Cephalosporium Stripe of Winter Wheat: Pathogen Virulence, Sources of Resistance, and Effect on Grain Quality

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ABSTRACT

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The virulence of 25 isolates of *Cephalosporium gramineum* from various areas in North America was determined on winter wheat. Most isolates caused more than a 40% yield reduction while a few isolates caused little yield reduction. Increased virulence was reflected in adverse effects on grain and flour quality; i.e., test weight, flour yield, and physical dough properties. However, these effects were not great enough to significantly affect loaf volume plus grain and texture. Over 1,000 red winter wheats from the U.S. Department of Agriculture World Collection and other sources were screened for resistance to *C. gramineum* in the field using oat kernel inoculum. In terms of the least reduction in yield, kernel weight, and kernels per head, when infected by highly virulent isolates, the four best lines were P. I. 278212, C. I. 11222, and Crest line row components 40 and 51.

The stripe disease of winter wheat (*Triticum aestivum* L.), which is caused by the fungus *Cephalosporium gramineum* Nisikado and Ikata, can reduce yields by up to 50% (8, 18). Some degree of control can be achieved by the use of cultural practices such as delayed seeding (11, 16, 17), stubble destruction by burning or deep plowing (16), and rotation with spring-sown crops (11). Since these methods are not always practical, the development of resistant cultivars is the most desirable control measure and would allow growers to make optimum use of their land.

In contrast to the numerous examples of resistance to wheat foliage pathogens, resistance to a variety of root- and crown-infecting fungi pathogenic to wheat has been located and used with only limited success. Bruehl et al. (4) screened most of the U.S. Department of Agriculture (USDA) World Collection of winter wheat for resistance to the snow mold fungi. A few lines showed moderate resistance and P. I. 181268 was used as the resistant parent in the development of the snow mold resistant cultivar, Sprague. Resistance to *Cercosporella herpotrichoides* Fron has been found in some northwestern European wheat types (3), and is characterized by reduced lesion size and decreased amount of lodging. In Europe, Cappelle-Desprez has been used for over 20 yr as a source of resistance to *C. herpotrichoides* (20). The resistance of Cappelle-Desprez, however, is only of an intermediate type so attempts have been made recently to use a higher level of resistance from *Aegilops ventricosa* Tausch (5). Resistance to common root rot of spring wheat, caused primarily by *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur, has been detected (10) and found to be a heritable character (19). Resistance may be governed by a major recessive gene, plus one or two minor genes (14). When over 7,500 spring wheat lines from various geographic areas were tested, no well-defined area was an obvious source of superior resistance (7).

Variation in susceptibility to Cephalosporium stripe has been noted by workers in Japan (21) and North America (2, 16). Such observations usually have been made following natural infection where inoculum density and pathogen virulence were unknown, or where uneven freezing and thawing of soil in the plots may have led to escapes due to inconsistent root damage. Wounds are believed to be necessary for infection. Recently, the addition of oat kernel inoculum to the row at the time of seeding has provided a tool for screening many wheat lines for resistance under field conditions (12). This technique was used in this study to determine: (i) the virulence of isolates of *C. gramineum* from various geographical areas, (ii) sources of resistance among winter wheats, and (iii) the effect of this disease on grain and flour quality.

MATERIALS AND METHODS

Isolates of *C. gramineum* were obtained from plant pathologists in Indiana, Kansas, Michigan, New York, Washington, and Alberta, Canada. Many isolates from Montana were tested, including one from *Bromus tectorum* L. growing in a severely infected wheat field. Cultures were maintained by mass transfer on potato-dextrose agar (PDA). Oat kernel inoculum of each isolate was prepared by growing it on autoclaved oat kernels and placing it in the furrow with the seed at planting as described (12). All isolates grew in a minimum of 95% of the oat kernels as determined by surface sterilization (0.5% NaOCl) and placement on PDA prior to use. Field tests were conducted at the Agronomy Field Research Laboratory near Bozeman, Montana.

Seed from the USDA World Collection of winter wheat was obtained from the Small Grain Collections, USDA, ARS, PGGI, Beltsville, MD 20705. Other lines or cultivars tested were from G. A. Taylor, Montana winter wheat breeder, or were available commercially.

Disease severity was scored first when the plants were elongating just prior to heading on a scale of 0 to 3 with 0 = no symptoms to 3 = numerous tillers with symptoms. White head readings were taken 3 wk after heading on a scale of 0 to 5 with 0 = no white heads, 1 = 1-20% white heads, 2 = 20-40% white heads, 3 = 40-60% white heads, 4 = 60-80% white heads, and 5 = 80-100% white heads.

Grain and flour quality characteristics were determined, including flour yield, flour ash, farinograph absorption, farinograph peak time, stability time, and volume, grain and flour protein content, the amount of water absorbed by the dough for optimum baking, the optimum dough mixing time, loaf volume, and grain and texture characteristics of the baked bread using standard procedures (1, 15).

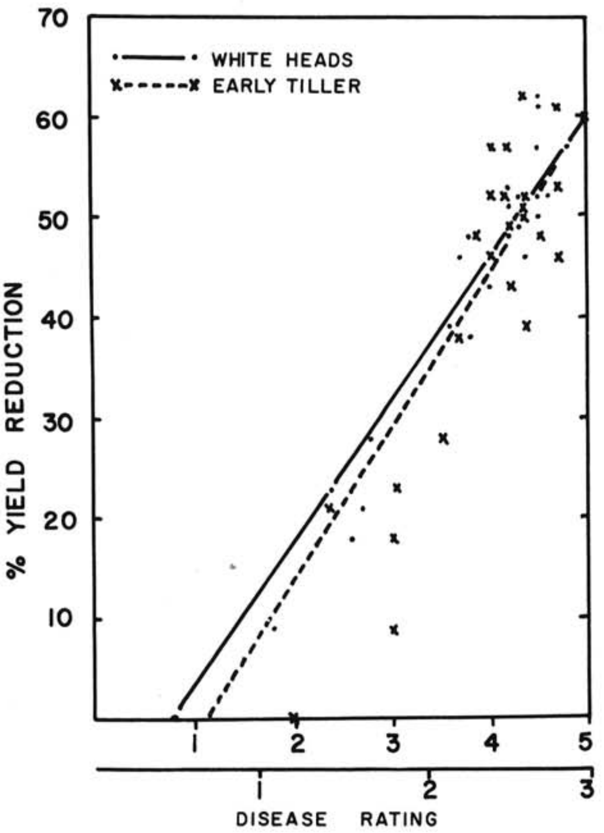

Fig. 1. Virulence level of 25 isolates of *Cephalosporium gramineum* to four lines of winter wheat (McCall, Winalta, Marias, P. I. 178383). Yield reduction averaged across the four wheat lines was plotted against the two different disease readings. Early tiller readings were taken just prior to heading and were rated on a scale of 0 to 3 with 0 = no symptoms to 3 = numerous tillers with symptoms. White head readings were taken 3 wk after heading on a scale of 0 to 5 with 0 = no white heads, 1 = 1-20% white heads, 2 = 20-40%, 3 = 40-60%, 4 = 60-80%, and 5 = 80-100%.

RESULTS

Virulence.—The virulence of 25 isolates of *C. gramineum* was determined in the field in the 1974-1975 growing season using four wheats [Marias (=C. I. 17595), Winalta (=C. I. 13670), McCall (=C. I. 13842), and P. I. 178383] that differed in susceptibility based on a previous test (12). The wheat lines were main plots in a split-plot design replicated three times. The subplots, which represented the different isolates, were 3.3 m long and consisted of three rows spaced 36 cm apart with the center row receiving the inoculum. Yield reductions were calculated using data from the inoculated row and an adjacent noninoculated row.

Most of the isolates were highly virulent with over half of them causing yield reductions of 40% or more using data averaged across the four wheat lines (Fig. 1). The highly virulent isolates originated from Indiana, Kansas, Michigan, Montana, Washington, and Alberta, Canada, and included the isolate from *Bromus tectorum*. The isolate (no. 5) used in a previous study (12) was intermediate in virulence, reducing yields by 28% across the four wheat lines. However, it caused a 47% yield reduction in the most susceptible wheat, Marias. A few isolates from Montana and New York were weakly virulent.

There was a fairly good agreement between yield reduction and the two methods of disease scoring (Fig. 1). The correlation was $r = 0.62$ for early disease readings vs. yield reduction and $r = 0.51$ for white head readings vs. yield reduction; both values were significant at $P = 0.01$.

Sources of resistance.—For the 1974-1975 growing season, lines of red winter wheat from a wide diversity of geographical areas were selected from the USDA's World Collection. These included 570 from Asia, 102 from southern Europe, 224 from northern Europe, and 105 from other areas. They were planted in single 3.3-m-long rows inoculated with isolate no. 5 (intermediate virulence). An early disease reading was taken during tiller elongation but prior to heading. Of the 1,001 lines tested, 178 winter-killed and 794 were moderately to highly susceptible to this isolate. The other 29 lines showed either a low infection percentage or restricted symptom development in infected plants and were selected for further testing to isolates of higher virulence. None was immune to the pathogen. Most of the selected lines were from Asia or northern Europe, but no one geographical area produced a high percentage of lines with moderate resistance.

In 1975-1976, the 29 lines selected above, plus other lines from the winter wheat development program in Montana that had shown some tolerance to isolate no. 5 were field tested for their reaction to three highly virulent isolates plus isolate no. 5. Several commercially grown cultivars were also included. A split-plot design was used with wheat lines or cultivars as the main plots, and isolates of the pathogen and a noninoculated control as subplots. Each subplot was a single row 3.3 m long and replicated three times. The first disease reading was taken approximately 1 wk prior to heading. The rows were scored from 1 = excellent growth with no visual symptoms on the leaves or culms to 5 = severe stunting with most plants exhibiting leaf symptoms. Approximately 1 mo after heading, the rows were rated for white heads. Plant height was taken at the time of the second disease rating. Just prior to harvest, five representative heads were harvested from each row of 24 selected lines and hand-threshed to allow a determination of seeds per head and thousand kernel weight (TKW).

The average yield reduction of the 48 lines ranged from 24.5% for P. I. 278212 to 77.8% for Marias (Table 1). The grain yield for lines inoculated with isolate no. 8 (high virulence) indicated that for most lines, those highest in grain yield also exhibited the lowest percent reduction in yield to the four isolates. However, several lines of intermediate reaction; e.g., P. I. 293002 and P. I. 347737, produced low yields probably as a result of some winter

TABLE 1. Reaction of various wheat lines inoculated with *Cephalosporium gramineum* under field conditions

Wheat line	Yield reduction[w] (%)	Yield isol. #8 (q/ha)	Disease score		Height reduction (%)
			Early[x] 1-5	White head[y] 1-5	
P. I. 278212	24.5 ab[z]	33.6	1.4	2.3	22.8
C. I. 11222	34.7 bcd	26.0	2.3	2.2	22.0
Crest LRC 40	39.3 bcde	31.3	1.8	2.5	16.1
Crest LRC 51	39.4 bcde	21.9	2.3	2.9	25.8
P. I. 092397	40.5 bcde	26.1	2.1	2.7	26.6
P. I. 094424	43.5 bcdef	30.6	2.3	2.8	24.9
P. I. 094422	46.9 cdefg	24.1	1.9	2.8	30.5
Crest LRC 34	48.0 cdefgh	25.0	2.1	2.8	19.3
C. I. 11247	48.5 cdefgh	14.9	2.4	3.1	35.6
P. I. 094530	49.2 cdefgh	20.4	2.0	2.9	34.1
C. I. 13837	49.5 cdefgh	29.4	3.1	2.2	30.9
Minter	52.1 cdefgh	19.9	1.9	3.0	25.7
P. I. 293002	52.1 defghi	8.1	2.8	...	33.7
C. I. 07638	53.3 defghi	13.2	2.1	2.3	24.3
P. I. 178383	53.6 defghi	18.6	2.1	...	19.2
C. I. 15227	53.8 defghi	20.9	2.3	3.2	29.2
P. I. 094426	54.0 defghi	16.4	2.1	3.3	31.1
MT 7420	54.0 defghi	28.6	2.4	3.3	27.4
MT 7217	54.2 defghi	17.4	2.3	3.0	29.7
P. I. 347737	54.3 defghi	11.0	2.5	3.7	18.3
Crest	54.5 defghi	20.2	2.2	3.0	18.1
Winalta	54.7 defghi	19.7	2.3	3.5	27.8
P. I. 061346	55.1 defghi	16.6	2.3	2.9	33.8
P. I. 201134	56.4 efghi	16.0	2.2	3.6	33.2
P. I. 094489	56.5 efghi	22.2	2.5	3.0	32.0
MT 7015	56.6 efghij	19.5	2.7	3.8	33.7
MT 7443Y	57.2 efghij	20.7	2.5	2.7	28.6
C. I. 08598	57.3 efghij	9.8	2.9	3.2	29.8
P. I. 184145	57.9 efghij	19.0	3.3	3.5	35.6
P. I. 094427	58.6 efghij	19.7	2.1	3.2	31.0
P. I. 094428	58.9 efghij	17.0	2.3	3.5	40.0
Centurk	59.0 efghij	21.7	2.0	3.5	24.0
Crest LRC 35	59.2 efghij	21.1	2.2	3.8	27.9
MT 7403	59.5 efghij	18.2	2.9	3.7	34.9
P. I. 094485	62.3 fghij	16.0	2.2	2.9	34.8
MT 7406	62.8 fghij	15.5	2.5	3.8	37.1
P. I. 316006	63.0 fghij	15.7	3.3	4.1	35.0
P. I. 094479	64.0 fghij	13.2	2.5	3.3	34.3
P. I. 094504	66.7 fghij	14.3	2.4	3.5	26.2
Trader	66.9 ghij	16.6	2.4	3.7	31.5
Homestead	67.5 ghij	11.9	2.1	3.6	18.7
MT 7402	68.0 ghij	15.3	2.9	3.8	35.9
Cheyenne	68.2 hij	14.3	2.0	4.0	31.6
Buckskin	68.6 hij	13.7	2.1	3.7	31.9
Warrior	71.1 hij	16.0	2.5	3.9	34.8
Bronze	72.6 ij	9.0	3.1	4.3	39.3
P. I. 317489	72.8 ij	9.8	3.3	...	23.8
Marias	77.8 j	6.4	2.9	4.1	43.5

[w] Average yield reduction for the four isolates used.

[x] Early disease reading taken just prior to heading; 1 = no symptoms to 5 = severe stunting and most leaves with symptoms.

[y] White head readings: 0 = no white heads; 1 = 1-20% of tillers with white heads; 2 = 20-40%; 3 = 40-60%; 4 = 60-80%; and 5 = 80-100% with white heads.

[z] Column means followed by the same letter are not significantly different at ($P = 0.05$) using Duncan's multiple range test.

killing or inherent low yielding ability. A measure of the difference between resistant and susceptible lines is seen in the three-fold difference in percent yield reduction (24.5% vs. 77.8%). This difference is even greater when grain yields are compared; the most resistant line produced 33.6 quintals (q)/ha and the most susceptible line produced 6.4 q/ha, a five-fold difference. All the lines were not affected similarly by the four isolates ($P = 0.05$). Some (e.g., Cheyenne and Crest) showed a high yield reduction when infected with the virulent isolates, but only a moderate or low yield reduction when infected with isolate no. 5 of intermediate virulence. Minter and C. I. 11222, on the other hand, were reduced an equal amount when infected by any of the four isolates. However, the majority of the lines reacted in a manner similar to Crest and Cheyenne.

The isolate intermediate in its ability to cause overall yield reduction also was intermediate in its ability to affect the number of seed per head and kernel weight. Kernel weight was more adversely affected than the number of seeds per head (Table 2). Yields were reduced because seeds per head and especially kernel weight were reduced, but not because number of heads were reduced. The lines in Table 2 were ranked according to the TKW averaged for the four *Cephalosporium* isolates used; their performance when inoculated with isolate no. 8 (highest virulence) is given. Kernel weight and/or seed number per head for C. I. 11222, P. I. 278212, and P. I. 347737 were the least affected by infection with isolate no. 8.

Effect on grain quality.—Samples from the four lines in the 1975 wheat line-isolate interaction test were processed by the Montana State University Cereal Quality Lab. The amount of grain available from any one isolate-host combination was in some cases insufficient to run the tests. Therefore, three categories of virulence level were established. A low-virulence category included isolates no. 11, 13, and 33; a medium-virulence category included isolates no. 4, 5, 14, and 37; and a high-virulence category included isolates no. 8, 17, 27, 28, 38, and 237. Grain of a cultivar infected with the isolates in each category was then bulked for the tests.

Cultivar means differed for each quality trait measured except bake absorption and grain and texture score of the baked loaf. Significant interactions were measured for test weight, flour yield, and farinograph absorption. Marias sustained a more severe reduction in test weight and flour yield from the pathogen than did either McCall or Winalta. At the low-virulence level, the farinograph absorption of Marias was reduced, but the farinograph absorption of McCall and Winalta was reduced only by the highest virulence level of *C. gramineum*.

When quality means of all cultivars were averaged, changes induced by the pathogen were noted in seven of the 13 quality traits (Table 3). A significant reduction in test weight resulted from the medium virulence level with an additional reduction from the high virulence level. Significant reductions in flour yield resulted from the medium- and high-virulence levels of *C. gramineum*. Flour ash, which consists mainly of calcium and phosphorus compounds, increased significantly with the medium- and high-virulence levels. As level of virulence increased, water absorption of the dough decreased whereas dough strength, as measured by peak time, stability, and the valorimeter, increased. A trend toward increased wheat protein was noted, but it was not significant. The baking properties remained unchanged although a trend toward lower loaf volume at the high virulence level was evident (Table 3).

TABLE 2. Effect of a highly virulent isolate (no. 8) of *Cephalosporium gramineum* on thousand-kernel weight and on number of seeds per head of winter wheat lines with varying degrees of resistance

Wheat line	Thousand-kernel weight			Seed number per head		
	Noninoc. (g)	Inoc. (g)	Reduction (%)	Noninoc. (g)	Inoc. (g)	Reduction (%)
C. I. 11222	44.6	25.8	42.2	31	29	6.5
P. I. 278212	36.2	28.9	20.2	30	19	36.7
P. I. 347737	39.7	26.0	34.5	46	33	28.3
Crest LRC 40	31.5	22.0	30.2	39	29	25.6
P. I. 178383	35.0	21.1	39.7	33	27	18.2
Crest LRC 51	31.1	21.4	31.2	41	33	19.5
C. I. 13837	34.6	21.5	37.9	52	43	17.3
Crest	32.7	18.9	42.2	39	32	18.0
C. I. 07638	33.7	19.2	43.0	37	25	32.4
Crest LRC 34	31.2	21.3	31.7	42	31	26.2
Winalta	36.0	18.1	49.7	31	26	16.1
Crest LRC 35	32.8	17.8	45.7	38	25	34.2
P. I. 094485	34.4	17.3	49.7	47	35	25.5
P. I. 094424	32.7	17.6	46.2	38	31	18.4
MT 7217	35.3	15.7	55.5	31	25	19.4
Trader	32.3	16.3	49.5	35	25	28.6
Centurk	31.6	16.1	49.1	36	26	27.8
Minter	30.0	13.9	53.7	32	18	43.8
P. I. 094422	28.3	14.3	49.5	44	31	30.0
Buckskin	35.5	14.1	60.3	32	20	37.5
Homestead	31.6	12.4	60.8	25	22	12.0
Cheyenne	35.5	13.3	62.5	30	24	20.0
Bronze	30.4	12.3	59.5	39	28	28.2
Marias	33.9	10.3	69.6	33	16	51.5

TABLE 3. Effect of virulence level in *Cephalosporium gramineum* on grain and flour quality of McCall, Winalta, and Marias winter wheats

Virulence level	Test[x] wt (kg/hl)	Flour		Farinograph				Protein		Bake		Loaf vol. (cc)	Grain and texture[y] (1-10)
		Yield (%)	Ash (%)	Abs. (%)	Peak (min)	Stab. (min)	Vol. (cc)	Wheat (%)	Flour (%)	Abs. (%)	Mix time (min)		
Uninoculated	82.0 a[z]	69.7 a	0.341 c	61.0 a	6.2 c	8.5 d	65 c	13.2 a	12.1 a	61.1 a	2.9 a	963 a	5.0 a
Low	81.4 a	69.3 a	0.353 bc	60.8 a	7.3 b	10.6 c	68 c	13.2 a	12.0 a	61.3 a	2.5 a	968 a	5.0 a
Medium	79.6 b	68.3 b	0.360 b	60.0 b	8.5 a	13.6 b	73 b	13.6 a	12.1 a	60.7 a	2.5 a	958 a	4.9 a
High	77.3 c	66.0 c	0.397 a	59.0 c	9.2 a	19.7 a	77 a	13.5 a	12.0 a	59.8 a	3.1 a	943 a	4.9 a

[x] All values are averaged across the three cultivars.

[y] Arbitrary scale of 1 = poor to 10 = excellent.

[z] Column means followed by the same letter are not significantly different at ($P = 0.05$) using Duncan's multiple range test.

DISCUSSION

Most isolates (18 out of 25), regardless of their geographic origin, caused yield reductions of more than 50% in susceptible wheats. Three of 25 isolates were of low virulence. Differences in virulence were also expressed in grain and flour quality tests (Table 3), with increased virulence resulting in an overall decrease in quality as measured by kernel weight, flour yield, and physical dough properties. However, quality decreases were not severe enough to affect baking properties. Similar effects on milling and baking properties of wheat grain were observed as the result of infection by wheat soilborne mosaic virus and wheat streak mosaic virus (6). The responses we observed are the type expected for grain produced on drought-stressed plants. However, a marked increase in protein content is usually observed in grain from such plants, a phenomenon we did not observe. Since farinograph stability time is a measure of protein quantity and quality in the dough, a marked increase in this value without a concomitant increase in protein content suggests that the protein quality of grain from infected plants has been altered in a manner similar to that expected as a result of drought stress (McGuire, *unpublished*). One measure of protein quality is lysine content, but it was not found to differ significantly ($P = 0.05$) between grain from healthy and infected plants (S. Johnston, *personal communication*).

A selected screening of the World Collection of red winter wheats revealed that most lines are highly susceptible, even to an isolate of intermediate virulence. Yunoki & Sakurai (21) reported similar results when they field-tested 171 lines of wheat in Japan. They found 16 lines with some resistance as expressed by a lower infection percentage. We obtained seven of these lines, but five were winter-killed under our conditions and the other two were of intermediate susceptibility.

Of the 29 lines selected for further testing from our World Collection studies, only a few had enough resistance to highly virulent isolates to be worth including in a breeding program. The most resistant line was P. I. 278212, which is called Feldsberger Irannen. It produced 33.6 q/ha (49.9 bu/ac) when inoculated with a highly virulent isolate, had a test weight of 80.1 kg/hl (62.2 lb/bu) and a protein content of 16.6%; these values are equal to or better than those for currently grown cultivars. Other lines with some resistance to virulent isolates were P. I. 092397, P. I. 094422, P. I. 094424, C. I. 13837, and C. I. 11222. Johnston (8) and Mathre (*unpublished*) earlier had shown that some of the line row components that were bulked to form the heterogeneous cultivar Crest differed in their susceptibility to isolate no. 5. These same line row components (no. 34, 40, and 51) were somewhat resistant to the highly virulent isolates. In addition, some of them carry good resistance to prevalent races of stripe rust (*Puccinia striiformis* West). Line C. I. 13837 resulted from a cross between Burt and P. I. 178383 while the Crest line row components were developed from a cross between Westmont and P. I. 178383. We previously indicated that P. I. 178383 had some resistance to an isolate of intermediate virulence (12). Although P. I. 178383 was moderately susceptible to the virulent isolates, lines originating from it may have a greater level of resistance via transgressive segregation.

Previous work in the greenhouse (9) indicated that the yield components of a susceptible cultivar (Lancer) most strongly affected by *C. gramineum* were number and weight of seeds per head, whereas number of heads per plant was not affected. We did not determine the effect of *C. gramineum* on number of heads per plant in the current study, but our field studies confirmed these greenhouse studies. Seed size, as indicated by kernel weight, was reduced more than was seed number per head. Yield components of some lines were differentially affected by infection; i.e., some lines with little reduction in number of seeds per head (e.g., C. I. 11222 and Homestead) still had greatly reduced kernel weights, whereas in other cases a line with a small reduction in kernel weight (P. I. 278212) suffered a 37% reduction in number of seeds per head. This suggests that wheat lines may react to the pathogen in different ways. Intercrossing lines with resistance to decreased kernel weight with those with resistance to decreased seed number per head may give rise to lines with both types of resistance. We are currently testing this possibility.

Several cultivars and lines tested to isolate no. 5 in a previous study (12) showed some resistance as indicated by low yield reductions. However, all of these lines with the hard red kernel characteristic; e.g., Trader, Crest, and P. I. 347737, were quite susceptible to the highly virulent isolates. Other currently grown cultivars of high susceptibility (e.g., Warrior, Homestead, Trader, Centurk, and Marias) all have Cheyenne in their pedigree, a cultivar released in 1922 as a selection from Crimean. High susceptibility of these lines, therefore, may be related to their Cheyenne background.

Resistance of the type currently exhibited by lines or cultivars of *T. aestivum* may be affected by inoculum density (13). Greenhouse tests indicated that P. I. 278212, P. I. 094422, and Crest LRC 34, lines moderately resistant or tolerant to field inoculation, were highly susceptible when their roots were severed and inoculated with a conidial suspension in water of 10^7 conidia/ml (Mathre, *unpublished*). However, lower inoculum levels (i.e., 10^3 conidia/ml, differentiated between resistant and susceptible lines. The latter inoculum level may approximate the levels encountered in the field. This suggests that under conditions of natural inoculum, lines performing well under our oat kernel inoculum may perform even better, since it is doubtful whether natural inoculum levels are as high as with oat kernel inoculum.

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