Prevalence of *Fusarium* crown rot pathogens of wheat in southern Queensland and northern New South Wales

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Abstract

Crown rot affected wheat crops in northern New South Wales and southern Queensland were surveyed during the 2001 and 2003 growing seasons. Sixteen species of *Fusarium* were isolated from crowns and basal stem nodes displaying crown rot symptoms. *Fusarium pseudograminearum* was the predominant crown rot pathogen isolated, followed by *F. crookwellense*, *F. graminearum* and *F. avenaceum*, respectively. A greater proportion of isolates from crowns were *F. pseudograminearum* than were isolates from stem nodes, while *F. graminearum* preferentially infected stem nodes relative to crowns. Future work will determine the genetic basis of specificity among the principal crown rot pathogens and the evolutionary mechanisms influencing their population structure.

Media summary

Surveys of wheat fields in northern NSW and southern Queensland have isolated 16 *Fusarium* species associated with the disease, crown rot.

Keywords

Gibberella coronicola, Gibberella zeae, Gibberella avenacea, Triticum aestivum, epidemiology

Introduction

Crown rot is a chronic disease of wheat in Australia, as well as in South Africa, Argentina and parts of the USA (Burgess *et al.* 2001). The costs associated with the disease in Australia have been estimated as A$ 56 million per annum (Brennan and Murray 1998). It is a disease of increasing importance to Australian wheat growers due to increases in the use of minimum tillage and stubble retention practises, allowing for greater carry over of inoculum between seasons. Crown rot is caused by many species within the genus *Fusarium*, including *F. pseudograminearum* (teleomorph *Gibberella coronicola*), *F. culmorum, F. crookwellense* (Liddell 1985) and *F. graminearum* (teleomorph *G. zeae*) (Akinsanmi *et al.* 2004). In Australia, the predominant crown rot pathogen is thought to be *F. pseudograminearum* (Akinsanmi *et al.* 2004; Akinsanmi *et al.* 2004), however comprehensive surveys of the major wheat growing areas have not been done. Knowledge of the genetic structure of pathogen populations, and the evolutionary processes that influence that structure, can provide indications of a pathogen's ability to adapt to various disease control strategies. However, no studies of Australian populations of crown rot pathogens have been reported to date.

This paper determines the relative prevalence of *Fusarium* species responsible for crown rot from two field surveys of wheat fields in northern New South Wales (NSW) and southern Queensland (Qld). This will serve as the baseline information for research on the population structure and evolutionary mechanisms that determine population structure of the major crown rot pathogens in this region.

Materials and methods

Pathogen survey

Surveys of wheat fields in northern NSW and southern Qld were conducted during the grain filling stage in the 2001 (October and November) and 2003 (October) growing seasons. In 2001 and 2003, 39 and 12 fields were surveyed, respectively. Fields were delineated into one to four transects, each containing four to five, evenly spaced, 1 m^2 quadrats. Transect and quadrat numbers varied depending on field area. Within each quadrat, up to seven wheat plants displaying the basal browning symptoms of crown rot infection, were arbitrarily sampled.

Three sections, approximately 2 mm in diameter, were taken from around each symptomatic crown (2001 and 2003) and basal stem node (2003 only). Sections were surface sterilised in 1 % available chlorine for 5?min, rinsed twice in sterile distilled water, and plated onto ? strength potato dextrose agar (PDA), containing 10 ?g/mL tetracycline hydrochloride and 100 ?g/mL streptomycin sulphate. Plates were incubated at 25 ?C for three to five days and single spore cultures obtained by streaking a spore suspension onto water agar, incubating for one day at 25 ?C, and sub-culturing a single macroconidia onto full strength PDA. Isolates were stored at –80 ?C on either PDA or Spezieller Nahrstoffarmer agar (SNA; 1.0 g/L KH_2PO_4, 1.0 g/L KNO_3, 0.5 g/L $MgSO_4.7H_2O$, 0.5 g/L glucose, 0.2 g/L sucrose), under 25 % glycerol.

Species identification

Species determination was conducted by initially screening isolates with species-specific PCR primers (Table 1). DNA was extracted using the CTAB extraction method(M?ller *et al.* 1992). Approximately 50?mg of mycelium, harvested either directly from 10-day-old PDA, or vacuum-filtered through sterile Mira cloth (Calbiochem Int.) from potato dextrose broth (PDB) and freeze-dried, was ground with a sterile pestle. Ground tissue was suspended in 500 ?L TES buffer (100 mM Tris pH 8.0, 10 mM EDTA, 2 % SDS), containing 50 ?g Proteinase K and incubated at 60 ?C for 1 h. Sodium chloride (140 ?L) and 10 % cetyltrimethlammonium bromide (0.1 vol.) were then added and suspensions incubated for 10 min at 65 ?C. One volume of chloroform:isoamyl alcohol (24:1) was added, suspensions centrifuged at 14,000 rpm for 10?min and the aqueous phase transferred to a fresh tube. DNA was precipitated by adding 0.1 vol. of 3 M sodium acetate (pH 5.2) and 0.6 vol. of isopropanol, and chilling on ice for 30 min. Suspensions were pelleted by centrifuging at 14,000 rpm for 10 min and the supernatant discarded. Pellets were washed twice with cold 70 % ethanol, resuspended in 100 ?L TE buffer (1 M Tris-HCl pH 8.0, 0.5 M EDTA). RNA was digested by the addition of 10 mg/mL RNAse A and incubating at 37 ?C for 45 min. Extractions were stored at -20 ?C.

PCR amplifications were conducted using a 25 ?L reaction mix containing PCR reaction buffer (67 mM Tris-HCl pH 8.8, 16.6 mM $(NH_4)_2SO_4$, 0.45 % Triton X-100, 0.2 mg/mL gelatin), 1.5 mM $MgCl_2$, 200 ?M of each dNTP, 240 nM of each primer, 1.5 U of *Taq* DNA polymerase (Biotech Int., Brussels, Belgium) and 25 ng of target DNA. PCR reactions were conducted with a temperature profile of initial denaturation at 94??C for 3?min, 35 cycles of denaturation at 94 ?C for 45 s, annealing for 45 s at primer dependant temperatures (Table 1), and extension at 72 ?C for 2 min, followed by a final extension at 72 ?C for 7 min. Amplicons were separated by gel electrophoresis on 1 % agarose in 0.5x TBE buffer (0.045 M Tris-borate, 1?mM EDTA), stained with ethidium bromide (10 mg/?L) and visualised under UV light.

Table 1. Species-specific primers used for identification of *Fusarium* spp.

Target species	Primer	Sequence 5'-3'	Amplicon size (bp)	Temp. (?C)[1]
F. graminearum	Fg16NF[2]	ACA GAT GAC AAG ATT CAG GCA CA	280	57

	Fg16NR[2]	TTC TTT GAC ATC TGT TCA ACC CA		
F. pseudograminearum	Fp1-1[3]	CGG GGT AGT TTC ACA TTT CCG	523	57
	Fp1-2[3]	GAG AAT GTG ATG ACG ACA ATA		
F. avenaceum	FA-ITSF[4]	CCA GAG GAC CCA AAC TCT AA	272	59
	FA-ITSR[4]	ACC GCA GAA GCA GAG CCA AT		
F. culmorum	Fc01F[2]	ATG GTG AAC TCG TCG TGG C	570	59
	Fc01R[2]	CCC TTC TTA CGC CAA TCT CG		
F. poae	Fp82F[5]	CAA GCA AAC AGG CCT CTT GAC C	220	57
	Fp82R[5]	TGT TCC ACC TCA GTG ACA GGT T		
F. acuminatum	FAC-F[6]	GGG ATA TCG GGC CTC A	602	50
	FAC-R[6]	GGG ATA TCG GCA AGA TCG		
F. oxysporum	PFO3[7]	CGG GGG ATA AAT GCG G	70	50
	PFO2[7]	CCC AGG GTA TTA CAC GGT		

[1]Annealing temperature, [4](Schilling *et al.* 1996), [7](Edel *et al.* 2000), [2](Nicholson *et al.* 1998), [5](Parry and Nicholson 1996), [3](Aoki and O'Donnell 1999), [6](Williams *et al.* 2002)

Isolates from the 2001 surveys that failed to react with any primer pair were identified based on morphological and cultural characteristics on PDA, SNA and water agar containing three to four pieces of γ-radiated carnation leaf (CLA) (Aoki and O'Donnell 1999; Burgess *et al.* 1994). Cultures were grown at 25??C for 30 days under alternating periods of 12 h of combined black light (F20T9BL-B 20W FL20S.SBL-B NIS, Japan) and standard fluorescent light (35098 F18E/33 General Electric, USA), and 12 h dark.

Results

From surveys in 2001, 241 isolates were obtained (Table 2), consisting of 16 *Fusarium* spp. Fifty eight percent of isolates were identified as *F. pseudograminearum*. Other prominent species isolated were *F.?crookwellense* (10 %), *F. graminearum* (8 %) and *F. avenaceum* (7 %). All other species (*F.*

acuminatum, F. culmorum, F. equiseti, F. poae, F. nygamai, F. torolosum, F. verticillioides, F. babinda, F. lateritium, F.?oxysporum, F. sporotrichioides and *F. tricinctum*) were isolated at rates of less than 2 %.

In 2003, from 105 isolates, 80 isolates were identified by PCR (Table 2), consisting of three species; *F.?pseudograminearum* (58 %), *F. graminearum* (14 %) and *F. avenaceum* (4 %). *Fusarium pseudograminearum* was isolated at higher rates from the crowns of wheat plants than from nodes, while *F.?graminearum* was isolated at higher rates from the nodes than from crowns.

Table 2. *Fusarium* species isolated from wheat tissues symptomatic for crown rot.

Species	2001	2003		
	Crown	Crown	Nodes	Combined
F. pseudograminearum	139 (57.7)[1]	27 (65.9)	34 (53.1)	61 (58.1)
F. crookwellense	24 (10.0)	na[2]	na	na
F. graminearum	19 (7.9)	3 (7.3)	12 (18.8)	15 (14.3)
F. avenaceum	17 (7.1)	1 (2.4)	3 (4.7)	4 (3.8)
other *Fusarium* spp.	23 (9.5)	na	na	na
unidentified	19 (7.9)	10 (24.4)	15 (23.4)	25 (23.8)
Total	241	41	64	105

[1]values in parentheses are percentages of total number of isolates
[2]not available; *F. crookwellense* identifications based on morphology

Discussion

This research identified 16 *Fusarium* species associated with crown rot of wheat in northern NSW and southern Qld, 10 of which, including *F. acuminatum, F. avenaceum, F. babinda, F. crookwellense, F.?graminearum, F. torulosum, F. tricinctum F. verticillioides* (Akinsanmi *et al.* 2004) and *F. culmorum* (Liddell 1985), have been confirmed to incite crown rot. The predominant crown rot pathogen isolated was *F. pseudograminearum,* with isolation rates consistent between 2001 and 2003, suggesting that approximately 60% of crown rot infections can be attributed to this species. Other major crown rot pathogens appear to be *F. crookwellense, F.?graminearum* and *F.?avenaceum*. Within these species some evidence for differing host tissue preference exists. *Fusarium graminearum* was isolated at over twice the rate from stem nodes than from crowns in 2003, while *F.?pseudograminearum* constituted a greater proportion of isolates from crowns, than from stem nodes. In addition, *F. crookwellense*, a minor pathogen of wheat heads in the region (Akinsanmi *et al.* 2004), was the second most prevalent pathogen isolated from crowns in 2001, constituting 10% of all isolates.

Ongoing work includes the identification of the remaining isolates from the 2003 survey via morphological and cultural characteristics. Species-specific primers are now available for *F. crookwellense* (Yoder and Christianson 1998), *F. torulosum* (Yoder and Christianson 1998), *F. verticillioides* (M?ller *et al.* 1999) and *F. equiseti* (Mishra *et al.* 2003), and these are being used in addition to morphological and cultural characters. The assessment the genetic and pathogenic diversity within field populations of the principal crown rot pathogens will follow speciations. Selectively neutral markers, including AFLP and restriction fragment length polymorphisms of selected regions of the *Fusarium* genome will lead to an understanding of population structure. In addition, pathogenic specialisation will be assessed by glasshouse bioassays using a range of wheat genotypes. The identification of genotype groupings will allow the monitoring of changes in the population structure over time in the field in response to management strategies. These studies will assess the influence of evolutionary mechanisms, such as recombination, selection, mutation and migration, on population structure for the major crown rot pathogens.

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