Wheat Crown Rot Pathogens *Fusarium graminearum* and *F. pseudograminearum* Lack Specialization

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ABSTRACT

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This article reports a lack of pathogenic specialization among Australian *Fusarium graminearum* and *F. pseudograminearum* causing crown rot (CR) of wheat using analysis of variance (ANOVA), principal component and biplot analysis, Kendall's coefficient of concordance (*W*), and κ statistics. Overall, *F. pseudograminearum* was more aggressive than *F. graminearum*, supporting earlier delineation of the crown-infecting group as a new species. Although significant wheat line–pathogen isolate interaction in ANOVA suggested putative specialization when seedlings of 60 wheat lines were inoculated with 4 pathogen isolates or 26 wheat lines were inoculated with 10 isolates, significant *W*

and κ showed agreement in rank order of wheat lines, indicating a lack of specialization. The first principal component representing nondifferential aggressiveness explained a large part (up to 65%) of the variation in CR severity. The differential components were small and more pronounced in seedlings than in adult plants. By maximizing variance on the first two principal components, biplots were useful for highlighting the association between isolates and wheat lines. A key finding of this work is that a range of analytical tools are needed to explore pathogenic specialization, and a statistically significant interaction in an ANOVA cannot be taken as conclusive evidence of specialization. With no highly resistant wheat cultivars, *Fusarium* isolates mostly differ in aggressiveness; however, specialization may appear as more resistant cultivars become widespread.

Additional keywords: κ statistics.

Species of *Fusarium*, including *Fusarium graminearum* and *F. pseudograminearum*, causing necrosis and dry rot of the crown, basal stem, and root tissue commonly known as crown rot (CR), are widespread in many cereal-producing countries, including Australia, South Africa, and the United States (7,16,28). *F. pseudograminearum* is the predominant CR pathogen in the 11-million-ha wheat-growing region in Australia (5) where, each year, nearly $80 million is lost from reduced grain yield and quality due to CR (22). Both *F. graminearum* and *F. pseudograminearum* can cause equally severe CR in bioassays (3). In the Pacific Northwest of the United States, *F. pseudograminearum* can reduce winter wheat yield by up to 61% (28). These and other *Fusarium* spp. are better known for recent Fusarium head blight (FHB) epidemics in Canada, China, Europe, South America, and the United States (13). CR inoculum survives for 2 years or longer in residues of infected winter cereals and grass hosts (7). As in FHB, widespread adoption of zero tillage has caused resurgence in CR in many cereal-producing countries, including Australia (7,8,16,28). In the absence of cultivars with a high level of resistance, tillage and stubble management (31), crop rotation (14,16), and planting partially resistant cultivars are used to manage CR.

In recent years, there has been a coordinated approach to develop CR-resistant wheat cultivars in Australia (8). In screening for disease resistance, it is essential to determine whether the host–pathogen association is differential or nondifferential. A differential association occurs when the severity of disease in-

duced by a particular pathogen genotype is dependent upon the host genotype (10). In contrast, a nondifferential association is defined when the severity of disease that results from a particular pathogen genotype is not influenced by the host genotype (10). A nondifferential interaction indicates a nonspecialized pathogen whereas differential interaction is a sign of specialization. A race of a specialized pathogen has the genetic ability, often termed virulence, to overcome genetically determined host resistance, which is effective against other races, whereas a nonspecialized pathogen uses aggressiveness to damage the host without regard to resistance genes (25). A single highly aggressive isolate would be sufficient for resistance screening if the pathogen is nonspecialized but all races would have to be used if there is specialization.

There is extensive genetic diversity among both *F. graminearum* and *F. pseudograminearum* in Australia and elsewhere (1,6,19), despite their distinct mode of reproduction, phylogeny, and lineage development (24,29). However, there is little pathogenic specialization in *F. graminearum* for FHB and isolates mostly differ in aggressiveness (30), although some studies, including those using Australian *F. graminearum* and *F. pseudograminearum*, reveal significant isolate–wheat cultivar interaction (2). Pathogenic specialization for CR was first suggested in 1967 (23), where forms of *F. graminearum* caused either FHB or CR. The crown-infecting *F. graminearum* group 1 was later named *F. pseudograminearum* (4). Although variation for CR aggressiveness among isolates has been recorded (20,27) no study has examined pathogenic specialization for CR in *F. pseudograminearum*.

A statistically significant host genotype–pathogen isolate interaction in an analysis of variance (ANOVA) has often been used as an indication of pathogenic specialization. It is essential to verify whether the interaction stems from a significant change

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in rank order of host genotypes by the pathogen isolates. Kendall's coefficient of concordance (W) (12) and κ statistics (15) can test for changes in rank order. Other analytical tools such as principal component and discriminant analysis can also be used to detect and classify pathogenic races in some instances (2,9). The primary aim of this study is to examine whether there is

pathogenic specialization for CR in selected Australian isolates of *F. pseudograminearum* and *F. graminearum*. By applying ANOVA, Kendall's W, and κ statistics for rank order and principal component and biplot analysis, this article also compares how each of these statistical tools explains host–pathogen interaction.

MATERIALS AND METHODS

Source of wheat germplasm and pathogen isolates. Wheat germplasm from the Australian Winter Cereals Collection at New South Wales Department of Primary Industries, Tamworth and from other sources are routinely screened for CR resistance in the Commonwealth Scientific and Industrial Research Organisation (CSIRO) (18). Based on these results, a subset of 56 hexaploid wheat and 4 durum wheat lines was selected to span the available range of CR-resistant to -susceptible lines (Table 1). Five mono-conidial isolates each of *F. pseudograminearum* (CS3096, CS3286, CS3321, CS3427, and CS3442) and *F. graminearum* (CS3005, CS3200, CS3187, CS3192, and CS3255) from a CSIRO collection were selected to span the available range of CR aggres-siveness based on previous studies (3,20). All isolates were collected from field surveys of infected wheat crops in different regions of eastern Australia.

CR reaction of wheat lines. All 60 wheat lines were screened using a highly aggressive and a weakly aggressive isolate, CS3096 and CS3442 of *F. pseudograminearum* and CS3005 and CS3200 of *F. graminearum*, respectively, using a CR bioassay (21). There were three replications and the replications were performed over time in July, August, and September 2005, respectively. Each time, six seedlings were grown in sterilized potting mix (50% sand and 50% peat) in 5-by-5-by-5-cm punnets with one seedling per punnet in seedling trays (Rite grow kwik pots, www.Gardencityplastics.com). The seedling trays were placed in a temperature- and relative humidity (RH)-controlled glasshouse with night and day temperatures of 15 and 25°C ($\pm$1°C), respectively, at 65% ($\pm$5%) RH. Ten days after emergence, seedlings were laid horizontally on their side and inoculated by placing a 10-μl droplet of a 10^6 macroconidia/ml suspension of a single isolate on the stem base, ≈0.5 cm away from the soil surface. Inoculated seedlings were incubated at near-saturated RH in darkness for 48 h inside a plastic tent (35-by-77-by-40 cm) positioned on a galvanized metal tray (37-by-80-by-5 cm) with a thin film of water. Following this, seedlings were placed upright in seedling trays, CR severity was assessed 35 days after inocu-lation and a CR severity index was calculated as follows: CR severity index = (length of stem discoloration/seedling height) × (number of leaf sheath layers with necrosis).

With a maximum of seven leaf sheath layers at the time of severity assessment, the CR severity index ranged from 0, for no infection, to 7, when the entire length of stem was discolored and all seven leaf sheath layers were infected.

Pathogenic specialization. All 10 isolates of *F. graminearum* and *F. pseudograminearum* were used to study pathogenic specialization. These included the four isolates used in CR screening of the 60 wheat lines. A subset of 22 hexaploid and 1 durum wheat lines was selected from the 60 lines, and 3 addi-tional wheat lines (Ernie, Fredrick, and Janz) were added to these (Table 2). All subsequent work involved these 26 lines and 10 isolates. Screening for CR severity in the glasshouse largely followed methods outlined in the previous section. There were three replications and the replications were performed over time with six seedlings at each time in November 2005, March 2006, and April 2006, respectively. Of the six seedlings, four were destructively sampled 35 days after inoculation, CR severity index was determined as described above, and data was averaged for the four seedlings and, henceforth, referred to as seedling severity. The two remaining plants from each time were repotted

TABLE 1. Eigenvectors for the first three principal components (PC1–PC3) from an analysis of square-root-transformed (severity + 0.5) crown rot severity index data on seedlings of 60 wheat lines inoculated with four *Fusarium* isolates[a]

No.	Wheat line	PC1	PC2	PC3
1	2-49	0.148	0.041	**0.175**
2	Abura Komugi	**0.169**	–0.002	–0.076
3	Aso Zairai	0.131	0.139	–0.092
4	Aso Zairai II	**0.157**	–0.053	0.123
5	Batavia	0.117	–0.137	**–0.158**
6	Baxter	0.025	**0.226**	–0.032
7	Chile	0.082	–0.127	**0.252**
8	Freedom	0.124	**–0.160**	–0.005
9	Frontana	0.085	**0.170**	**0.166**
10	Gala	0.135	0.139	–0.054
11	Giles	0.030	**0.223**	0.060
12	Gluyas Early	**0.150**	–0.081	–0.124
13	Itou Komugi	0.149	–0.112	–0.047
14	Kagoshima	0.136	–0.138	0.049
15	Kamilaroi	0.047	**–0.218**	0.058
16	Kennedy	**0.167**	–0.055	–0.028
17	Kikuchi	0.113	–0.147	0.146
18	Lang	0.093	**0.170**	–0.146
19	Mulgara	0.113	**0.169**	–0.063
20	Ning 7840	0.113	**0.159**	–0.113
21	Norm	0.077	**0.194**	–0.108
22	Nyuubai	0.120	–0.120	**0.181**
23	Pioneer 2375	0.014	0.136	**0.295**
24	Pretel	0.123	**0.159**	0.043
25	Puseas	0.048	**0.193**	**0.171**
26	QT10198	**0.155**	0.053	–0.138
27	QT10776	0.124	**–0.154**	–0.064
28	Qiamai	0.143	–0.001	**–0.205**
29	Rowan	0.146	–0.027	**–0.192**
30	SVP 72017	**0.151**	–0.109	–0.024
31	Shiro Nankin	0.150	–0.111	–0.037
32	Shou Komugi II	0.140	–0.131	0.054
33	Soba Komugi IC	0.103	**0.184**	–0.013
34	Sotome A	**0.159**	–0.031	–0.135
35	Strezleki	0.087	0.115	**0.259**
36	Sumai 3	0.114	**–0.163**	0.088
37	Sunbrook	0.117	**0.157**	–0.098
38	Sunco	**0.169**	0.043	–0.015
39	Sunlin	0.130	0.100	**0.180**
40	Sunstate	0.110	0.079	**0.253**
41	Sunvale	0.059	**0.211**	–0.072
42	Tamaroi	–0.129	0.123	–0.144
43	Wallaroi	0.137	–0.113	–0.130
44	Yallaroi	0.039	**0.212**	0.113
45	Zairai Yuubou	0.130	–0.096	**0.187**
46	Sotome	**0.172**	0.004	–0.021
47	Nobeokabou Komugi	**0.161**	–0.064	–0.082
48	CSCR1	**0.158**	0.084	0.056
49	CSCR2	**0.168**	–0.040	0.058
50	CSCR3	**0.160**	–0.012	0.136
51	CSCR4	0.141	0.117	–0.100
52	CSCR5	0.150	0.109	0.050
53	CSCR6	**0.160**	0.070	–0.082
54	CSCR7	**0.171**	0.002	–0.043
55	CSCR8	**0.155**	–0.099	0.032
56	CSCR9	**0.155**	0.096	–0.051
57	CSCR10	0.149	–0.112	0.038
58	CSCR11	0.139	0.105	–0.139
59	CSCR12	0.036	0.115	**0.309**
60	CSCR13	0.118	**–0.167**	0.013
	Variation (%)[b]	…	56	87	100

[a] Values >0.15 are in bold.
[b] Cumulative percent variation explained.

in pots (5 cm^2 in area and 12 cm deep) 35 days from inoculation, and plants were grown to maturity in the glasshouse. At harvest, each yielding tiller was separately assessed for its length and the length of stem browning from crown. For adult plants, only the proportion of stem browning for each tiller was used as a measure of CR severity because the number of infected leaf sheath layers could not be correctly determined.

Data analysis. Pathogenic specialization was explored using ANOVA, rank order, and principal component analysis (PCA). The SAS software (version 9.1; SAS Institute Inc., Cary, NC) was used in all analyses. For ANOVA, seedling data on the CR severity index of 60 wheat lines and both seedling and adult plant severity of the 26 lines were square root transformed (severity + 0.5) to stabilize variance. The fixed effects of wheat line and isolate and their interaction were determined using the MIXED procedure. When the wheat line–isolate interaction was significant, the isolate effect on each wheat line and the wheat line effect on each isolate were tested using the SLICE option on the least square means in PROC MIXED. Kendall's W and κ statistics were used to examine whether significant line–isolate interaction from ANOVA was due to significant changes in rank order. The '*magree*' macro available from the SAS user library (http://support.sas.com/kb/25/006.html) was used to compute W and κ statistics after ranking observations using the RANK procedure in SAS. This macro tests for agreement among multiple raters (isolates) for their ranking of wheat lines. A high degree of agreement among isolates signifies a lack of specialization.

For PCA, square-root-transformed data on CR severity were averaged over replicates to form a mean value for each isolate, and each line was considered as a separate variate. This generated a data matrix with 4 isolates (columns) and 60 lines (rows) for the first experiment and 10 isolates and 26 lines for the second experiment. Principal component scores were calculated from the correlation matrix of observations (isolates) and variates (wheat lines) using the PRINCOMP procedure in SAS. Correspondence analysis, which is a form of weighted PCA to compute scores on

each dimension for each row and column in a contingency table, was initially applied using PROC CORRESP to the square-root-transformed data on 60 lines. This analysis provides a χ^2 statistic as a measure of association between rows and columns and inertia, which is proportional to the χ^2 and conceptually similar to variance in PCA (17).

Biplots were generated to explore the association between isolate and wheat lines (32). Data on CR severity, averaged over replicates to form a mean value for each line and each isolate, which were used in PCA, were transposed for biplot analysis. This generated a data matrix with 60 observations and 4 variates for the 60 lines and 26 observations and 10 variates for the 26 lines. To generate biplots, PROC PRINQUAL with the MDPREF option was used to monotonically transform the data to maximize

TABLE 3. Analysis of variance using square-root-transformed (severity + 0.5) crown rot severity index data on seedlings or adult plants of wheat lines inoculated with *Fusarium* isolates

Effect	df	F value	$P > F$
Seedlings			
60 lines[a]			
Isolate	3	109.20	<0.0001
Line	59	7.47	<0.0001
Line–isolate	177	2.37	<0.0001
26 lines[b]			
Isolate	9	85.03	<0.0001
Line	25	10.09	<0.0001
Line–isolate	225	1.97	<0.0001
Adult plants			
26 lines[c]			
Isolate	9	7.76	<0.0001
Line	25	34.2	<0.0001
Line–isolate	198	1.15	0.099

[a] Seedlings of 60 lines inoculated with four isolates.
[b] Seedlings of 26 lines inoculated with 10 isolates.
[c] Adult plants of 26 lines inoculated with 10 isolates.

TABLE 2. Eigenvectors for the first five principal components (PC1–PC5) from an analysis of square-root-transformed (severity + 0.5) crown rot severity data of seedling and adult plants of 26 wheat lines inoculated with 10 *Fusarium* isolates[a]

Wheat line	Seedling severity index					Adult plant severity				
	PC1	PC2	PC3	PC4	PC5	PC1	PC2	PC3	PC4	PC5
2-49	0.22	0.11	–0.21	–0.19	0.09	0.21	–0.21	–0.03	0.10	**0.38**
Aso Zairai	0.22	0.07	0.09	0.11	–0.13	0.23	0.20	–0.13	–0.07	0.02
Baxter	0.18	**0.25**	0.20	0.05	–0.18	0.18	0.10	–0.12	–0.13	**0.35**
Chile	0.22	–0.06	–0.07	**0.30**	**0.28**	0.20	0.09	–0.02	–0.21	**–0.31**
CSCR1	0.19	–0.22	–0.24	0.09	–0.02	0.16	–0.02	0.10	**0.49**	0.06
CSCR12	0.22	–0.15	0.00	0.18	0.04	0.17	**–0.33**	–0.02	–0.02	–0.01
CSCR2	0.15	0.09	**–0.41**	0.15	**0.33**	0.24	0.13	0.11	0.03	0.19
CSCR3	0.23	–0.09	0.03	0.06	**0.26**	0.17	0.24	0.03	–0.21	**–0.30**
CSCR7	0.20	–0.23	0.16	–0.20	0.07	0.23	0.05	–0.23	0.22	0.05
CSCR8	0.22	–0.18	0.10	0.10	0.09	0.24	–0.04	–0.09	–0.07	–0.23
Ernie	0.21	–0.21	0.01	–0.16	–0.16	0.16	0.14	**0.37**	**0.25**	–0.01
Fredrick	0.21	–0.22	–0.02	–0.15	0.13	0.14	–0.13	**0.44**	–0.11	0.06
Frontana	0.22	0.11	0.03	–0.12	**–0.32**	0.21	–0.03	–0.19	**–0.26**	–0.02
Giles	0.19	0.11	**–0.35**	0.08	0.14	0.22	0.21	–0.05	–0.10	**0.32**
Gluyas Early	0.15	**0.28**	0.04	**–0.51**	0.24	0.21	–0.21	–0.04	–0.18	–0.24
Janz	0.20	–0.22	0.07	–0.10	**–0.28**	0.12	0.00	**–0.30**	**0.37**	–0.13
Kennedy	0.20	–0.19	0.24	–0.11	–0.07	0.23	–0.21	0.17	0.08	0.01
Kikuchi	0.17	**–0.33**	–0.06	–0.20	–0.03	–0.06	**0.43**	0.01	0.22	0.04
Lang	0.20	–0.02	–0.08	**0.39**	**–0.33**	0.23	0.08	–0.12	–0.19	0.10
Mulgara	0.20	**0.25**	–0.13	0.08	–0.17	0.25	0.19	–0.01	0.08	–0.08
Pioneer 2375	0.14	**0.35**	**0.26**	–0.01	–0.01	0.19	–0.16	**0.28**	0.01	–0.07
Rowan	0.08	–0.04	**0.53**	0.20	**0.46**	0.20	0.21	**–0.25**	0.03	0.04
Sotome A	0.20	**0.25**	–0.13	–0.01	0.02	0.22	–0.11	0.24	–0.02	–0.09
Sumai 3	0.24	0.05	–0.03	–0.01	–0.11	0.18	**–0.25**	–0.06	0.17	0.19
Sunvale	0.20	0.22	0.00	**–0.27**	0.07	0.19	0.14	0.10	0.25	**–0.40**
Tamaroi	0.18	0.22	**0.28**	**0.27**	–0.04	0.10	0.22	**0.28**	**–0.28**	0.21
Variation (%)[b]	65	79	89	93	95	47	62	74	82	87

[a] Values >0.25 are in bold.
[b] Cumulative percent variation explained.

the proportion of variance accounted for by the first two principal components. PROC FACTOR was used on the monotonically transformed data to perform a PCA and the biplot was created using the %PLOTIT macro available as part of the SAS autocall library.

RESULTS

CR reaction of wheat lines. ANOVA results of CR severity index of four isolates infecting 60 wheat lines in a glasshouse bioassay showed significant effect of wheat line, isolate, and their interaction (Table 3), suggesting a differential host–pathogen response. When tested using the SLICE option, there was a significant difference in CR severity among the 60 lines for each of the four isolates (data not shown). In contrast, there was no significant difference between the four isolates for 26 of the 60 lines: 2-49, Aso Zairai, Chile, CSCR4, CSCR5, CSCR8, CSCR9, CSCR11, CSCR12, Freedom, Frontana, Kamilaroi, Kikuchi,

Mulgara, Norm, Nyuubai, Rowan, Shou Komugi II, Soba Komugi IC, Sotome A, Sunco, Sunstate, Tamaroi, Wallaroi, Yallaroi, and Zairai Yuubou.

Inertia and χ^2 decomposition from a correspondence analysis showed that 29 dimensions were necessary to explain 90% of the total χ^2 and inertia. Each wheat line explained only a small component of the variation. In contrast, the first three components explained 100% of the variation in a PCA, indicating that variation in CR reaction of the 60 lines had three major dimensions. Because the emphasis of this article is on the specialization of isolates rather than host resistance, PCA made more biological sense and was used in all subsequent analyses. The first principal component (PC1) explained 56% of the variation and its eigenvector had positive weights for all the wheat lines except for the highly susceptible durum cv. Tamaroi (Table 1). Due to its overall nondifferential nature, PC1 can be considered the aggressiveness (25) component of the isolates. The second eigenvector (PC2), explaining a further 31% of variation, had large (>15%)
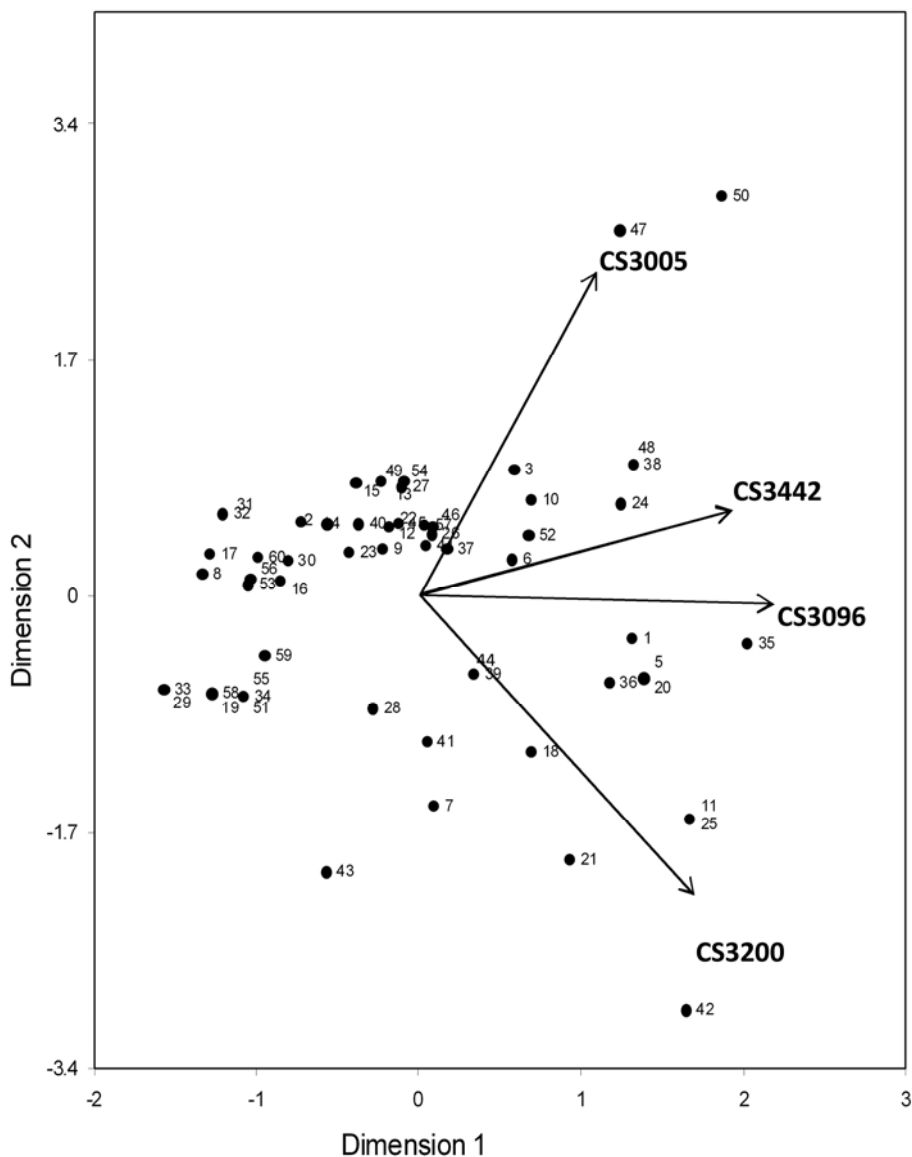

Fig. 1. Biplot of crown rot severity index data on 60 wheat lines inoculated with two isolates each of *Fusarium pseudograminearum* (CS3096 and CS3442) and *F. graminearum* (CS3005 and CS3200). Wheat lines 1, 2-49; 2, Abura Komugi; 3, Aso Zairai; 4, Aso Zairai II; 5, Batavia; 6, Baxter; 7, Chile; 8, Freedom; 9, Frontana; 10, Gala; 11, Giles; 12, Gluyas Early; 13, Itou Komugi; 14, Kagoshima; 15, Kamilaroi; 16, Kennedy; 17, Kikuchi; 18, Lang; 19, Mulgara; 20, Ning 7840; 21, Norm; 22, Nyuubai; 23, Pioneer 2375; 24, Pretel; 25, Puseas; 26, QT10198; 27, QT10776; 28, Qiamai; 29, Rowan; 30, SVP 72017; 31, Shiro Nankin; 32, Shou Komugi II; 33, Soba Komugi IC; 34, Sotome A; 35, Strezleki; 36, Sumai 3; 37, Sunbrook; 38, Sunco; 39, Sunlin; 40, Sunstate; 41, Sunvale; 42, Tamaroi; 43, Wallaroi; 44, Yallaroi; 45, Zairai Yuubou; 46, Sotome; 47, Nobeokabou Komugi; 48, CSCR1; 49, CSCR2; 50, CSCR3; 51, CSCR4; 52, CSCR5; 53, CSCR6; 54, CSCR7; 55, CSCR8; 56, CSCR9; 57, CSCR10; 58, CSCR11; 59, CSCR12; and 60, CSCR13.

positive coefficients for 13 lines and large (>15%) negative coefficients for 5 lines (Table 1), indicating differential susceptibility of these lines to the four isolates. Similarly, the third eigenvector (PC3), explaining the remaining 13% of variation, contrasts large positive responses on 2-49, Chile, Frontana, Nyuubai, Pioneer 2375, Puseas, Strezleki, Sunlin, Sunstate, Zairai Yuubou, and CSCR12, with large negative responses on Batavia, Qiamai, and Rowan.

The association between isolates and lines can be better visualized from the biplot, where the wheat lines are shown as points plotted with the first two principal components as coordinates (Fig. 1). The isolate vectors emanate from the origin of this two-dimensional space, their direction indicating the most favored association with wheat lines based on coefficients for the first two principal components. The 60 lines are generally distributed within the two-dimensional space, and 42 are located to the left of origin of the isolate vectors and cannot be associated with specific isolates (Fig. 1). Except for Nobeokabouzu Komugi (line no. 47) and CSCR3 (no. 50), showing clear association with isolate CS3005; Strezleki (no. 35) with CS3096; and Tamaroi (no. 42) with CS3200, association of the wheat lines with isolates was

generally weak. The wheat lines did not show any distinct grouping. This general lack of association between lines and isolates and the absence of clusters of wheat lines point to a lack of specialization in the four isolates.

Wheat lines were ranked based on the square-root-transformed CR severity index for each isolate and were found to have significant agreement in rank correlation among isolates according to W (Table 4). The κ statistic for the 60 wheat lines was also significant, indicating agreement in rank (Table 4). Despite a significant wheat line–isolate interaction in the ANOVA, a significant κ and W showed no significant change in rank order, indicating a lack of specialization among the four isolates toward the 60 lines.

Pathogenic specialization. To further explore specialization, an expanded set of 10 isolates, including the previous 4, were screened against 26 lines. These included 11 of the 60 lines—CSCR1, CSCR2, CSCR3, CSCR7, 2-49, Chile, Giles, Lang, Sunvale, Sumai 3, and Tamaroi—based on their differential association with the four isolates from PCA, plus an additional 12 lines—Aso Zairai, Baxter, CSCR8, CSCR12, Frontana, Gluyas Early, Kennedy, Kikuchi, Mulgara, Pioneer 2375, Rowan, and Sotome A—to represent the full range of CR severity among the

TABLE 4. Kendall's coefficient of concordance (W) and κ statistic for rank order of wheat lines inoculated with different isolates based on square-root-transformed crown rot severity of seedling and adult plants

Experiment	Kendall's W			κ Statistic		
	W	F	$P > F$	κ	Z	$P > Z$
Seedling severity 60 lines inoculated with 4 isolates	0.52	3.29	0.0001	0.014	1.75	0.04
Seedling severity 26 lines inoculated with 10 isolates	0.35	4.90	0.0001	0.008	1.32	0.09
Adult plant severity 26 lines inoculated with 10 isolates	0.60	13.73	0.0001	0.047	7.91	0.0001

TABLE 5. Square-root-transformed (severity + 0.5) crown rot severity of adult plants and seedling severity index of 26 wheat lines inoculated with five isolates each of *Fusarium graminearum* and *F. pseudograminearum* and $P > F$ for the isolate effect on each wheat line and the wheat line effect on each isolate tested using the SLICE option on least square means in PROC MIXED

Species	Isolate, line	Mean severity, seedling	$P > F$	Mean severity, adult plant	$P > F$
F. graminearum	CS3005	1.02	0.0004	0.83	<0.0001
F. graminearum	CS3187	0.95	0.0001	0.82	<0.0001
F. graminearum	CS3192	0.98	<0.0001	0.83	<0.0001
F. graminearum	CS3200	0.71	1	0.78	0.001
F. graminearum	CS3255	1.12	<0.0001	0.83	<0.0001
F. pseudograminearum	CS3096	1	0.0122	0.84	<0.0001
F. pseudograminearum	CS3286	0.81	0.9992	0.8	<0.0001
F. pseudograminearum	CS3321	1.33	<0.0001	0.87	<0.0001
F. pseudograminearum	CS3427	1.45	<0.0001	0.88	<0.0001
F. pseudograminearum	CS3442	1.03	0.0032	0.83	<0.0001
	2-49	0.99	0.0005	0.84	0.5751
	Aso Zairai	1.01	<0.0001	0.97	<0.0001
	Baxter	0.97	0.4013	0.8	0.9208
	Chile	0.95	<0.0001	0.98	0.0579
	CSCR1	0.77	0.1586	0.98	0.5829
	CSCR12	1.04	<0.0001	0.86	0.1928
	CSCR2	1.07	<0.0001	0.83	0.3792
	CSCR3	1.1	<0.0001	0.82	0.3874
	CSCR7	1.03	<0.0001	0.97	0.0032
	CSCR8	1.01	<0.0001	0.95	0.0247
	Ernie	0.92	0.0008	0.94	0.0203
	Fredrick	1.12	<0.0001	0.84	Not determined
	Frontana	1.13	<0.0001	0.84	0.7227
	Giles	0.87	0.0688	0.77	0.7999
	Gluyas Early	1.04	<0.0001	0.82	0.343
	Janz	1.03	<0.0001	0.84	0.0316
	Kennedy	1.16	<0.0001	0.81	0.0603
	Kikuchi	1.07	<0.0001	0.97	0.0008
	Lang	0.95	0.0004	0.78	0.6341
	Mulgara	1.08	<0.0001	0.77	0.6533
	Pioneer 2375	0.99	<0.0001	0.77	0.8827
	Rowan	0.89	0.2438	0.77	0.9912
	Sotome A	1.23	<0.0001	0.83	0.0104
	Sumai 3	1.33	<0.0001	0.82	0.3033
	Sunvale	1.02	0.0004	0.78	0.3389
	Tamaroi	1.04	0.0058	0.8	0.5738

60 lines. Three other lines—Ernie, Fredrick, and Janz—that were not in the original 60 but showed a high level of CR resistance in subsequent screening were also included.

CR severity index of seedlings. Isolate, wheat line, and their interaction were significant in an ANOVA of the square-root-transformed CR severity index of 26 lines inoculated with 10 isolates (Table 3). Isolates of *F. pseudograminearum* were generally more aggressive than *F. graminearum* (Table 5). Of the six most aggressive isolates, four were *F. pseudograminearum* (CS3427, CS3321, CS3442, and CS3096). *F. graminearum* CS3200 and *F. pseudograminearum* CS3286 were the least aggressive and all isolates, except these two, caused significantly different levels of severity on the 26 lines (Table 5). The CSIRO selection CSCR1 and Giles, Rowan, Ernie, Chile, Lang, and Baxter were among the most resistant lines, and the susceptibility of four of these did not change significantly with the isolate (Table 5). The susceptibility of all other lines changed significantly with the isolate, suggesting potential specialization.

Kendall's *W* was significant, indicating no change in rank order of lines among the 10 isolates, but κ was not significant at *Pr* < 0.05 (Table 4).

The first five components explained >95% of variance in a PCA. The eigenvector of PC1, with a positive weight on all 26 wheat lines, explaining 65% of the variation (Table 2), was higher than the nondifferential aggressiveness component (56%) of the 60 wheat lines inoculated with 4 isolates (Table 1). PC2 and PC3 explained another 14 and 10% of variation, respectively, while PC4 and PC5 each explained <5% of variation. In all, 22 wheat lines had large (≥25%) positive or negative coefficients for the first five principal components (Table 2, PC1–PC5). Of these,

seven wheat lines (Chile, CSCR2, Gluyas Early, Lang, Pioneer 2375, Rowan, and Tamaroi) had large coefficients for two of the principal components.

Isolates can be grouped into four broad groups from the biplot (Fig. 2). Group one, with five isolates (CS3005, CS3096, CS3187, CS3321, and CS3442), shows an association with 10 wheat lines: CSCR2 (line no. 7), CSCR3 (no. 8), CSCR7 (no. 9), Gluyas Early (no. 15), Janz (no. 16), Kennedy (no. 17), Fredrick (no. 12), Sumai 3 (no. 24), Sotome A (no. 23), and Tamaroi (no. 26). The second group of two isolates (CS3192 and CS3427) has association with Mulgara (no. 20) and Sunvale (no. 25). Group three, with two isolates (CS3200 and CS3255), shows some association with Baxter (no. 3), Frontana (no. 13), Giles (no. 14), Pioneer 2375 (no. 21), and Rowan (no. 22); and the isolate CS3286 shows a putative association with Chile (no. 4). Aso Zairai (no. 2), CSCR1 (no. 5), CSCR7 (no. 9), CSCR8 (no. 10), CSCR12 (no. 6), Ernie (no. 11), Kikuchi (no. 18), or Lang (no. 19) cannot be easily associated with any of the 10 isolates.

CR severity of adult plants. Both the isolate and wheat line main effects were significant but their interaction was not significant in the ANOVA (Table 3). The reduced degrees of freedom for the interaction term was due to some inoculated winter wheat lines, including Fredrick, failing to reach maturity. The overall difference between the two *Fusarium* spp. was not as pronounced as with the seedling data but *F. pseudograminearum* CS3427 and CS3321 were the most aggressive and *F. pseudograminearum* CS3286 and *F. graminearum* CS3200 the least aggressive isolates in both seedling and adult plant tests (Table 5). Although each of the 10 isolates caused significantly different levels of severity on the 26 lines (Table 5), the overall severity
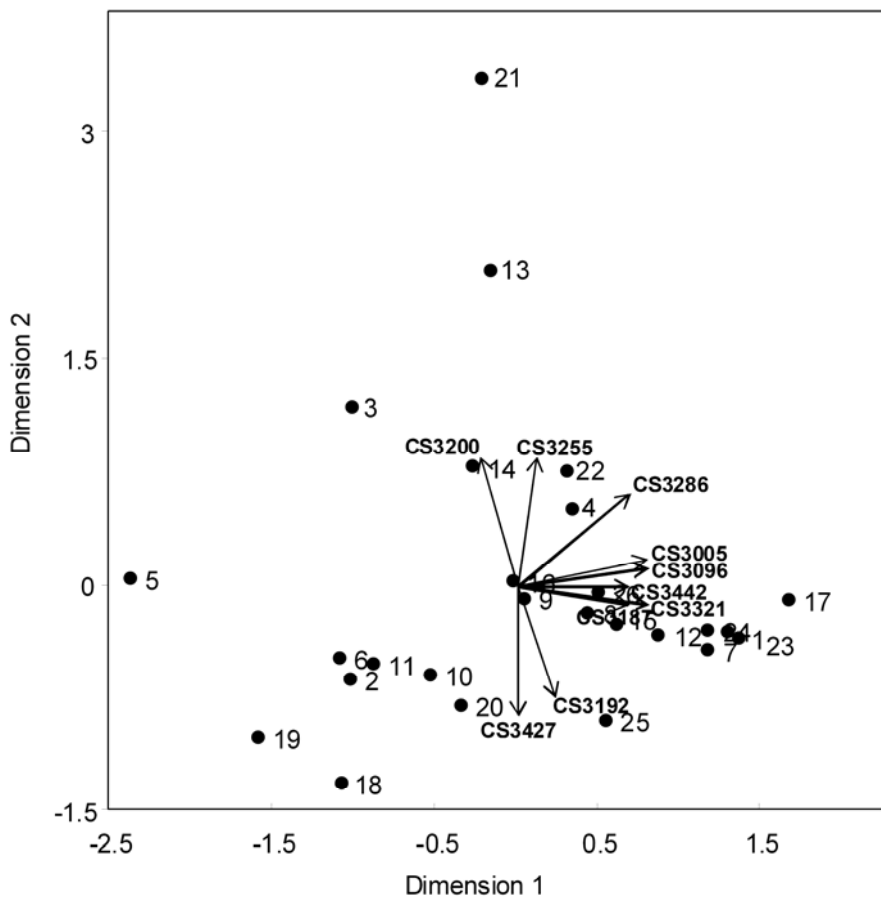

Fig. 2. Biplot of seedling crown rot severity index of 26 wheat lines inoculated with five isolates each of *Fusarium pseudograminearum* (CS3096, CS3286, CS3321, CS3427, and CS3442) and *F. graminearum* (CS3005, CS3200, CS3187, CS3192, and CS3255). Wheat lines 1, 2-49; 2, Aso Zairai; 3, Baxter; 4, Chile; 5, CSCR1; 6, CSCR12; 7, CSCR2; 8, CSCR3; 9, CSCR7; 10, CSCR8; 11, Ernie; 12, Fredrick; 13, Frontana; 14, Giles; 15, Gluyas Early; 16, Janz; 17, Kennedy; 18, Kikuchi; 19, Lang; 20, Mulgara; 21, Pioneer 2375; 22, Rowan; 23, Sotome A; 24, Sumai3; 25, Sunvale; and 26, Tamaroi.

was low. The overall low levels of severity in adult plants showed that all wheat lines were more resistant to CR and the difference between lines was not pronounced. Also, in contrast to the seedling data, the CR severity of 18 of the 25 lines did not change significantly ($Pr < 0.05$) with the isolate, indicating a lack of specialization.

Both W and κ were significant (Table 4) and higher than corresponding values for seedlings, indicating no host–pathogen specificity in adult plants.

The first five principal components explained 87% of variance, lower than for seedling data (Table 2). The mostly nondifferential PC1, with positive weight on all lines except Kikuchi, explained only 47% of variation, as opposed to 65% for seedlings. Of the 20 lines with large ($\geq 25\%$) coefficients for PC1–PC5, 3 lines (Ernie, Janz, and Tamaroi) had large coefficients for two of the principal components (Table 2). Twelve lines (Baxter, Chile, CSCR3, Frontana, Giles, Janz, Kikuchi, Lang, Pioneer 2375, Rowan, Sunvale, and Tamaroi) had large coefficients for both seedling and adult plant severity.

Wheat lines Aso Zairai (no. 2), CSCR1 (no. 5), CSCR7 (no. 9), CSCR8 (no. 10), Ernie (no. 11), Frontana (no. 13), and Kikuchi (no. 18) showed putative association with one or more isolates (Fig. 3). The remaining 19 lines cannot be easily associated with specific isolates.

DISCUSSION

This work has found a lack of pathogenic specialization among *F. graminearum* and *F. pseudograminearum* isolates for CR severity in wheat lines according to ANOVA, PCA, biplot analysis, Kendall's W, and κ statistics. Although there was significant wheat line–isolate interaction for seedling severity when 60 lines were inoculated with 4 isolates or 26 lines inoculated with 10 isolates, there was generally no significant change in rank order of lines according to Kendall's W or κ statistics, indicating a lack of pathogenic specialization. For CR severity of adult plants, a lack of pathogenic specialization was clear from a nonsignificant wheat line–isolate interaction in ANOVA, and a lack of any significant change in rank order from Kendall's W or κ statistics. In PCA, the first principal component had generally positive weights on all wheat lines and accounted for up to 65% of variation. This strong PC1 component can be considered as isolate aggressiveness due to its nondifferential nature (25). The differential effects in PC2–PC5 were relatively small and more pronounced in seedlings than in adult plants. Most wheat lines were generally distributed in biplots without showing any distinct grouping or clustering and most could not be associated with specific isolates. This general lack of association between lines and isolates and the absence of clustering
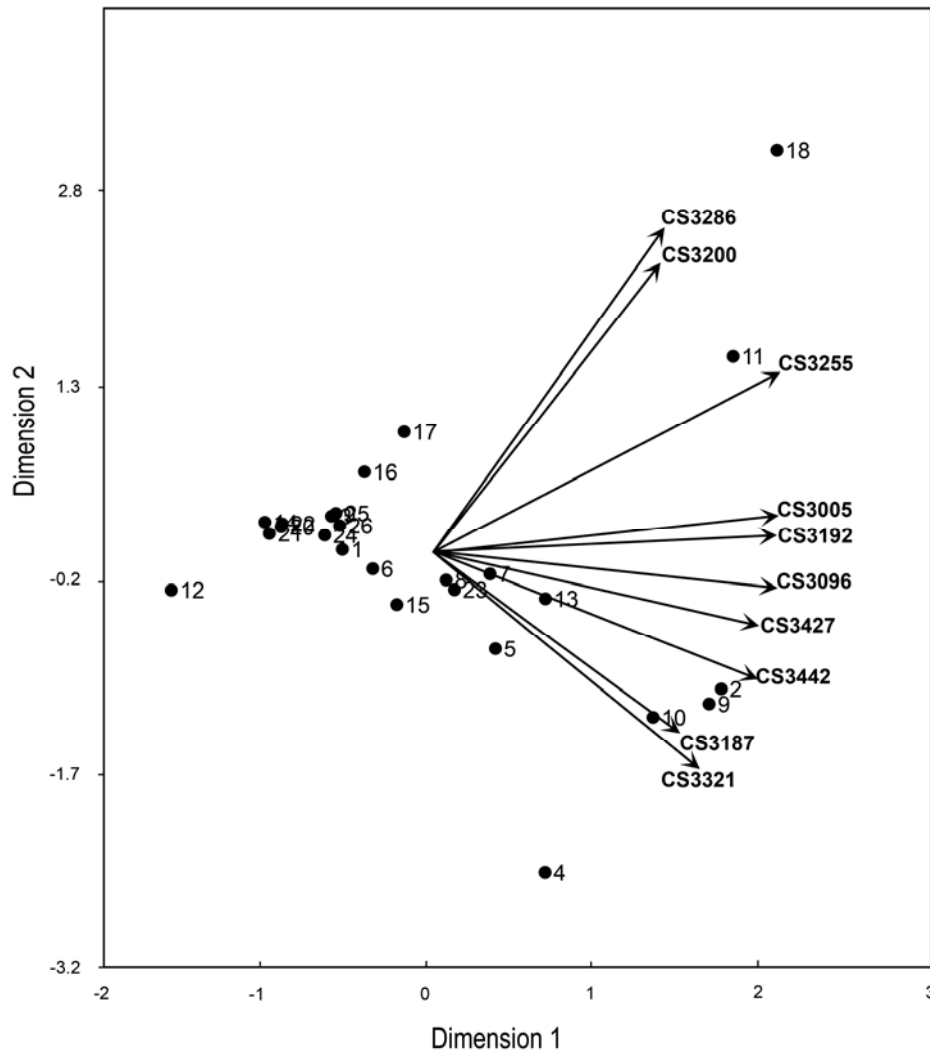

Fig. 3. Biplot of adult plant crown rot severtiy of 26 wheat lines inoculated with five isolates each of *Fusarium pseudograminearum* (CS3096, CS3286, CS3321, CS3427, and CS3442) and *F. graminearum* (CS3005, CS3200, CS3187, CS3192, and CS3255). Wheat lines 1, 2-49; 2, Aso Zairai; 3, Baxter; 4, Chile; 5, CSCR1; 6, CSCR12; 7, CSCR2; 8, CSCR3; 9, CSCR7; 10, CSCR8; 11, Ernie; 12, Fredrick; 13, Frontana; 14, Giles; 15, Gluyas Early; 16, Janz; 17, Kennedy; 18, Kikuchi; 19, Lang; 20, Mulgara; 21, Pioneer 2375; 22, Rowan; 23, Sotome A; 24, Sumai3; 25, Sunvale; and 26, Tamaroi.

of wheat lines further point to a lack of specialization in the two species.

In the limited number of studies on the CR pathogen, all have found major differences in isolate aggressiveness, with only a minor differential component (2,21,27). Similarly for FHB, a strong differential effect has not been found in the vast majority of studies. In studies where a significant isolate–line interaction was reported, interaction patterns were not stable over experiments and genotype ranking was only slightly influenced by isolates; and, when major sources of variation were accounted for, there had been little or no differential effect (30). Our results support these earlier findings to show a lack of pathogenic specialization for CR where isolates of the two *Fusarium* spp. mostly differed in their nondifferential aggressiveness. Overall, *F. pseudograminearum* was more aggressive than *F. graminearum* and, although CR severity was reduced in the adult plants, the two most aggressive isolates common to seedling and adult plants were both *F. pseudograminearum*. These findings support earlier delineation of the crown-infecting group of *F. graminearum* as a separate species (23).

A key finding of this work is that a range of analytical tools are needed to explore pathogenic specialization, and a statistically significant interaction in an ANOVA cannot be taken as conclusive evidence of specialization (2). PCA proved useful in separating out the nondifferential component, which explained 56 to 65% of the variation in seedling assays, from the differential components, which explained ≤31% of variation. Applying PCA to FHB severity caused by *Fusarium* spp. has similarly found only a relatively small differential effect (2). However, when PCA was used to determine whether specialization existed in strains of *Colletotrichum gloeosporioides* causing anthracnose of *Stylosanthes scabra*, it was unable to do so, whereas discriminant analysis was able to classify isolates into races (9).

A biplot is a scatter plot that approximates and graphically displays a matrix by both row and column factors to allow simultaneous visualization of relationships between row and column factors and their underlying interactions (32). In agriculture, biplots have been used to analyze genotype–environment data, including a study examining wheat genotypes for susceptibility to various diseases and agronomic traits (26). Both PCA and biplot use a common mathematical tool, and by maximizing variance on the first two principal components, biplots make it easy to visualize groupings and association between rows and columns. In our study, biplots showed a lack of clear-cut association between isolates and wheat lines, suggesting an overall absence of specialization among the isolates used.

Both Kendall's *W* and κ statistics offered an easy way to test whether a significant isolate–wheat line interaction in ANOVA resulted from significant changes in rank order. Both rank order statistics had low values for CR severity of seedlings while the highest coefficient was obtained for adult plant rankings, and all except the κ statistic for the ranking of 26 lines for seedling severity were significant at $P < 0.05$. These show that any differential effect was small and more pronounced in seedlings than in adult plants. However, the coefficients are not easy to interpret. Kendall's *W* is easy to interpret when it is either 0, indicating no agreement, or 1, for perfect agreement, but not for intermediate values. A $W = 0.49$, for instance, indicates that the variance of the total ranks is 49% of the maximum possible; however, whether it is an acceptable level of agreement is difficult to decide from a significance test (12). A degree of agreement has been suggested to interpret κ values (15): ≤0 = poor, 0 to 0.2 = slight, 0.2 to 0.4 = fair, 0.4 to 0.6 = moderate, 0.6 to 0.8 = substantial, and 0.8 to 1 = almost perfect. Based on this, there is only a "slight" agreement between rankings of wheat lines by the 10 isolates, pointing to potential specialization.

The absence of a strong evidence of pathogenic specialization can be partly due to the limited number of isolates and wheat lines used in our work. Also, the current Australian wheat cultivars are partially resistant to CR, and resistance does not discriminate between pathogen isolates. If the intensified search for CR resistance in Australia and elsewhere leads to wheat cultivars with specific resistance genes offering near-immunity and these cultivars are deployed right across the Australian wheat belt, this may increase selection pressure on the pathogen to develop specialization. Similarly, sexual reproduction between moderately aggressive *F. graminearum* parents can give rise to highly aggressive individuals through transgressive segregation (11). Regular monitoring of pathogen population will provide early warning of any pathogenic specialization.

ACKNOWLEDGMENTS

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