Quantification of common root rot symptoms in resistant and susceptible barley by image analysis[1]

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Abstract: A series of tests were carried out to determine the effect of common root rot, caused by *Cochliobolus sativus*, on root growth and discolouration in the moderately resistant cv. Bonanza and the susceptible cv. Gateway of barley. An image analyser was used to quantify the growth and discolouration (mean intensity) of the roots and the subcrown internode (SCI) of individual plants. The plants were also visually rated for root rot severity and their root dry weight was determined. In the absence of disease, no differences in root discolouration or growth were observed between the two barley cultivars. In plants grown in naturally infested soil, visual root rot ratings and mean intensity values indicated that the SCIs of Bonanza were less discoloured than those of cv. Gateway. In cv. Gateway, higher levels of disease were usually associated with a smaller root system than in cv. Bonanza, but the effect was significant ($P < 0.01$) only in the first experiment. The root system of cv. Gateway had lower ($P > 0.05$) mean intensity values (i.e., darker) than those of cv. Bonanza in the first experiment. Seedling emergence of cv. Gateway was lower in naturally infested soil than in fumigated soil, but this was not evident in cv. Bonanza. There was a strong inverse relationship between the mean intensity and visual rating of the SCI and a positive association between total root area as measured using image analysis and root weight. Image analysis also determined that in the severe root rot category, plants of cv. Gateway had SCIs that were more discoloured than those of cv. Bonanza. Discolouration of the SCI of root rot susceptible barley appeared to adversely influence the growth and discolouration of the root system, but this effect was not always significant.

Key words: common root rot, image analysis, barley, *Cochliobolus sativus*.

Résumé : Une série de tests ont été effectués pour déterminer l'effet de la pourriture sèche, causée par le *Cochliobolus sativus*, sur la croissance et la couleur des racines des cultivars d'orge moyennement résistant cv. Bonanza et sensible cv. Gateway. Un analyseur d'images a été utilisé pour quantifier la croissance et l'altération de la couleur (intensité moyenne) des racines et de l'entrenoeud subcoronal (ESC) de plantes individuelles. De plus, l'intensité de la pourriture des racines a été déterminée visuellement et la matière sèche des plantes a été mesurée. En absence de maladie, aucune différence d'altération de la couleur ou de croissance n'a été observée entre les deux cultivars d'orge. Pour les plantes cultivées en sol infecté naturellement, les évaluations visuelles et les valeurs moyennes d'intensité ont indiqué que la couleur des ESC de cv. Bonanza était moins altérée que celle de cv. Gateway. Chez cv. Gateway, les niveaux de maladie plus élevés étaient généralement associés à un système racinaire plus petit que celui de cv. Bonanza, mais cette différence n'a été significative ($P > 0,01$) que lors du premier essai. Lors de ce premier essai, des valeurs plus faibles d'intensité moyenne (c.-à-d. plus foncé) ont été calculées ($P > 0,05$) pour le système racinaire de cv. Gateway que pour celui de cv. Bonanza. La levée des semis de cv. Gateway en sol naturellement infecté était plus faible que dans le sol fumigé, mais ce n'était pas évident pour cv. Bonanza. Il y avait une relation inverse entre l'intensité moyenne et la note visuelle de l'ESC, et une relation positive entre la surface racinaire totale, telle que mesurée par analyse d'images, et le poids des racines. L'analyse d'images a aussi déterminé que dans la catégorie de pourriture grave, la couleur des ESC des plantes de cv. Gateway était plus altérée que celle de cv. Bonanza. L'altération de la couleur de l'ESC de l'orge sensible à la pourriture des racines a semblé être défavorable à la croissance et à la couleur du système racinaire, mais cet effet n'a pas toujours été significatif.

Mots clés : pourriture sèche, analyse d'images, orge, *Cochliobolus sativus*.

Introduction

Common root rot, caused primarily by *Cochliobolus sativus* (Ito & Kurib.) Dreschl. ex Dastur (syn. *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem.), is one of the most prevalent diseases of barley (*Hordeum vulgare* L.) on the Canadian prairies (Piening et al. 1976). On average, common root rot has been estimated to reduce barley yields by 10–11% (Piening et al. 1976; Bailey et al. 1997). Reductions in yield are primarily due to lower tiller number in in-

Accepted November 26, 1999.

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fected plants (Piening 1973; Grey et al. 1991; Duczek and Jones-Flory 1993). Duczek (1997) reported that common root rot was responsible for a reduction in leaf growth in the early stages of plant development and this might explain later reductions in tiller number caused by the disease. Common root rot has its greatest impact on plant growth under dry conditions when the diminished root system of severely diseased plants is unable to supply the increased moisture demand (Grey et al. 1991).

The development of root rot resistant cultivars has been recommended for reducing yield losses in barley (Mathre 1982); yet several studies have shown that moderately resistant cultivars do not always outyield susceptible cultivars, despite large differences in root rot severity as measured by discolouration of the subcrown internode (SCI) (Piening 1973; Tinline and Ledingham 1979; Grey and Mathre 1984; Duczek and Jones-Florey 1993; Duczek and Wildermuth 1993; Bailey et al. 1997). Cultivars that are severely diseased and yet do not experience major yield losses are considered to be root rot tolerant.

While the name of the disease suggests it is primarily a root problem, most studies on common root rot have been confined to an examination of discolouration of the SCI (Piening et al. 1976; Tinline et al. 1994). Until recently, it was difficult to obtain reliable assessments of the effects of disease on the growth and discolouration of the roots of cereal crops, but the use of image analysis now makes accurate measurement of these variables possible (Kokko et al. 1995). Image analysis has been proven to be a reliable and objective technique to quantify disease based on densitometric measurements (Kokko et al. 1993, 1995). An image analysis study of common root rot in hard red spring wheat (Kokko et al. 1995) demonstrated that the disease had little effect on crown and seminal root discolouration. Only severe discolouration of the SCI resulted in a significant decrease in root area. However, barley is generally considered to be more susceptible to common root rot than wheat (Ledingham et al. 1973; Piening et al. 1976; Conner et al. 1996), so there could be a marked difference in the response of barley to root rot from that of wheat.

This study was conducted to examine the effects of common root rot on root growth and discolouration of moderately resistant and susceptible cultivars of barley using image analysis.

Materials and methods

The barley cultivars were the moderately resistant cv. Bonanza and the susceptible cv. Gateway (Conner et al. 1996). The study was carried out in two experiments that were conducted in metal containers measuring 61 × 88 × 18 cm and filled to a soil depth of 15 cm. Both experiments were conducted in a greenhouse set at a constant temperature of 21 ± 3°C with supplemental lighting to provide a 16-h photoperiod.

Experiment 1: Growth and discolouration of root systems in naturally infested soil

The first experiment was conducted to determine whether common root rot differentially affected root growth in moderately resistant and susceptible barley cultivars. The experiment was conducted in naturally infested soil collected from a field near Lethbridge, Alberta, where barley had been grown continuously for 13 years and common root rot was known to be severe (Conner et al. 1996). Isolations from surface sterilized SCIs that were plated on potato dextrose agar had shown that *C. sativus* and to a lesser extent *Fusarium* spp. were the principal causes of disease at this dryland site (R.L. Conner, unpublished data). A randomized block design with five replications was used and the experiment was conducted as two separate tests (i.e., tests 1 and 2). There were two plots (experimental units) in each container, one for each of the barley cultivars, with each plot consisting of three rows of plants with 10 cm-row spacings. Seeds were planted 6.5 cm below the soil surface to promote long SCI development. Plants were watered when the top 3 cm of soil was dry.

Experiment 2: Growth and discolouration of root systems in naturally infested and fumigated soil

A second experiment was conducted in naturally infested and fumigant-treated soil to determine whether differences among barley cultivars in growth and discolouration of the root system were caused by their response to common root rot or inherent differences in root growth. The naturally infested soil and fumigated soil came from the same field site as used for experiment 1. The fumigated soil was treated with Vapam (380 $g \cdot L^{-1}$ metam-sodium, ICI Chipman/Zeneca), as described by Bailey et al. (1997), 3 years before the study. The barley cultivars were planted in two-row plots and cv. Gateway was seeded as a guard row around the edge of each of the metal containers. Plots were seeded at the same soil depth and row spacing as in experiment 1. A split-plot design was used with soil treatments as the whole units arranged in three randomized blocks and cultivars as the subunits. Experiment 2 was conducted as two separate tests (i.e., tests 3 and 4). After the first test, the disease-free soil was replaced with a fresh batch of soil from the original Vapam-treated source. In test 4, plant numbers were thinned to ensure that all the plots within a replication had the same number of plants to avoid any confounding effects due to differences in plant number.

Root rot assessment

Seven weeks after seeding, the plants were carefully removed from the soil and the root systems were thoroughly washed. The aboveground portion of each plant was detached and the SCI and roots were placed on filter paper in a plant press and left to dry for 1 week in a cabinet at 60°C, and then stored at 21 ± 2°C until they were examined. The dried root systems were separated from the plant crown and SCI and weighed. The SCI from each plant was visually rated for disease severity on a 1 to 4 scale (1 = healthy, 2 = less than 25% discolouration, 3 = 25–50% discolouration, and 4 = 50–100% discolouration), as described by Ledingham et al. (1973). The SCI and the root system from each plant were stored together in a glassine bag.

Image analysis

Image analysis was carried out separately for the SCI and the root system of each plant. The procedure was used to determine that the total area and mean intensity (discolour-

Table 1. The effect of barley cultivar on plant number (N) and growth and discolouration of the subcrown internode (SCI) and roots in soil naturally infested with *Cochliobolus sativus* for experiment 1.

		Roots			SCI		
Cultivar	N	Total area	Mean intensity[a]	Weight (mg)	Total area	Mean intensity	Visual rating[b]
Bonanza	44.8±0.5	184±7	188±1	261±13	21.1±0.3	124±2	3.31±0.05
Gateway	35.4±0.5	136±7	183±1	184±13	23.7±0.4	102±2	3.74±0.04
P_C	***	**	*	**	***	***	***
P_{CT}	**	ns	ns	ns	ns	*	**

Note: Values shown are mean ± standard error (8 df). P_C and P_{CT} refer to the P values for the cultivar effect and the cultivar × test interaction, respectively. ns, *, **, *** indicate not significant, significant at $P = 0.05$, 0.01, and 0.001, respectively.

[a]Mean gray scale intensity was assessed using an image analyser (0 = black, 255 = white).

[b]Visual ratings of discolouration of the SCI were based on a 1–4 scale (1 = healthy, 4 = 50–100% discolouration).

ation) of the sample were the same as those described by Kokko et al. (1995), except that the seminal and crown roots were combined together as one sample. The image analyser measured mean intensity on a grey scale ranging from 0 (black) to 255 (white). Images were acquired with a Dage 81 B/W video camera (DAGE-MTI, Inc. Michigan City, Ind.) mounted on a Bencher M2 photomacrography stand (Bencher, Inc., Chicago, Ill.). Images were input or digitized with 512 × 512 pixel resolution in a Tracor Northern 8502 image analyser (Noran, Inc., Middleton, Wis.).

Statistical analyses

Analyses of variance were carried out for each experiment to determine the effects of cultivar and disease on root and SCI variables (Steel and Torrie 1980). Within each experiment, analyses were carried out over the two tests, with tests being regarded as a fixed effect. Since the number (N) and variance (V) of plant observations of root and SCI variables in a plot were generally not homogeneous, weighted least squares analysis (Draper and Smith 1981) using weights N/V were performed on the plot means. Analyses of covariance were performed, with the number of plants in the experimental unit being used as a covariate in an attempt to adjust for competition effects among plants within a container. Analyses of variance were also carried out to compare the SCI mean intensities among the cultivars for the disease assessment categories. Covariance analyses were carried out over tests for each experiment to study the effect of mean intensity of the SCI on root growth and root and SCI discolouration. SAS software was used for the statistical analyses (SAS Institute Inc. 1989).

Results

Analyses of variance over tests for experiment 1 indicated that the main effect of cultivar was generally highly significant ($P < 0.01$) for the plant number and the root and SCI response variables (Table 1). The cultivar × test interaction was significant ($P < 0.05$) for the plant number and mean intensity and visual rating of the SCI variables. However, the interaction terms for these variables were small in magnitude compared with the main effects, and correspondingly, cultivar effects were similar for the tests. In the naturally infested soil, the moderately resistant cv. Bonanza had a greater root area ($P < 0.01$), root mean intensity (i.e., whiter) ($P < 0.05$), root weight ($P < 0.01$), and a smaller SCI ($P < 0.001$) than the susceptible cv. Gateway. Cultivar Bonanza had whiter SCIs than cv. Gateway, as indicated by the visual rating and mean intensity of the SCIs. In experiment 1, there were fewer ($P < 0.001$) plants of cv. Gateway than of cv. Bonanza.

For experiment 2, there were highly significant ($P < 0.001$) cultivar main effects and soil treatment × cultivar interactions for SCI mean intensity and visual rating (Table 2). The mean intensity of the SCI was much higher in cv. Bonanza than in cv. Gateway in all the tests in the naturally infested soil ($P < 0.01$). The total area of the SCI of cv. Gateway was larger ($P < 0.05$) than that of cv. Bonanza when averaged over both soil treatments.

The total area, weight, and mean intensity of the roots were similar ($P > 0.05$) for cv. Bonanza and cv. Gateway in the naturally infested and fumigated soils (Table 2), except in test 4, where root area of cv. Bonanza was greater ($P > 0.05$) than that of cv. Gateway (327 ± 12 and 288 ± 14 for cv. Bonanza and cv. Gateway, respectively). The mean intensity of the roots of cv. Bonanza tended ($P < 0.10$) to be higher (i.e., whiter) than cv. Gateway in the naturally infested soil.

There were fewer plants of cv. Gateway in comparison to cv. Bonanza in the naturally infested soil in test 3, but not in test 4, where plant numbers were adjusted by thinning the plants. In fumigated soil, the number of plants was similar for both cultivars in the two tests. The inconsistent cultivar effect in test 3 and thinning of plants in test 4 led to the significant ($P < 0.05$) soil × cultivar × test interaction (Table 2).

The SCI mean intensities were higher (i.e., whiter) ($P < 0.01$) and visual ratings were lower in the fumigated soil than in naturally infested soil, although the magnitude of the differences was less for cv. Bonanza than for cv. Gateway (Table 2). The area of the SCI was greater ($P < 0.05$) in the naturally infested soil than in the disease-free soil (24.2 ± 0.3 and 22.3 ± 0.3, respectively) in test 3, but not in test 4, where the SCI areas for the two soil treatments were similar. There was little evidence of an effect of soil treatment on root area and weight, but in test 4 the root mean intensities were higher (whiter) ($P < 0.05$) in the disease-free than in the pathogen-infested soil (181 ± 4 and 164 ± 5, respectively). Plant numbers for cv. Gateway were lower in root rot compared with fumigated soil in test 3, where

Table 2. The effect of barley cultivar on plant number (N) and the growth and discolouration of the subcrown internode (SCI) and roots in naturally infested and fumigated soil for experiment 2.

			Roots			SCI		
Soil	Cultivar	N	Total area	Mean intensity[a]	Weight (mg)	Total area	Mean intensity	Visual ratings[b]
FM	Bonanza	61.3±2.3	231±9	184±1	294±12	25.1±0.5	187±1	1.09±0.02
	Gateway	59.8±2.3	222±11	183±1	302±17	26.6±0.7	188±1	1.11±0.02
NI	Bonanza	61.7±2.3**	228±10	178±1	273±15	25.1±0.6*	122±1***	3.33±0.04***
	Gateway	48.2±2.3	209±12	174±2	258±16	27.4±0.7	101±2	3.75±0.03
P_S		*	ns	ns	ns	ns	***	***
P_C		*	ns	ns	ns	*	***	***
P_{SC}		*	ns	ns	ns	ns	***	***
P_{ST}		*	ns	*	ns	**	*	ns
P_{CT}		ns	ns	ns	ns	ns	ns	ns
P_{SCT}		*	*	*	ns	ns	ns	ns

Note: Values shown are mean and standard error (8 df). P_S, P_C, P_{SC}, P_{ST}, P_{CT}, and P_{SCT} refer to the P values for the soil treatment effect, cultivar effect, soil × cultivar interaction, soil × test interaction, cultivar × test interaction, and soil × cultivar × test interaction, FM refers to fumigated soil, NI refers to naturally infested soil, and ns, *, **, *** indicate not significant, significant at $P = 0.05$, 0.01, and 0.001, respectively.

[a]Mean grey scale intensity was assessed using an image analyser (0 = black, 255 = white).

[b]Visual ratings of discolouration of the SCI were based on a 1–4 scale (1 = healthy and 4 = 50–100% discolouration).

Table 3. Slopes and standard errors for linear regressions of area, mean intensity, and weight of barley roots on mean intensity of the subcrown internode for experiments 1 and 2.

		Regression variables[a]		
Experiment	Test	TRA vs. SMI	RMI vs. SMI	RWT vs. SMI
1[b]	1+2	1.06±0.56	0.25±0.06***	1.74±1.04
		(0.17)	(0.52)	(0.14)
2	3	–0.34±0.22	–0.05±0.04	–0.26±0.34
	4	1.06±0.34**	0.21±0.04***	1.79±0.49**
		(0.38)	(0.65)	(0.41)

Note: TRA refers to total root area, SMI refers to subcrown internode mean intensity, RMI refers to root mean intensity, and RWT refers to root weight. Values in parentheses are the portion of the error variation in the experiment accounted for by the regression. *, **, *** Slope is significant at $P = 0.05$, 0.01, and 0.001, respectively.

[a]Mean gray scale intensity and root area were assessed using an image analyser.

[b]Average within test slope and standard error is given for experiment 1 since the slopes for the tests were homogeneous ($P > 0.05$).

plants had not been thinned. Root rot levels in the fumigated soil were low in both tests of experiment 2.

It was of interest to determine whether there was a difference in the mean intensity of the SCIs for cv. Bonanza and cv. Gateway when they both had the same visual classification. Categories 1 (healthy) and 4 (severe) had the most plants for this comparison. In experiments 1 and 2, SCIs of cv. Bonanza in category 4 had mean intensities of 107 ± 1.6 and 108 ± 0.8, respectively, which were significantly greater ($P < 0.001$) than the corresponding mean intensities of 95 ± 1.4 and 94 ± 0.7 for SCIs of cv. Gateway in the same category. Since fumigated soil was not used in experiment 1, there were insufficient plants in category 1 to allow a cultivar comparison. However, in experiment 2, the mean intensities of the SCIs in category 1 was 189 ± 1.3 for cv. Bonanza and 190 ± 1.4 for cv. Gateway, which were not significantly different ($P > 0.05$).

The covariance analyses indicated that slopes of the regressions of area, mean intensity, and weight of barley roots on mean intensity of the SCI were homogeneous ($P > 0.05$) for the tests in experiment 1, but not for those in experiment 2 ($P < 0.01$). The relationship between mean intensity of the SCI and the root area and weight was significant ($P < 0.01$) for test 4 in experiment 2 (Table 3) and indicated that root areas and weights rose with increases in the mean intensity of the SCI. The mean intensity of the roots increased ($P < 0.001$) as the mean intensity of the SCI increased in experiment 1 and in test 4 of experiment 2.

There was a close relationship between SCI mean intensity and visual rating of the SCI as indicated by the high correlation ($r = -0.97$, $P < 0.001$, $N = 44$) that was obtained using the mean values for the experimental units over tests (data not shown). A high correlation was also observed between total root area and root weight ($r = 0.96$, $P < 0.001$) (data not shown).

The addition of the number of plants in a plot as a covariate in the above analyses was generally not significant ($P > 0.05$) and did not affect the conclusions presented here.

Discussion

This study demonstrated that in the absence of common root rot, cv. Gateway and cv. Bonanza had similar amounts of root growth. Based on this observation, it appears that any differences in root growth between the two cultivars in the naturally infested soil could be primarily attributed to their response to common root rot. In the naturally infested soil, root area and weight in cv. Gateway were reduced in comparison to that of cv. Bonanza, although this effect was not statistically significant in experiment 2.

The results of this study indicate that under certain conditions, common root rot can have an adverse effect on root growth, especially in susceptible cultivars such as cv. Gateway. Root rot is most severe under dry conditions (Grey et al. 1991), so the extent of differences in root growth between the two barley cultivars and the response to soil treatment would be greater if the plants were grown under high moisture stress.

Common root rot on the SCI does not seem to have had a major effect on the discolouration of the root system of barley. The resistant cv. Bonanza had whiter roots than the susceptible cv. Gateway in naturally infested soil in both experiments, but the difference between cultivars was significant ($P < 0.05$) only in experiment 1. Soil treatment had no overall effect on the mean intensity of the roots. These results are similar to those from an earlier image analysis study of root rot in wheat (Kokko et al. 1995), in which the disease was shown to have no effect on the root mean intensity. In Australia, Fedel-Moen and Harris (1987) observed that *C. sativus* could be readily isolated from the roots of barley, but occurred predominantly on the SCIs and crowns. They also found several species of *Fusarium* that could be regularly isolated from the roots and were responsible for discolouration of root cortical tissue and a reduction in dry root weight. In the present study, any root infection that occurred, whether it was caused by *C. sativus* or *Fusarium* spp., did not greatly affect root discolouration.

Cochliobolus sativus has been reported to cause seedling mortality in both wheat (Hanson and Christensen 1953) and barley (Mathre 1982), but reductions in seedling survival were usually related to infected seed rather than infested soil. Grey et al. (1991) reported that under a variety of moisture regimes, inoculation of the soil with *C. sativus* reduced barley stands in comparison with the disease-free checks or inoculation with *Fusarium culmorum* (W.G.Sm.) Sacc., but reductions in plant numbers did not reduce grain yield. The current study suggests that the number of plants in susceptible cultivars could be substantially reduced in naturally infested soils. Any decrease in interplant competition caused by reductions in plant number of cv. Gateway did not appear to affect the amount of root growth or discolouration. Adjusting for differences in plant number generally did not improve the relationship between the mean intensity of the SCI and root area or discolouration. In test 4, adjusting plant numbers by thinning the seedlings also did not change the response to root rot in the two cultivars in comparison to the other tests.

Total area of the SCI was greater in cv. Gateway than in cv. Bonanza. Differences in the length of the SCI among wheat (Kokko et al. 1993) and barley cultivars (Dofing and Schmidt 1984) have been previously observed. Cultural factors such as deep seeding are known to increase the length of the SCI, making barley plants more prone to common root rot (Duczek and Piening 1982). Sallans (1961) noted cultivar differences in depth of crown was a direct function of the length of the SCI.

The use of image analysis provides a precise, unbiased method for quantifying the effects of plant diseases on root growth and discolouration. Image analysis procedures have been criticized as being too involved for routine use (Tinline et al. 1994). However, roots and the SCI of a plant could be washed in less than a minute and evaluated with the image analyser in the same length of time, which is not excessive if a high level of precision is required. This study found that a close relationship existed between total root area as measured by the image analyser and root weight. There was also strong agreement between the mean intensity of the SCI and its visual rating. This indicates that image analysis can accurately measure SCI discolouration, but provides greater objectivity and precision than does visual assessment. It was interesting to note that there was a significant cultivar difference in the mean intensity of SCIs in the severe category. Even though the SCIs of both cultivars had most of their surface covered by root rot lesions, the SCIs of cv. Gateway were more deeply discoloured. It would be interesting to determine if this difference in colouration was due to either later infection or less extensive hyphal growth of *C. sativus* on the SCIs of cv. Bonanza.

The barley cultivars in this study were selected because previous research had indicated that cv. Bonanza was highly resistant to common root rot (Piening 1973; Tinline and Ledingham 1979) and cv. Gateway was highly susceptible and its yield was reduced by as much as 42.3% by severe infection (Piening 1973). Other susceptible barley cultivars have not shown a similar negative yield response to severe root rot infection and for this reason are considered to be root rot tolerant (Piening 1973; Tinline and Ledingham 1979; Duczek and Wildermuth 1993; Bailey et al. 1997). It seems likely that an examination of root growth using image analysis would provide new insights into the way tolerant cultivars minimize or prevent yield losses caused by common root rot.

Acknowledgments

The authors thank B.J. Nishiyama for his assistance with the statistical analysis of this study. We also thank R.M. Clark, A.J. Lamb, and N.J. Thurston for their technical assistance.

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