Screening Wheat Lines as Seedlings for Resistance to *Cephalosporium gramineum*

S. L. VAN WERT, Graduate Research Assistant, A. V. RAVENSCROFT, Technician, and D. W. FULBRIGHT, Associate Professor, Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824-1312

ABSTRACT

Van Wert, S. L., Ravenscroft, A. V., and Fulbright, D. W. 1984. Screening wheat lines as seedlings for resistance to *Cephalosporium gramineum*. Plant Disease 68:1036-1038.

Seedlings of winter wheat lines with known field disease reactions to *Cephalosporium gramineum* were assayed in controlled-environment chambers for resistance to strains of *C. gramineum*. This procedure differentiated between highly susceptible and highly resistant lines but was less effective for discriminating between lines with intermediate reactions. This assay may be useful for rapid selection of *C. gramineum*-resistant winter wheat germ plasm before field trials.

Cephalosporium leaf stripe, caused by *Cephalosporium gramineum*, is the only known vascular disease of wheat (2). Field resistance and tolerance in *Triticum aestivum* L. have been observed in only a few breeding lines. Mathre et al (6) observed variations in yield components among wheat lines in field inoculations. Morton and Mathre (7) suggested that intercrossing wheat lines showing tolerance to *C. gramineum* may give rise to lines showing resistance to the pathogen and that monitoring kernel weight and seed number per head in field plots could be an effective means of identifying resistance in *C. gramineum*-infected plants. Their field plot studies identified wheat lines with three distinct types of resistance to the pathogen: 1) reduced number of diseased plants in a population, 2) reduced number of diseased tillers within a plant, or 3) reduced severity and rate of disease symptom development within a plant (8).

This study reports a method to rapidly screen wheat lines in controlled-environment chambers for resistance to *C. gramineum* by seedling inoculation. Wheat lines with known field reactions to *C. gramineum* were inoculated to test the procedure. Isolates of *C. gramineum* with varying degrees of virulence were used as inoculum to observe a range of disease symptoms in each line. Portions of this work have been published (11).

MATERIALS AND METHODS

C. gramineum **isolates.** Cultures (Table 1) were grown and maintained on potato-dextrose agar plus 100 μg of streptomycin per milliliter (PDA + S) or grown in potato broth (PB) to produce inoculum (10). Wild-type isolates had a mycelial growth habit and mutant isolates had a yeastlike growth habit on PDA + S (3,11).

Plants. Wheat lines provided by D. E. Mathre (Montana State University, Bozeman) and the New York cultivar Yorkstar were hand-planted in short 30-cm rows in a wheat field on the East Lansing campus of Michigan State University that was known to contain high populations of *C. gramineum*. These lines, and their natural field-disease reactions obtained by randomly counting the striping in 100 tillers, are listed in Table 2.

Seedling assay. Wheat seedlings were grown for 10 days in autoclaved sand in a controlled-environment chamber with a 14-hr photoperiod (9×10^4 ergs/cm^2 · sec) and a temperature regime of 21 C. Pots were fertilized after 3 and 9 days with a solution of Rapid Gro (25% N, 19% P, 17% K; 15 ml/4.5 L). After 10 days, seedlings were removed and all but 2.5 cm of the root was cut and washed free of sand particles. Conidial suspensions were prepared by growing the wild-type and mutant isolates of *C. gramineum* in 50 ml of PB for 6 days on a reciprocal shaker, 96 strokes per min, at 24–25 C. Six seedlings were placed in each conidial suspension (10^7–10^9 conidia per milliliter, depending on the isolate) for 15 min, then transferred to a pot containing autoclaved soil. The remaining conidial suspension was poured into the holes in which seedlings were planted. Pots were placed in a controlled-environment chamber with a 15-hr photoperiod (9×10^4 ergs/cm^2 · sec) at 17 C and were fertilized as described previously, 7 days after inoculation. Symptoms were rated after 24 days. If no symptoms appeared, the plants were kept in the chamber for another week to make certain no symptoms were produced.

The experiment was a factorial design with wheat lines and isolates as factors. The isolate factor had two levels (nonautoclaved and autoclaved shake cultures). A total of 15 wheat lines and seven isolates, including a PB-only

Table 1. *Cephalosporium gramineum* strains used in this study

Isolate	Virulence on Yorkstar[a]	Source[b]
CG-82	Very high	Michigan field isolate
M-13	Very high	Michigan field isolate
CG-18	Moderate	UV mutant of M-13
N20	High	NTG mutant of CG-18
E53	Low	EMS mutant of CG-18
E67	Very low	EMS mutant of CG-18

[a] Yorkstar is a soft white winter wheat commonly grown in Michigan.
[b] UV = ultraviolet light-induced (3), NTG = *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced (11), and EMS = ethyl-methanesulfonate-induced (11).

Table 2. Wheat lines and their disease reactions to natural populations of *Cephalosporium gramineum* in a Michigan field plot[a]

Wheat line	Disease reaction[b]	Source[c]
Highly resistant		
Agrotritichum[d]	0	Seed Research Inc., Kansas
CI 07638	4	Russia
CI 11222	5	England
Moderately resistant		
UT 89099	10	Utah
PI 178383	13	Turkey
F6-870	14	Montana
Lenore	14	Idaho
Moderately susceptible		
PI 278212	21	Czechoslovakia
Marias	28	Montana
MT 77077	28	Montana
Highly susceptible		
LRC 40	30	Montana
Lancer	34	Montana
Yorkstar	41	New York
PI 094424	42	Russia
PI 347738	43	Japan

[a] Disease reactions for the 1980–1981 growing season in a Michigan State University field plot.
[b] Percentage of tillers with striping symptoms at growth stage 5 on the Feekes' scale (4).
[c] D. Mathre, *personal communication.*
[d] Cross between *Agropyron elongatum* and an unknown hard red winter wheat (D. Mathre, *personal communication*).

Michigan Agricultural Experiment Station Journal Article 11347.

Accepted for publication 8 August 1984 (submitted for electronic processing).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked *"advertisement"* in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

control, were used. The screening procedure for the seedlings was performed twice.

Symptoms were rated visually using a scale of 1–15 (Table 3) for each individual plant and transformed to the disease severity index by multiplying by 6.66. Each plant was considered a replicate, making a total of six replicates per treatment. The following arbitrary disease severity index was used: 0–25 = no disease or very mild, 26–45 = mild, 46–60 = moderate, 61–85 = severe, and 86–100 = very severe.

RESULTS

Field disease reactions indicated that in a nonreplicated test, three wheat lines, Agrotritichum, CI 07638, and CI 11222, were highly resistant to *C. gramineum* (Table 2). These lines also had the greatest resistance to the various isolates used in the seedling assay (Fig. 1). Agrotritichum was symptomless in field tests but was not immune in the seedling assay (Fig. 1). The wheat lines Yorkstar, Marias, PI 347738, MT 77077, and LRC 40 were highly susceptible in both the field tests (Table 2) and in the seedling assay (Figs. 1 and 2). Both PI 094424 and Lancer, which were highly susceptible under Michigan field conditions (Table 2), had resistance to wild-type and mutant isolates of *C. gramineum* except M-13 and CG-82, respectively. The remaining wheat lines, F6-870, PI 278212, PI 178383, UT 89099, and Lenore had intermediate levels of resistance to wild-type and mutant isolates of *C. gramineum* in the seedling assay although their field disease reaction varied from intermediate to susceptible.

The high disease severity caused by isolates E53 and E67 on wheat line LRC 40 and by E53 on PI 178383 and PI 094424 was due to severe disease ratings (11–15) in one of the tests, which raised the disease severity rating averaged from the two replicates. These isolates were usually low in virulence and the reason for high disease severity observed with these isolates is not known.

DISCUSSION

Inoculations of wheat seedlings with wild-type and mutant isolates of *C. gramineum* resulted in differential reactions among wheat lines. Although inoculation to determine disease resistance to *C. gramineum* in winter wheat has been used before (5,6), its use has been limited to vernalized winter wheat seedlings in the field. The use of nonvernalized winter wheat plants for fungal inoculation has been discouraged because it was assumed that nonvernalized plants provided an atypical environment for the pathogen (9). Spring cultivars of wheat are susceptible to the pathogen but may not become infected because their roots are not freeze-stressed (1) or because entry points may be absent since spring plantings escape root breakage caused by frost heaving (13).

Mathre et al (6) contend that lower inoculum levels of *C. gramineum* differentiate best between resistant and susceptible varieties because these levels approximate those encountered in the field (10^3 conidia per milliliter). Use of high inoculum levels (10^7–10^9 conidia per milliliter) did not appear to interfere with evaluation of resistance in this study. The differences in disease severity produced by the two wild-type isolates CG-82 and M-13 on Agrotritichum, PI 178383, PI 278212, PI 094424, and Lancer suggests that races of *C. gramineum* may exist.

The screening procedure described in this paper differentiated well between the field disease reactions of highly resistant and highly susceptible wheat lines, but that differentiation was questionable for wheat lines showing intermediate resistance. The seedling assay ratings for F6-870 and PI 278212, wheat lines of intermediate resistance, are in agreement with field disease reactions. However,

Table 3. Symptom rating system used in the seedling assay to evaluate disease severity in winter wheat plants inoculated with *Cephalosporium gramineum*

Rating	Symptoms
1	No symptoms
2	Faint, general chlorosis
3	First or second leaf chlorotic
4	First and second leaves chlorotic
5	First, second, or third leaf striping
6	First or second leaf chlorotic and striping
7	First and second leaves striping
8	First leaf chlorotic, second leaf striping; or first leaf striping, second leaf chlorotic
9	First leaf chlorotic and striping, second leaf chlorotic or striping; or first leaf chlorotic or striping, second leaf chlorotic and striping
10	First and second leaf chlorotic and striping
11	First leaf dead
12	First leaf dead, second leaf chlorotic
13	First leaf dead, second leaf striping
14	Entire plant nearly dead, stem green
15	Entire plant dead, no green tissue

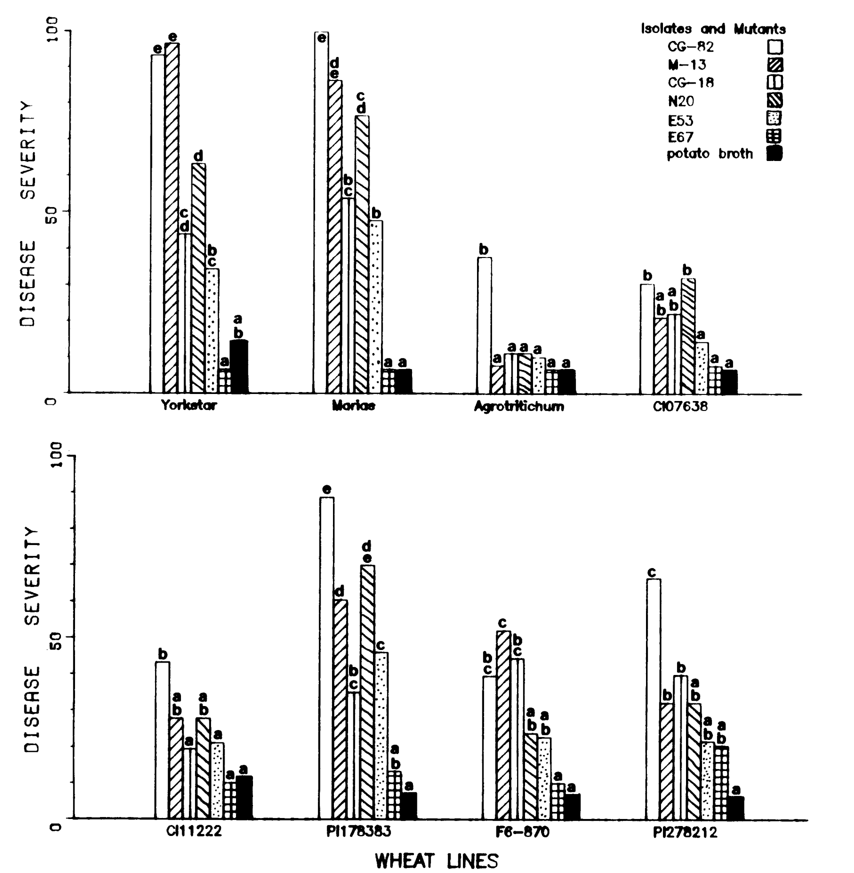

Fig. 1. Disease severity of winter wheat lines inoculated with various isolates of *Cephalosporium gramineum*. Cultivars Yorkstar and Marias were used as susceptible controls. Isolates with the same letter do not differ significantly ($P = 0.05$) within a wheat line according to the LSD test.

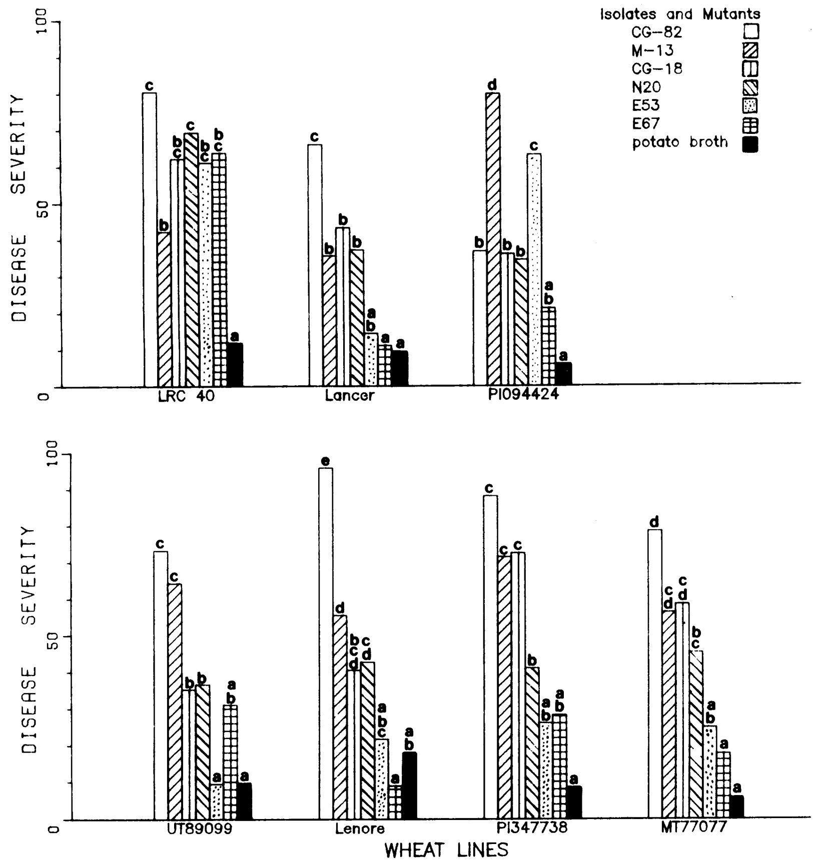

Fig. 2. Disease severity of winter wheat lines inoculated as seedlings with various isolates of *Cephalosporium gramineum*. Isolates with the same letter do not differ significantly ($P = 0.05$) within a wheat line according to the LSD test.

differential evaluation of UT 89099 and PI 178383 in seedling assays and field trials demonstrated that the seedling assay may eliminate lines with intermediate field resistance before field trials. The wheat lines PI 094424 and Lancer showed little resistance to certain strains of the pathogen in the seedling assay and appeared to be susceptible in field tests. These lines, however, showed some resistance to all the other strains tested in the seedling assay. This demonstrates the necessity of using more than one isolate of the pathogen.

Seedling assay may serve as a rapid means of identifying wheat germ plasm with intermediate and high resistance to *C. gramineum*. Such wheat lines should undergo further evaluation in field plots. For both screening processes, *C. gramineum* isolates of different but known virulence should be included, because plant lines may differ in disease reactions to various isolates.

ACKNOWLEDGMENTS

This work was supported by USDA Grant 59-2261-1-1-644-0. We thank D. Mathre for wheat lines and Marianne La Haine for typing the manuscript.

LITERATURE CITED

1. Bailey, J. E., Lockwood, J. L., and Wiese, M. V. 1982. Infection of wheat by *Cephalosporium gramineum* as influenced by freezing of roots. Phytopathology 72:1324-1328.
2. Bruehl, G. W. 1957. Cephalosporium stripe disease of wheat. Phytopathology 47:641-649.
3. Fulbright, D. W., and Ravenscroft, A. V. 1981. Mutants of *Cephalosporium gramineum*. (Abstr.) Phytopathology 71:217.
4. Large, E. C. 1954. Growth stages in cereals. Illustration of the Feekes scale. Plant Pathol. 3:128-129.
5. Mathre, D. E., and Johnston, R. H. 1975. Cephalosporium stripe of winter wheat: Procedures for determining host response. Crop Sci. 15:591-594.
6. Mathre, D. E., Johnston, R. H., and McGuire, C. F. 1977. Cephalosporium stripe of winter wheat: Pathogen virulence, sources of resistance, and effect on grain quality. Phytopathology 67:1142-1148.
7. Morton, J. B., and Mathre, D. E. 1980. Physiological effects of *Cephalosporium gramineum* on growth and yield of winter wheat cultivars. Phytopathology 70:807-811.
8. Morton, J. B., and Mathre, D. E. 1980. Identification of resistance to Cephalosporium stripe in winter wheat. Phytopathology 70:812-817.
9. Morton, J. B., Mathre, D. E., and Johnston, R. H. 1980. Relation between foliar symptoms and systemic advance of *Cephalosporium gramineum* during winter wheat development. Phytopathology 70:802-807.
10. Tuite, J. 1969. Plant Pathological Methods. Fungi and Bacteria. Burgess Publishing Co., Minneapolis, MN.
11. Van Wert, S. L. 1983. Virulence mutants of *Cephalosporium gramineum*. M.S. thesis. Michigan State University, East Lansing.
12. Van Wert, S. L., and Fulbright, D. W. 1983. Bioassays to evaluate wheat lines for resistance to Cephalosporium leaf stripe. (Abstr.) Phytopathology 73:844.
13. Wiese, M. V. 1977. Compendium of Wheat Diseases. American Phytopathological Society, St. Paul, MN.