83 |
| | SC599 | 5 | 15 | 33 |
| | BKS45 | 5 | 12 | 67 |
| | B1778 | 4 | 10 | 70 |
| F-01183 | Wheatland I | 5 | 11 | 73 |
| | Wheatland II | 5 | 11 | 73 |
| | SC599 | 5 | 10 | 100 |
| | BKS45 | 5 | 10 | 70 |
| | B1778 | 5 | 13 | 77 |
| F-01244 | Wheatland I | 5 | 10 | 10 |
| F-01292 | Wheatland I | 5 | 10 | 90 |
| F-01321 | Wheatland I | 5 | 10 | 80 |
| F-01383 | Wheatland I | 5 | 10 | 100 |

[x] No isolate recovered from the control plants was the same as any isolate used in this study.
[y] Two isolates recovered from two different control plants were F-00728, but no other isolate was the same as any isolate used in this study.
[z] One isolate recovered was F-00728, but no other isolate was the same as any isolate used in this study.

ability in *G. fujikuroi* mating population F as measured by the number of VCGs (12), combined with a relative scarcity of female-fertile strains (15), may limit the amount of genetic exchange within this group and prevent the synthesis through meiosis of strains that would be more aggressive than their counterparts.

With main lesion length as a measure of resistance, the four cultivars were segregated into resistant and susceptible as previously described by Bramel-Cox et al. (2). The use of total lesion length gave a more confusing picture. B1778 would still be resistant, and BKS45 would still be susceptible; however, SC599 would now be susceptible, and Wheatland could be classified as either resistant or susceptible. In the absence of a cultivar by isolate interaction in the analysis, cultivar resistance appears to be general across the species rather than strain- (and presumably VCG-) specific.

Based on this study, main lesion length seems to be a more appropriate measure of aggressiveness than total lesion length because of the reduction in variability. We observed that much of the variability in measuring total lesion length occurred when the fungus entered the vascular system, where it quickly moved away from the point of inoculation. Mechanically, the main lesion was also easier to identify and measure, because it was not necessary to follow the infestation along the length of the stalk in one or a few vascular bundles. We also observed differences in mean lesion lengths between the two repetitions of the isolate aggressiveness and the cultivar resistance experiments. Lesion lengths were correlated with the time of year when the experiments were conducted. In both instances, both the main and total lesion lengths were shorter when the plants were grown under cooler greenhouse conditions.

Our tests were conducted with plants that were not significantly drought-stressed during the experiment. Trimboli and Burgess (26) have previously found that under greenhouse conditions grain sorghum plants grown in soil infested with *F. moniliforme* showed symptoms of greater basal stalk rot and root rot when plants were subjected to moisture stress between flowering and mid-dough stages. It is possible that if stressed plants had been used, then differences between resistant and susceptible cultivars or aggressive and nonaggressive fungal isolates might have been easier to discern.

A confounding problem that appears to be very difficult to control is that of plant infections with strains of *Fusarium* other than the targeted ones. According to results with our uninoculated controls, less than 10% (3 of 45) of the isolates were the same as those used to inoculate the test plants. Similar "contamination" problems were reported by Trimboli and Burgess (26). Controls for this problem would seem to be imperative for this type of study under both field and greenhouse conditions. Possible anomalous results attributable to this type of contamination are probably a major concern. In our case, the fungal inoculum leading to this contamination cannot be attributed to the strains used in our study, which implies that the major source of the contamination observed is from other sources, such as airborne or insect-borne spores brought into the greenhouse from other locations.

In summary, we found no significant differences in aggressiveness among the common Kansas VCGs of *G. fujikuroi* mating population F. However, the aggressiveness of less common VCG isolates should be evaluated before concluding definitively that aggressiveness to sorghum is a property of the mating population rather than of a few widespread clones. We believe that reliable estimates of *Fusarium* resistance and susceptibility among sorghum cultivars can be made under greenhouse conditions. Precautions will need to be taken to minimize cross-contamination with external *Fusarium* strains, however, and more discriminatory results might be obtained by using drought-stressed plants. Finally, the pathogenicity of *G. fujikuroi* mating population F isolates to maize and mating population A isolates to sorghum need to be evaluated to determine the importance of strain selection for use in breeding programs.


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