Quantitative Disease Assessment of Wheat Seedling Leaves Inoculated with *Fusarium roseum* 'Culmorum'

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ABSTRACT

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Wheat seedling leaves were wounded and inoculated with agar disks containing microconidia of *Fusarium roseum* 'Culmorum.' Seedlings were grown in a growth chamber under moisture stress conditions of −5 or −10 bars for 3 wk before inoculation. Relative comparisons between cultivar pairs were made by measuring lesion length 1 wk after inoculation. There were no differences in lesion size among seedlings of the same cultivar grown under the two different soil matric potentials. Significant lesion size differences occurred between cultivars in all paired comparisons when seedlings were grown under moisture stress and lesions were measured 7 days after inoculation. This quantitative method of measuring disease reaction between wheat cultivars may be useful in identifying general resistance (horizontal resistance sensu Vanderplank). Identification of general resistance could significantly aid breeders in selecting for increased resistance to Fusarium foot or root rot of wheat.

Foot and root rots of cereals commonly occur throughout the major wheat growing areas of the world (1). Environmental stress (cold, dry winters and/or low summer moisture) enhances the disease and increases yield losses (1,4,6). *Fusarium roseum* Lk. ex Fr. 'Culmorum' [*F. culmorum* (W. G. Smith) Sacc.] is one of the major foot and root rot causal agents in the Pacific Northwest (2,4) and western Nebraska (6). Other fusaria and *Cochliobolus sativus* [(Ito and Kurib) Dreschsl. ex Dastur] may induce the disease, either alone or in a complex (2,7,9,11), and the importance of these causal agents may vary from region to region. Fenster et al (6) described foot and root rot as ". . . an insidious, persistent, and inconspicuous disease that each year reduced wheat yields to some degree." The disease, in some instances, can be extremely destructive, resulting in considerable localized losses (2,6).

Control of foot and root rot through genetic resistance has generally not been effective. No vertical resistance (sensu Vanderplank) against any of the causal agents has been identified (1), although susceptibility among some cultivars is variable. General resistance (horizontal resistance sensu Vanderplank) against some of the causal agents has been reported (8,15), but difficulties in identifying and manipulating this resistance persist. An accurate quantitative disease measurement method sensitive enough to detect small resistance differences has not been developed. Such a method would provide the means to identify, select, and breed for increased resistance to foot and root pathogens.

Cook and Papendick (5) demonstrated that moisture stress is important in the interaction of wheat and *F. roseum* 'Culmorum.' Previous studies (12,13) indicated differences in lesion size among cultivars when wheat seedlings were grown under moisture stress and wounded stems were inoculated with *F. roseum* 'Culmorum.' With the stem-wound method it was difficult to keep the inoculum in contact with the wound and many plants were required to detect statistically significant differences among cultivars. The leaf wound method alleviates these problems.

The purpose of this study was to determine if inoculations in leaf wounds with *F. roseum* 'Culmorum' of wheat seedlings grown under moisture stress would reveal cultivar differences in disease expression (lesion size).

MATERIALS AND METHODS

Fort Collins clay loam (10) was air-dried 3 days, sifted through a 4-mm mesh screen, and added at the rate of 508 g per 15-cm-high × 11-cm-diameter plastic pot. Matric potentials of −5 or −10 bars were established in the soil by adding water. The amount of water needed to establish the matric potentials was determined from the matric potential versus soil moisture curve for Fort Collins clay loam (10). The matric potential was maintained by weighing the pots every 2 days and adding the amount of water needed to reestablish the original weight. Six wheat (*Triticum aestivum* L.) seeds were sown in each pot. Each pot was covered with a clear plastic bag to enhance seed germination. Pots were placed in a Percival plant growth chamber (Percival Refrigeration and Manufacturing Co., Boone, IA 50036) programmed for 21 ± 1 C and 16 hr of both fluorescent and incandescent light (~5,000 lx) each day. After 5 days, the plastic bags were removed and the seedlings were thinned to four plants per pot. The plants were inoculated when they were ~3 wk old.

A culture of *F. roseum* 'Culmorum' (supplied by R. J. Cook, USDA, ARS, Pullman, WA) was maintained on potato-dextrose agar (PDA) by single-spore transfers. Off types were discarded after each transfer. The original culture was preserved in sterile soil at 3–4 C and periodically cultured for comparison with the cultures in use. Inoculum was prepared by calibrating a suspension to contain 10^4 macroconidia per milliliter of distilled water and adding 1 ml of this spore suspension to 20 ml of warm, melted (50 C), sterilized water agar in petri dishes. Disks were cut from the solidified agar medium with a 5.5-mm-diameter sterilized cork borer. Agar disks, each containing ~35 conidia, were used to inoculate seedlings. A sterilized dissecting needle was used to puncture the leaf and an agar disk was secured over the wound with cellophane tape. Two leaves per plant were inoculated for a total of eight inoculations per pot. Infection was enhanced by individually covering the pots with plastic to maintain high humidity, and the pots were returned to the growth chamber. The plastic was removed after 5 days, and the lesion length was measured 2 days later. No infection occurred in 10–30% of the inoculations, and there was only a very small necrotic area surrounding the wounds. *F. roseum* 'Culmorum' routinely could be isolated from surface-disinfested lesions cultured on PDA.

Each randomized experiment was designed to compare two cultivars and consisted of: 10 pots of cultivar 1 at −5 bars; 10 pots of cultivar 1 at −10 bars; 10 pots of cultivar 2 at −5 bars; and 10 pots of cultivar 2 at −10 bars. There were four experiments comparing cultivars Scout and Baca, and one experiment each comparing

TABLE 1. Lesion size comparison 7 days after inoculation within nine separate experiments, each consisting of two wheat seedling cultivars whose primary leaves were inoculated with *Fusarium roseum* 'Culmorum'

Comparison	Cultivar	Mean lesion length (mm)		Cultivar	Mean lesion length (mm)	F ratio
1	Scout	16.6	vs	Baca	20.6	61.09*[a]
2	Scout	19.5	vs	Baca	25.8	86.99*
3	Scout	25.4	vs	Baca	29.2	39.81*
4	Scout	20.3	vs	Baca	23.5	5.58*
5[b]	Scout	17.1	vs	Baca	17.6	0.22
6	Vona	24.1	vs	Newton	26.7	17.10*
7	Scout	20.6	vs	Calvin	28.7	154.65*
8	Baca	19.7	vs	Calvin	28.2	125.94*
9	Vic	22.3	vs	Calvin	26.5	27.03*

[a] Asterisks indicate statistical significance, $P = 0.05$.
[b] No moisture stress.

cultivars Scout and Calvin, Newton and Vona, Baca and Calvin, and Vic and Calvin. Lesions were measured 5, 6, and 7 days after inoculation in one of the Scout and Baca comparisons. A separate experiment was done to compare Scout and Baca under −5 bars and under no water stress (ample moisture). The data for each experiment were analyzed with a two-way analysis of variance (14).

RESULTS

The first experiment compared Scout and Baca under matric potentials of −5 and −10, and the analysis of variance of these data revealed highly significant differences. Differences between cultivars were evident, whereas differences within the same cultivar under the two matric potentials were slight. To determine where significant differences existed, each combination of the cultivar and matric potential factors was analyzed by a one-way analysis of variance. There were no significant differences between identical cultivars under different matric potentials, but all differences between cultivars were significant. The disease measurements of each cultivar under the different matric potentials were pooled and the comparison was made between cultivars. Matric potential also had little effect on disease rating in all the other experiments. Therefore, the lesion size measurements of both matric potentials for each cultivar were pooled, and these comparisons were analyzed for each experiment. The analyzed data are presented in Table 1.

Significant differences between cultivars in lesion size were found at 7 but not at 5 and 6 days after inoculation. No significant lesion size differences between Scout and Baca were found when seedlings were grown under adequate moisture. The lesion sizes of both cultivars were significantly larger when seedlings were grown under moisture stress and the difference between cultivars was significant.

DISCUSSION

Results of previous studies (12,13) demonstrated that *F. roseum* 'Culmorum,' in similar stem-wound inoculation experiments, produced larger lesions under −5 and −10 than under −1/3 and −1 bars. These lower matric potentials (−5 and −10 bars) were used in this leaf-wound study because more disease (larger lesions) was consistently observed under these conditions. No significant lesion size differences between cultivars occurred when plants were grown with ample moisture. The lesions on the nonstressed plants were significantly smaller than on the moisture stressed plants. This is consistent with results from inoculations in stem wounds (12,13) and Cook and Papendick's (5) observations that moisture stress enhances infection and disease severity caused in cereals by foot and root rotting *Fusarium* species.

Lesion size was recorded 5, 6, and 7 days after inoculation during one experiment with cultivars Scout and Baca. The 5- and 6-day comparisons revealed no significant differences. This indicates significant lesion size differences may be expressed only at least 7 days after inoculation. Lesion size variation between experiments on the same cultivars was observed, which demonstrates the necessity of making cultivar comparisons only within and not among experiments.

Cook (3) reported that wheat plants can sustain mild root and culm rot due to infection by *F. roseum* 'Culmorum,' but without moisture stress no severe foot rot occurred. Physical factors, such as depth of rooting, leaf area, and number of stomata per unit area may affect the rate of soil water use. These factors would help determine the relative moisture stress a cultivar sustains under various environmental conditions. Wheat cultivars having the ability to maintain high leaf water potentials may sustain the least Fusarium foot rot (3). Variation in field "resistance" may only be due to variation of stress factors, or an interaction of these factors with resistance to the pathogen. For these reasons, resistance identified by the leaf inoculation method may or may not correlate with field resistance. Additional studies are needed to determine if this resistance identified in the laboratory is effective in the field and can be correlated with yield loss.

Calvin and Vic are durum wheat cultivars reported to be susceptible and resistant, respectively, to foot and root rot in North Dakota field tests (J. S. Quick, *personal communication*). Foot and root rot losses in North Dakota may be due more to *C. sativus* than *Fusarium* species, although *Fusarium* species are involved in the disease complex (15). The lesion size comparisons of cultivar Calvin and Vic correlated with their reported field reactions. This does not necessarily indicate that resistance to *C. sativus* and *F. roseum* 'Culmorum' are under the same genetic control. *Fusarium* species, or specifically *F. roseum* 'Culmorum,' may be more involved in the North Dakota disease complex than previously thought.

Cultivar variation affecting expression of disease caused by *F. roseum* 'Culmorum' was demonstrated in the laboratory with a leaf inoculation method and moisture stress conditions. The small cultivar differences that were observed may be due to resistance mechanisms under quantitative genetic control. Moisture stress conditions simulate natural water stress in the field and are necessary for the expression of cultivar differences in lesion size. This method is less expensive and faster than tests in the field. Another advantage is that the plant survives and can be grown to maturity as a seed source. The leaf inoculation method may be adaptable to breeding programs since it would enable workers to identify and select for increased resistance to Fusarium foot and root rot causal agents. Such a quantitative method of measuring resistance would be extremely helpful in developing commercial cultivars with increased resistance to Fusarium foot and root rot of wheat.

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