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Characterisation of a population of *Fusarium oxysporum* f. sp. *vasinfectum* causing wilt of cotton in Australia

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Abstract

Following the discovery of fusarium wilt in Australian cotton crops in 1993, isolates of *Fusarium oxysporum* f. sp. *vasinfectum* were collected from 6 cotton farms on the Darling Downs of Queensland. Using a range of procedures the Australian isolates could not be differentiated from each other, but they did differ from foreign isolates of the pathogen in a number of characteristics. Pathogenically, the isolates behaved similarly to race 6 of the pathogen when inoculated onto differential lines. Using aesculin hydrolysis tests, however, it was difficult to match local isolates with any of the known races. Additionally, none of the foreign isolates examined produced detectable volatile compounds when grown on a starch substrate, while all Australian isolates produced a distinctive odour during these tests. The local strain was not vegetatively compatible with any of the foreign isolates and belonged in a single, unique vegetative compatibility group. It is speculated that the Australian strain arose locally, perhaps from a minor population becoming prominent in response to wide-scale planting of highly susceptible cotton cultivars. These findings have significant implications for control of the disease and spread of the pathogen in Australia.

Additional keywords: Fusarium wilt, cotton, VCG, aesculin tests, volatile compounds, soil-borne pathogen.

Introduction

Fusarium wilt of cotton (*Gossypium* L. spp.), caused by *Fusarium oxysporum* Schlecht. f. sp. *vasinfectum* (Atk.) Snyd. & Hans. (*Fov*), has been recorded in most of the major cotton-growing areas of the world, but until recently had not been recorded in Australia (Kochman 1995). This vascular wilt disease has been responsible for substantial yield losses in some developing countries (Kelman and Cook 1977; Hillocks 1984) and remains important in the USA, Egypt, Tanzania, and India (Smith *et al.* 1981) and China (Chen *et al.* 1985).

Cotton production in Australia is concentrated in 2 States, New South Wales and Queensland. Dryland cotton production is increasing, but the main cotton-growing areas are irrigated systems in central and north-western New South Wales, and southern, south-western, and central Queensland (Fig. 1). During maturation of the 1993 cotton crop, wilted plants were observed in several fields on the Darling Downs (Kochman 1995). Although verticillium wilt occurs on these heavy clay soils, the symptoms were not typical of that disease. Plants became stunted and chlorotic, and often died. A *Fusarium* species was consistently

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isolated from vascular tissue of affected plants and was subsequently identified as *Fusarium oxysporum*. Tests confirmed that the isolates were pathogenic to cotton, establishing the first record of *Fov* in Australia (Kochman 1995). Fusarium wilt was subsequently found in most cotton crops growing in the Brookstead and Cecil Plains area and in several crops in the Dalby region. An isolated recording was also made from a crop near Goondiwindi (Kochman 1995) (Fig. 1). The disease caused plant losses and consequent yield depressions in these areas.

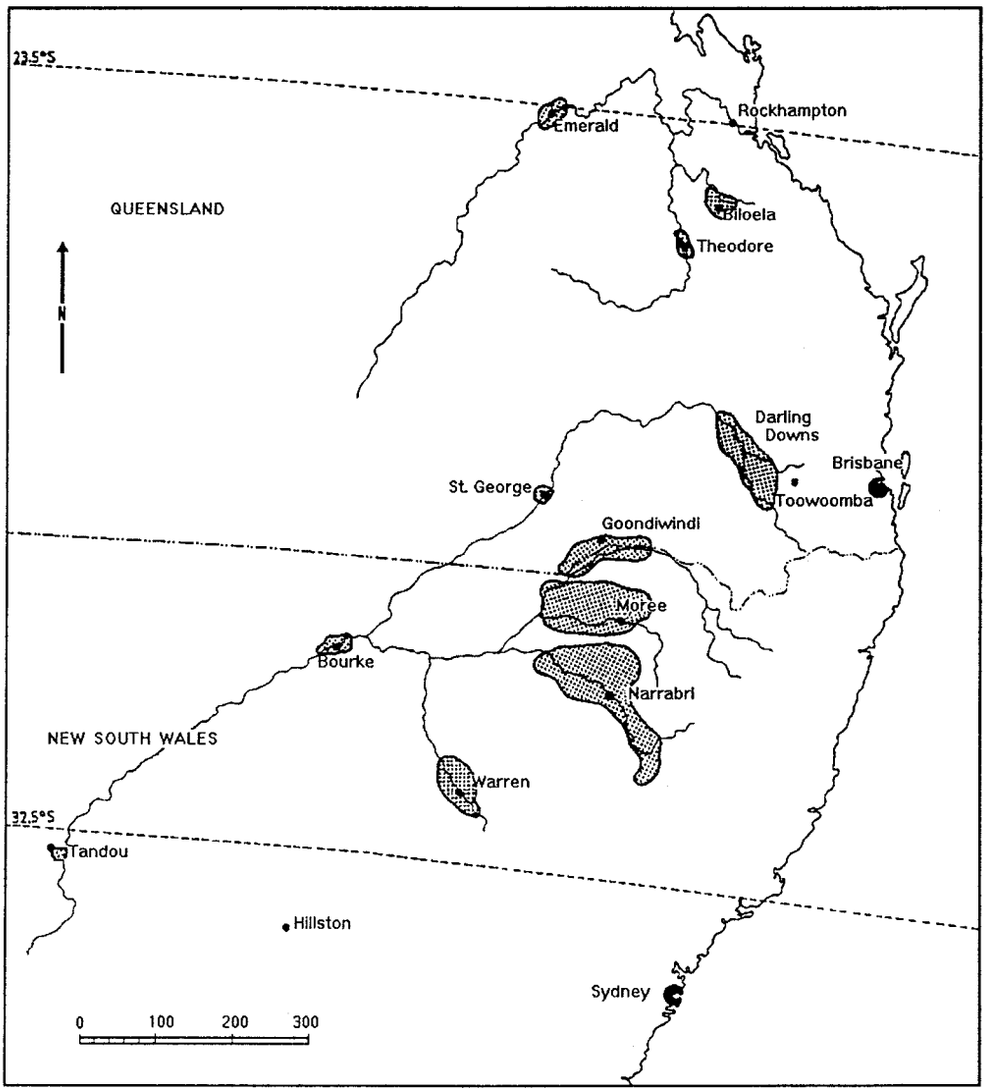

Fig. 1. Major cotton-growing regions of Australia, indicated by shading. Isolates of *Fusarium oxysporum* f. sp. *vasinfectum* were collected from the Darling Downs.

Currently, up to 8 races of *Fov*, most of which are geographically separated, are recognised worldwide. The basis for determining races of *Fov* depends on their virulence to a differential set of cotton (*Gossypium*) lines and species and up to 5 non-cotton hosts. Armstrong and Armstrong (1960, 1978) used *Glycine max* L. (soybean), *Nicotiana tabacum* L. (tobacco), and *Lupinus luteus* L. (yellow lupin) in their differential host sets to identify races 1 and 2 in the USA, race 3 in Egypt, race 4 in India, and race 6 from Brazil. Race 5 was described from Sudan (Ibrahim 1966) based on pathogenicity to cotton alone. Chen *et al.* (1985) reported races 7 and 8 from China by examining the reactions of their isolates

on cotton, tobacco, alfalfa (*Medicago sativa* L.), okra [*Abelmoschus esculentus* (L.) Moench], and soybean. Recently Assigbetse *et al.* (1994) confirmed that races 1, 2, and 6 could not be differentiated in pathogenicity tests using cotton cultivars alone, and put foward a race A construction to include these 3 former races in a single group.

Assigbetse *et al.* (1991) suggested that a relationship may exist between vegetative compatibility groups (VCGs) and physiologic races of *Fov.* They characterised 6 VCGs from a collection representing 6 races and 7 geographic regions. Katan and Katan (1988) examined race 3 isolates from Israel, and found them all to belong to a single VCG. More recently, Bridge *et al.* (1993) confirmed that the race 3 isolates they tested comprised a common VCG, whereas their race 1 and 2 isolates belonged to a single, second VCG. Fernandez *et al.* (1994) have since established 10 VCGs (0111–01110), 8 of which contained race A strains (VCGs 0111, 0112, and 0115–01110). All 12 isolates of race 3 and the 9 race 4 isolates used in their study were placed in VCGs 0113 and 0114, respectively.

In interpreting the results of restriction fragment length polymorphism (RFLP) analysis of mitochondrial DNA of *Fov*, Bridge *et al.* (1993) could not distinguish banding pattern differences between race 1 and race 2 isolates, and suggested, in view of the result of their VCG tests, that these 2 races could be derived from a common population. However, both Bridge *et al.* (1993) and Rutherford *et al.* (1993) found that races 1 and 2 differed significantly in their utilisation of coumarin on aesculin amended media.

In a study of Australian isolates of the banana wilt pathogen *F. oxysporum* f. sp. *cubense* (E. F. Smith) Snyd. & Hans. (*Foc*)., Moore *et al.* (1991) found good correlation existed between race, VCG, and production of volatile compounds from a starch substrate. Race 1 and 2 isolates of *Foc* and non-pathogenic isolates of *F. oxysporum* from the banana rhizosphere did not produce detectable volatile compounds when grown on this substrate, whereas all race 4 isolates produced distinctive volatile odours.

Isolates of *Fov* from wilted cotton plants in Australia were characterised in this study using vegetative compatibility analysis, pathogenicity tests, aesculin hydrolysis, relationships between growth and temperature, and volatile compound production, with a view to characterising the Darling Downs population of this pathogen and establishing its relatedness to some overseas isolates of *Fov*.

Materials and methods

Isolates

Twelve isolates were recovered from wilted cotton plants from 6 farms on the Darling Downs in Queensland (Fig. 1) from mid 1993 to mid 1994 (Table 1). Isolates were also recovered from glasshouse-grown plants which wilted when grown in soil collected from these farms.

Isolations were made from discoloured vascular tissue in the upper stems of wilted cotton plants. Monoconidial cultures derived from sporodochial-type cultures were prepared for all isolates. Cultures were grown on half-strength potato dextrose agar (PDA) medium for no longer than 4 days to check morphology. Cultures were otherwise maintained on Carnation Leaf Agar (CLA) (Burgess *et al.* 1988) or as colonised filter paper cultures (Correll *et al.* 1986). Lyophilised CLA cultures were also prepared for all isolates, and one of these (isolate 24232) has been lodged as a type culture with the American Type Culture Collection, Maryland, USA (ATCC number unavailable).

Table 1. Australian isolates of *Fusarium oxysporum* f. sp. *vasinfectum* obtained during 1993–94

Isolate accession no.	*Gossypium hirsutum* cultivar	Experiments in which isolate included[A]	Isolate accession no.	*Gossypium hirsutum* cultivar	Experiments in which isolate included[A]
24230	Siokra 1–4	2, 3, 4, 5, 6	24291	Deltapine 90	2, 3, 6
24232	Siokra 1–4	1, 2, 3, 6	24292	Deltapine 90	2, 3, 6
24233	Siokra 1–4	1, 2, 3, 6	24294	Deltapine 90	3, 4, 5, 6
24288	Unknown	1, 2, 3, 6	24299	CS 189+	1, 3, 4, 5, 6
24289	Unknown	2, 3, 6	24300	CS 7S	1, 3, 4, 5, 6
24290	Siokra 1–4	3, 6	24301	CS 189+	1, 3, 4, 5, 6

[A] 1, pathogenicity experiment 1; 2, pathogenicity experiment 2; 3, vegetative compatibility analysis; 4, aesculin hydrolysis experiment; 5, growth–temperature relationships; 6, volatile production analysis.

International isolates of *Fov* (Table 2) were included in tests where quarantine restrictions permitted.

Pathogenicity test: experiment 1

Six isolates of *Fusarium oxysporum* (*Fo*) obtained from soil or wilted cotton plants (Table 1) were grown for 10 days on CLA at 26°C. The culture plates were flooded with sterile tap water and the conidial suspensions of each isolate were standardised at 10^6 spores/mL. Seed of 11 *Gossypium hirsutum* L. cultivars and breeding lines were sown in vermiculite. After 14 days, the seedlings were carefully removed, and the roots washed and immersed in inoculum suspension for 1 min. The inoculated plants were then transplanted to pasteurised potting mix in 12-cm pots. A pot contained 5 plants of a single line or cultivar inoculated with the same isolate of *Fo*. The treatments were completely randomised on benches in a glasshouse (18–30°C). Control plants were treated with sterile tap water.

The plants were assessed at weekly intervals for 6 weeks for the presence or absence of symptoms. Isolation of *Fo* from discoloured vascular tissue in the hypocotyl confirmed the visual disease assessments. These isolates were referred to as *Fov*.

Pathogenicity test: experiment 2

Seven isolates of *Fov* (Table 1) were examined for pathogenic specialisation. Inoculum was prepared by autoclaving sorghum seed (at 121°C and 103·4 kPa for 20 min) twice, then inoculating with monoconidial cultures of *Fov* that had been growing on CLA. When fully colonised (10 days), the inoculated sorghum was mixed with pasteurised UC potting mix (v/v 1:10 sorghum : potting mix) in plastic bags and incubated for 4 weeks. The colonised sorghum–UC mix was then added to more pasteurised potting mix (v/v 1:1) and distributed evenly into 20-cm pots. The differential cotton cultivars Acala 44 (*G. hirsutum*), Ashmouni and Sakel (*G. barbadense* L.) and Roseum (*G. arboreum* L.), as well as local cv. Siokra 1–4 (*G. hirsutum*), were grown from seed in the inoculated potting mix. One plant of each cultivar was grown in each pot and there were 4 replications (pots) of each treatment (isolate of *Fov*).

Single-plant pots of tobacco (cv. Gold Dollar), yellow lupin (cv. Weiko 3), and soybean (cv. Yelredo) were also raised in the same inoculated sorghum–UC mix.

Vegetative compatibility analysis

Nitrate non-utilising (*nit*) mutants were generated for each isolate using the method developed by Cove (1976) and modified by Puhalla (1985) and Correll *et al.* (1987). Briefly, monoconidial wild-type cultures were transferred to potato sucrose medium amended with 1·5% potassium chlorate (KPS medium) and incubated at 25°C. Chlorate-resistant mutants, which emerged as fast-growing sectors from the restricted colonies on the KPS medium after 5–10 days, were subcultured onto minimal medium (MM) (Puhalla 1985) to determine their status as *nit* mutants. The phenotype of the *nit* mutants was determined using the method of Correll *et al.* (1987). Mutants with either *nit* 1 or *nit* 3 phenotype were used for compatibility

tests with Nit M mutants generated from 2 Australian isolates (24230, 24299) and 4 foreign isolates (IMI 141146, IMI 292248, ATCC 16611, IMI 325576). At least 3, and up to 6, *nit* mutants from each isolate (Tables 1 and 2) were paired with the Nit M testers on plates of MM, which were incubated at 25°C. After 5 days, the plates were inspected for heterokaryon development along the line of contact between the mycelium of the *nit* mutants and the advancing margin of the tester Nit M mutant. If no heterokaryon growth had developed by 14 days, the isolate was scored as being vegetatively incompatible with the Nit M tester strain. If heterokaryon growth developed between isolates, the isolates were scored as being vegetatively compatible and were, therefore, assigned to the same VCG.

Table 2. Isolates of *Fusarium oxysporum* f. sp. *vasinfectum* obtained from overseas for experimentation under quarantine

Isolate accession no.	Origin	Race	Experiments in which isolate was included[A]
IMI 141146	USA	1	3, 4, 6
IMI 292248	Tanzania	1	3, 5, 6
IMI 141148	USA	2	3, 4, 6
ATCC 16611	USA	2	3, 5, 6
ATCC 16612	USA	3	3, 4, 5, 6
IMI 141112	USA	4	3, 5, 6
IMI 325576	Sudan	5	3, 4, 6
ATCC 36198	Brazil	6	3, 5, 6
IMI 195176	Tanzania	Putative race 1	3, 4, 5, 6

[A] 3, vegetative compatibility analysis; 4, aesculin hydrolysis experiment; 5, growth-temperature relationships; 6, volatile production analysis.

Aesculin hydrolysis

Czapek Dox medium was modified (m-CZA) to contain (g/L), 5 sucrose, 3 aesculin, and 0·2 ferric citrate (Patterson and Bridge 1994). Pronounced darkening of this medium indicates fungal β-glucosidase activity as the aesculin is hydrolysed, giving glucose and aesculetin, which in turn reacts with the ferric citrate to produce a dark pigment.

Ten isolates of *Fov*, including 5 obtained under quarantine from other countries (Tables 1 and 2), were examined on 3 occasions. The inoculated plates of m-CZA were colour-assessed on the reverse at 7 and 14 days after inoculation. Uninoculated medium colour was also assessed prior to, and throughout, the experiment. Working separately under fluorescent light, 3 assessors described the colour of the plates according to colours illustrated in the Methuen Handbook of Colour (Kornerup and Wanscher 1967). Each plate was also viewed under a near-UV (black) light for evidence of fluorescence.

Growth temperature relations

The growth rates of 5 Australian isolates of *Fov* (Table 1) were compared with the rates recorded for isolates from the USA (races 2, 3, and 4), Tanzania (race 1 and putative race 1), and Brazil (race 6) (Table 2) at 10 temperatures of 13–35·5°C.

Two replications were used in each of 3 separate examinations of these isolates. A 3-mm-diameter plug of actively growing fungal culture was placed at the centre of a PDA plate. After 3 days' growth at 26°C base radii were established for each colony by averaging 2 measurements taken at right angles for each plate. The plates were then incubated at their treatment temperatures for 3 days, and the same radii were measured again. The growth differentials were used to calculate the daily radial growth rates.

Volatile analysis

Sterile, steamed rice medium was used as a starch substrate to culture isolates for volatile production. The media was prepared in the manner described by Moore *et al.* (1991): 30 mL of white rice and 90 mL of distilled water added to 250-mL Erlenmeyer flasks. The flasks

were then plugged with cotton wool and covered with aluminium foil before being steamed in an autoclave at 103°C for 1 h on each of 2 consecutive days.

Two pieces of colonised agar (approx. 10 by 20 mm) taken from monoconidial, sporodochial-type cultures of each isolate of *Fov* (Tables 1 and 2) were used to inoculate flasks of sterile, steamed rice. The cultures were then grown at 23–27°C on the laboratory bench for 12–14 days before being assessed for production of volatile compounds. Included in each experiment for comparison were 2 isolates of *Foc* (22615 and 23532) that had been used in studies of volatile production by Moore *et al.* (1991) and Moore (1994), and represented volatile-producing and non-volatile-producing strains of *Foc*, respectively. Uninoculated flasks of sterile rice media were also included in each experiment as controls.

After 12–14 days, the cotton wool plugs were removed from individual flasks and the head space above the cultures was assessed for the presence of volatile compounds.

Results

Pathogenicity test: experiment 1

All 6 isolates of *Fo* obtained from wilted cotton plants were pathogenic on inoculated glasshouse-grown cotton accessions.

Symptoms usually, though not always, commenced with one or both cotyledons becoming flaccid during the warm period of the day and eventually failing to recover as conditions became cooler. In older plants, the lower leaves became partly chlorotic or mottled, and wilted after several days. Sometimes vein darkening in 1 or more leaves became apparent as the plants aged. Vein clearing, as described by Ibrahim and Nirenberg (1993), was never observed. Most seedlings and young plants, which developed symptoms up to the third true leaf stage, died within a week. Brown vascular discoloration was evident in the stems. Vascular material from the uppermost discoloured internode yielded *Fov* on isolation.

The most virulent isolates (24232 and 24233) produced symptoms on more than 89% of the test plants and the least virulent (isolate 24288) wilted 58% of the plants. The remaining 3 isolates infected between 76 and 78% of the inoculated plants. There was no isolate×cultivar interaction to indicate any pathogenic specialisation among the 6 isolates on these Australian cotton cultivars.

Table 3. Percentage of plants with symptoms of fusarium wilt at three and six weeks after inoculation with *Fusarium oxysporum* f. sp. *vasinfectum*

Cultivar	3 weeks	6 weeks	Cultivar	3 weeks	6 weeks
Siokra 1–4	73·3	86·7	CS 7S	60·0	93·3
Siokra S324	56·7	86·7	CS 50	63·3	90·0
Siokra L22	10·0	63·3	CS 189+	13·3	76·7
Siokra L23	26·7	80·0	DP 90	20·0	70·0
Sicala 34	36·7	93·3	Pima	10·0	66·7
Sicala V-1	26·7	63·3			

The most susceptible accessions were Sicala 34, CS 7S, and CS 50, which sustained overall plant infections of ≥90% at the end of the experiment, 6 weeks after inoculation (Table 3). The latter 2 cultivars and Siokra 1–4 sustained more plant losses (≥60%) at 3 weeks than the others. Sicala V-1 and Siokra L-22 were the least affected; however, with final infections at 6 weeks of >60%, neither was considered resistant.

Pathogenicity test: experiment 2

The 7 Australian isolates of *Fov* were pathogenic on both *G. hirsutum* cultivars (Siokra 1–4 and Acala 44) and both *G. barbadense* cultivars (Ashmouni and Sakel). No symptoms were apparent in *G. arboreum* cv. Roseum. The remaining differentials (tobacco, lupin, soybean) were not visually affected by any isolate in this test (Table 4).

Symptoms appeared in the plants over a 12-week period commencing 3 weeks after sowing, and vascular discoloration was observed in at least three-quarters of the stem length at the final assessment. External symptoms were similar to those recorded in Expt 1 and appeared by week 3 in Siokra 1–4 and Sakel plants, by week 7 in Acala 44 plants, and not until week 10 in Ashmouni plants. There was no visual evidence of vascular invasion in any of the cv. Roseum plants or the other differentials. The fungus was recovered from the vascular tissue of all the visually affected plants and the apparently healthy tissue in one plant of cv. Roseum. Isolations from all remaining symptomless plants were negative.

Vegetative compatibility analysis

All of the Australian isolates of *Fov* were compatible with the Nit M testers derived from isolates 24230 and 24299, including isolates 24230 and 24299. These Australian isolates therefore belong to a common vegetatively compatible group. Vegetative compatibility could not be demonstrated among any of the foreign isolates, and none of them formed heterokaryons with the Australian group of isolates used in this study.

Aesculin hydrolysis

Fresh, uninoculated m-CZA medium plates were described by the assessors as almost colourless to yellowish grey (Methuen Handbook of Colour, Chart 2, Colour B2). The colour of the uninoculated control medium did not vary during the 14-day incubation period of the experiment.

At the first critical assessment 7 days after inoculation, all 5 Australian isolates had discoloured the medium to olive brown (Chart 4, Colour E8), as had the race 5 isolate (from Sudan) and a race 2 isolate from the USA. Two other isolates (races 1 and 3 from the USA) produced a yellowish brown (Chart 5, Colour E8) or light brown (Chart 5, Colour D7) colour change in the medium, while the third (a putative race 1 from Tanzania) did not discolour the medium. The Australian isolates did not fluoresce when viewed under black light at 7 days; however, the putative race 1 isolate from Tanzania and the uninoculated control fluorescenced strongly. Dull fluorescence was associated with the remaining 4 isolates from Sudan and the USA.

At 14 days, the medium colour of the Australian isolates had not changed, while most of the others had darkened slightly from their previous colours. Dull fluorescence was apparent in the control and the putative race 1 isolate plates. The remaining plates did not fluoresce.

The same results were recorded when this experiment was repeated on 2 subsequent occasions.

Table 4. Reactions (R, resistant; S, susceptible) of a differential set of *Gossypium* and other genera (*Nicotiana, Lupinus, Glycine*) to inoculation by Australian isolates of *Fusarium oxysporum* f. sp. *vasinfectum* and the reactions of known races of the pathogen

Isolate accession no. or race[A]	*G. hirsutum* cv. Siokra 1–4	*G. barbadense* cv. Acala 44	*G. barbadense* cv. Ashmouni	*G. barbadense* cv. Sakel	*G. arboreum* cv. Roseum	*N. tabacum* cv. Gold Dollar	*L. luteus* cv. Weiko 3	*Glycine max* cv. Yelredo
24230	S	S	S	S	R	R	R	R
24232	S	S	S	S	R	R	R	R
24233	S	S	S	S	R	R	R	R
24288	S	S	S	S	R	R	R	R
24289	S	S	S	S	R	R	R	R
24291	S	S	S	S	R	R	R	R
24292	S	S	S	S	R	R	R	R
Race 1	?	S	S	S	R	R	S	R
Race 2	?	S	S	S	R	S	S	S
Race 3	?	R	R	S	S	R	?	?
Race 4	?	R	R	R	S	R	?	?
Race 5	?	R	S	S	S	?	?	?
Race 6	?	S	S	S	R	R	R	R

[A] Denotes pathogen–host interactions according to Hillocks (1992).

Growth temperature relations

All isolates grew slowly below 18°C (< 2·5 mm/day), and very little growth occurred at 35°C. Consequently, the mean growth rates recorded at 25, 28, and between 23 and 30°C only were used to compare isolate growth rates. There were no differences among any of the Australian isolates, which recorded mean growth rates of 3·86–4·20 mm/day and grew optimally at 28°C (Table 5).

Table 5. Mean growth rates (GR, mm/day) of isolates of *Fusarium oxysporum* f. sp. *vasinfectum* recorded at 23, 25, 28, and 30°C and their optimum temperatures for growth

Means followed by the same letter are not significantly different at $P = 0·05$

Isolate accession	Mean GR	Optimum temp. (°C)	Isolate accession	Mean GR	Optimum temp. (°C)
24230	4·20a	28	ATCC 16611	3·89abcd	30
24294	3·86abcd	28	ATCC 16612	3·30de	25
24299	4·10ab	28	IMI 141112	3·30de	28
24300	3·92abc	28	ATCC 36198	3·23e	28
24301	3·88abcd	28	IMI 195176	3·53bcde	28
			IMI 292248	3·36cde	30

Volatile analysis

The volatile odours produced were strong and distinctive, and independent assessors had no difficulty in separating isolates as either volatile-producing or non-volatile-producing.

All of the Australian isolates of *Fov* produced the same volatile odour as the known volatile-producing isolate of *Foc* (22615). None of the international isolates of *Fov* tested produced volatile compounds when cultured on rice media. The known non-volatile isolate of *Foc* also failed to produce any odour.

Discussion

Isolates of *Fov* obtained from 6 crops in the Brookstead and Cecil Plains areas of Queensland proved to be pathogenic to all commercially popular cotton cultivars grown in Australia. In most cases, the results of these glasshouse tests have since been confirmed in field trials (J. K. Kochman, unpublished data). The crops were growing on heavy clay soils, and no evidence of nematode activity was found in infected plants or in soil samples assayed for their presence (G. R. Stirling, pers. comm.), although *Meloidogyne* Goeldi spp. are often associated with fusarium wilt infections elsewhere (Kappleman and Nash-Smith 1981).

Although these isolates appear pathogenically similar to race 6 of *Fov* from Brazil, we question the rationale of determining races using a differential set containing secondary, non-cotton hosts. The set was developed initially by Armstrong and Armstrong (1968) and expanded by Chen *et al.* (1985). This has lead to some confusion in the number of races currently recognised. Hillocks (1992) reported that there were 6 races; however, there are apparently an additional 2 races (7 and 8) in China (Chen *et al.* 1985), where a further 2 non-cotton hosts (alfalfa and okra) were added to the differential set. By using pathogenicity

testing in conjunction with RAPD analysis, Assigbetse *et al.* (1994) described 3 races from a world-wide collection of isolates. They constructed a race A which groups isolates previously designated as races 1, 2 and 6. Their races 3 and 4 corresponded with those previously described. We believe it would be preferable to confine the differential host set to *Gossypium* spp. containing different genes for resistance to *Fov.*

In addition, the question of comparing pathogenicity results determined using several techniques should be addressed. The stem injection technique favoured by Bugbee and Sappenfield (1972), Kappelman (1981), and Hillocks (1984, 1992) did not produce consistent and reliable results when examined by us in preliminary work. Similarly, Ibrahim and Nirenberg (1993) concluded that the stem injection method failed to produce any symptoms in cultivars inoculated with their race 5 pathogen, but the root dip method produced consistent results with both races 1 and 5. Besides the inoculation method there are several other factors that should be established and standardised, including acceptable temperature ranges, light intensities, inoculum concentrations, and assessment techniques, before pathogenicity testing alone can be relied on to distinguish races of *Fov.*

Considering this uncertainty, we are not prepared to designate these Australian isolates as race 6, even though their reaction to the differentials in our experiment indicates a similarity to this South American race. As the term 'race' implies a genetic relationship between pathogen and host, there is then a need to review the international differential set in relation to the non-*Gossypium* spp. hosts and standardise its application in conjunction with other tests to separate the races of *Fov.*

Vein-clearing symptoms as described by Ibrahim and Nirenberg (1993) were not observed in any interaction. They showed that this symptom was confined to race 5 interactions, regardless of cultivars or species of *Gossypium*, while their race 1 isolates always produced vein-darkening symptoms. Vein-darkening was also occasionally seen in our experiments. Isolates of races 1 and 5 in Egypt have been differentiated using these criteria (Nirenberg *et al.* 1994).

Assigbetse *et al.* (1991) suggested that there may be a relationship between vegetative compatibility groups (VCGs) and physiologic races of *Fov.* They found 6 VCGs among 25 uncharacterised isolates from 7 geographical regions. Three of these VCGs (from isolates from the Ivory Coast, Peru, Argentina, Paraguay, and China) were able to pair successfully with American Type Culture Collection isolates representing 3 known races, but the remaining 3 VCGs (identified in isolates from Benin, India, and the Ivory Coast) did not form heterokaryons with known races. Katan and Katan (1988) examined race 3 isolates from Israel and found them all to belong in a single VCG. More recently Bridge *et al.* (1993) confirmed that 3 race 3 isolates of unknown origin were complementary, but in turn were incompatible with race 1 isolates from Tanzania and Zimbabwe. Significantly, they also reported that race 2 isolates from Zimbabwe belonged in the same VCG as the race 1 isolates from Tanzania.

Fernandez *et al.* (1994) have adopted a different race structure in *Fov* (races A, 3 and 4). They, in contrast, did not find any isolates representing different races that were vegetatively compatible with each other in a world-wide collection of 52 isolates. They identified 10 VCGs (VCG 0111–VCG 01110), indicating unusually wide diversity for a *forma specialis* of *F. oxysporum.* The Australian

strains used in our experiments were subsequently examined by D. Fernandez and placed in VCG 01111, a new *Fov* vegetative compatiblity group (D. Fernandez, unpublished data). This by itself does not necessarily mean that the Australian strains represent a new race, since within race A there have been 8 VCGs so far determined (Fernandez *et al.* 1994). It can be assumed, however, that this Australian population is represented by a genetically homogenous group of strains.

Aesculin hydrolysis was used by Rutherford *et al.* (1993) to separate races 1, 3, and 6 from race 2 on a sucrose-free agar medium. They also distinguished race 1 from race 3 isolates on a medium that contained sucrose. Bridge *et al.* (1993) found differences between a group of isolates representing races 2, 3, and 4 and a group of strains including race 1 and unidentified races. Their former group significantly darkened the medium, while the latter did not discolour it. Our results indicated that the Australian isolates did colour the medium, but differed from the foreign isolates we tested (representing races 1, 2, 3, and 5) in the intensity of the colour change. It is difficult to make comparisons with the general colour descriptions given by Rutherford *et al.* (1993) and Bridge *et al.* (1993), but since none of the Australian isolates we tested cleared the medium after an initial colour change, it is unlikely they belong to races 1, 2 or 6 according to the criteria put by Rutherford *et al.* (1993).

In vitro growth was compared between Australian and foreign isolates of *Fov.* Most of the isolates grew optimally at 28°C, but one grew better at 25°C and 2 preferred 30°C. This range is consistent with the ranges found for optimum growth of *Fusarium oxysporum.* Ploetz (1990) indicated that growth rates of *Foc* differed among VCGs of that pathogen.

The local isolates were the only ones to produce a volatile odour associated with growth on steamed rice. This result provides further evidence of diversity within populations of the cotton pathogen and another characteristic apparently unique to the Australian strains. Moore *et al.* (1991) established that in Australian populations of *Foc*, all race 4 isolates produced volatile odours, while the race 1 pathogens did not. *Fusarium oxysporum* isolates that caused wilting in host plants were assessed and separated into 2 groups by Nash-Smith *et al.* (1994). These 2 groups, as well as differing in their ability to sporulate on high glucose agar without added biotin, were also easily separated by the presence or absence of an easily detectable volatile aroma. The volatile-producing characteristic of isolates of *Fov* will continue to be used in further characterising the Australian pathogen.

Although the first recording of fusarium wilt in Australian cotton was made in 1993, the disease may have been present and undetected for several years (Kochman 1995). Seedborne transmission of *Fov* was first recognised by Elliot (1923) and more recently by Hillocks (1983), who found that even seed from highly resistant cultivars could contain infection. However, it would seem unlikely that the initial outbreak in Australia could be linked to infected seed. Commercial cotton seed is produced in New South Wales at sites removed from the infected areas, and strict quarantine procedures apply to seed importations from overseas for research and plant breeding purposes. It is also unlikely the pathogen was introduced in contaminated soil. Our work has indicated the Australian pathogen is not identical to any of the foreign isolates of *Fov* that we have tested, and supports these conclusions.

Ebbels (1975) described the host range of *Fov* as being moderately wide with the pathogen capable of infecting 12 host families. If the Australian pathogen is considered regional, it may have originated from an indigenous Darling Downs population of *F. oxysporum* (saprophytic or parasitic), and existed initially without a major host. An increase in this population could have resulted from the widespread and continued planting of particularly susceptible cotton cultivars.

There are still major cotton production areas in New South Wales and Queensland where fusarium wilt has not been observed. It is important to extend our knowledge of *Fov* in Australia by examining further isolates if outbreaks of the disease occur in these regions. This can be achieved along similar lines to the work reported here, combined with examination of the isolates at the molecular level. DNA fingerprinting of the local isolates has begun (S. Bentley, pers. comm.). Successful control strategies, particularly the development of resistance in commercially acceptable local cultivars, will ultimately depend on the outcomes of these studies.

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