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Characterisation of Australian isolates of *Fusarium oxysporum* f. sp. *cubense* by DNA fingerprinting analysis

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Abstract. Genetic variation among Australian isolates of the fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*), which causes Fusarium wilt in banana, was examined using DNA amplification fingerprinting (DAF). Ninety-four isolates which represented Races 1, 2, 3, and 4, and vegetative compatibility groups (VCGs) 0120, 0124, 0125, 0128, 0129, 01211, 01213/16, and 01220 were analysed. The genetic relatedness among isolates within each VCG, and between the 8 different VCGs of *Foc* present in Australia was determined. The DNA fingerprint patterns were VCG-specific, with each VCG representing a unique genotype. The genetic similarity among isolates within each VCG ranged from 97% to 100%. Among the different VCGs of *Foc*, 3 major clusters were distinguished which corresponded with race. All Race 1 and 2 isolates (VCGs 0124, 0125, 0128, and 01220) were closely related and clustered together, the Race 3 isolates from *Heliconia* clustered separately, and all Race 4 isolates (VCGs 0120, 0129, 01211, and 01213/16) clustered together.

Fifteen isolates from Alstonville, NSW, were characterised because although they were classified as Race 2 based on their recovery from cooking banana cultivars, they belonged in VCG 0124, which had previously contained only Race 1 isolates. The occurrence of more than one race within a VCG means that vegetative compatibility grouping cannot be used to assign pathotype to pathogenic race as previously thought. It was possible to distinguish the Race 1 and Race 2 isolates within VCG 0124 using DNA fingerprinting, as each race produced a unique DNA fingerprint pattern. Among the Australian isolates, DNA fingerprinting analysis identified 9 different VCGs and genotypes of *Foc*.

Additional keywords: banana, polymerase chain reaction, vegetative compatibility group.

Introduction

Banana (*Musa* spp.) is Australia's most valuable tropical fruit crop with an annual production for domestic consumption valued at approximately $300 million (Pegg *et al.* 1996). The major banana production areas in Australia are situated in the wet semi-tropics of northern Queensland in the coastal area between Cardwell and Cairns, and in the subtropical region between Bundaberg in southern Queensland and Coffs Harbour in northern New South Wales. Production areas are also located at Carnarvon and Kununurra in Western Australia, and near Katherine and Darwin in the Northern Territory (Pegg *et al.* 1996). Cavendish (AAA) cultivars are the most widely grown, with Williams representing 95% of total production. Lady finger (AAB) is also grown, mostly in the southern parts of Queensland and in northern New South Wales.

Fusarium wilt (or Panama disease) of banana is caused by the fungus *Fusarium oxysporum* Schlect. f. sp. *cubense* (E. F. Smith) Snyd. & Hans. (*Foc*), and is most commonly spread through infected planting material (rhizomes or suckers). Persistence of the fungus in soil renders plantations non-viable once infested and it is only the planting of disease-free areas which allows continued expansion of pro-

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duction areas. Fusarium wilt is regarded as one of the most serious threats to banana production in Australia since disease resistant replacements for Cavendish cv. Williams and Lady finger are not yet widely available.

Isolates of *Foc* have traditionally been classified into 4 races based on pathogenicity to a small number of differential host cultivars in the field. All 4 races of *Foc* are present in Australia. Race 1 of *Foc* affects Lady finger and Gros Michel (AAA) cultivars, and Race 2 affects Bluggoe (ABB) and related cultivars. Races 1 and 2 of *Foc* have been recovered from banana in both northern and southern Queensland, and northern New South Wales. Race 1 has also been isolated from banana in Western Australia. The subtropical banana industry in Australia is in jeopardy due to its heavy reliance on the Lady finger cultivar, which many consumers prefer but is limited in availability due to lower productivity and extreme susceptibility to fusarium wilt. Race 3, a pathogen of *Heliconia* spp. which was only mildly pathogenic on banana, was first reported in 1963 from Colombia, Costa Rica, Honduras, and Panama (Waite 1963). In 1995, Race 3 isolates of *Foc* were recovered from *Heliconia* spp. in the Northern Territory, Australia (J. Duff, pers. comm.). Race 4 of *Foc* was first described in 1977 from Taiwan (Su *et al*. 1977), and is capable of attacking Cavendish cultivars as well as all cultivars affected by Races 1 and 2. Within Australia, Race 4 has been found in northern New South Wales and southern Queensland. Although damage to Cavendish was first reported in the Canary Islands in the 1920s (Ashby 1926), until recently only Cavendish cultivars in subtropical regions of Australia, South Africa, and Taiwan were seriously affected by Race 4 of *Foc* (now referred to as 'subtropical' Race 4). However, strains of *Foc* capable of attacking Cavendish cultivars under tropical conditions have been identified in Malaysia and Indonesia and have been referred to as 'tropical' Race 4. Recent outbreaks of 'tropical' Race 4 in the Northern Territory have heightened awareness of the threat that this strain poses to the Australian banana industry.

Race classification is an artificial grouping since the genetics of virulence and resistance are poorly understood in *Foc* and its banana hosts. The term 'race' is used for convenience only and is in need of better definition for this pathosystem. Numerous methods other than race have been used in an attempt to classify both Australian and world populations of *Foc*, including: vegetative compatibility (Ploetz and Correll 1988; Brake *et al*. 1990; Ploetz 1990; Moore *et al*. 1993; Moore 1994), production of volatiles (Brandes 1919; Stover 1962; Moore *et al*. 1991), electrophoretic karyotyping (Miao 1990; Boehm *et al*. 1994), random amplified polymorphic DNA (RAPD) analysis (Bentley *et al*. 1995), restriction fragment length polymorphism (RFLP) analysis (Koenig *et al*. 1997), DNA amplification fingerprinting (DAF) analysis (Bentley and Bassam 1996; Bentley *et al*. 1998), and DNA sequencing of nuclear and mitochondrial genes (O'Donnell *et al*. 1998). These methods have been useful for indicating pathotype, as no reliable small-plant pathogenicity test currently exists. Field testing for pathogenicity is expensive and inefficient due to the limited number of strains in any given field, and the use of exotic isolates is restricted by quarantine regulations.

Isolates of *Foc* have been divided into vegetative compatibility groups (VCGs) that comprise genetically isolated subpopulations. Isolates within the same VCG are assumed to be clonally derived populations of the pathogen, since sexual recombination is not known to occur in *Foc* (Leslie 1993). At least 21 VCGs of *Foc* have been characterised world-wide (Ploetz 1990; Moore 1994; Ploetz and Pegg 1999). Brake *et al*. (1990) used vegetative compatibility to subdivide Australian populations of *Foc* into 6 VCGs and found that VCG was correlated with virulence on specific cultivars in the field. Moore *et al*. (1993) confirmed these results and found that Race 1 isolates belonged to VCGs 0124 and 0125, Race 2 isolates belonged to VCG 0128, and Race 4 isolates belonged to VCGs 0120, 0129, and 01211. In 1992, a unique population of *Foc* that has been designated VCG 01220 was identified in Carnarvon, Western Australia (Pegg *et al*. 1995; Shivas *et al*. 1995). The Carnarvon isolates of *Foc* were classified as Race 4 because they were isolated from Cavendish; however, characterisation by volatile production, vegetative compatibility, and pectic enzyme and RAPD-PCR analyses indicated that these isolates were more similar to Races 1 and 2 of *Foc* than to Race 4 (Pegg *et al*. 1995).

Many molecular methods have been used to assess genetic relationships among the VCGs and races of *Foc*. Boehm *et al*. (1994) divided isolates of *Foc* into 2 broad groups based on their electrophoretic karyotype, chromosome number, and genome size. Group I contained isolates in VCGs 0124, 0125, 0124/5, 01210, and 01214, which typically had a higher chromosome number and larger genome size than Group II, which contained isolates in VCGs 0120, 0121, 0122, 0123, 0129, and 01213. Bentley *et al*. (1995) also divided isolates of *Foc* into 2 major groups based on RAPD-PCR fingerprinting. Group 1 included all isolates in VCGs 0120, 0121, 0122, 0126, 01210, 01211, and 01212. Group 2 contained all isolates belonging in VCGs 0123, 0124, 0124/5, and 0125. Koenig *et al*. (1997) further differentiated the 2 major groups of *Foc* into 10 clonal lineages based on RFLP analysis using anonymous single-copy probes. Among 165 isolates, 72 haplotypes were identified, 5 of which accounted for nearly half of the isolates examined. O'Donnell *et al*. (1998) resolved 5 lineages among 23 isolates of *Foc* based on the DNA sequences for translation elongation factor 1α and the mitochondrial small subunit ribosomal RNA genes. Bentley *et al*. (1998) identified 9 clonal lineages among 341 isolates of *Foc* based on DNA fingerprinting analysis. A total of 33 different genotypes were identified within *Foc*, including 14 new genotypes that did not belong to any of the currently described VCGs. There

Table 1. Australian isolates of *Fusarium oxysporum* f. sp. *cubense* analysed in this study (DFG, DNA fingerprinting group; VCG, vegetative compatibility group)

Acc. No.	Geographic origin	Host genotype[A]	Race	DFG	Acc. No.	Geographic origin	Host genotype[A]	Race	DFG
		VCG 0120					*VCG 0128*		
22615	Byron Bay, NSW	Cavendish (AAA)	4	I	22993	South Johnstone	Blue Java	2	IV
23486	Wamuran, Qld	Cavendish	4	I	22994	South Johnstone	Bluggoe	2	IV
23516	Wamuran	Inarnibal (AA)	4	I	23909	Kamerunga, Qld	Bluggoe	2	IV
23539	Beerwah, Qld	Lady finger (AAB)	4	I	23996	Kamerunga	'Sugar' (?)	2	IV
23550	Wamuran	*Musa jackeyi*	4	I	24246	South Johnstone	Monthan (ABB)	2	IV
23551	Wamuran	SH3362 (AA)	4	I	24247	South Johnstone	Tuu Gia (AA)	2	IV
23897	Wamuran	Cavendish	4	I	24250	South Johnstone	Kluai Nui Mue Nang (ABB)	2	IV
23598	Wamuran	Cavendish	4	I	24251	South Johnstone	Blue Java	2	IV
23599	Wamuran	Cavendish	4	I	24253	South Johnstone	Silver Bluggoe	2	IV
23607	Wamuran	SH3362	4	I	24255	South Johnstone	Tukuru (ABB)	2	IV
5631N	Landsborough, Qld	Cavendish	4	I			*VCG 0129*		
–42F	Wamuran	Cavendish	4	I	8617	Mooloolah, Qld	Cavendish	4	I
W91-307	Eungella, NSW	Lady finger	4	I	23509	Gunalda, Qld	Lady finger	4	I
W91-345	Mullumbimby, NSW	Lady finger	4	I	23510	Gympie, Qld	Lady finger	4	I
MD401	Wamuran	Cavendish	4	I	23512	Wappa Dam, Qld	Lady finger	4	I
		VCG 0124			23518	Kin Kin, Qld	Lady finger	4	I
23485	Mena Creek, Qld	Lady finger	1	IV	23540	Wamuran	Pisang Rajah	4	I
23532	Ormeau, Qld	Lady finger	1	IV	23549	Wamuran	Mysore (AAB)	4	I
23534	Ormeau	Lady finger	1	IV	23565	Oakwood, Qld	Lady finger	4	I
23536	Brookfield, Qld	Ducasse (ABB)	1	IV	23566	Valdora, Qld	Lady finger	4	I
23538	Agnes Waters, Qld	Lady finger	1	IV	23606	Wamuran	Japaraka (AA?)	4	I
23567	Innisfail, Qld	Ducasse	1	IV	23608	Wamuran	Cavendish	4	I
23568	Innisfail	Ducasse	1	IV	23610	Wamuran	Japaraka	4	I
23603	Tallebudgera, Qld	Lady finger	1	IV	23611	Wamuran	Kokopo (AAA)	4	I
23734	Ormeau	Lady finger	1	IV	23615	Wamuran	Japaraka	4	I
24409	Alstonville, NSW	Bluggoe (ABB)	2	IV	23619	Wamuran	Pacific Plantain (AAB)	4	I
24410	Alstonville	Blue Lubin (ABB)	2	IV	23620	Wamuran	IC2 (AAAA)	4	I
24411	Alstonville	Blue Torres (ABB)	2	IV	23621	Wamuran	Kokopo	4	I
24412	Alstonville	Blue Torres	2	IV	23622	Wamuran	Waimara (AA?)	4	I
24413	Alstonville	Bluggoe	2	IV	23624	Wamuran	Waimara	4	I
24414	Alstonville	Bluggoe	2	IV	23625	Wamuran	Cavendish	4	I
24415	Alstonville	Bluggoe	2	IV	23626	Wamuran	*M. acuminata* subspecies *banksii* X *M. schizocarpa*	4	I
24416	Alstonville	Bluggoe	2	IV					
24417	Alstonville	Blue Java (ABB)	2	IV	24234	Mooloolah	Cavendish	4	I
24418	Alstonville	Blue Java	2	IV	N5446	Cooloolabin, Qld	Lady finger	4	I
24219	Alstonville	Blue Java	2	IV			*VCG01211*		
24220	Alstonville	Blue Java	2	IV	23631	Wamuran	SH3142 (AA)	4	I
24421	Alstonville	Pisang Rajah (AAB)	2	IV			*VCG 01213/16*		
24422	Alstonville	Pisang Rajah	2	IV	23371	Darwin, NT	Cavendish	4	III
24425	Alstonville	Silver Bluggoe (ABB)	2	IV			*VCG01220*		
		VCG 0125			24200	Carnarvon, WA	Cavendish	4	IV
8605	Tallebudgera	Lady finger	1	IV	24208	Carnarvon	Plantain var.	4	IV
8611	Tomewin, Qld	Lady finger	1	IV	24218	Carnarvon	Cavendish	4	IV
22468	Tomewin	Lady finger	1	IV	24219	Carnarvon	Cavendish	4	IV
23477	Tallebudgera	Lady finger	1	IV	24220	Carnarvon	Cavendish	4	IV
23480	Tallebudgera	Lady finger	1	IV	24221	Carnarvon	Cavendish	4	IV
23482	Currumbin, Qld	Lady finger	1	IV	24223	Carnarvon	Cavendish	4	IV
23487	Currumbin	Lady finger	1	IV			*Heliconia F. oxysporum*		
23488	Tallebudgera	Lady finger	1	IV	22212	Darwin	*Heliconia chartacea*	3	IX
23529	South Johnstone, Qld	Ducasse	1	IV					
23604	Petsch Creek, Qld	Lady finger	1	IV					
23906	Pimpama, Qld	Lady finger	1	IV					
M5386	Mareeba, Qld	Ducasse	1	IV					

[A] Banana cultivars are interspecific and intraspecific hybrids of *Musa acuminata* (A) and *Musa balbisiana* (B).

was good correlation between each of the groups of *Foc* defined by these different methods.

In this study, the genetic relatedness among each of the different races and VCGs of *Foc* that occur in Australia was determined using DNA fingerprinting. An understanding of the genetic diversity within the fungus *Foc* is essential for breeding or selection of banana cultivars with resistance to fusarium wilt, so that new cultivars can be tested against the widest possible range of pathogen variants. Knowledge of the distribution of pathogen variants is also essential in designing effective quarantine regulations to limit the spread of this disease within Australia.

Materials and methods

Fungal isolates

Ninety-four Australian isolates of *Foc* were analysed that represented Races 1, 2, 3, and 4, and VCGs 0120, 0124, 0125, 0128, 0129, 01211, 01213/16, and 01220 (Table 1). Isolates were prepared as monoconidial cultures that had been either lyophilised or grown on sterile, moist filter paper then dried and stored on silica gel at 4°C. Each isolate was cultured on carnation leaf agar plates at 25ºC for 4–5 days. These cultures were used to inoculate 250-mL Erlenmeyer flasks containing 200 mL of quarter-strength potato dextrose broth, and incubated at room temperature without shaking for no longer than 7 days. Fungal mycelium was harvested by filtration through Miracloth (Calbiochem Inc.) and stored at –70ºC until used for DNA extraction. Each isolate was analysed at least twice by DNA fingerprinting.

DNA fingerprinting analysis

The methods used for DNA extraction and DNA fingerprinting analysis have been described previously (Bentley and Bassam 1996; Bentley *et al*. 1998). The modified DAF system incorporates optimised PCR reaction, thermocycling, and electrophoresis conditions that tolerate variation and consistently generate reproducible DNA fingerprints (Bentley and Bassam 1996). DNA amplification fingerprinting system DNA was amplified using each of 4 oligonucleotide primers (EHKJ 5´ GCTCACGA 3´; IMBE 5´ GAAACGCC 3´; NROI 5´ CCTGGTGG 3´; and RKMI 5´ CCCGTCGT 3´). Primers were synthesised by Integrated DNA Technologies (Coralville, IA, USA). The similarity between different isolates of *Foc* was determined using Molecular Analyst Software Fingerprinting Plus v. 1.0 (Bio-Rad Laboratories, Hercules, California, USA). A total of 234 fragments were scored for all 4 primers; 174 were polymorphic. To determine the relationships between the different VCGs based on all primers, a similarity matrix that represented the average of the values from the 4 similarity matrices was clustered by the unweighted pair group method with arithmetic means (UPMGA) using the SAHN module of NTSYSpc v. 2.0. The goodness-of-fit of the phenogram was determined by computing a cophenetic value matrix using the COPH module and comparing this matrix with the SAHN tree matrix using the MXCOMP module. A cophenetic correlation of $r > 0.9$ is considered a very good fit.

Results

Genetic variation within each VCG

All isolates within each VCG generally produced the same DNA fingerprint for each of the primers used, regardless of the geographic location or host cultivar from which they were collected. Isolates within VCG 0120 all produced an identical DNA fingerprint with the exception of isolate 23607. This isolate differed from the other isolates in VCG 0120 in that it was missing one DNA fragment (at 225 bp) using primer IMBE and 2 DNA fragments (at 150 bp and 600 bp) using primer RKMI. All isolates in VCG 0120, including 23607, produced an identical DNA fingerprint with primers EHKJ and NROI. The genetic similarity among isolates within VCG 0120 was 99% (Table 2).

All Race 1 isolates in VCG 0124 produced an identical DNA fingerprint using primers EHKJ, NROI, and RKMI. Using primer IMBE, isolate 23603 differed slightly from the other Race 1 isolates in VCG 0124 in that one DNA fragment was produced at a lower intensity. All Race 2 isolates in VCG 0124 from Alstonville, NSW, produced an identical DNA fingerprint pattern to each other with each of the primers used. However, the Alstonville isolates were identical to the Race 2 isolates in VCG 0128, not the Race 1 isolates in VCG 0124. The average genetic similarity of all isolates within VCG 0124 was 98%.

All isolates within VCG 0125 produced the same DNA fingerprint with primer EHKJ, except that a group of isolates (22468, 23477, 23480, 23488, 23604, and 23906) produced 2 extra DNA fragments at 375 bp and 575 bp. Several isolates within this VCG also differed by the absence of a single DNA fragment; 8605 was missing a DNA fragment at 695 bp, 8611 and M5386 at 690 bp, and 23477 at 300 bp. Using primer RKMI, all isolates within VCG 0125 produced the same DNA fingerprint except that isolates 22468 and 23488 were both missing a DNA fragment at 625 bp, and isolate 23477 was missing a DNA fragment at 150 bp. Using primers IMBE and NROI, all isolates within VCG 0125 produced an identical DNA fingerprint. The average genetic similarity among isolates within VCG 0125 was 97% (Table 2).

All isolates within VCG 0128 produced an identical DNA fingerprint with each of the 4 primers used, therefore the genetic similarity among isolates within VCG 0128 was 100%.

All isolates within VCG 0129 produced an identical DNA fingerprint pattern with primer IMBE (Fig. 1) and primer NROI. However, using primer RKMI, isolates 23615 and

Table 2. Genetic similarity (%) among isolates within each vegetative compatibility group (VCG) of *Fusarium oxysporum* f. sp. *cubense*

VCG	No. of isolates analysed	Average genetic similarity (%)
0120	15	99
0124	24	98
0125	12	97
0128	10	100
0129	23	99
01211	1	—[A]
01213/16	1	—[A]
01220	7	99
Heliconia F. oxysporum	1	—[A]

[A] Genetic similarity could not be determined as only one isolate was examined from each of these groups.

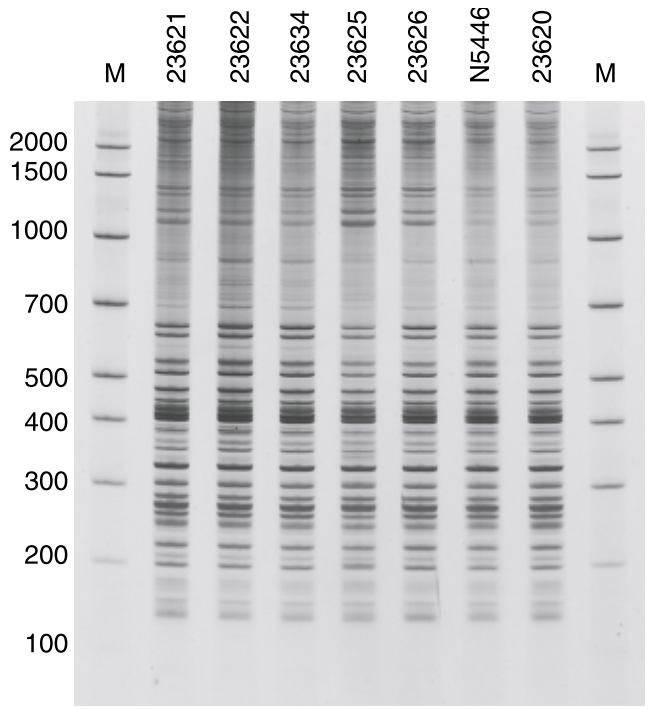

Fig. 1. Genetic similarity of isolates within vegetative compatibility group (VCG) 0129 of *Fusarium oxysporum* f. sp. *cubense*. DNA fingerprints were generated using primer IMBE. The isolate accession numbers are indicated at the top of the figure. The sizes in base pairs of the molecular weight marker are indicated to the left of the figure.

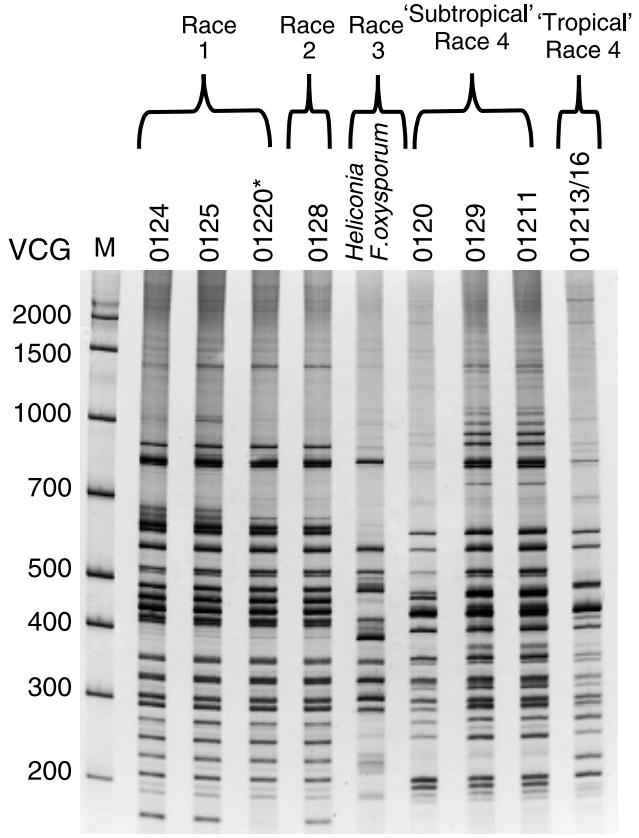

Fig. 2. Genetic variation between different Australian vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f. sp. *cubense*. DNA fingerprints were generated using primer RKMI. The VCG codes and races are indicated at the top of the figure. The sizes in base pairs of the molecular weight marker are indicated to the left of the figure.

23621 produced an extra DNA fragment at 700 bp, and with primer EHKJ, isolate 24235 produced an extra DNA fragment at 600 bp. The average genetic similarity among isolates in VCG 0129 was 99%.

All isolates within VCG 01220 produced an identical DNA fingerprint pattern with each of the primers used, except isolate 24200 which produced one extra DNA fragment at 330 bp with primer EHKJ, and one extra DNA fragment at 150 bp with primer RKMI. The genetic similarity among isolates within VCG 01220 was 99%.

The genetic similarity within VCGs 01211 and 01213/16, and the strain of *F. oxysporum* from *Heliconia* could not be determined, as only one isolate was examined from each of these groups.

Genetic variation between VCGs and races

As there was little to no genetic variation among isolates within each VCG, one isolate was selected to represent each VCG, and the relationships between the VCGs and their race designation were determined by comparing these representative isolates using the 4 different primers. Each of the 4 primers grouped the isolates similarly, based on the respective DNA fingerprints they generated (Fig. 2). Each VCG represented a unique genotype that could be distinguished by the presence or absence of one or more DNA fragments. However, isolates within VCGs 0124 and 0125 (Race 1), and VCGs 0129 and 01211 ('subtropical' Race 4), produced identical DNA fingerprint patterns to each other with most of the primers used.

To determine the relationships between the VCGs based on all primers, a similarity matrix that represented the average of the values determined for each of the 4 primers was clustered by the UPGMA method using NTSYS pc v. 2.0 (Table 3, Fig. 3). The cophenetic correlation value of $r = 0.98$ indicated that the UPGMA cluster analysis was statistically significant. Three major clusters were distinguished: all Race 4 isolates within VCGs 0120, 0129, 01211, and 01213/16 clustered together, all Race 1 and 2 isolates within VCGs 0124, 0125, 01220, and 0128 also clustered together, and the Race 3 isolates from *Heliconia* clustered separately (Fig. 3). The average genetic similarity among the Race 4 VCGs (0120, 0129, 01211, and 01213/16) ranged from 76% to 96%, whereas the average genetic similarity among the Race 1 and 2 VCGs (0124, 0125, 01220, and 0128) ranged from 93 to 99%. The average genetic similarity between the Race 4 cluster and the Race 1 and 2 cluster was 63%. The average genetic similarity between the Race 3 and Race 4 clusters was 63%, and the average genetic similarity between the

Table 3. Genetic similarity (%) between different vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f. sp. *cubense*

Each value is the average of the values determined for the 4 different primers using the Jaccard similarity coefficient

VCG	VCG								
	0129	01211	0120	01213/16	0124	0125	01220	0128	*Heliconia F. oxysporum*
0129	100								
01211	96	100							
0120	85	86	100						
01213/16	79	76	84	100					
0124	64	63	61	61	100				
0125	66	65	59	59	99	100			
01220	65	66	63	61	93	94	100		
0128	67	67	65	63	93	94	98	100	
Heliconia F. oxysporum	67	62	62	61	64	64	68	68	100

Table 4. Australian genotypes of *Fusarium oxysporum* f. sp. *cubense* identified by DNA fingerprinting analysis

Genotype identified by DNA fingerprint pattern[A]	Races represented	Clonal lineages represented[B]
0120	4	I
0124	1 and 2	IV
0125	1	IV
0128	2	IV
0129	4	I
01211	4	I
01213/16	4	III
01220	4?	IV
Heliconia F. oxysporum	3	IX

[A] Genotypes were referred to by their vegetative compatibility group codes.

[B] Clonal lineages within *F. oxysporum* f. sp. *cubense* were previously described by Bentley *et al*. (1998).

Race 3 and Race 1 and 2 clusters was 66% (Fig. 3). The Race 4 cluster (VCGs 0120, 0129, 01211, and 01213/16) corresponded to clonal lineages I and III, the Race 1 and 2 cluster (VCGs 0124, 0125, 0128, and 01220) corresponded to lineage IV, and the Race 3 cluster corresponded to lineage IX described by Bentley *et al*. (1998) (Table 4).

Characterisation of isolates of Foc *from Alstonville, NSW*

The 15 Race 2 isolates of *Foc* from Alstonville, NSW, belonging in VCG 0124, were compared specifically to isolates from VCGs 0124 and 0128, because previously only Race 1 isolates belonged in VCG 0124. All of the isolates from Alstonville produced an identical DNA fingerprint pattern to each other with each of the primers used. The DNA fingerprint pattern of these Alstonville isolates was also identical to the Race 2 isolates belonging in VCG 0128 for each of the primers used (Fig. 4). Therefore, the genetic similarity between the Alstonville VCG 0124 isolates and the VCG 0128 isolates was 100%.

Discussion

In this study, the genetic variation among Australian isolates of *Foc* was examined using DNA amplification fingerprinting. There was little or no variation among isolates of *Foc* within each VCG, although the isolates were from many different host cultivars and geographical locations. The lack of genetic variation within a VCG is to be expected because each VCG is thought to be a genetically isolated group that is clonally derived (Leslie 1993; Kistler 1997). The genetic similarity of isolates within a VCG has been previously reported for VCGs of *Foc* (Bentley *et al*. 1995; Koenig *et al*. 1997; Bentley *et al*. 1998) and VCGs of other *F. oxysporum* formae speciales (Kistler 1997).

Although there was generally little or no genetic variation among isolates within each VCG, there was considerably more variation among isolates within VCGs 0124 and 0125. The genetic variation among isolates within each of these VCGs ranged from 97% to 100%. Several isolates within each of these VCGs differed by the presence or absence of one or more DNA fragments. Some isolates in VCGs 0124, 0125, 0128, and 01220 also produced identical DNA fingerprint patterns to each other with some of the primers used. Bentley *et al*. (1998) previously reported that isolates in VCGs 0124, 0125, 0128, 01212, and 01220 formed a VCG complex where some isolates in the same VCG produced different DNA fingerprints, and some isolates belonging in different VCGs produced identical DNA fingerprints. VCGs 0124 and 0125 have been shown to contain 'bridging' or 'cross compatible' isolates (Brake *et al*. 1990; Ploetz 1990; Moore *et al*. 1993; Moore 1994). The bridging isolates are capable of forming heterokaryons with Nit M testers representing more than one VCG. The high degree of genetic similarity identified between VCGs 0124, 0125, 0128, and 01220, emphasises the need to involve multiple Nit M testers or use a promiscuous tester for identification of these VCGs using vegetative compatibility analysis.

Isolates from Alstonville, NSW, were placed in VCG 0124 when analysed by vegetative compatibility. Previously, in

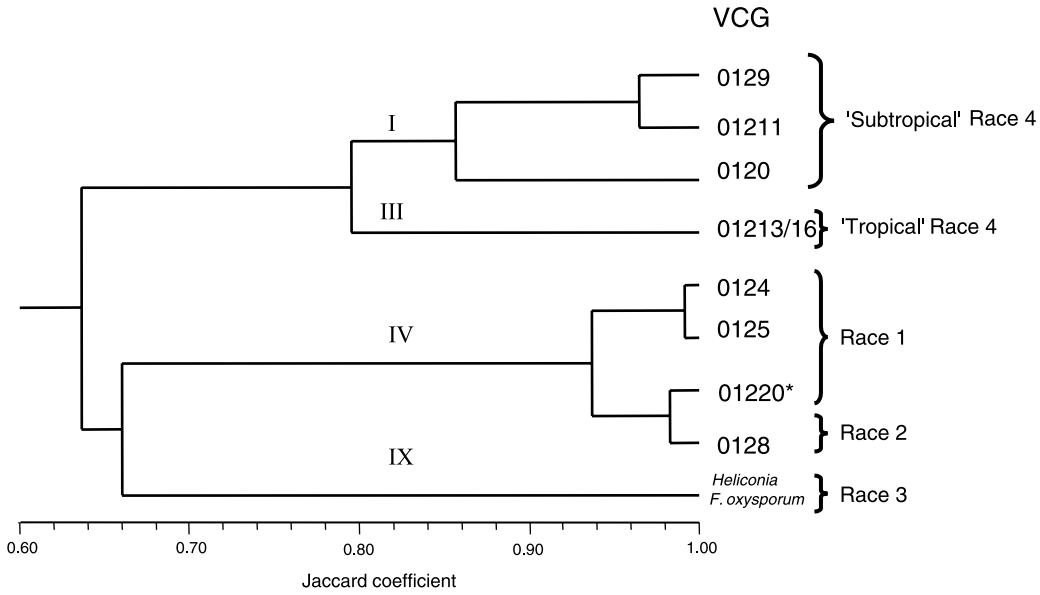

Fig. 3. Genetic similarity among the Australian genotypes of *Fusarium oxysporum* f. sp. *cubense*. Phenogram represents UPGMA cluster analysis of the average similarity values determined for 4 arbitrary primers using the Jaccard similarity coefficient. DNA fingerprint groups (DFGs) are indicated on each branch of the phenogram. *Isolates in VCG 01220 from Carnarvon were isolated from Cavendish and therefore called Race 4; however, they are more genetically similar to Race 1 and 2 isolates of *F. oxysporum* f. sp. *cubense* (Pegg *et al*. 1995).

Australia, although more than one VCG has been associated with a race, multiple races have not been associated with only a single VCG. Until now, all Australian isolates of *Foc* belonging in VCG 0124 were placed in Race 1 since only Lady finger and other Race 1 susceptible cultivars were affected (Brake *et al*. 1990; Moore *et al*. 1993). However, the VCG 0124 isolates from Alstonville were recovered from only Race 2 susceptible cultivars. DNA fingerprinting analysis revealed that the Alstonville isolates in VCG 0124 were identical to other Race 2 isolates that belonged in VCG 0128. All prior studies have shown that race and VCG classification of *Foc* in Australia have correlated well with each other. The identification of 2 races within VCG 0124 by DNA fingerprinting analysis indicates that VCG phenotyping cannot always be used to assign pathogenic race as previously thought.

Three major groups were distinguished among the Australian VCGs of *Foc*. All Race 1 and 2 isolates in VCGs 0124, 0125, 0128, and 01220 clustered together, the Race 3 isolates from *Heliconia* clustered separately, and all Race 4 isolates in VCGs 0120, 0129, 01211, and 01213/16 also clustered together. The Race 1 and 2, and the Race 4, clusters correlate with the 2 major groups of *Foc* previously distinguished by volatile production (Moore *et al*. 1991), electrophoretic karyotyping (Boehm *et al*. 1994), RAPD-PCR fingerprinting (Bentley *et al*. 1995; Bentley and Bassam 1996), RFLP analysis (Koenig *et al*. 1997), and DAF analysis (Bentley *et al*. 1998). In this study, characterisation of the isolates of *F. oxysporum* from *Heliconia* represented a third major group within *Foc*. These particular isolates from *Heliconia* were not pathogenic to banana (J. Duff, pers. comm.) and therefore by definition should be excluded from the forma specialis '*cubense*'.

There are 2 hypotheses for the origin of *Foc*. Stover (1962) first proposed that the pathogen co-evolved with edible bananas and their wild diploid progenitors in Asia, and then has been distributed to other countries with infected banana plants. Simmonds (1966) then proposed that *Foc* evolved independently from local populations in different countries to attack an introduced host plant. The close relationships among Australian isolates in VCGs 0124, 0125, 0128, and 01220 suggest that these VCGs may have a common progenitor (Ploetz and Pegg 1997; Bentley *et al*. 1998). The VCGs 0124 and 0125 are widespread in most banana growing regions of the world (Ploetz *et al*. 1990) and it is likely that these strains have been introduced to Australia with infected banana plants. The VCG 01220 isolates from Carnarvon were identical to isolates recovered from an unidentified AA banana plant that was used as a windbreak in Carnarvon (Pegg *et al*. 1995). Isolates of *Foc* obtained from these windbreak plants in the mid-1960s and deposited in the herbarium at the Western Australian Department of Agriculture were also identical to the current VCG 01220 strain, indicating that the population has been present in the area for at least 30 years. Furthermore, this genotype (VCG 01220) has also been identified from Indonesia by DNA fingerprinting analysis (S. Bentley, unpubl. data), which is con-

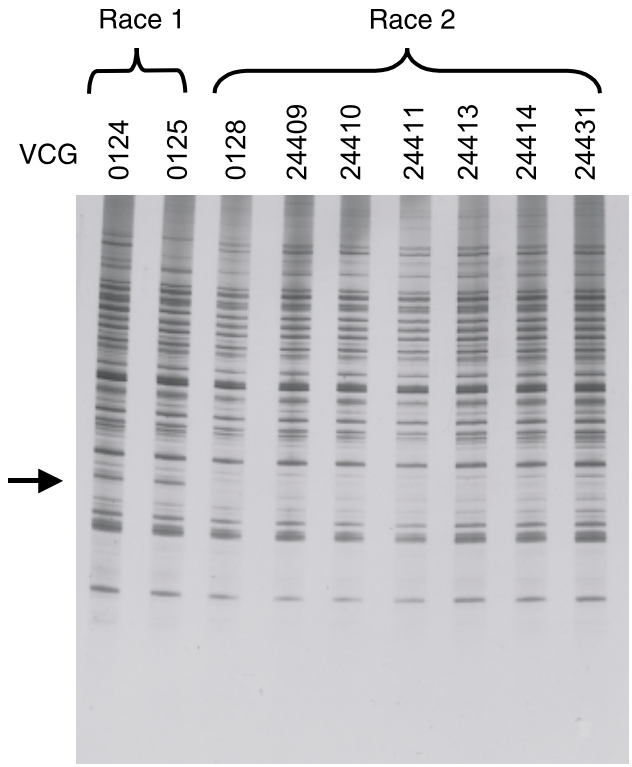

Fig. 4. Genetic variation between isolates of *Fusarium oxysporum* f. sp. *cubense* from Alstonville and other Australian Race 1 and 2 isolates from vegetative compatibility groups (VCGs) 0124, 0125, and 0128. DNA fingerprints were generated using primer NROI. The isolate VCG code, race, and accession number are indicated at the top of the figure. The arrow indicates the major band differences that distinguish Race 1 isolates and Race 2 isolates.

sistent with the introduction of many cultivars of banana directly into the north-west of Western Australia from Java and Singapore before 1930 (Shivas *et al*. 1995).

The genetic similarity of the 'subtropical' Race 4 strains of *Foc* (VCGs 0120, 0129, and 01211) suggests that these populations are also closely related. Pegg *et al*. (1993) reported that some isolates from VCGs 0120 and 0129 were cross-compatible with isolates from VCG 01211, so it was possible that VCGs 0129 and 01211 had arisen from a diverging VCG 0120 population as the isolates lost the ability to form heterokaryons consistently. Since VCGs 0129 and 01211 are yet to be found in Asia, it is possible that these VCGs may have also evolved locally from introduced strains of VCG 0120. The 'tropical' Race 4 strain of *Foc* (VCG 01213/16) is genetically distinct (genetic similarity of 80%) from the 'subtropical' Race 4 strains of *Foc* (VCGs 0120, 0129, and 01211). 'Tropical' Race 4 was first recorded in the early 1990s in Cavendish plantations in Sumatra, Halmahera, and Java, in Indonesia, and in peninsular Malaysia. Since then the pathogen has also been recorded in Australia, Irian Jaya, India, and Taiwan. The first outbreak of 'tropical' Race 4 in Australia was recorded in 1997 in Darwin, NT, in Cavendish cv. Williams. Although the origin of this strain is unclear, it is possible that the pathogen may have been introduced many years ago with bananas grown by immigrants from south-east Asia.

As the best way to combat this disease is by the use of resistant genotypes, the information provided in this study on pathogen genotypes and their distribution is extremely important. A new resistant hybrid is urgently needed to replace Lady finger in subtropical production areas where only Race 1 populations of the pathogen are present. It is expected that International Breeding Programs will provide suitable replacements for Lady finger. However, clearly of greatest concern to the Australian banana industry is the presence of 'tropical' Race 4 in the Northern Territory. Populations of this race cause wilt in Cavendish growing under optimal conditions in tropical production areas. Previously, fusarium wilt of Cavendish in Australia had only been found in subtropical production areas where plants are predisposed to disease by low winter temperatures. Unfortunately, a resistant genotype with acceptable horticultural and post-harvest performance is not currently available to replace Cavendish. The detection of 'tropical' Race 4 in the Northern Territory emphasises the need to observe quarantine laws to prevent its spread to other areas.

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