Genetic Structure of *Fusarium pseudograminearum* Populations from the Australian Grain Belt

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Accepted for publication 20 September 2007.

ABSTRACT

Bentley, A. R., Leslie, J. F., Liew, E. C. Y., Burgess, L. W., and Summerell, B. A. 2008. Genetic structure of *Fusarium pseudograminearum* populations from the Australian grain belt. *Phytopathology* 98:250-255.

Crown rot, caused by the fungus *Fusarium pseudograminearum* (teleomorph *Gibberella coronicola*) is a major disease of wheat in the Australian grain belt. However, there is little information available on the population structure of this pathogen. We measured genetic diversity as assessed with amplified fragment length polymorphism (AFLP) analysis within and between populations of *F. pseudograminearum* from northeastern, south central, and southwestern regions of the Australian grain belt. Amongst the 217 isolates, 176 haplotypes were identified and grouped

into two main clusters. One cluster contained isolates from populations in northeastern Australia, and the other cluster contained isolates from populations in south central and southwestern Australia. The southern populations were distinguished from the northeastern populations by higher levels of population differentiation (G_{st}) between them and genetic identity amongst the regional populations. We hypothesize that the *F. pseudograminearum* populations from northeastern and southern Australia are independent, which could result from different founding events or from geographic isolation and the accumulation of genetic differences due to genetic drift and/or selection.

Additional keywords: soilborne diseases, *Triticum aestivum*.

Crown rot, caused by the fungus *Fusarium pseudograminearum*, has become the major constraint to wheat production in the grain belt of northeastern Australia over the past two decades (9) following the widespread adoption of conservation tillage and the increased popularity of highly susceptible durum wheat cultivars. The pathogen has become increasingly important across the entire Australian grain belt, where it is a critical component of the multi-species crown and root disease complexes in south central Australia (5,46), and in the winter cereal cropping regions of southwestern Australia, where it causes localized losses (24). *F. pseudograminearum* also can be found in other wheat growing regions of the world (28,30,38); however, there is relatively little information on the genetic diversity in these populations or their relationship to populations from Australia.

Durable resistance to *F. pseudograminearum* is critical for the long-term management of crown rot in Australia (41,44,45), but there are no definitive sources of resistance known in Australia, or elsewhere (29). Resistant varieties must be effective against a majority of the variants in the pathogen population (6,37,42). Thus, characterization and quantification of genetic diversity, which may or may not represent pathogenic variation, is an essential first step towards ensuring that breeding materials are tested against representative isolates, so that when resistant host lines are identified they are known to be resistant to a number of genetically diverse pathogen isolates.

Previous studies, often with a phylogenetic emphasis, utilized primarily strains from culture collections (8,28,30,35) and found limited variation in nuclear DNA as assessed by restriction fragment length polymorphism (RFLP) analysis (8), relatively few unique haplotypes (28), and no correlation between genetic

relatedness and either host (8) or geographic origin (8,30). However, isolates from the North and South Islands of New Zealand can be differentiated by using amplified fragment length polymorphisms (AFLPs) and gene genealogies (7,30), suggesting that the geographic separation of these islands is an effective barrier to gene flow. High levels of gene and genotypic diversity also have been reported in a population study of *F. pseudograminearum* (71 isolates) collected from seven sites in the wheat producing area of northeastern Australia (2). Genetic variation in this case was consistent with high levels of gene flow between populations and low levels of population differentiation within a single geographical area (2). The present study extends the previous work by expanding the breadth of the sampling area to include three relatively large, but geographically distinct, areas of the Australian grain belt, by increasing the number of isolates representing populations, and by utilizing multiple molecular loci to evaluate the genetic variation present.

The Australian grain belt is not geographically continuous, as a large desert separates the southwestern portion from the remainder of the grain belt. Climatic conditions, crop rotations, and crop management strategies also differ markedly between the three regions. Our objective was to characterize the genetic structure of eight populations of *F. pseudograminearum* representing three key regions of the Australian grain belt in northeastern, south central, and southwestern Australia, and to estimate the genetic differentiation within and between these populations. Our working hypothesis was that these populations are genetically distinguishable due to geographic separation and restricted gene flow. This study will determine whether these populations are distinct enough to warrant a search for differences in pathogenicity between them.

MATERIALS AND METHODS

Sample collection. Samples of mature spring wheat were collected from eight sites representing three regions of the

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doi:10.1094/PHYTO-98-2-0250

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Australian grain belt in either November 2003 or 2004 (Fig. 1; Table 1). Three sites each in northeastern and south central Australia, and two sites in southwestern Australia were sampled. Only two sites in southwestern Australia were sampled because the disease is less common in this region and diseased fields often are difficult to find. Sites were selected based on the presence of a physiologically mature, pre-harvest crop with an estimated level of crown rot infection >50%, as determined by basal stem browning. Wheat plants within a site were collected arbitrarily based on a zigzag transect beginning 20 m from the edge of the field. Within the transect, each horizontal line of the zigzag was 5 m in length, with five plants collected each time the direction in the zigzag line was changed. The total number of stems collected from each site ranged from 70 to 100 (Table 1) based on the estimated percentage of infection of the crop, with lower stem numbers corresponding to higher levels of disease incidence.

The outer leaves and sheath tissue of the leading tiller of each plant were removed and discarded and stems were washed with tap water before being surface sterilized with 70% ethanol for 3 min and dried on tissue paper for 15 min. Single stem sections, ≈ 2 to 3 cm in length, including the crown and the first node were removed aseptically from each stem and plated on half strength potato dextrose agar (PDA), amended with broad-spectrum antibiotics (23). Plates were incubated under standard conditions at 25°C with 12 h each of alternating light and dark for 7 days (23).

Red colonies developing after 7 days of incubation were sub-cultured aseptically to carnation leaf agar (CLA) (14) and were

incubated as for the PDA plates. After 9 days incubation, all cultures growing on CLA were purified by subculturing single germinated conidia (23). The purified cultures were identified to species level based on morphology (4,23). Four hundred and seventy-five morphologically identified isolates of *F. pseudograminearum* were accessioned in the Fusarium Research Laboratory culture collection at The University of Sydney and stored in 15% glycerol (vol/vol) at -70°C (Region, Location [FRL accession number/s]): northeastern Australia, Collarenebri (F16962-F16995, F16997-F17003, F17005-F17010, F18536-F18647), Goondiwindi (F16651, F16653-F16654, F16656, F16659-F16668, F16670-F16676, F16678-F16716), Walgett (F17011-F17015, F17017-F17020, F17022-F17044, F17046-F17053, F17055-F17057, F18546-F18557, F18648-F18652); south central Australia, Cambrai (F17058-F17109, F17111-F17118), Wharminda (F16500, F16504, F16506, F16509, F16512-F16540, F16542-F16543, F16545-F16553, F16555-F16558, F18992), Roseworthy (F16065, F16074, F16083, F16092-F16110, F16112-F16145, F16147-F16150); southwestern Australia, Jerramungup (F16385, F16393, F16417-F16454, F17234-F17250) and Narembeen (F14843-F14859, F14861-F14868, F14870, F14872-F14884, F14886-F14902, F14904-F14915, F15334).

DNA isolation and AFLP reactions. Of the 475 isolates recovered, 217 isolates (Table 2) representing the eight populations from northeastern, south central, and southwestern Australia were evaluated for AFLPs. These isolates were grown on PDA at 25°C in the dark for 5 days. Mycelium was harvested and aseptically transferred to a sterile 1.5 ml microcentrifuge tube for DNA extraction with a FastDNA Kit (Qbiogene, Inc., Irvine, CA) according to the manufacturer's instructions.

AFLPs (43) were generated as previously described (23). Three primer pairs were used to generate AFLP fingerprints of the *F. pseudograminearum* isolates: *EcoRI*+GG/*MseI*+CT, *EcoRI*+AA/*MseI*+AT and *EcoRI*+TG/*MseI*+TT. Each set of 96 reactions included four positive, and at least two negative controls. AFLPs were resolved on polyacrylamide gels. Bands between 100 and 800 bp in size were scored manually as either 1 (presence of a band) or 0 (absence of a band). Isolates with $\geq 97\%$ similarity (as determined by the DICE coefficient) were considered to be putative clones. Loci with alleles with a frequency of <5% were censored from the final analysis to avoid any bias that they might contribute. Repeated AFLPs of the positive controls on multiple gels isolated from multiple cultures of the same strain indicate little (<3% variation) in banding patterns in different assays. These repeated control isolates were used for direct comparison of the results from different AFLP gels with one another and to specify the % similarity value cut-off we used to identify putative clones.

Mating type determination. Mating type was determined by using the degenerate oligonucleotide primers fusALPHAfor, fusALPHArev, fusHMGfor, and fusHMGrev developed for asexually reproducing *Fusarium* species (21). Multiplex polymerase chain reaction (PCR) reactions were performed in a final reaction volume of 20 μ l containing 1 $\times$ PCR buffer (Bioline (Aust) Pty.,

Fig. 1. Map of Australia showing approximate location of wheat collection sites in northeastern (three sites), south central (three sites), and southwestern (two sites) Australia from which populations of *F. pseudograminearum* were obtained. Sampling sites – C: Collarenebri; Ca: Cambrai; G: Goondiwindi; J: Jerramungup; N: Narembeen; R: Roseworthy; W: Walgett; and Wh: Wharminda.

TABLE 1. Geographic origin within Australia, host wheat variety and collector of samples from which *Fusarium pseudograminearum* populations were recovered and percentage recovery from stems plated at each site

Population	Site	Year	Variety	Collector ^a	Stems plated	% <i>F. pseudograminearum</i> recovery
Northeastern	Collarenebri	2004	Wedgetail	ARB	70	91
	Goondiwindi	2004	Unknown	LWB	75	92
	Walgett	2004	Wedgetail	ARB	80	89
South central	Cambrai	2004	Unknown	ARB	100	88
	Wharminda	2004	Wyalkatchem	ME	95	51
	Roseworthy	2004	Unknown	HW	80	99
Southwestern	Jerramungup	2004	04MT72	JD	70	79
	Narembeen	2003	Westonia	LWB	90	74

^a Collector ARB: Alison R. Bentley, LWB: Lester W. Burgess, ME: Margaret Evans, HW: Hugh Wallwork, JD: Judy Devenish.

Ltd., Alexandria, NSW, Australia), 1.5 mM MgCl₂ (Bioline), 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Astral Scientific, Pty., Ltd., Caringbah, NSW, Australia), 0.25 µM of each primer, 1 unit of BIOTAQ DNA polymerase (Bioline), and ≈20 ng of fungal genomic DNA. Initial denaturation was done at 94°C for 30 s, followed by 30 cycles of 30 s at 94°C, 30 s at 55°C and 90 s at 72°C and a final extension at 72°C for 1 min following the last amplification cycle. PCR amplification products were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide and visualized under UV light. Isolates from which a 200-bp fragment was amplified were classified as *MAT-1* and isolates from which a 260-bp fragment was amplified were classified as *MAT-2* (21).

Population genetic analyses. AFLP banding patterns were analyzed by using NTSYSpc version 2.10q (Applied Biostatistics, Inc., Setauket, NY). Genetic similarities amongst the isolates were calculated by using the DICE coefficient (13,33) and clustered with the Unweighted Pair Group Method of Arithmetic mean clustering algorithm (UPGMA) (39). Ten isolates from each of the eight fields collectively representing the range of diversity in AFLP haplotypes (data not shown) and <97% similar to the others were used to create an UPGMA dendrogram. Two isolates of *F. graminearum* lineage 7 from Kansas State University (KSU99-21, KSUY47) were used as outgroups (4). Bootstrapping with 1,000 replications was performed on the binary data matrix with phylogenetic analysis using parsimony (PAUP) version 4.0b10 (Sinauer Associates, Sunderland, MA).

Measures of genetic diversity were calculated for both the original AFLP data and the clone censored data (data adjusted so all but one representative isolate of each haplotype retained) for all 217 isolates. Nei's mean gene diversity (31) and the proportion of polymorphic loci were calculated for each population with POPGENE version 1.31 (University of Alberta, Alberta, Canada). POPGENE also was used to calculate pairwise measures of Nei's unbiased genetic identity and genetic distance (32) and genetic differentiation (fixation index [G_{st}]). Individuals in each population also were analyzed by parametric genetic mixture analyses based on a Bayesian clustering approach in Structure version 2.0 (Department of Human Genetics, University of Chicago, IL) to assign the individuals to populations based on their AFLP haplotypes. In Structure, nine values of K were tested in duplicate with conditions of admixture and correlated alleles defined based on Monte Carlo Markov chain (MCMC) runs of 100,000 iterations following a burn-in period of 100,000 iterations. Further, $K = 7$ and $K = 8$, which had the lowest estimated natural log probability of data ($\ln(P|D)$) in the initial analyses (data not

shown) were run in triplicate based on MCMC runs of 900,000 iterations following a burn-in period of 100,000 iterations. The estimated ancestry coefficients (Q) for $K = 7$ across all three runs were correlated to ensure robust assignment of individuals to the $K = 7$ populations. The results of population assignment were visualized using Distruct (36).

Arlequin version 2.000 (University of Geneva, Geneva, Switzerland) was used for analysis of molecular variance (AMOVA) to estimate variance components between populations and regions based on whole haplotypes. The index of multilocus association (I_A) and linkage disequilibrium (RbarD algorithm) for each of the eight populations were calculated with MultiLocus version 1.2 (Department of Biology, Imperial College, Silwood Park, United Kingdom) and compared with data generated from 1,000 randomizations with the expectation of panmixis (1). Mating type ratios for each of the populations were tested with χ^2 against an expected ratio of 1:1, and also were used to calculate the effective population number based on mating type ($N_{e(mt)}$) (22).

RESULTS

Population structure. One hundred loci were scored for the three AFLP primer pairs: 29 for primer pair *EcoRI*+GG/*MseI*+CT, 34 for *EcoRI*+AA/*MseI*+AT, and 37 for *EcoRI*+TG/*MseI*+TT. Eighty-eight loci (88%) were polymorphic across all 217 isolates. In the dendrogram based on the AFLP data for individual isolates, two clusters can be identified (data not shown). Cluster I includes all of the *F. pseudograminearum* isolates from the three northeastern Australian populations, while Cluster II includes all of the isolates from the south central and southwestern Australian populations. Within each cluster, isolates shared at least 78% DICE similarity. Members of clusters I and II are <73% similar, but bootstrap support for the separation of the clusters was not signifi-

Fig. 2. Assignment of individual *Fusarium pseudograminearum* isolates from three regions to $K = 7$ populations based on Structure analysis with 900,000 iterations of the Monte Carlo Markov chain (MCMC) followed by 100,000 iterations of burn-in.

TABLE 2. Summary of statistics comparing populations of *Fusarium pseudograminearum* from northeastern, south central, and southwestern Australia

Population	N ^a	N _{adj} ^b	PLP ^c	h^d	h_{adj}^e	$I_A^f(P)$	RbarD ^g (P)	MT($P[\chi^2]$) ^h	$N_{e(mt)}^i$
Northeastern Australia									
Collarenebri	29	29	70	0.2	0.2	2.88 (<0.01)	0.04 (<0.01)	>0.05	>99%
Goondiwindi	30	26	47	0.14	0.14	1.60 (<0.01)	0.04 (<0.01)	>0.05	>99%
Walgett	30	26	53	0.14	0.15	1.56 (<0.01)	0.03 (<0.01)	>0.05	89%
South central Australia									
Cambrai	28	17	45	0.11	0.14	2.91 (0.23)	0.07 (<0.01)	>0.05	>99%
Wharminda	24	22	47	0.13	0.14	1.43 (0.05)	0.03 (0.05)	>0.05	75%
Roseworthy	30	17	36	0.07	0.09	1.17 (0.17)	0.04 (<0.01)	>0.05	96%
Southwestern Australia									
Jerramungup	23	18	45	0.12	0.12	2.00 (<0.01)	0.05 (<0.01)	>0.05	98%
Narembeen	23	21	54	0.16	0.16	2.20 (<0.01)	0.04 (<0.01)	>0.05	98%

^a Number of isolates analyzed.

^b Number of isolates after adjustment for clonal haplotypes (at ≥97% DICE similarity).

^c Proportion of polymorphic loci.

^d Nei's gene diversity (31).

^e Nei's gene diversity adjusted for clonal haplotypes.

^f Index of multilocus association (P value based on data generated from 1,000 randomizations).

^g Linkage disequilibrium (P value based on data generated from 1,000 randomizations).

^h Significance test for chi-squared goodness of fit test for deviation of mating type ratio from 1:1.

ⁱ Effective population number based on mating types (22).

cant (all bootstrap values <50). Both Cluster I and II were 50% similar (bootstrap support value = 100%) to the two isolates of *F. graminearum*.

The use of model-based clustering in Structure 2.0 enabled the assignment of $K = 7$ populations to the AFLP data (Fig. 2). $\text{Ln}(\text{P}|\text{D})$ values were lowest at $K = 7$ and $K = 8$ based on initial duplicate runs. When run with 1,000,000 iterations in triplicate $K = 7$ had the lowest overall $\text{Ln}(\text{P}|\text{D})$ (approximately -4950; data not shown). The ancestry coefficients between runs were correlated at >0.9995. The isolates from individual populations within regions were distinct, with the exception of the populations from Goondiwindi and Collarenebri in northeastern Australia, which were classified as a single population. All seven populations had varying levels of genetic contributions (based on their AFLP haplotypes) to or from other populations (Fig. 2).

We identified 176 haplotypes amongst the 217 isolates examined, indicating a high level of genotypic diversity in the Australian *F. pseudograminearum* populations (Table 2). The proportion of polymorphic loci for each population ranged from 36% (Roseworthy, south central Australia) to 70% (Collarenebri, northeastern Australia). The highest level of gene diversity was recorded for the Collarenebri population ($h = 0.20$), and the lowest recorded at Roseworthy ($h = 0.07$ [clone adjusted $h = 0.09$]) (Table 2). The index of association (I_A) for all of the populations from northeastern and southwestern Australia, and the Wharminda population in south central Australia were significantly different from the expectation of random mating ($P \leq 0.05$ in all cases) (Table 2). The I_A values for the Cambrai and Roseworthy populations from south central Australia were not significantly different from the expectation of random mating ($P = 0.23$ and 0.17 , respectively) with these two populations having the fewest number of haplotypes and the lowest genetic diversity values ($h = 0.11$ [clone adjusted $h = 0.14$] and 0.07 [clone adjusted $h = 0.09$], respectively) (Table 2). Linkage disequilibrium values (R_{barD}) for all populations indicated that they were all significantly different from the expectation of random mating ($P \leq 0.05$ in all cases).

Population differentiation, as estimated by G_{st} (Table 3) indicates that there is much less genetic differentiation between the populations in south central and southwestern Australia compared to the populations from northeastern Australia. Gene flow estimates between populations based on G_{st} calculations in each geographic region (northeastern Australia N_m from 1.84 to 3.73; south central Australia N_m from 0.78 to 2.03; southwestern Australia $N_m = 2.08$) indicate gene flow is an important factor in regional population structure. The AMOVA indicates that approximately 50% of the variation recorded occurred within populations, with a further 32% of variation occurring between populations, and the remaining 18% occurring among populations within regions (Table 4).

Mating type. A single band, representing a single *MAT* idiomorph, corresponding to either an *MAT-1* or an *MAT-2* allele, was amplified from all 217 isolates indicating that *F. pseudo-*

graminearum is heterothallic. The χ^2 test indicated that the 1:1 ratio of mating types could not be rejected at a 95% confidence level for all eight populations. The effective population numbers based on mating type allele ratios varied between populations within and between regions, except for the southwestern Australian populations ($N_{\text{e(mt)}}$ >98% in both cases) (Table 2). In northeastern Australia, two of the populations (Collarenebri and Goodiwindi) had high effective population numbers (>99% of the count), but the third population (Walgett) had a lower value (89% of the count). Similarly, in south central Australia, two of the populations, Cambrai and Roseworthy, had high effective population numbers, (>99% and 96%, respectively), while the Wharminda population had a N_e of 75% of the count (Table 2).

DISCUSSION

Our results from the current study are consistent with the working hypothesis that regional differentiation occurs in populations of *F. pseudograminearum* in the Australian grain belt. Similar differentiation was observed in the geographically isolated *F. pseudograminearum* populations from the South and North Islands of New Zealand (7,30). Despite being almost equidistant from both the northeastern and southwestern Australian populations ($\approx 1,800$ and $2,000$ km, respectively), the south central populations of *F. pseudograminearum* are similar (genetic identity range from 0.81 to 0.96) to the populations from southwestern Australia, with 61 to 89% of the observed genetic variation in the five populations from these regions shared in a common gene pool. There are low levels of genetic differentiation between the south central and southwestern populations (G_{st} from 0.11 to 0.39). As the Structure analysis is consistent with the existence of predominantly distinct populations within and between populations, we hypothesize that the south central and southwestern populations are all members of a single, larger metapopulation (i.e., a spatial network of populations that interact at some level) (18). Early records of wheat production in Australia (10,19) indicate that south central Australian wheat breeders and producers frequently supplied both grain and hay to southwestern Australia, which could explain the interconnectedness of the *F. pseudograminearum* populations from these areas in spite of their apparent geographic isolation (19).

There is, however, clear genetic differentiation between the southern populations and the three field populations of *F. pseudograminearum* from northeastern Australia. This finding is incon-

TABLE 4. Analysis of molecular variation of northeastern, south central, and southwestern Australian populations of *Fusarium pseudograminearum*

Source of variation	df	Variance component	<i>P</i>	Percentage of variation
Within country	2	1.7	0.05	32%
Within regions	7	0.9	0.05	18%
Within populations	222	2.6	0.05	50%

TABLE 3. Nei's unbiased genetic identity (32) and pairwise fixation index (G_{st}) values between *F. pseudograminearum* populations from northeastern, south central, and southwestern Australia^a

Population	Northeastern Australia			South central Australia			Southwestern Australia	
	Collarenebri	Goondiwindi	Walgett	Cambrai	Wharminda	Roseworthy	Jerramungup	Narembeen
Collarenebri	*	0.95	0.91	0.76	0.84	0.76	0.83	0.81
Goondiwindi	0.12	*	0.91	0.73	0.83	0.73	0.78	0.78
Walgett	0.18	0.21	*	0.82	0.80	0.82	0.83	0.86
Cambrai	0.39	0.49	0.38	*	0.88	0.95	0.87	0.96
Wharminda	0.27	0.34	0.37	0.29	*	0.84	0.81	0.86
Roseworthy	0.44	0.54	0.42	0.20	0.39	*	0.85	0.96
Jerramungup	0.30	0.39	0.32	0.26	0.32	0.33	*	0.89
Narembeen	0.30	0.38	0.28	0.11	0.26	0.14	0.19	*

^a Genetic identity values are given above the diagonal and pairwise G_{st} values between populations below.

sistent with earlier reports that suggest limited genetic differentiation and high gene flow between populations on both regional and continental scales (2,8,28). The results from the northeastern populations, however, are consistent with the results of Akinsanmi et al. (2) who found high levels of gene flow between local *F. pseudograminearum* populations from this region. These authors reported an N_m value of 14 (compared to an approximated N_m of 4 in the current study) for six populations representing a similar area to that classified as northeastern Australia in the current study. The cause for the differentiation of populations of *F. pseudograminearum* from northeastern and southern Australia could be founder effects attributable to separate founding events. Alternatively, the populations could have a common origin and have diverged with time due to differences in drift and/or selection. Limited long-distance dispersal of the primarily asexually propagating *F. pseudograminearum* could reduce the mixing of these regional populations. Further work is required to determine likely sources/origins of the strain(s) that founded the regional populations.

The results of the current study also indicate that there are high levels of gene and genotypic diversity present in populations of *F. pseudograminearum* in Australia. Similar levels of genetic diversity have been reported for a number of *Fusarium* species collected from a range of hosts on both local and global scales, including *F. pseudograminearum* (2), *F. verticillioides* (G. *fuji-kuroi* MPA) (12), *F. graminearum* (11,16,25,27,48,49), *F. culmorum* (17,25,26), and *F. proliferatum* (34), although the inferred causes of the diversity within these species differ.

Sexual recombination is an important evolutionary force, and may be a key contributor to the genotypic diversity observed in *F. pseudograminearum* populations (2,28) within regions. The significance of sexual reproduction in the life cycle of *F. pseudograminearum*, other than its existence, is unknown, as the teleomorph *Gibberella coronicola* has been reported only occasionally in the field (15,40) and under laboratory conditions (3). In this study, we used PCR amplification of conserved portions of the mating type idiomorph to confirm that *F. pseudograminearum* has a dimictic mating system. The linkage disequilibrium in the eight populations indicates that sexual recombination has not occurred at a sufficient level for the populations to be modeled as a randomly mating population. Thus there is less genotypic diversity than expected within the populations we evaluated than would be predicted by Akinsanmi et al. (2) based on populations of *F. pseudograminearum* from northeastern Australia (2). Seemingly contradictory results for the index of association and linkage disequilibrium from two of the populations in south central Australia (Cambrai and Roseworthy) may be due to the small size of the clone-censored populations. Low values of N_e for the Wharminda and Walgett are consistent with the observed skewed mating type ratios in both of these populations. The remainder of the subpopulations had relatively even mating type ratios ($N_e \geq 96\%$ of the count). These populations may have been more recently established or may have experienced a severe bottleneck at founding or some other relatively recent event. The high values of N_e indicate that when sexual recombination occurs, relatively little of the genetic variation should be lost to genetic drift. Further work with more isolates is required to confirm these hypotheses.

The genetic divergence of the southern and northeastern populations observed in the current study has implications for crown rot management in Australia. For example, novel genotypes with novel character combinations could be generated by sexual recombination. If the parental strains differ in pathogenic capabilities or mycotoxigenic potential, then strains with novel pathogenic or mycotoxigenic characters could be produced (20). Thus it is important to screen representative isolates from the southern and northeastern populations for economically significant traits, to determine the potential risk such recombinant strains might pose to Australian agriculture.

Under field conditions, recombination probably occurs with sufficient frequency that inter-regional hybrids could form, at least occasionally, if strains from both regions were present at the same location. The role and precise frequency of sexual recombination under field conditions remains unknown. Developing female-fertile tester strains would enable N_e to be estimated based on female fertility (22), which is more accurate than the estimate based on mating type frequencies that we used in this study, and would provide an opportunity to estimate the relative frequency of sexual reproduction. The ability of the pathogen to be dispersed regularly across long distances also remains unproven, even though there is frequent movement of humans, livestock, and agricultural products throughout the Australian grain belt, and this movement could vector the observed gene flow between the populations. Our results suggest that such movement to date has not resulted in sufficient gene flow to overcome the preexisting differences in these populations.

In summary, we found relatively high levels of gene and genotypic diversity in eight populations of *F. pseudograminearum* from the Australian grain belt. The *F. pseudograminearum* populations from northeastern Australia were readily distinguished from those in south central and southwestern Australia, leading us to accept the working hypothesis that geographic separation and apparently restricted continental gene flow influence population structure. The populations in south central and southwestern Australia could belong to a single metapopulation due to high levels of genetic identity that resulted from a single founder event or from gene flow between populations in these regions. The southern populations were clearly distinguishable from the populations collected in northeastern Australia, and the differentiation may have several causes including: selection, genetic drift, population bottlenecks, and founder effects. The limited long-distance dispersal abilities of the pathogen suffice to prevent homogenization of the differentiated populations, and act as a barrier to gene flow into northeastern Australia. Further work is required to determine the likely cause underlying the observed population differentiation and to determine if this differentiation was accompanied by changes in *F. pseudograminearum* pathogenicity in the Australian grain belt.

ACKNOWLEDGMENTS

The senior author was supported by a Grains Research and Development Corporation Postgraduate Research Scholarship. We thank H. Wallwork, M. Evans, V. Vanstone, R. Loughman, D. Wright, J. Devenish, B. Coleman, the Walgett Sustainable Agriculture Group, J. Smith-White, and P. Caster for assistance in collecting wheat samples, B. Ramundo, A. Saleh, and M. Kabbage for technical assistance with the AFLP analyses, and I. Mackay for assistance with Structure analyses. Contribution no. 07-79-J from the Kansas Agricultural Experiment Station, Manhattan.

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