POTENTIAL FUSARIUM PATHOGENS OF COTTON ASSOCIATED WITH NATIVE *GOSSYPIUM* SPECIES

B. Wang, C. L. Brubaker and J. J. Burdon
CSIRO Plant Industry; GPO Box 1600; Canberra ACT 2601

Introduction

Fusarium wilt of cotton, caused by the soil-borne fungus – *Fusarium oxysporum* formae specialis *vasinfectum* (*Fov*), is a worldwide disease of great economic importance. In Australia, it was first reported in 1993 in the Brookstead and Cecil Plains areas in the Darling Downs of Queensland (Kochman et al, 1995). Now found in almost all major Australian cotton-growing regions, Fusarium wilt has become a serious problem, causing substantial losses.

Australian *Fov* strains have been demonstrated to be genetically distinct from all the overseas isolates screened to date (unpublished data). One possible explanation is that it is indigenous, previously occurring saprophytically in soil or parasitically on alternative hosts, and has recently arisen as a cotton pathogen as a consequence of unintentional selection for pathogenicity under cotton monoculture or host shifting. There are 17 native *Gossypium* species or wild cottons in Australia, four of which (*G. australe*, *G. bickii*, *G. nelsonii*, *G. sturtianum*) are widely distributed in the eastern and central areas where the major cotton-growing regions are located. As the closest relatives to the cultivated cottons, these native *Gossypium* species might have been associated with the *Fov* strains now present in the cotton-growing regions for a long period either by providing preferable habitats or acting as alternative hosts.

In this study we aimed to determine whether there are any Fusarium wilt pathogens (*Fov*) occurring in association with these native *Gossypium* species. Furthermore, we also asked whether these native cottons are infected by other *Fusarium* species that in turn may be potential future threats to the cotton industry.

Materials and methods

During 2001, 919 stem samples and 426 soil samples were collected from 78 native *Gossypium* populations in western Queensland (Mt Isa region), central Northern Territory (Alice Springs-Tennant Creek region), and southern South Australia (Flinders Ranges region). Stems were collected by cutting a section (5–15 cm in length) from the basal end of five lateral shoots from 5 to 20 plants in each population. Soil samples were collected by taking about 200 g of soil from the rhizosphere of each of five scattered plants after the top 2 to 3 cm surface layer was removed. Stem and soil samples were air-dried in the glasshouse at 15–25 °C; the soil was ground, if necessary, and passed through a 710-μm sieve. Samples were stored at 4 °C until assayed.

Fusarium species were isolated from stem samples incubated on Komada medium (Komada, 1975). Stem sections were surface-sterilized in 0.5% NaOCl for 5 min and peeled aseptically. Three small pieces were cut from the vascular tissue of each stem and placed on one plate. The plates were incubated at 25 °C for 1 week and the fungi growing out of the tissue pieces were transferred to 10% potato dextrose agar (PDA) slopes for further analysis.

Fusarium oxysporum (*Fo*) was isolated from 0.5 g soil samples sprinkled directly on Komada medium and Peptone PCNB agar plates (Burgess et al, 1995). Five plates of each medium were used for each sample. The plates were incubated at 25 °C for 1 week and all the *Fo* colonies were transferred to 10% PDA slopes for further analysis.

All the *Fusarium* isolates were subcultured from single spores and grown on carnation leaf agar plates at 25 °C under variable light. They were identified following the approach of Burgess et al. (1995), which is based on the morphological characteristics of colonies, micro- and macro-conidia, and conidiophores. Single spore isolates were grown 10% PDA slopes at 25 °C for 1 week. Conidia were washed off in 1.5 ml of sterile 15% glycerol and stored in 2.0-ml Cryogenic vials at –80 °C.

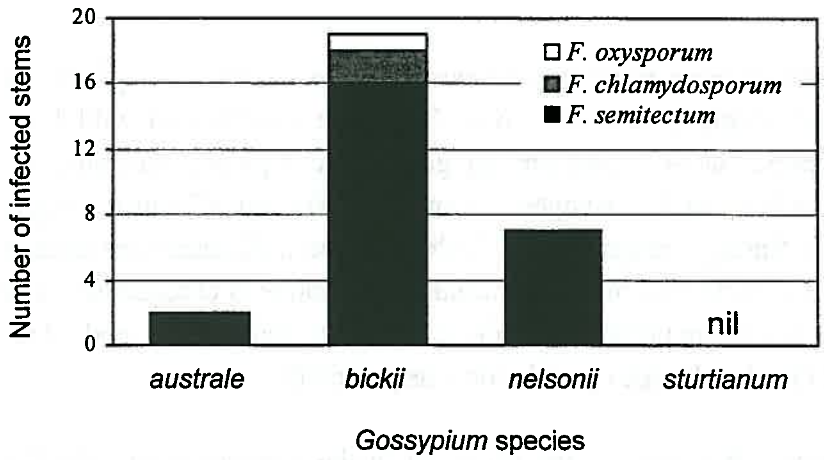
The pathogenicity of wild *Fo* isolates was determined by challenging seedlings of the highly susceptible cotton cultivar, Siokra 1-4. Isolates were grown in 75 ml of 25% potato dextrose broth in 150-ml Erlenmeyer flasks, which were placed on an orbital shaker at 150 rpm at room temperature (18–23 °C) for 1 week. Inocula were prepared by adding 75 ml of distilled water to each of the cultures. Final conidial concentrations determined using haemocytometer counts ranged from 2.0×10^5 to 8.6×10^7 conidia/ml. Two-week-old seedlings were inoculated by suspending bare rooted seedlings in the inocula for 5 min. Inoculated plants were transplanted into fresh potting mix (compost and perlite; 50:50) and grown at 18–23 °C in the glasshouse. Nine seedlings were used for each isolate with three plants per pot. Isolates causing vascular discoloration were re-tested.

Disease symptoms were assessed 6 weeks after inoculation. Isolates capable of inducing vascular discoloration in at least one plant in both trials were considered putative pathogens and, therefore, are referred to as wild *Fov*.

Results

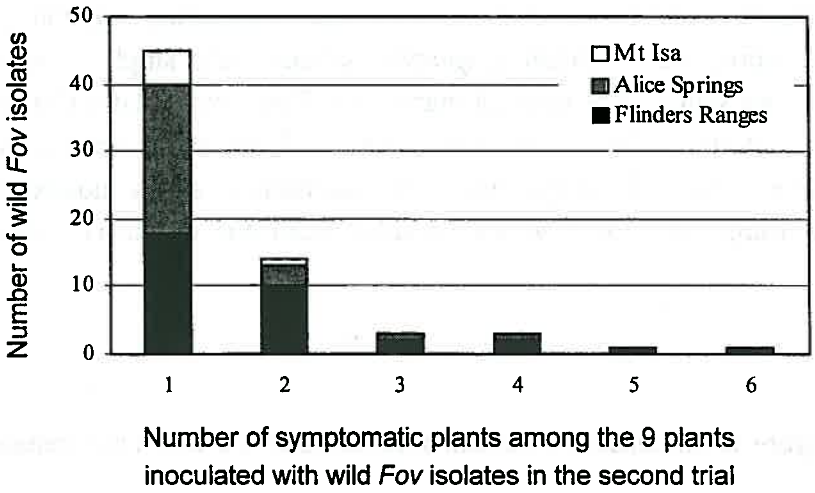
Three *Fusarium* species were isolated from 28 of the 919 stem samples – *F. semitectum*, *F. chlamydosporum* and *F. oxysporum*. *Fusarium semitectum* was the most common (25 isolates), while two *F. chlamydosporum* isolates and a single *F. oxysporum* were found in *G. bickii* stems. The greatest numbers of Fusarium isolates (19) were obtained from *G. bickii* stems. The *G. australe* and *G. nelsonii* stems hosted two and seven isolates, respectively. Notably, while *G. sturtianum* stems hosted a variety of endovascular fungi, no *Fusarium* was isolated from any of the *G. sturtianum* stems (Figure 1).

Figure 1. Incidence of *Fusarium* species in stems of four native cottons.



Of the 437 *F. oxysporum* isolates extracted from the soil samples collected from 49 native *Gossypium* populations, 132 (30%) were symptomatic in the first trial, but only 67 (15%) were symptomatic in both trials. These 67 putative wild *Fov* isolates induced mild Fusarium wilt symptoms, such as vascular discoloration and growth suppression, on the plants of Siokra 1-4, one of the most susceptible cotton cultivars in Australia. Some isolates caused foliar necrosis, but none of the plants died during the assay period. The most virulent and greatest number of pathogenic isolates originated from the Flinders Ranges following by Alice Springs. The five pathogenic isolates from Mt Isa only caused symptoms in one or two plants (Figure 2).

Figure 2. Variation in virulence among the wild *Fov* isolates from three regions.



Of the 49 native *Gossypium* populations with *Fo* in the rhizosphere, 31 (63%) also contained pathogenic isolates, *i.e.*, wild *Fov*. The incidence of wild *Fov* in the native *Gossypium* populations varied among geographic regions, occurring in 91% of the populations in the Flinders Ranges, but only in 50% and 57% of the populations in Mt Isa and Alice Springs, respectively (Table 1). The differences are even bigger if only the incidence of wild *Fov* in *G. sturtianum* populations is considered. It was not found in those the Mt Isa populations, while it was detected in 44% and 91% of the Alice Springs and Flinders Ranges populations, respectively.

A similar geographic pattern was observed in the percentage of wild *Fo* isolates that were pathogenic: 27 % of the *Fo* isolates extracted from the Flinders Ranges samples were pathogenic, while only 14% of the Alice Springs samples and 5% of the Mt Isa samples were pathogenic (Table 1).

Table 1. Incidence of wild *Fov* in the populations of native cottons and of the *Fo* isolates.

Region	Native cottons	Populations			Isolates		
		Fo	*Fov*	*Fov*/*Fo* (%)	*Fo*	*Fov*	*Fov*/*Fo* (%)
Mt Isa	*G. australe*	3	3	100	47	4	9
	G. nelsonii	2	1	50	20	2	10
	G. sturtianum	3	0	0	48	0	0
	Sub total	***8***	***4***	***50***	***115***	***6***	***5***
Alice Springs	*G. australe*	10	6	60	62	9	15
	G. bickii	7	5	71	49	8	16
	G. nelsonii	4	2	50	41	3	7
	G. sturtianum	9	4	44	46	7	15
	Sub total	***30***	***17***	***57***	***198***	***27***	***14***
Flinders Ranges	*G. sturtianum*	11	10	91	124	34	27
	Sub total	***11***	***10***	***91***	***124***	***34***	***27***
Total		**49**	**31**	**63**	**437**	**67**	**15**

Discussion

Compared with most of the cotton-growing countries in the world, Australia does not have a long history of cotton cultivation. However, native *Gossypium* species have existed on this continent for millions of years. Therefore, they are more likely to host high densities of pathogenic organisms. Little is known about the microflora, particularly *Fusarium* species, associated with these wild cottons. The present study is the first to characterise the diversity of *Fusarium* species associated with the Australian *Gossypium* species.

Various vascular discolorations, often indicative of endovascular fungi, were observed in most of the *Gossypium* stem samples collected from wild populations. A variety of filamentous fungi were isolated from 281 stems (data not provided), but *Fusarium* species were only isolated from 28 stems. A single *Fusarium oxysporum* isolate was extracted from a *G. bickii* stem; however, this isolate was not capable of causing wilt symptoms in cultivated cotton. It is also notable that no endophytic *Fusarium* infection was found in any of the *G. sturtianum* stems, although *G. sturtianum* is more susceptible to other endovascular fungi (unpublished data) and had the greatest rhizosphere exposure to *F. oxysporum*. These results suggest that while the native *Gossypium* species are frequent hosts to endovascular fungi and are growing in soils in which *F. oxysporum* is a common resident, *Fusarium oxysporum* only rarely invades the stems. This further raises the possibility that the native *Gossypium* species may contain resistance that would be effective against the *Fov* occurring in the cotton fields.

That some of the wild *Fusarium* isolates cause mild wilt symptoms on a susceptible cotton cultivar suggests that the *de novo* origin of the cotton field pathogens could have occurred as a result of unintentional selection for pathogenic clones under continual cotton monoculture. While the possibility that *Fusarium* crop pathogens could have arisen in this manner has been suggested, it has never been documented unambiguously, and the accidental introduction of an overseas *Fov* or a host switch from an agricultural weed cannot be ruled out (Gordon and Martyn, 1997). Ongoing molecular genotyping of the wild *Fusarium* isolates will improve our understanding of the origin of the *Fov* strains occurring in the cotton growing regions of Queensland and New South Wales. This will be an important component of disease management strategies that will reduce the risk of new pathogenic strains of *Fov* arising.

Variation in virulence suggests that the wild *Fov* isolates may represent distinct lineages. This is consistent with the asexual mode of reproduction that characterizes *Fusarium*. Preliminary work has been initiated to elucidate the genetic structure of the wild *Fusarium* isolates as well as the genetic relationships between the wild *Fov* and the *Fov* identified in the cultivated cotton fields. If the *Fov* in the cultivated cotton fields arose from an indigenous strain, we expect to find a closely related wild lineage.

Acknowledgements

This work was supported by a grant from CRDC. We thank Ms Alison Fraser and Mr Jim Grace for their assistance in sample collection and Mr Matt Woods for his technical assistance in this study.

References

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. University of Sydney.

Gordon, T. R., and Martyn, R. D. (1997). The evolutionary biology of *Fusarium oxysporum* Annual Review of Phytopathology 35:111-128.

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

Komada, H. (1975). Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Review of Plant Protection Research* 8:114-125.