ORIGIN AND DISTRIBUTION OF FUSARIUM WILT PATHOGENS IN AUSTRALIAN COTTON FIELDS

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Introduction

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*), is a devastating disease in many cotton-producing countries of the world. In Australia, this disease, found first in 1993, has become a serious problem, occurring in most major cotton-growing regions and causing substantial losses (Kochman 1995). It is attributable to two genotypes of *Fov*, each of which belongs to a distinct vegetative compatibility group, VCGs 11 and 12, respectively (Bentley et al. 2000). Not only are the two Australian *Fov* strains different from the overseas *Fov* races in DNA fingerprints, but they have unique aesculin hydrolysis and volatile characteristics, implying that they were not introduced (Davis et al. 1996; Bentley et al. 2000).

Cotton was introduced to Australia with European settlement in 1788. However, 17 *Gossypium* species, also known as wild cottons, are indigenous, four of which (*G. australe*, *G. bickii*, *G. nelsonii*, *G. sturtianum*) are commonly present in the eastern and central parts of the country where the majority of cotton is currently grown. In a survey of *Fusarium* species associated with these native *Gossypium* populations, some *F. oxysporum* isolates recovered from the rhizosphere soil were able to cause mild but typical symptoms of Fusarium wilt on cotton (Wang et al. 2004). This suggests that pathogenic *F. oxysporum* (designated wild *Fov*) may have existed in Australia before cotton was introduced and implies that the *Fov* found in Australian cotton fields may have arisen *de novo*.

The two *Fov* strains arose simultaneously in different regions in Australia. VCG 11 was first found in 1993 in the Brookstead and Cecil Plains regions of QLD and VCG 12 was found the following year in Boggabilla (NSW). They behave similarly under field conditions, but most of recently reported occurrences of this disease are attributable to VCG 11, suggesting that the two *Fov* strains may have different distribution patterns in Australian cotton fields.

The objective of this study was to clarify the identity of the *Fov* found in Australian cotton fields. This was achieved by isolating indigenous *F. oxysporum* from the rhizosphere soil of native *Gossypium* populations and determining if the two Australian cotton field *Fov* strains are closely related to any of the genetic lineages of indigenous *F. oxysporum* isolates identified by amplified fragment length polymorphism (AFLP) fingerprints. A complementary survey of *Fov* isolates extracted from diseased cotton stems was also undertaken to determine the extent to which the two *Fov* strains have spread beyond the sites in which they were first discovered.

Materials and methods

Soil was collected from 90 native *Gossypium* populations in Mt Isa (QLD), Longreach-Theodore (QLD), Alice Springs-Tennant Creek (NT), and Leigh Creek-Arkaroola (SA) as well as from 18 native vegetation sites close to cotton fields in Brookstead (QLD) and Narrabri (NSW) during 2001 and 2002. Isolation of *F. oxysporum* was conducted on Komada and peptone PCNB

media. Five plates were employed for each sample, which were inoculated with 0.5 g of soil sprinkled onto the surface and incubated at 25°C for 1 week. *Fusarium oxysporum* isolates were selected based on the morphological characteristics of colonies. All isolates were subcultured from single spores and confirmed as *F. oxysporum* following Burgess et al. (1995).

Symptomatic plants were collected from 24 cotton farms in Warren, Moree, and Boggabilla (NSW) and Cecil Plains, Pampas, Bourke, and St George (QLD) during 2002 and 2004. Isolation of *Fov* was conducted on peptone PCNB agar. Stem sections were surface-sterilized in 0.5% sodium hypochlorite for 5 min and peeled under aseptic conditions. Three small pieces were cut from the discoloured vascular tissue of each stem and placed on a plate. Plates were incubated at 25°C for 1 week, after which *Fov* mycelia growing out of the tissue pieces were subcultured.

AFLP fingerprints were generated following Vos et al. (1995). Genomic DNA was extracted from 3-day-old mycelia (25°C in potato dextrose broth) using Qiagen DNeasy Plant kits. Total DNA was digested with *Eco*RI and *Mse*I. Pre-selective amplification was performed with non-selective primers. Selective amplification was performed with *Eco*RI+AC (end-labelled with ^{33}P) and *Mse*I+A. The reaction products were resolved on polyacrylamide gels and autoradiographs were obtained by exposing films to dried gels.

The *F. oxysporum* isolates from the soil of *Gossypium* populations were grouped into genetic lineages based on the similarity of their AFLP fingerprints using a single primer combination (*Eco*RI+AC: *Mse*I+A). In addition, representatives of these lineages, selected VCG 11 and VCG 12 isolates, and representatives of the eight overseas races of *Fov* were genotyped using two AFLP primer combinations (*Eco*RI+ACC: *Mse*I+A & *Eco*RI+AGG: *Mse*I+A). The AFLP bands were scored as binary characters and UPGMA topologies and bootstrap (1000 replicates) values were generated using the Dice estimator of genetic distance (Nei and Li 1979). The *Fov* isolates extracted from diseased cotton stems were assigned to VCGs based on their genetic similarity to a set of standard VCG 11 and 12 isolates (provide by N. Moore of QDPI).

Results

Diversity and distribution of indigenous F. oxysporum *isolates*

A total of 695 *F. oxysporum* isolates were recovered from the soil collected from 74 (69%) of the 108 populations sampled. The AFLP fingerprints placed 660 (95%) of these isolates in one of five genetic lineages, designated A-E. The remaining 35 isolates were all genetically unique and could not be assigned to discrete lineages. The distribution of Lineages A to E varied among *Gossypium* species and geographic locations (Fig. 1). Lineage B was the most prevalent lineage, irrespective of species, in four of the five regions. Lineages A and E occurred in four of the five regions, but the majority of both lineages were isolated from the soil of *G. sturtianum* populations in Leigh Creek-Arkaroola (SA). Lineage C appears to be limited in Mt Isa (QLD) where it is the predominant lineage. Lineage B was the only lineage found in the soil of *G. bickii* populations. Lineage D occurs infrequently – only seven isolates were obtained, but they were recovered from two regions – Mt Isa (QLD) and Alice Springs-Tennant Creek (NT).

The lineage groupings were consistent with the UPGMA phenogram (Fig. 2). Pairwise genetic similarities among the lineages were less than 50%, and the isolates of each lineage clustered together with bootstrap values ranging from 99 to 100. Evident in the phenogram is that Lineages A and E are more closely related to the cotton-field *Fov* VCG 11 and VCG 12 strains than are the

other three lineages. Notably, the VCG 11 and 12 isolates are embedded in the Lineage A cluster. The pairwise similarities of the VCG 11 and 12 isolates to the Lineage A isolates are over 80%, which are significantly higher than the similarities between the VCG 11 and 12 isolates and any of the overseas *Fov* races. Lineage E and the eight overseas *Fov* races cluster together, suggesting a common, although probably ancient, genetic origin (Fig. 2).

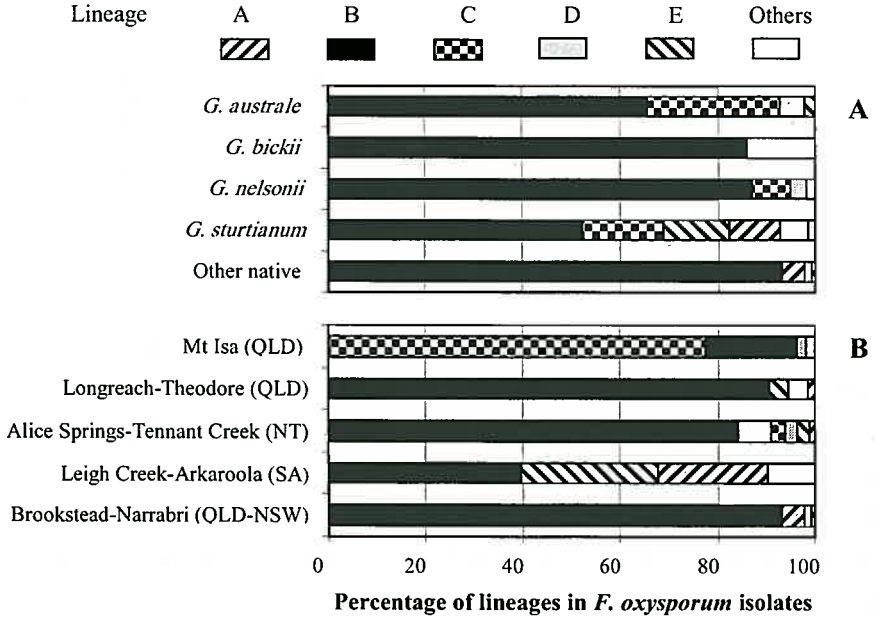

Fig. 1. Relative frequencies of indigenous *F. oxysporum* lineages (A-E) associated with four *Gossypium* species (A) and by geographic region (B).

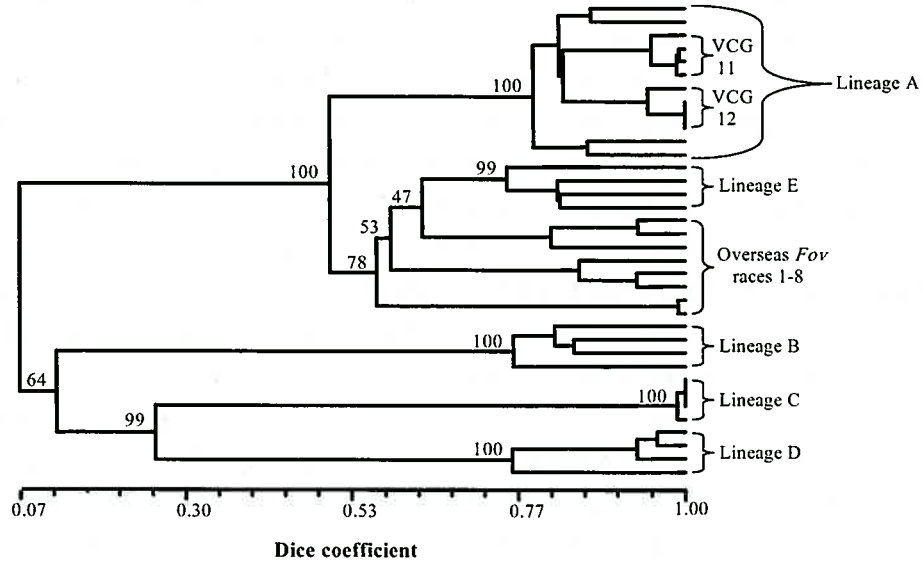

Fig. 2. UPGMA dendrogram derived from AFLP fingerprints of representatives of 5 *Fov* lineages indigenous to Australia, representative Australian cotton-field *Fov* pathogens (VCG 11 and VCG 12), and the eight *Fov* races found in cotton fields outside Australia. The UPGMA phenogram obtains from the pairwise genetic similarities estimated from 254 polymorphic AFLP bands using a Dice coefficient (Nei and Li, 1979). Numbers at the nodes of major clusters represent bootstrap values (%) generated by 1000 replicates.

Table 1. Incidence of VCG 11 and VCG 12 of *Fov* in 24 cotton farms of QLD and NSW based on the isolates from symptomatic plants collected during 2002 and 2004.

State	City	Number of farms			
		Sampled	Only VCG 11	Only VCG 12	Both VCGs
QLD	Cecil Plains	6	6	0	0
	Pampas	2	2	0	0
	Bourke	3	3	0	0
	St George	3	3	0	0
NSW	Warren	2	2	0	0
	Moree	4	4	0	0
	Boggabilla	4	2	0	2
Total		24	22	0	2

Distribution of VCGs 11 and 12 across the Queensland and New South Wales cotton districts

A total of 1382 *Fov* isolates were recovered from symptomatic cotton plants, from which 348 (25%) were randomly selected and fingerprinted using AFLPs. VCG 11 was the predominant strain, accounting for 92% (319) of the isolates. VCG 11 was widely distributed in Australian cotton fields, occurring in all farms sampled. In contrast, VCG 12 was limited to the Boggabilla area where it was first detected and never as the sole genotype (Table 1). VCG 11 genotypes appear to have successfully invaded fields where VCG 12 was assumed to be dominant.

Discussion

This study extends our knowledge of the extent to which *F. oxysporum* is associated with native *Gossypium* species in Australia. *Fusarium oxysporum* is a common component of the soil of native *Gossypium* populations, occurring in 69% of the populations sampled. Indigenous *F. oxysporum* are diverse genetically, comprising five distinct genetic lineages. The diversity of *F. oxysporum* from Arkaroola-Leigh Creek (SA) was greater than in the other regions (Fig. 1).

The most important finding of this study was that the two cotton-field *Fov* strains devastating the Australian cotton fields (VCGs 11 and 12) are clearly related to the Lineage A isolates and are genetically distinct from the eight *Fov* races found outside Australia (Fig. 2). This suggests that the appearance of Fusarium wilt of cotton in Australian cotton fields has resulted from recent change (either in virulence or prevalence) in indigenous *F. oxysporum* populations in response to continuous, wide-scale cropping of susceptible cotton cultivars. This has significant implications for further development in the pathogen and therefore for the industry. The appearance of novel Fusarium wilt pathogens from existing low frequency pathogens or local populations of non-pathogenic strains has also been observed in *F. oxysporum* strains causing Fusarium wilt of melon and tomato (Appel and Gordon 1994; Katan et al. 1994). In ongoing research we hope to clarify the diversity of indigenous *F. oxysporum* genotypes occurring in Australian cotton field soils and the evolutionary processes that promote the development and spread of pathogenic *F. oxysporum* genotypes.

The distributions of Fusarium wilt across all the major cotton-growing regions in Australia are clearly attributable to the spread of VCG 11, which was detected in all 24 farms. In contrast, VCG 12 appears to be restricted to the Boggabilla region (NSW). The underlying reasons for this exceptional difference between the distributions of VCG 11 and VCG 12 are not understood. Elucidating the underlying factors could facilitate improved disease management protocols. The prevalence of VCG 11 in Australian cotton fields should be taken into account when selecting *Fov* isolates for evaluating novel germplasm in Fusarium wilt resistance breeding programmes.

Acknowledgements

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References

Appel, D. J., and Gordon, T. R. 1994. Local and regional variation in populations of *Fusarium oxysporum* from agricultural field soils. Phytopathology 84:786–791.

Bentley, S., Kochman, J. K., Moore, N. Y., Pattermore, J. A., Gulino, L., and O'Neill, W. T. 2000. DNA diagnostics for fusarium wilt of cotton. Proceedings of the 10th Australian Cotton Conference, pp. 455–461.

Burgess, L. W., Summerell, B. A., Bullock, S., Gott, K. P., and Backhouse, D. 1994. Laboratory Manual for Fusarium Research (third edition), pp. 1–133.

Davis, R. D., Moore, N. Y., and Kochman, J. K. 1996. Characterisation of a population of *Fusarium oxysporum* f. sp. *vasinfectum* causing wilt of cotton in Australia. Australian Journal of Agricultural Research 47:1143–1156.

Katan, T., Katan, J., Gordon, T. R., and Pozniak, D. 1994. Physiologic races and vegetative compatibility groups of *Fusarium oxysporum* f. sp. *melonis* in Israel. Phytopathology 84:153–157.

Kochman, J. K. 1995. Fusarium wilt in cotton – a new record in Australia. Australasian Plant Pathology 24:74.

Nei, M. and Li, W. I. 1979. Mathematical models for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Science, USA 76:5269-5273.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research 23:4407–4414.

Wang, B., Brubaker, C. L., and Burdon, J. J. 2004. *Fusarium* species and Fusarium wilt pathogens associated with native *Gossypium* populations in Australia. Mycological Research 108: 35–44.