If you are participating in the presentations this year, please provide a written report and a copy of your final report presentation by 31 October. If not, please provide a written report by 30 September.

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: **03CSP012**

Project Title: Soil impacts on the incidence and evolution of Fusarium wilt

Project Commencement Date: 1/07/2007 **Project Completion Date:** 30/06/2010

CRDC Program: 2. Farming Systems

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Part 3 – Final Report Guide (due 31 October 2008)

(The points below are to be used as a guideline when completing your final report.)

Background

1. Outline the background to the project.

Aggressiveness and saprophytic ability are key life history features that determine patterns of disease incidence and severity for many soil pathogens, including Fov. Selection for these traits can be strongly influenced by interactions with other micro-organisms, agronomic practices, and soil abiotic factors.

Fusarium wilt is patchily distributed in Australian cotton-growing regions. No disease occurs in Narrabri, and even within severely infested areas, i.e. the Darling Downs and Boggabilla, many fields stay disease-free despite the sharing of recycled irrigation water with nearby diseased fields. Pilot studies showed that when disease-free soils were inoculated with Fov, some had significantly lower disease incidence, suggesting variation in Fov aggressiveness among different environments. Fov strains are also likely to differ in saprophytic ability to cope with other micro-organisms and various soils. For example, one possibility is that shifts in saprophytic ability may be responsible for the significant differences in soil prevalence of VCG 11 strains (originating from the Darling Downs) between the Darling Downs (23%) and Moree (<2%). Given the lack of differences in disease incidence and severity between Fov isolates from the Darling Downs and Moree when tested in pot trials in the glasshouse, this suggests that Fov aggressiveness may have increased in Moree soils relative to the Darling Downs. This also suggests the possibility of a trade-off between saprophytic ability and aggressiveness in Fov as reported for other fungal pathogens such as *Phytophthora* spp.

Quantification of the extent to which aggressiveness and saprophytic ability in Fov populations varies among different soils is directly applicable to the development of disease control strategies. This project has the aims of increasing our overall understanding of the incidence, ecology and evolution of Fov in Australian cotton growing regions. It also complements the work by other groups (e.g. Linda Smith and colleagues at QDPI on management practices) aimed at quantifying other key pathways relating agronomic practices to disease.

Objectives

2. List the project objectives and the extent to which these have been achieved.

The objectives of this project were to determine the soil impact on the incidence and evolution of Fusarium wilt. It contained the following four components that have been completed now:

I. Collection and characterisation of soils and quantification of Fov prevalence
II. Characterization of Fov populations from different soils
III. Determination of soil impacts on the evolutionary potential of Fov
IV. Determination of soil impacts on the incidence and severity of Fusarium wilt

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

<u>I. Collection and characterisation of soils and quantification of Fov prevalence</u>

Nine fields were sampled in Moree, Boggabilla, and the Darling Downs (3 fields per region) in August 2007 (Table 1). From each field, bulk soils (50-150 kg) were collected from both disease-

Table 1. Nine fields sampled in the study

Date	Region	Farm	Field	Disease status		GPS
				Fov-infested	*Fov*-free	
30-Aug	Moree	Glen Prairie	Field 19		v	29°24'02.6"S/149°49'07.2"
				v		29°24'08.0"S/149°48'46.7"
		Sappa	Field 2		v	29°22'01.7"S/149°50'03.4"
				v		29°21'58.7"S/149°49'44.0"
		Red Mill	Field 13		v	29°24'13.4"S/149°57'48.9"E
				v		29°24'02.3"S/149°57'30.5"E
30-Aug	Boggabilla	Korolea	Field 7		v	28°38'25.9"S/150°16'31.5"
				v		28°38'20.0"S/150°16'11.8"
		Yambocully	Field 1		v	28°26'14.4"S/150°09'41.4"
				v		28°26'15.7"S/150°09'18.1"
		Carbucky	Field 17N		v	28°37'33.2"S/149°58'26.3"
				v		28°37'33.2"S/149°58'12.8"
28-Aug	Darling Downs	Rainbow Valley	Circle 1		v	27°22'46.4"S/151°37'28.0"E
				v		27°22'27.8"S/151°37'15.4"E
		Keeley farm	Steve's trial field		v	27°41'10.3"S/151°22'20.2"E
				v		27°41'16.6"S/151°22'29.8"E
		Bud Kelly	CSD trial field		v	27°47'31.2"S/151°24'57.8"E
				v		27°47'16.8"S/151°25'24.4"E

infested and disease-free spots, respectively. Soils were air-dried in the glasshouse and stored at –4ºC till use.

Soil tests were carried out by Nutrient Advantage Laboratory in Werribee, VIC. Sub-samples of the soils were prepared and delivered according to the company instructions.

Soil Fov prevalence was determined using the method of Wang *et al*. (2004). Briefly, *Fusarium oxysporum* was isolated on peptone-PCNB plates. For each sample, 5 plates were inoculated with 0.2 ml of a 1:100 dilution. Plates were incubated at 25°C for 1 week, and *Fusarium oxysporum*-like colonies were subcultured. The pathogenicity of obtained isolates was determined by challenging cotton cultivar Sicot 71. Two-week-old seedlings were inoculated by dipping their roots in spore suspensions of individual isolates (5×10^6 cfu/ml) for 5 min. Inoculated plants were transplanted into fresh potting mix (compost and perlite; 50/50, v/v) and grown at 18–23°C in the glasshouse for 6 weeks. For each isolate, 9 plants were used with 3 plants per pot. Isolates causing Fusarium wilt symptoms were considered to be putative Fov. For each soil, Fov density was calculated based on the dilution and number of Fov colonies on the plates.

Disease incidence in the soils was determined by conducting bioassays on cotton cultivar Sicot 71 in the glasshouse. Fifty seeds were sown in 10 kg soils in a plastic tray and 3 replicates were used for each soil sample. Disease incidence was assessed 8 weeks after sowing. In addition, Fov was isolated from randomly chosen diseased plants by growing symptomatic stem sections on peptone-PCNB plates at 25°C for 1 week. Relationships between disease incidence and soil Fov prevalence were analysed; additionally, key soil abiotic variables influencing Fov prevalence were determined using correlation analyses.

II. Characterization of Fov populations from different soils

Genetic analyses were conducted using AFLPs to identify genotypic variation among Fov isolates from different soils and to determine whether the distribution of genotypes showed spatial structure. Genomic DNA was extracted from lyophilised mycelia using DNeasy Plant kits (Qiagen Pty Ltd, Clifton Hill, Australia). DNA (250 ng) was co-digested with *Mse*I and *Eco*RI at 37°C for 2 h and oligomer adapters were ligated to DNA fragments at 37°C for 3 h in 40 µL of digestion-ligation buffer. Pre-selective amplification was performed with 5 µL of digestion-ligation reaction in 50 µL of PCR buffer containing non-selective primers *Mse*I+0 and *Eco*RI+0 (20 cycles of 30 s at 94°C, 60 s at 56°C, and 60 s at 72°C). Selective amplification was performed with 5 µL of 1:30 diluted pre-selective amplification reaction in 20 µL of PCR buffer containing selective primers *Mse*I+A and ^{33}P-labelled *Eco*RI+AGG (one cycle of 30 s at 94°C, 30 s at 65°C,

and 60 s at 72°C; 12 cycles of 72°C with annealing temperature lowered by 0.7°C during each cycle; and 23 cycles of 30 s at 94°C, 30 s at 65°C, and 60 s at 72°C). Finally, DNA fragments were separated on a 6% polyacrylamide gel electrophoresed at 50 W for 2.5 h. and autoradiographs were generated by exposing the Kodak BioMax film to dried gels. Unambiguous polymorphic bands ranging in size from 69 to 455 bp were manually scored as biallelic loci (present or absent) and recorded in a binary data matrix. Loci of >5% frequency were used to estimate pair-wise genetic relationships using NTSYSpc 2.02j (Exeter Software, Setauket, NY), based on which the number of genotypes was determined following Saleh *et al*. (2003). Genotype representatives were used to generate a similarity matrix based on the Dice coefficient, from which a UPGMA dendrogram of genotypes was constructed. Bootstrap values were calculated using Winboot (International Rice Research Institute, Manila, Philippines).

Seventeen representatives of dominant genotypes were selected and tested for aggressiveness on cotton cultivar Sicot 71 using the same method described above (Table 2). Their saprophytic ability was determined by testing both mycelial growth and spore production. For growth tests, agar plugs (0.5-cm diameter) were removed from fungal cultures and placed on PDA plates. Inoculated plates were incubated at 25°C for 1 week and colony diameters were measured. Each isolate was tested on 5 plates and the test was conducted 3 times. For spore production tests, 125 ml PDB in 250-ml flasks were inoculated with one agar plug and incubated at 25°C for 1 week on an orbital shaker at 150 rpm. Fungal cultures were diluted and spore concentrations were determined based on haemocytometer counts. Each isolate was tested in 4 flasks and the test was conducted twice. Relationships among aggressiveness, mycelial growth, and spore production were analysed.

Table 2. 17 representatives of dominant Fov genotypes used in the study

Isolate	Aggressiveness		Colony diameter (cm)	Spore production ($X10^6$ spores/ml)
	Dis. incidence (%)	Dis. severity		
07/0066	70.4	2.30	6.10	7.56
07/0073	85.2	2.11	6.23	11.38
07/0075	25.9	0.67	5.78	16.63
07/0090	81.5	2.48	6.21	10.63
07/0135	81.5	2.26	6.21	10.69
07/0158	55.6	1.56	6.15	9.94
07/0168	77.8	2.26	6.09	12.31
07/0175	85.2	2.56	6.18	11.81
07/0202	51.9	1.63	6.15	12.50
07/0225	85.2	2.41	6.08	8.94
07/0235	55.6	1.41	5.87	15.88
07/0270	77.8	2.26	6.25	12.25
07/0285	81.5	2.37	6.16	14.56
07/0323	44.4	1.15	5.97	16.56
07/0324	55.6	1.67	6.03	16.25
07/0333	63.0	1.67	6.06	13.06
07/0380	63.0	1.52	6.09	16.81

III. Determination of soil impacts on the evolutionary potential of Fov

Six isolates, 2 from each region, were selected and used as inoculum in the trial to determine the impact of abiotic soil factors on Fov evolution (Table 3). These isolates differed in AFLP fingerprints so that they could be identified in isolates recovered from diseased plants generated in the trial. Inocula were produced on wheat medium. Wheat grains were treated in a water bath at 60°C for 2 hr, washed twice with tap water, distributed in 2-litre flasks plugged with cheesecloth and cotton and sterilised at 121°C for 1 hr on each of 2 consecutive days. Each flask was inoculated with 5 agar plugs of mycelia prepared as described above and incubated at 25°C until the fungal hyphae colonised all grains (about 4 weeks). The grains were removed from flasks, air-

Table 3. Six isolates used in the evolution trial

Isolate	Origin / Field	Recovery method	Genotype	Aggressiveness
07/0036	Moree / 3B	Directly from soil	11	L
07/0225	Moree / 2B	Baited out using Sicot 71	11	H
07/0270	Boggabilla / 5B	Baited out using Sicot 71	11-mutant-b	M
07/0073	Boggabilla / 4B	Directly from soil	11-mutant-a	H
07/0351	Darling Downs / 7B	Baited out using Sicot 71	11-mutant-c	L
07/0168	Darling Downs / 8B	Directly from soil	11-mutant-d	M

L=Dis. Incidence ranges 60.0-69.9
M=Dis. Incidence ranges 70.0-79.9
H=Dis. Incidence ranges 80.0-89.9

dried completely at 20–25°C, and triturated using a grinder. The Fov concentration was determined using the serial dilution and agar plate counting technique and adjusted to 5×10^7 cfu/g. Inoculum was eventually prepared by mixing 6 isolates at equal proportions. Disease-free soils from Moree, Boggabilla, and the Darling Downs were inoculated by introducing 100 g inoculum into 10 kg soil in a plastic tray. There were 3 replicates for each soil. Inoculated soils were left in a naturally lit glasshouse for 2 months before use for the Fov inoculum to allow good establishment in the soils. Three successive plantings of cotton cultivar Sicot 71 were carried out in these soils. For each growth cycle, 50 seeds were sown in each tray and grown in the glasshouse for 13 weeks. For each cycle, disease was assessed, Fov was re-isolated from all plants and the isolates obtained were genotyped using AFLPs as described above. A minimum 2-month fallow period was imposed between growth cycles.

The impact of soil on the trade-off between saprophytic ability and aggressiveness of Fov was determined in the Moree and the Darling Downs soils (Msoil and Dsoil respectively). Six Fov isolates, 3 from each region, were selected because they show identical AFLP fingerprints and similarly low to medium aggressiveness level (Table 4). Fungal inocula were produced on wheat grains using the same method as described above. The 3 isolates from the same region were bulked to generate inocula of Moree Fov (MFov) and the Darling Downs Fov (DFov), respectively. The Fov density of inocula were quantified using the method as described above and adjusted to 5×10^7 cfu/g. The experimental design is outlined in Fig 1 below. For the soil from each region, 60 kg were inoculated by introducing the inoculum of Fov from the same region, while another 60 kg was similarly treated with the inoculum of Fov from different region. Inoculated soils were distributed into plastic trays with 1 kg per tray. Cotton (cv. Sicot 71) and wheat (cv. Sunstate) were grown in the soils with 3 trays used for each soil×Fov origin×crop combination. Three 13-week crop growth cycles were successively conducted in the soils and there was an at least 2-month fellow period between growths. After the final growth, soil Fov density was determined using the same method as described above.

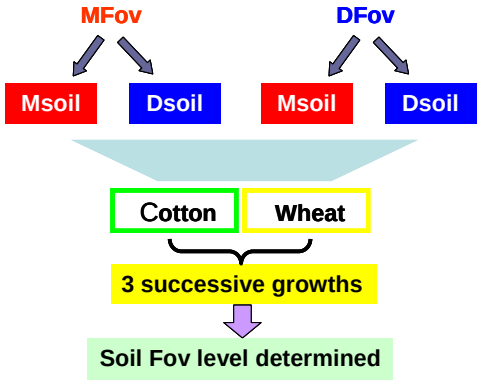

Fig. 1. Diagram showing experimental design to determine trade-off between saprophytic ability and aggressiveness of Fov

Table 4. Six isolates used in the trade-off trial

Isolate	Origin / Field	Recovery method	Genotype	Aggressiveness
07/0021	Moree / 2B	Directly from soil	11	L
07/0036	Moree / 3B	Directly from soil	11	L
07/0130	Darling Downs / 7B	Directly from soil	11	M
07/0159	Darling Downs / 8B	Directly from soil	11	M
07/0406	Darling Downs / 9B	Baited out using Sicot 71	11	M
07/0201	Moree / 1B	Baited out using Sicot 71	11	M

L=Dis. Incidence ranges 60.0-69.9
M=Dis. Incidence ranges 70.0-79.9
H=Dis. Incidence ranges 80.0-89.9

Table 5. 14 fields sampled in 2008 for the study of soil calcium/magnesium ratio on disease

Date	Region	Farm	Field	GPS
5-Aug	Goondiwindi	Carrington	#27	28°36'04.8"S/150°27'39.0"E
	Goondiwindi	Carrington	#33	28°36'23.7"S/150°30'13.4"E
	Boggabilla	Morella	?	28°38'43.8"S/150°20'12.5"E
	Boggabilla	Morella	?	28°38'40.1"S/150°20'13.5"E
	Boggabilla	Carbucky	#6	28°37'15.6"S/150°03'07.0"E
	Boggabilla	Carbucky	#7	28°37'26.9"S/150°02'40.3"E
6-Aug	Pampus	Michael McNamera Farm	Field #2	27°45'23.1"S/151°20'39.5"E
	Pampus	Carbrita	Field #4	27°42'33.5"S/151°20'40.6"E
	Norwin	Behendorff	Field N5	27°32'52.7"S/151°21'03.1"E
	Norwin	Bonnington	Field B3	27°35'36.9"S/151°17'42.2"E
7-Aug	Moree	Morecott	Field 21B	29°26'52.6"S/149°38'27.2"E
	Moree	Blairmore	?	29°27'04.3"S/149°34'44.1"E
	Moree	Woodbine	Field #1	29°21'33.7"S/149°30'35.7"E
	Moree	Moreton Plains	Field #1	29°31'26.3"S/149°41'07.8"E

Table 6. Nine isolates used in the study of soil calcium/magnesium ratio on disease

Isolate	Origin / Field	Recovery method	Genotype	Aggressiveness
07/0036	Moree / 3B	Directly from soil	11	L
07/0201	Moree / 1B	Baited out using Sicot 71	11	M
07/0225	Moree / 2B	Baited out using Sicot 71	11	H
07/0250	Boggabilla / 4B	Baited out using Sicot 71	11	L
07/0286	Boggabilla / 5B	Baited out using Sicot 71	11	M
07/0090	Boggabilla / 6B	Directly from soil	11	H
07/0380	Darling Downs / 8B	Baited out using Sicot 71	11	L
07/0406	Darling Downs / 9B	Baited out using Sicot 71	11	M
07/0135	Darling Downs / 7B	Directly from soil	11	H

L=Dis. Incidence ranges 60.0-69.9
M=Dis. Incidence ranges 70.0-79.9
H=Dis. Incidence ranges 80.0-89.9

IV. Determination of soil impacts on the incidence and severity of Fusarium wilt

The impact of soil calcium/magnesium (Ca/Mg) ratio on Fusarium wilt of cotton was further studied by comparing disease incidence in soils showing natural variation in the ratio. Soils were collected in 2008 from 14 different farms/fields (Table 5) and tested for their Ca/Mg ratio as described above. Nine Fov isolates (Table 6) were selected because of their identical AFLP fingerprints and different geographic origins and aggressiveness levels (Table 6). They were mixed to generate a bulked inoculum as described above. Soils were inoculated at a rate of 10 g inoculum per kg. Three replicates of 10 kg soils in a plastic tray were used for each soil and the trial was conducted twice. Fifty seeds of cotton cultivar Sicot 71 were sown in each tray and disease incidence was assessed 8 weeks after sowing. Statistical analysis was carried out to evaluate whether there was a correlation between disease incidence and soil Ca/Mg ratio.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

Relationships between soil Fov prevalence and disease incidence

No significant correlation was observed between soil Fov density and disease incidence in the 9 disease-infested soils (P=0.1281), suggesting significant impacts of soil conditions on disease. For example, Fov density was comparable in the Boggabilla soil B2 and Moree soil M1, however, disease incidence in soil B2 was significantly higher than that in soil M1 (Fig. 2). This suggests that some other soil factor may have played a more important role than Fov density in shaping disease incidence, although the presence of Fov is critical to the disease.

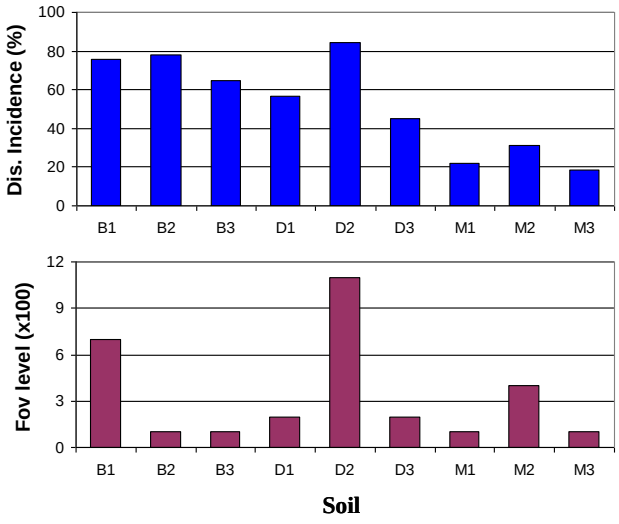

Fig. 2. Relationships between soil Fov level and disease incidence

Correlations between disease incidence and soil abiotic conditions

A range of chemical tests were conducted on the soils and a total of 22 soil characters were determined. Simple correlation analysis found that 3 parameters showed a significant correlation to disease incidence, including sodium, electric conductivity, and Ca/Mg ratio, of which Ca/Mg ratio had the lowest P value (i.e. was most significant) (Table 7).

Furthermore, stepwise regression analysis also found significant regressions of disease incidence to soil abiotic conditions. A total of 7 parameters were selected by the analysis, of which Ca/Mg ratio and chloride were the most important (Table 8). Consistent with the results from the correlation analysis, the regression analysis also showed that soil Ca/Mg ratio was negatively related to disease incidence.

Both correlation and stepwise regression analyses suggest that soil Ca/Mg ratio has a significant negative impact on disease incidence, based on which another experiment was designed to test the efficacy of manipulating soil Ca/Mg ratio in controlling Fusarium wilt under glasshouse conditions.

Table 7. Correlations between disease incidence and abiotic conditions of the 9 soils

Soil condition	Co-efficient	*P*
pH (1:5 Water)	– 0.0078	0.9763
pH (1:5 CaCl2)	0.0416	0.8741
Organic Carbon	0.1310	0.6164
Nitrate Nitrogen	0.2797	0.2768
Sulfate Sulfur (KCl40)	0.3574	0.1590
Phosphorus (Colwell)	0.3290	0.1973
Potassium	– 0.4053	0.1066
Calcium	0.1046	0.6894
Magnesium	0.4378	0.0788
Sodium	0.4976	0.0421
Chloride	0.4123	0.1001
Elec. Conductivity (EC)	0.5413	0.0248
Copper	0.0107	0.9674
Zinc	– 0.3422	0.1788
Manganese	0.0973	0.7103
Iron	0.3006	0.2410
Cation Exch. Cap. (CEC)	0.3250	0.2031
Calcium/Magnesium Ratio	– 0.6674	0.0034
Elec. Cond. (Sat. Ext.)	0.3330	0.1915
Sodium % of Cations (ESP)	0.3338	0.1905
Phosphorus Buffer Index	– 0.0912	0.7278

Table 8. Results of stepwise regression analysis of the relationships between disease incidence and soil abiotic conditions

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	7	2.6667	0.38096	7.43	0.004
Residual	9	0.4615	0.05128		
Total	16	3.1282	0.19551		

Seven parameters selected by stepwise regression analysis

Parameter	estimate	s.e.	t(9)	t pr.
Calcium_Magnesium_Ratio	-0.4890	0.1110	-4.41	0.002
Chloride	0.0038	0.0011	3.38	0.008
Potassium	-0.0034	0.0021	-1.67	0.129
Zinc	-0.0074	0.0040	-1.87	0.095
Elec_Cond_Sat_Ext	0.0476	0.0280	1.7	0.123
Sodium	-0.2190	0.1130	-1.93	0.086
Organic_Carbon	0.3710	0.3550	1.05	0.323

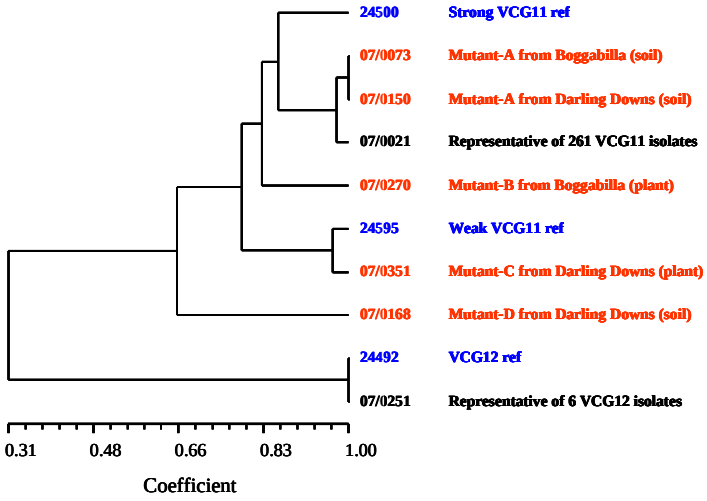

Fig. 3. UPGMA showing genetic relationships among Fov isolates collected in this study

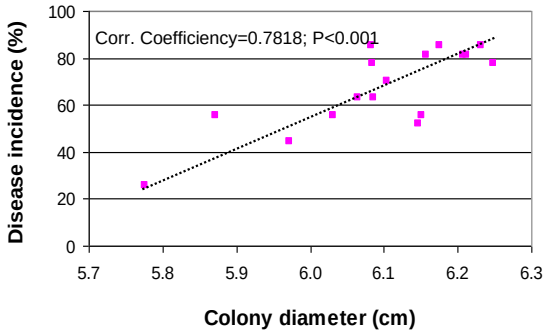

Fig. 4. Correlation between disease incidence and colony diameter of 17 Fov isolates

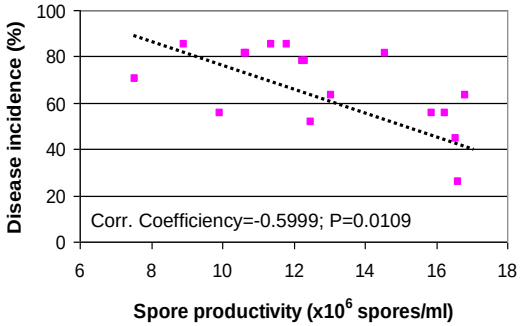

Fig. 5. Correlation between disease incidence and spore productivity of 17 Fov isolates

Genetic relationships among Fov isolates collected in this study

A total of 272 Fov isolates (28 isolated directly from the soils and 244 baited out using cotton cultivar Sicot 71) were recovered in this study. Based on the genetic similarity revealed by AFLP, these isolates can be divided into 2 major groups that well correspond to the 2 known VCGs. The majority of the isolates (266) belonged to VCG 01111, while the remaining 6 isolates belonged to VCG 01112. Isolates of VCG 01111 included 4 mutant genotypes (A, B, C and D); mutant A was represented by 2 isolates while the others were represented by single isolates (Fig. 3). There was no clear pattern in terms of distribution of Fov genotypes in different soils due to the lack of genetic variation except that VCG 01112 isolates were only recovered from the Boggabilla soils.

Relationships between saprophytic ability and aggressiveness of Fov

Observations based on the 17 representatives of Fov isolates suggest strong correlations between saprophytic ability and aggressiveness. A significant positive correlation was found between disease incidence and colony diameter of Fov isolates measured on PDA plates (P<0.001; Fig. 4), suggesting that fast-growing Fov isolates are usually more aggressive than slow-growing ones. In contrast, a significant negative correlation was observed between disease incidence and spore production of Fov isolates in PDB (P=0.0109; Fig. 5), suggesting that a trade-off exists between aggressiveness and spore producing ability in Fov, i.e., more aggressive Fov isolates usually tend to produce less spores in comparison with those less aggressive isolates.

Soil impact on the evolutionary potential of Fov

Significant differences were observed in the recovery frequency of Fov genotypes regardless of soils although they had the same soil density in the initial trial (Fig. 6), suggesting competition among Fov genotypes and soil impact on this competition. Aggressiveness of Fov may also play a role in this competition because genotypes of high aggressiveness levels, i.e., 11 and a, appeared to occur at higher frequencies, compared to those of low or moderate aggressiveness levels (Table 3).

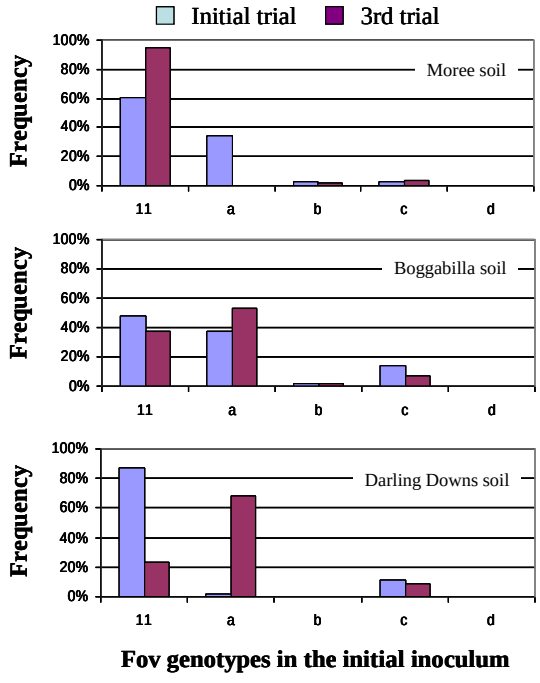

Fig. 6. Recovery frequency of Fov genotypes in isolates from the evolution trials

Significant soil impacts on the evolutionary potential of Fov were observed when the recovery frequencies of a specific Fov genotype from the initial and the 3rd trials were compared (Fig. 6). For example, because isolates of genotype 01111 in the inoculum derived from the Moree soil, its recovery frequency tended to increase in the Moree soil but decrease in the soils from the other 2 regions during the process of these trials. Similarly, mutant genotype 'a' occurred in both Boggabilla and the Darling Downs (Fig. 3), as a result, it showed higher recovery frequencies in the soils from Boggabilla and the Darling Downs, while it was not detected in the Moree soil in the 3rd trial although it occurred at a comparable recovery frequency in the initial trial. These observations suggest that there may be some degree of local adaptation of Fov to the soil environment of its origin as it is usually more aggressive in the soil of origin, than in non-local soils.

Soil impacts on the relationships between saprophytic ability and aggressiveness of Fov

After 3 successive growth cycles of different crops, soil Fov density was significantly lower in the cotton treatments than in the wheat treatments suggesting a trade-off between saprophytic ability and aggressiveness of Fov (*P*=0.024, Table 9; Fig 7A), i.e., Fov may show enhanced saprophytic growth in the soil at the absence of cotton host.

The trade-off was significantly related to the interaction between Fov and soil origins (*P*=0.03, Table 9). Consistent to what was found in the evolution trial (i.e. that Fov isolates are more aggressive in the soil where they originated), here the saprophytic ability of Fov appears to be somewhat suppressed in the soil from which it derives. For example, in this trade-off experiment, Fov from Moree occurred at lower density in the Moree soil relative to that in the Darling Downs soil. The same tendency was also seen on Fov from the Darling Downs (Fig. 7B).

Table 9. ANOVA results of soil Fov density in the trade-off trial

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Fov	1	1.0417	1.0417	3.16	0.095
Host	1	2.0417	2.0417	6.19	0.024
Soil	1	0.1437	0.1437	0.44	0.519
Fov.Host	1	1.0417	1.0417	3.16	0.095
Fov.Soil	1	1.8784	1.8784	5.69	0.03
Host.Soil	1	0.7151	0.7151	2.17	0.16
Fov.Host.Soil	1	0.0417	0.0417	0.13	0.727
Residual	16	5.2789	0.3299		
Total	23	12.1828			

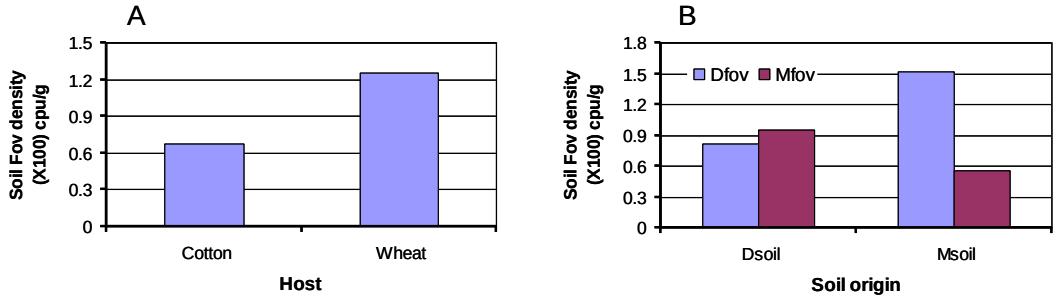

Fig 7. Difference in soil Fov density showing how the saprophytic ability of Fov is influenced by host (A) and interaction between Fov and soil origins

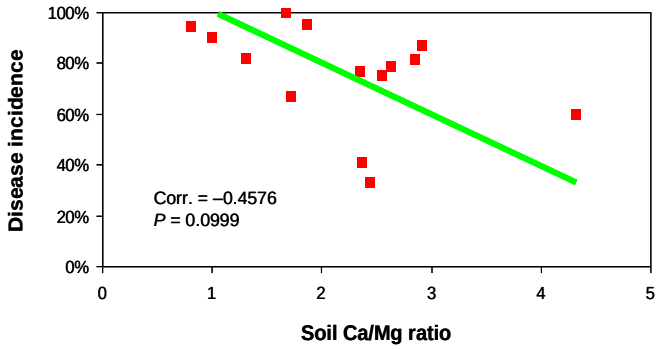

Fig. 8. Negative correlation between disease incidence and soil Ca/Mg ratio observed in 14 soils inculated with the same level of Fov inoculum

Effect of soil Ca/Mg ratio on the disease

The negative correlation between disease incidence and soil Ca/Mg ratio observed in naturally infested field soils was confirmed by results from different soils inoculated with the same level of Fov inoculum in the glasshouse (Fig. 8). However, the correlation was only marginally significant here probably because the soil impacts on Fov settlement and aggressiveness were not taken into consideration in this trial.

Outcomes

5. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

- Results from this project show that Fov persistence varies among fields but disease incidence is not strictly related to soil Fov density because abiotic soil factors are likely to play a more important role in shaping patterns of disease. This will assist growers to better understand why Fusarium wilt occurs patchily and differences in the incidence and severity of disease among fields.
- Results from this project suggest a trade-off between saprophytic ability and aggressiveness in Fov. This should be taken into account when rotation is used as a control measure since soil Fov density may increase as a result of enhanced saprophytic ability in response to the absence of cotton in the fields. In addition, such a trade-off can be affected by the interaction between Fov and soil conditions. These findings will help make growers aware that changes in agronomic management (e.g. cropping practices) as well as differences in soils can influence variation in Fov life history traits, e.g. saprophytic ability and aggressiveness.
- Results from this project highlight the potential for Fov to evolve to become locally adapted to different soil conditions. Further insights into the impact of soil factors on the evolutionary potential of Fov will facilitate the development of more effective, cost-efficient and sustainable disease control strategies.
- Results from this project also suggest the possibility that amendment of soil conditions by lifting Ca/Mg ratio could be an added disease control measure. Although the efficacy of such amendments needs to be tested under field conditions, both glasshouse trials in this project revealed significant negative correlations between soil Ca/Mg ratio and disease incidence. With further work, this result may assist growers to choose suitable chemicals in routine agronomic practices.

6. Please describe any:-

   a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);
   b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and
   c) required changes to the Intellectual Property register.

   None.

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

   The occurrence of Fusarium wilt of cotton is influenced by soil conditions. Disease incidence is negatively correlated to soil calcium/magnesium ratio. Bioassays in 14 soils inoculated with the same level of Fov inoculum showed that disease was less severe in soils of high Ca/Mg ratio (>2) compared to those of low ratio (<2). A trade-off between spore production and aggressiveness was observed on 17 representatives of Fov isolates when tested on agar plates in the laboratory. Results suggest that aggressive isolates tend to produce fewer spores than less aggressive ones. This trade-off was also observed in the soil when saprophytic ability was assessed based on Fov prevalence in the absence of cotton. Interaction between Fov and soil origins was involved in the trade-off process. The evolutionary potential of Fov is significantly influenced by soil conditions. Recovery frequency of Fov on diseased plants is greater in the soil from which it derives than in so-called alien soils, suggesting the tendency of Fov to evolve to become soil- or region-specific. Overall, results from this project provide insights into soil impacts on persistence, evolution, and control of Fov, which will facilitate the development of integrated control strategies.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:
   (a) to further develop or to exploit the project technology.
   (b) for the future presentation and dissemination of the project outcomes.
   (c) for future research.

   None.

9. A. List the publications arising from the research project and/or a publication plan.

   (NB: Where possible, please provide a copy of any publication/s)

   - A research paper titled 'Local origin of two vegetative compatibility groups of *Fusarium oxysporum* f. sp. *vasinfectum* in Australia' is in press on Evolutionary Applications.
   - A manuscript titled 'Host resistance mediated inter-genotype competition and temporal variation in *Fusarium oxysporum* f. sp. *vasinfectum'* is in review on European Journal of Plant Pathology.

   B. Have you developed any online resources and what is the website address?

   None.

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

Fusarium wilt is a limiting factor for cotton production in Australia. As a soil-borne fungal pathogen, epidemiological, ecological and genetic features of Fov populations are strongly influenced by soil conditions. This includes key fungal life-history traits crucial to managing the disease such as saprophytic ability and aggressiveness. The objectives of this project were to provide a better understanding of how Fov copes with different soils in the perspective of aggressiveness, saprophytic ability and evolution.

Soil samples were collected from Moree, Boggabilla, and the Darling Downs regions and disease incidence, Fov density, and soil abiotic conditions were tested. Results showed that Fov density varied among fields and disease incidence was not significantly correlated to Fov density (P=0.1281). However, correlation and stepwise regression analyses found significant relationships between disease incidence and soil conditions like calcium/magnesium (Ca/Mg) ratio, sodium, chloride, and electric conductivity, among which only soil Ca/Mg ratio showed a negative relationship. This observation was confirmed by further studies in soils inoculated with the same level of Fov inoculum, suggesting that lifting soil Ca/Mg ratio could be used as an alternative disease control measure provided the efficacy is verified under field conditions in future studies.

Studies of 17 representative Fov isolates clarified that fast-growing Fov isolates are more aggressive than slow-growing ones and aggressive isolates usually produce few spores than less aggressive ones, suggesting trade-off between spore producing ability and aggressiveness in Fov. Consistently, the trade-off was also detected in the soil when the same Fov population was associated with cotton and wheat, respectively. Compared with cotton soils, Fov density was significantly greater in wheat soils due to enhanced saprophytic ability as a result of the absence of cotton. Furthermore, a significant impact of the interaction between Fov and soil origins on the trade-off was also noticed.

Difference in Fov recovery frequency on diseased plants grown in different soils suggests a strong soil impact on Fov evolution. When the recovery frequencies of a Fov isolate from different soils are compared, it is usually higher in the soil from which it derives than in those so-called alien soils. This suggests that Fov can evolve to become soil- or region-specific due to local adaptation and this should be taken into consideration when a suitable cotton cultivar is chosen for different soils.

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