Testing Wheat Seedlings for Resistance to Crown Rot Caused by *Fusarium graminearum* Group 1

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ABSTRACT

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Resistance to crown rot of wheat caused by *Fusarium graminearum* Group 1 is currently measured in mature plants that have been grown in inoculated soil in the field. This technique is laborious and time-consuming, and a faster technique is desired. Seedlings of different cultivars of wheat were grown in steam-air treated soil inoculated with a band of wheat-barley grain colonized by *F. graminearum*. Plants were grown at 13, 19, and 25 C; and after 22 days the sheaths of leaves 1, 2, and 3 were rated for the extent of necrotic lesions. At each temperature, this rating was positively correlated with disease ratings of the same cultivars grown in the field. At 25 C, the correlation coefficient between seedling and mature plant ratings of 28 cultivars was 0.78 ($P \leq 0.01$). This close relationship gives confidence that seedlings can be used to measure resistance to crown rot. Use of seedlings will speed the selection of resistant progeny in wheat breeding programs where resistance to crown rot is an objective.

Additional keywords: field test, leaf sheaths, seedling test, soilborne disease, wheat cultivars

Crown rot of wheat (*Triticum* spp.) caused by *Fusarium graminearum* Schwabe Group 1 (*Fg* G1) (6) (teleomorph *Gibberella zeae* (Schwein.) Petch) occurs in many parts of the Australian wheat belt and is particularly damaging in New South Wales and Queensland. Symptoms of crown rot caused by *Fg* G1 have also been reported from Washington State (3), Argentina (2), and South Africa (14).

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Crown rot can cause losses in yield (4,7). Use of resistant cultivars is one of the principal means by which the damaging effects of this disease can be reduced. Resistance is normally measured in mature plants that have been grown in inoculated rows in the field (4,5,13,15). These plants are collected from the field and rated for disease in the laboratory. If this technique was used in a breeding program designed to produce resistant cultivars, it would be laborious and time-consuming.

Selection for plants resistant to foliar diseases such as stem rust caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. and yellow spot caused by *Pyrenophora tritici-repentis* (Died.) Drechs. is usually made on seedlings. However, there are relatively few examples where seedling tests have been used for testing resistance to root or crown rots of cereals. Macer (8) showed that resistance to eyespot caused by *Pseudocercosporella herpotrichoides* (Fron) Deighton could be detected in seedlings grown in compost. Miedaner (9) found that resistance to *Fusarium culmorum* (Wm. G. Sm.) Sacc. could be detected by differences between dry weights of inoculated and noninoculated seedlings. In contrast, no correlation could be found between seedling tests and field reaction of plants infected with *F. culmorum* (9,12), with *Rhizoctonia solani* Kühn (10), or with *Fg* G1 (13). Purss (13) stated that seedling blight reaction in the glasshouse gave no guide to field tolerance but could be of value if true resistance was found. Despite this assertion, Klein et al (7) claimed that seedling tolerance to *Fg* G1 in wheat was correlated with adult plant tolerance in six of eight cultivars assessed. In addition, Brewster and Burgess (1) reported that differences in basal browning of 6-wk-old seedlings were found among wheat, barley, and oats, and between two wheat cultivars.

This paper reports on experiments designed to determine the effect of temperature and placement of inoculum on disease caused by *Fg* G1 in wheat seedlings. It also shows the positive relationship between the susceptibility or resistance of cultivars measured as adult plants in field tests and that measured in seedlings grown in a controlled environment.

MATERIALS AND METHODS

Soil. The soil was a shallow, brown clay-loam classified as Gn 3.22 (11) with the following characteristics: particle size distribution <2 μm, 33.0%; 2–20 μm, 25.3%; >20 μm, 41.7%; pH 7.0 (1:5 water). Before use, the soil was moistened to 35% (w/w) for approximately 16 hr, heated at 70 C with a steam-air mixture for 30 min, and air-dried.

Inoculum. Inoculum was wheat-barley grain colonized by *Fg* G1. Wheat and barley grain (ratio 1:1) were mixed and placed in water in Erlenmeyer flasks for 12 hr. The water was decanted, and the grain was autoclaved for 20 min at 121 C on two successive days. Czapek Dox agar colonized by *Fg* G1 was added to the grain and incubated for 21 days at 25 C (4). The grain was air-dried and ground in a laboratory mill to pass through a 2-mm sieve.

Seedling test. The reaction of spring wheat cultivars to infection by *Fg* G1 was tested by growing seedlings in pots (500 cm^3). Air-steam treated soil (300 g, 8.6% moisture) was added to each pot and moistened to 37.5% (−0.1 bar), and 12 seeds were placed on the soil surface. One hundred grams of the same soil (screened to pass a 6.25-mm sieve) were placed above the seed, and 0.35 g of inoculum of *Fg* G1 were added to cover the surface (63.6 cm^2) of the soil. An additional 100 g of soil was placed above the inoculum. The entire pot was moistened to 37.5% by addition of water to the surface of the pot 7 days after commencement. The soil was maintained at this moisture content for the duration of the experiment by adding an appropriate weight of water each day.

Disease measurement. Disease was assessed on coleoptiles, subcrown internodes, leaf sheaths, and whole seedlings. Coleoptiles, subcrown internodes, and leaf bases were assessed for disease on a 0–4 scale where 0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis (Fig. 1).

Whole plants were also assessed with a 0–4 scale where 0 = completely healthy, 1 = coleoptile and/or subcrown internode partially or wholly necrotic but no necrosis in the leaf bases, 2 = coleoptile and/or subcrown internode and leaf sheaths partially or completely necrotic, 3 = same as 2 with reduction in plant size, and 4 = whole plant severely to completely necrotic.

Effect of temperature on crown rot in seedlings. The development of crown rot in the cultivars Puseas, Vasco, Hartog, Gala, and line 2-49 was compared in seedling tests conducted at 13, 19, and 25 C with a 12-hr photoperiod. The line 2-49 was a resistant selection from plants of the cross Gala/Gluyas Early. Seedlings were removed from pots after 22 and 42 days, washed, and assessed for severity of disease. Plants recovered after 22 days were rated for disease on the coleoptiles, subcrown internodes, leaf sheaths of leaves 1, 2, and 3, and whole plants. Similar assessments were made on plants recovered after 42 days, except that the coleoptiles were not examined and disease in leaf sheaths of leaves 4 and 5 was also recorded.

Inoculum placement. Severity of crown rot was compared in five cultivars that were inoculated either by banding the inoculum (as described previously) or by dispersing it in the soil. Two banding treatments, 0.4 g and 0.7 g per pot, were included. To disperse the inoculum, soil was added to a plastic bag, and sufficient inoculum was added so that the concentration would be 0.25 g per pot. This concentration had previously been shown to cause severe disease (15). The contents of the plastic bag were shaken for 30 sec before they were dispensed to pots.

Comparison of field and seedling tests. Twenty-eight cultivars were compared for their reactions to crown rot in field tests and in a seedling test. Field tests were conducted by opening a furrow in a black earth, adding ground colonized grain (2.0 g m^{-1} of row) along the furrow, and planting seed dusted with benomyl (Benlate 50WP) at a rate of 0.225 g a.i. kg^{-1} of seed. At maturity, plants in each row were collected, and tillers were separated from each other and assessed for presence or absence of disease; tillers with a honey brown to dark brown discoloration were rated as diseased, and the percent diseased tillers was calculated. An index of the susceptibility of each cultivar/line relative to the highly susceptible cultivar Puseas (4,5,15) was calculated according to the equation (% diseased tillers of test cultivar/% diseased tillers of Puseas) × 100. A mean value over all experiments for each cultivar/line was then determined.

The same 28 cultivars/lines were included in a seedling test conducted at 25 C, and the severity of disease in the leaf sheaths of leaves 1, 2, and 3 was recorded.

Design and statistical analysis. Treatments were replicated three times in each experiment and arranged in a randomized block design. Data were subjected to analysis of variance, and means were compared using Duncan's multiple range test at the $P = 0.05$ level of significance. As well, a regression between relative susceptibility of cultivars to crown rot (independent variable) and seedling rating (dependent variable) was established.

RESULTS

Temperature. Disease assessments of coleoptile (COL), subcrown internode (SCI), leaf sheaths, and whole plants after 22 days were significantly higher ($P \leq 0.01$) at 19 than at 13 C; and in all plant parts except coleoptiles assessments were significantly higher ($P \leq 0.01$) at 25 than at 19 C. At 13 C, there was no significant difference in disease rating; whereas at 19 C, cultivars could be separated into three groups and at 25 C into four groups according to their susceptibility (Table 1). The latter separation is similar to that reported for field tests (4,13,17).

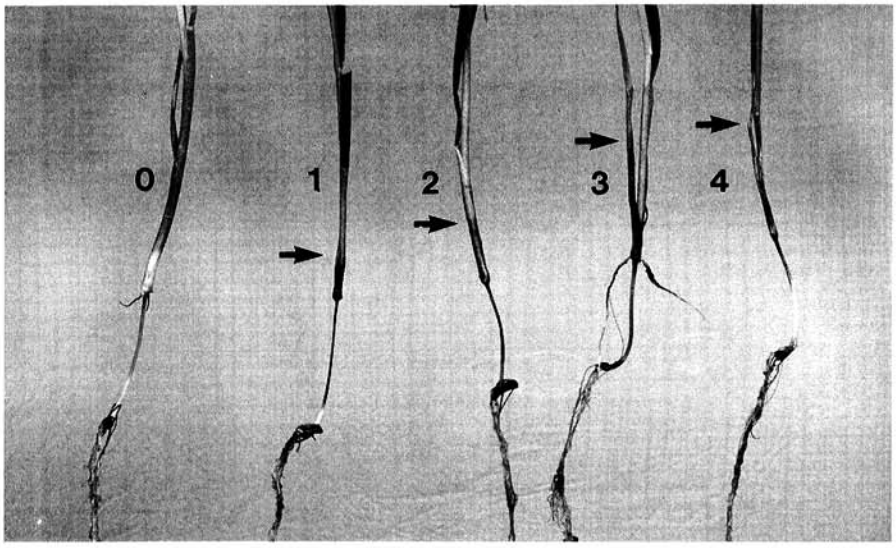

Fig. 1. Rating system for severity of crown rot in the first leaf sheath of seedlings: 0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 50–75% necrosis, and 4 = greater than 75% necrosis. Arrow indicates extent of necrosis on leaf sheath.

Disease severity in different plant tissues. After 22 days, disease had developed extensively in coleoptiles, subcrown internodes, and leaf sheaths of leaves 1 (LS1), 2 (LS2), and 3 (LS3) of seedlings. All plants sustained disease symptoms in one or more tissues.

There were no differences among cultivars in the development of disease in COLs (Table 2). Disease development in SCIs was significantly higher in Puseas than in Vasco, Hartog, Gala, and 2-49; the lowest disease rating was in Hartog. Disease severity in LS1 was higher than in LS2, which was higher than LS3. Combinations of LS1, LS2, and LS3 severity ratings in all possible ways, with or without the COL and SCI measurements, were calculated to determine which would give similar differences among cultivars to those obtained from field tests (Table 2). All of the leaf sheath measurements fulfilled this requirement, and LS1 + LS2 + LS3 was selected for use in further experiments.

The whole-plant ratings showed that Puseas sustained significantly more disease than the other cultivars, whereas 2-49 had significantly less disease than Puseas and Vasco. Differences among cultivars were less with plant ratings than with leaf sheath ratings.

After 42 days, Puseas had the highest level of disease in the SCI, and 2-49 had the lowest level. However, disease levels in Vasco were not significantly greater than those in 2-49. All assessments of disease in leaf sheaths showed cultivar differences similar to those found in field tests. Relative differences in disease levels among cultivars at 42 and 22 days were similar (Table 3). This experiment has been repeated numerous times with similar differences among susceptible and partially resistant cultivars or lines.

Inoculum placement. Whether banded or dispersed inoculum was used, disease severities in Puseas and Vasco were significantly greater than those of other cultivars (Table 4). As well, the disease level in Hartog was significantly greater than that of 2-49, and in banded treatments, was also greater than that of Gala. Similar differences among cultivars were also expressed in seedlings inoculated with a band of inoculum of either 0.4 or 0.7 g per pot. Banding of inoculum at or near the lower rate was the method adopted in further experiments.

Comparison of field and seedling tests. Data from field experiments on the susceptibility of cultivars/lines relative to Puseas are presented in Table 5. The data for each cultivar are a mean of the relative susceptibility value of each experiment in which the cultivar was included. Values varied from 100% (Puseas) to 18.6% (Gluyas Early). Twenty-one of the 28 cultivars/lines were in the 50–100% range of relative susceptibility. Disease severity in leaf sheaths of leaves 1, 2, and 3 in seedlings varied in a similar manner to that of the field test. Values varied from 4.9 in Puseas to 0.7 in Gluyas Early.

Correlations between relative susceptibility from field tests and seedling parameters showed that the highest correlation was between relative susceptibility and disease severity of LS1 + LS2 + LS3 and the lowest was with SCIs (Table 6) ($P < 0.01$). The regression

Table 1. Rating for extent of necrosis in leaf sheaths of leaves 1, 2, and 3 of wheat seedlings grown for 22 days at 13, 19, and 25 C in soil inoculated with a band of wheat-barley inoculum of *Fusarium graminearum* Group 1

Temperature (C)	Puseas	Vasco	Hartog	Gala	2-49	Mean of cultivar
13	1.0[y] g[z]	0.6 g	0.5 g	0.4 g	0.2 g	0.5 A
19	5.0 c	3.3 de	2.3 ef	3.2 def	2.3 f	3.2 B
25	7.8 a	6.7 b	4.6 c	4.1 cd	3.2 def	5.3 C

[y] Rating scale of 0–4, where 0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis. Each value is the mean of three replicates.
[z] Figures with similar letters do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

Table 2. Rating for extent of necrosis in subcrown internodes and leaf sheaths of seedlings of five wheat cultivars 22 days after inoculation with *Fusarium graminearum* Group 1

Cultivar/line	Relative field susceptibility[v]	COL[w]	SCI[w]	LS1[w]	LS2[w]	LS3[w]	LS1 + LS2	LS1 + LS3	LS2 + LS3	LS1 + LS2 + LS3	Plant rating[x]
Puseas	100	3.7[y] a[z]	2.5 a	2.3 a	1.5 a	0.8 a	3.8 a	3.1 a	2.3 a	4.6 a	2.5 a
Vasco	97.9	3.8 a	1.5 bc	2.3 a	0.9 b	0.3 b	3.2 b	2.6 b	1.2 b	3.5 b	1.9 b
Hartog	72.3	3.8 a	1.4 c	1.9 b	0.4 c	0.2 c	2.3 c	2.1 c	0.6 c	2.5 c	1.8 bc
Gala	55.0	3.7 a	1.7 b	2.0 b	0.5 c	0.1 c	2.5 c	2.1 c	0.5 c	2.6 c	1.8 bc
2-49	48.8	3.7 a	1.5 bc	1.5 c	0.3 c	0.1 c	1.8 d	1.6 d	0.4 c	1.9 d	1.7 c

[v] Relative field susceptibility = (% diseased tillers of test cultivar/% diseased tillers of Puseas) × 100.
[w] COL = coleoptile, SCI = subcrown internode, LS1 = leaf sheath of leaf 1, LS2 = leaf sheath of leaf 2, LS3 = leaf sheath of leaf 3.
[x] Rating scale of whole plants is 0–4, where 0 = completely healthy, 1 = coleoptile and/or subcrown internode partially or wholly necrotic but no necrosis in the leaf bases, 2 = coleoptile and/or subcrown internode and leaf sheaths partially or completely necrotic, 3 = same as 2 with reduction in plant size, and 4 = whole plant severely to completely necrotic. Each value is the mean of three replicates.
[y] Rating scale is 0–4, where 0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis. Each value is the mean of three replicates.
[z] Figures with similar letters do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

Table 3. Rating for extent of necrosis in subcrown internodes and leaf sheaths of seedlings of five wheat cultivars 42 days after inoculation with *Fusarium graminearum* Group 1

Cultivar/line	SCI[x]	LS1[x]	LS2[x]	LS3[x]	LS4[x]	LS5[x]	LS1 + LS2	LS1 + LS2 + LS3	LS1 + LS2 + LS3 + LS4	LS1 + LS2 + LS3 + LS4 + LS5
Puseas	3.8[y] a[z]	3.7 a	3.1 a	2.4 a	2.0 a	1.6 a	6.8 a	9.3 a	11.2 a	12.8 a
Vasco	2.3 bc	3.6 a	2.0 b	0.9 b	0.4 b	0.1 b	5.6 b	6.5 b	6.9 b	7.0 b
Hartog	2.5 b	3.6 a	1.9 b	0.8 bc	0.3 bc	0.1 b	5.5 bc	6.3 bc	6.5 bc	6.6 bc
Gala	2.5 b	3.3 b	1.6 b	0.6 cd	0.2 bc	0.1 b	5.0 c	5.5 c	5.7 c	5.8 c
2-49	2.3 c	2.9 c	1.0 c	0.3 d	0.1 c	0.0 b	3.9 d	4.2 d	4.3 d	4.3 d

[x] SCI = subcrown internode, LS1 = leaf sheath of leaf 1, LS2 = leaf sheath of leaf 2, LS3 = leaf sheath of leaf 3, LS4 = leaf sheath of leaf 4, and LS5 = leaf sheath of leaf 5.
[y] Rating scale is 0–4, where 0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis. Each value is the mean of three replicates.
[z] Figures with similar letters do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

Table 4. Severity of crown rot in leaf sheaths of seedlings after inoculation with banded or dispersed inoculum of *Fusarium graminearum* Group 1

Type of inoculation	Inoculum[x] (g)	Puseas	Vasco	Hartog	Gala	2-49
Banded	0.4	5.3[y] bc[z]	6.7 b	4.0 cd	1.1 ef	1.2 ef
Banded	0.7	8.1 a	6.4 b	3.9 cd	1.9 ef	1.9 ef
Dispersed	0.25	5.6 bc	6.0 b	3.0 de	2.3 de	0.3 f

[x] Amount of inoculum per pot.
[y] Rating scale is 0–4, where 0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis. Each value is the mean of three replicates.
[z] Figures with similar letters do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

Table 5. Crown rot field index and rating for necrosis in leaf sheaths of leaves 1, 2, and 3 of seedlings of 28 cultivars and lines

Cultivar/line	Relative susceptibility[u] (field, %)	No. of field tests	Disease severity in seedlings (LS1 + LS2 + LS3)
Puseas	100	16	4.9[v] abc[w]
King	98.2	2	2.4 fgh
Vasco	97.9	4	3.5 def
Sunstar	97.1	4	5.4 ab
Cunningham	95.9	2	4.4 abcd
Banks	90.0	5	5.6 a
Torres	88.1	2	4.6 abcd
Perouse	84.1	2	4.6 abcd
Sunkota	80.9	1	2.9 ef
Flinders	78.7	3	5.1 ab
Harrier	76.6	1	4.2 bcde
Sunelg	73.5	3	3.0 ef
Suneca	72.5	2	0.4 i
Hartog	71.4	6	1.0 i
Diaz	64.9	4	3.5 def
Gala	62.7	16	1.5 ghi
Bass	60.9	2	2.6 fg
Kite	59.8	4	1.5 ghi
Frontiera	59.2	2	0.7 i
QT2997[x]	58.4	2	0.7 i
Cook	53.8	6	2.6 fg
QT4118[x]	47.4	1	0.4 i
Sunco	45.5	2	2.4 fgh
2-49[y]	45.0	10	0.9 i
IRN538[z]	37.7	2	1.0 i
IRN497[z]	32.1	2	0.5 i
Mexico 234	28.9	2	0.5 i
Gluyas Early	18.6	2	0.7 i

[u] Relative susceptibility = (% diseased tillers of test cultivar/% diseased tillers of Puseas) × 100.
[v] Rating scale is 0–4, where 0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis. Each value is the mean of three replicates.
[w] Figures with similar letters do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.
[x] Lines from the Queensland Wheat Research Institute breeding program.
[y] A selection for crown rot resistance from the cross Gala/Gluyas Early.
[z] Lines from the 1965 International Spring Wheat Rust Nursery.

Table 6. Correlations between a field index for susceptibility to crown rot (% of Puseas) and disease symptoms in the subcrown internodes and leaf bases of seedlings of 24 cultivars and lines

	Seedling test					
	SCI[x]	LS1[y]	LS2[y]	LS3[y]	LS1 + LS2	LS1 + LS2 + LS3
Relative susceptibility (field)	0.34 NS[z]	0.72**[z]	0.75**	0.6**	0.77**	0.78**

[x] SCI = subcrown internode.
[y] LS1 = leaf sheath of leaf 1, LS2 = leaf sheath of leaf 2, and LS3 = leaf shealth of leaf 3.
[z] NS = not significant, and ** = statistically significant ($P < 0.01$).

equation with leaf sheaths was: Relative susceptibility = 42.0 + 9.8 (disease severity of LS1 + LS2 + LS3), R^2 = 0.6 (Fig. 2).

DISCUSSION

Severity of crown rot can be assessed in many ways. It can be assessed with emergence, percent infection by *Fg* G1, discoloration of tiller bases, numbers of whiteheads, yield (13), number of healthy seedlings (7), percent diseased tissue and percent yield loss (4). Not all of these methods permit detection of resistant cultivars/lines.

Seedlings have been used to determine resistance to stem and leaf rust in wheat and barley for many years. Klein et al (7) counted numbers of healthy seedlings as an index of seedling tolerance to crown rot and found that this was correlated with adult tolerance in six of eight cultivars. In their tests, measurements of Banks, a susceptible cultivar, and Cook, a partially resistant cultivar, were not significantly different until plants had grown for 102 days. At this time, they would have been past the seedling stage. In our tests, most plants were diseased to some extent, and thus the method of Klein et al could not be adopted.

Purss (13) stated that field tolerance to crown rot could not be measured by seedling blight reactions in the glasshouse. In our experiments, seedling blight occurred, particularly in the more susceptible cultivars. However, it was not used alone as a measure of disease level but was incorporated into the rating scale for disease severity. Our results showed that seedlings can be used to measure partial resistance to crown rot.

The precision of the seedling test was enhanced, and the duration was shortened by conducting it at 25 C. At temperatures below 25 C, development of disease was slower, and some differences among cultivars known from field tests were not evident.

Differences in susceptibility to crown rot among cultivars were expressed with either banded or dispersed inoculum. One advantage of banded inoculum is the increased likelihood of an emerging seedling contacting inoculum and the resistance or susceptibility of that seedling being determined. This would minimize the possibility of disease escapes in screening of progeny for crown rot in a breeding program.

Both crown rot and strawbreaker foot rot (eyespot), caused by *Pseudocercosporella herpotrichoides,* attack similar areas of the crown and lower internodes of wheat plants. Macer (8) found that resistance to eyespot could be measured by the rate of penetration of successive leaf sheaths by the fungus. In a similar way, we have found that the degree of necrosis on the leaf sheaths of leaves 1, 2, and 3 is a useful measure of the resistance or susceptibility of a cultivar/line

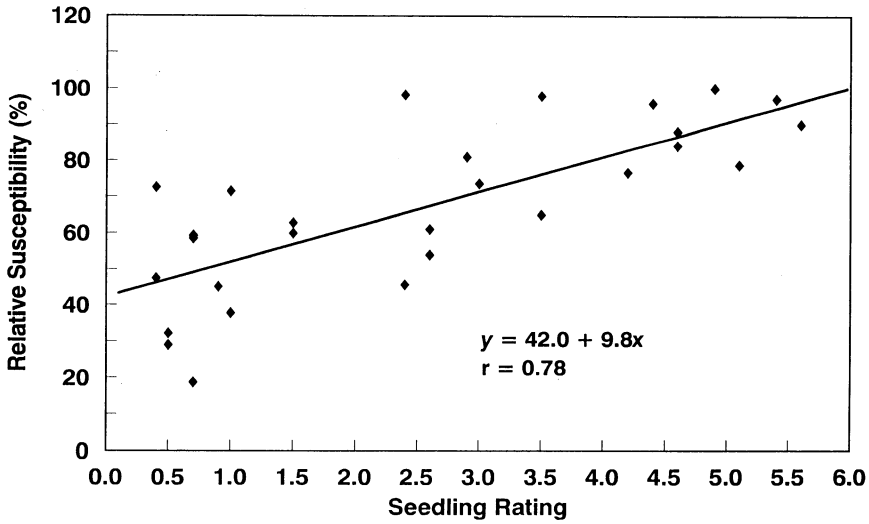

Fig. 2. Regression of relative field susceptibility (independent variable) to crown rot on seedling disease rating (dependent variable) for 28 cultivars/lines of wheat. Relative susceptibility = (% diseased tillers of test cultivar/% diseased tillers of Puseas) × 100. Percent diseased tillers were obtained from disease rating of mature plants grown in inoculated soil in the field. Seedling rating scale is 0–4, where 0 = completely healthy, 1 = less than 25% necrosis, 2 = 25–50% necrosis, 3 = 51–75% necrosis, and 4 = greater than 75% necrosis. Data for relative susceptibility and seedling rating are the mean of three replicates. Seedlings were grown at 25 ± 1 C in a growth cabinet and assessed after 3 wk.

to crown rot. Although differences among some cultivars were shown by disease levels in the subcrown internodes, more precision was obtained by using leaf sheaths.

The relative susceptibilities of the cultivar Gluyas Early and the lines IRN497, IRN538, and Mexico 234 confirm previous studies by Wildermuth and Purss (7). Cultivars/lines with relative susceptibilities less than Gala (4,5,13,16,17) could be regarded as partially resistant. These cultivars/lines also had low levels of disease in the leaf sheaths of seedlings. The high correlation between seedling and field tests indicates that the seedling test is an accurate means by which the susceptibility or resistance of a cultivar/line can be determined. It also shows that the seedling technique could be used as an efficient method to screen progeny in a breeding program designed to produce cultivars with resistance to crown rot.

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