Eur J Plant Pathol (2010) 128:541–551
DOI 10.1007/s10658-010-9690-1

Host resistance mediated inter-genotype competition and temporal variation in *Fusarium oxysporum* f. sp. *vasinfectum*

B. Wang · C. L. Brubaker · P. H. Thrall · J. J. Burdon

Accepted: 13 September 2010 / Published online: 9 October 2010
© KNPV 2010

Abstract Temporal variation in *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*) populations was determined by comparing the genetic diversity of pathogen isolates recovered from three consecutive cotton crops (2002, 2004 and 2006) in the Boggabilla area of New South Wales, Australia. A total of 288 isolates were collected, among which 25 distinct AFLP genotypes were identified. These genotypes were classified into two main groups corresponding to known vegetative compatibility groups (VCG)—01111 and 01112. The *Fov* populations were dominated by four genotypes (I-A, I-B, II-A, II-B) that accounted for 87.5% of the isolates. Significant temporal variation was observed in both sampled fields with 6.8% and 10.7% of total genetic variation being attributed to differences among collections in different years. Genetic diversity based on Nei's gene diversity and the Shannon-Wiener index increased over time. Significant changes in the frequency of the dominant *Fov* genotypes were observed in one field, where genotype I-A declined from 84.8% to 40.0% over the study period (2002–2006), while genotype I-B increased from 7.6% to 35.4%. Strong inter-genotype competition was detected in glasshouse bioassays with 93.4% of symptomatic plants sampled from dual inoculation trials being infected by single genotypes. Competition was differentially mediated by cotton cultivars as the competitive ability of pathogen genotype I-B was enhanced on the resistant cultivar Sicot 189 relative to the susceptible cultivar Siokra 1–4. This suggests that host-mediated inter-genotype competition may play an important role in temporal variation in *Fov* populations in the field.

Keywords *Fusarium* · Cotton · Bioassay · Temporal variation · Competition

B. Wang (✉) · C. L. Brubaker · P. H. Thrall · J. J. Burdon
CSIRO Plant Industry,
G.P.O. Box 1600, Canberra ACT 2601, Australia
e-mail: Bo.Wang@csiro.au

Present Address:
C. L. Brubaker
Bayer BioScience N.V.,
Technologiepark 38,
9052 Ghent, Belgium

Introduction

Temporal variation in populations is a critical aspect in the evolution of plant pathogens. It can be affected by many evolutionary and demographic factors, but epidemiological interactions and co-evolution with host populations are key determinants (Burdon and Thrall 1999; Zhan et al. 2001). Mathematical models suggest that, in agricultural systems, predominant strains of pathogen populations can change rapidly following the release of novel resistant host cultivars (van den Bosch and Gilligan 2003; Pietravalle et al. 2006). Indeed, following the widespread deployment of resistance genes effective against races 1 and 2 of *Fusarium oxysporum* f. sp. *lycopersici* in tomato

cultivars, substantial genetic change was observed in populations in Florida and Georgia with the originally widespread vegetative compatibility group (VCG) 0030 being replaced by the novel VCG 0033 (Gale et al. 2003). Similarly, deployment of the *Ph* gene in tobacco cultivars resulted in a rapid increase of race 1 in populations of *Phytophthora parasitica* in North Carolina (Sullivan et al. 2005); while the frequency of race T in *Bipolaris maydis* dramatically declined when corn cultivars lacking Texas male-sterile cytoplasm were widely grown in the U.S. (Ullstrup 1972).

Natural host-pathogen interactions also provide evidence of co-evolution (Burdon and Jarosz 1991; Thrall and Burdon 2003) with genetic changes in virulence or other pathogen life-history traits resulting from strong selection pressure exerted by host populations (Clay and Kover 1996; Burdon and Thrall 2001). However, there are also some reports of high degrees of genetic stability over years in pathogen populations of large effective size in which host-mediated selection is limited (Borchardt et al. 1998; Zhan et al. 2001).

Direct evidence regarding the relative importance of different mechanisms by which genetic or numerical changes in host populations underpin temporal variation in pathogen populations is limited, although observations from field surveys (Kolmer 1989; Ferguson and Carson 2007) and theoretical models (Lipsitch et al. 1996; Rogoes et al. 2000) suggest a strong influence of host genotype on the evolution of pathogen populations. Temporal variation in genetic structure is believed to reflect the local adaptive process of pathogen populations in response to selection pressure from host populations (Zhan et al. 2002; Montarry et al. 2006) with adaptation being manipulated by hosts through differential virulence or competitive fitness of pathogen genotypes (Lebreton et al. 1999; Abang et al. 2006). Zhan et al. (2002) compared the frequencies of *Mycosphaerella graminicola* isolates occurring on different wheat cultivars over several generations of asexual reproduction under field conditions and found that temporal changes in the genetic structure of pathogen populations occurred more slowly on resistant than on susceptible cultivars.

Fusarium oxysporum f. sp. *vasinfectum* (*Fov*) is a soil-borne fungal pathogen that causes vascular wilt disease of cotton world-wide. In Australia, it was first reported during the 1993–94 growing season in the Darling Downs of Queensland (Kochman 1995). Australian *Fov* consists of two distinct clonal lineages belonging to VCGs 01111 and 01112 that differ from all others found elsewhere, and phylogenetic analyses show that they have evolved from a lineage of indigenous *F. oxysporum* (Davis et al. 1996; Kim et al. 2005; Wang et al. 2006; 2007; 2010). There is evidence suggesting that considerable changes in genetic structure may have occurred in the Australian *Fov* population over the past decade as many previously widespread, dominant *Fov* genotypes have not been found in recent collections (B. Wang, *unpublished data*). It is hypothesized that this shift is associated with increasing levels of resistance of widely deployed commercial cotton cultivars, although precisely how the changes in pathogen population structure may be mediated by host resistance remains unclear.

The main objectives of this study were: (a) to determine patterns of temporal genetic variation of *Fov* in Australian cotton fields by comparing population composition in three consecutive cotton growing seasons within a 5-year period; and (b) to explore the role of increasing resistance in cotton cultivars in driving this process by evaluating the outcome of competitive interactions between pathogen genotypes in glasshouse trials.

Materials and methods

Initial occurrences of *Fov* in the Boggabilla area of New South Wales in 1995 included two adjacent farms where cotton has been grown in rotation with wheat or a fallow, for decades. Two neighbouring fields on these farms were surveyed during three consecutive cotton years (2002–2004–2006), and temporal variation in the genetic structure of the *Fov* populations determined. In addition, dual inoculations of representative isolates of dominant *Fov* genotypes were conducted on susceptible and putatively resistant cotton cultivars in the glasshouse. The impact of host resistance on inter-genotype interactions of the pathogen was examined by assessing recovery frequencies of *Fov* genotypes on different cotton cultivars.

Fungal isolates

A total of 288 *Fov* isolates were collected from two fields (#7 and E2; as used in Wang et al. 2006)

affected by Fusarium wilt in the Boggabilla area of New South Wales, where four cotton cultivars were grown over the 5-year timeframe of the study (Table 1). These fields were chosen because one of the first records of occurrence of the disease was reported there in 1995. No attempt was made to grow the same cotton cultivar during the experimental period as the use of different cultivars reflected the increasing trend of Fusarium resistance in the commercial cotton crop. Furthermore, at the time all planting seed was supplied from one of two companies both of which produced seed in *Fov*-free areas and subsequently treated it before sale. As far as could be ascertained the agronomic practices applied to the two fields were the same.

Cotton plants showing Fusarium wilt symptoms were collected from the same plots in February 2002, 2004, and 2006. Fewer samples were collected in 2004 due to low disease incidence resulting from the exceptionally hot and dry conditions that year. Stems were surface-sterilized in 0.5% sodium hypochlorite for 5 min and peeled under aseptic conditions. Small pieces of vascular tissue were placed on Peptone PCNB agar plates (Burgess et al. 1994) and incubated at 25°C for 1 week. Colonies growing out of tissue pieces were deemed putative *Fov* and subcultured. All isolates were re-grown from single spores on 25% potato dextrose agar (Difco Laboratories, Detroit, MI) and stored at −80°C. Their pathogenicity against cotton was tested using the method of Wang et al. (2004). Isolates were grown in 75 ml of 25% PDB in 150-ml flasks, placed on an orbital shaker at 150 rpm, at 18–23°C for 1 week. Nine two-week-old seedlings were inoculated by dipping their roots in raw fungal cultures 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 a naturally lit glasshouse. Isolates of *Fov* were identified on the basis of their ability to cause wilt symptoms.

Table 1 Number of isolates of *F. oxysporum* f. sp. *vasinfectum* (cotton cultivar grown in the field) recovered from diseased plants collected in fields #7 and E2 in 2002, 2004, and 2006, respectively

Field	2002	2004	2006	Total
#7	66 (Sicot 70)	30 (Sicot 71)	65 (Sicot 71BR)	161
E2	56 (Sicot 189)	19 (Sicot 71)	52 (Sicot 71)	127
Total	122	49	117	288

Four *Fov* isolates (24500 and 24595 of VCG 01111 and 24492 and B/96/02 of VCG 01112), originating from the Darling Downs of Queensland were used as reference strains for the two known VCGs in Australia (these were provided by N. Moore and W. O'Neil, Queensland Department of Primary Industries and Fisheries, Indooroopilly, Australia).

DNA extraction

Isolates were grown in 12 ml of 80% potato dextrose broth (Difco) in 15-ml test tubes at 25°C for 3 days. Fungal mycelia were harvested by centrifuging cultures at 2800 g for 15 min and transferring the precipitate to Whatman No.1 filter paper to remove excess water. Total DNA was extracted from lyophilised mycelia using DNeasy Plant kits (Qiagen Pty Ltd, Clifton Hill, Australia) according to manufacturer instructions. Concentrations of extracted DNAs were determined using a GeneQuant II spectrophotometer (Pharmacia Biotech, Cambridge, England) and adjusted to 50 ng/μl.

AFLP fingerprinting

AFLP fingerprints were generated using the protocol described by Vos et al. (1995). DNA (250 ng) was co-digested with *Mse*I and *Eco*RI at 37°C for 2 h and oligomer adapters were ligated to restricted DNA 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). Finally, 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).

DNA fragments were separated on a 6% polyacrylamide gel electrophoresed at 50 W for 2.5 h on an AFLexpress automatic sequencer (Amersham Pharmacia Biotech, Roosendaal, the Netherlands). Autoradiographs were obtained by exposing the Kodak BioMax film (Eastman Kodak Co., Rochester, NY) to dried gels. Consistent profiles created from

different DNA extractions of the same isolates proved reproducibility of AFLP fingerprints.

Virulence comparisons among dominant *Fov* genotypes

Six representative *Fov* isolates, two each from a dominant genotype (isolates 02-061108 and 02-061122 of genotype I-A, 04-241101 and 04-241114 of genotype I-B, and 04-261106 and 04-261117 of genotype II-A; Fig. 1) were tested for virulence on two cotton cultivars—Siokra 1–4 (susceptible) and Sicot 189 (moderately resistant relative to Siokra 1–4; note resistance to *Fov* is based on quantitative traits). Isolates were grown using the same method as described above, however, fungal mycelia were removed by passing cultures through six layers of Kimwipes tissue (Kimberly-Clark, Milsons Point, Australia), and inocula containing 5.0×10^6 spores ml^{-1}, based on haemocytometer counts, were prepared by adding distilled water to spore suspensions. Distilled water was used for non-inoculated controls.

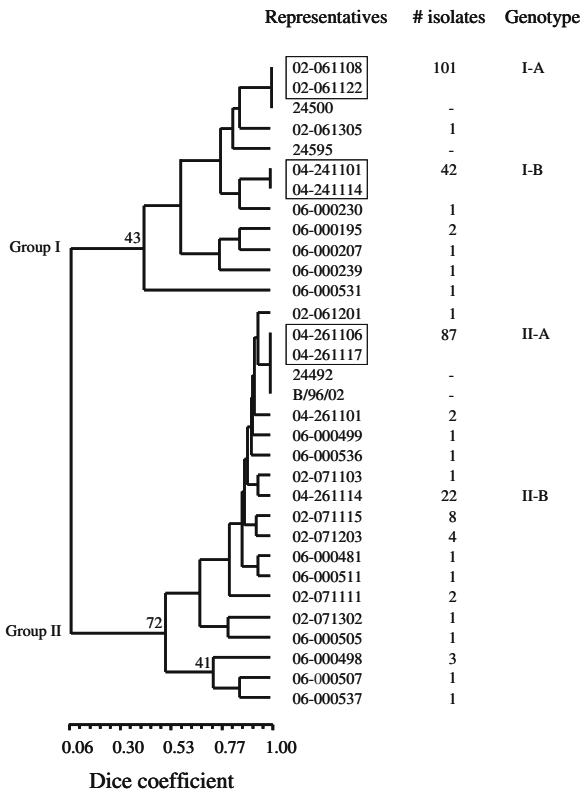

Fig. 1 UPGMA dendrogram illustrating genetic relationships among 288 isolates of *F. oxysporum* f. sp. *vasinfectum* recovered from diseased plants. It was constructed based on the Dice coefficient generated by cluster analysis of 13 polymorphic AFLP loci from representatives of 25 genotypes and four reference *Fov* isolates (24500 and 24595 of VCG 01111; 24492 and B/96/02 of VCG 01112). Bootstrap values (>40%) from 10,000 replicates analysis are shown above nodes. The clustering of genotypes, the number of isolates per genotype, and the four dominant genotypes discussed in the text are also indicated. Six isolates in rectangular boxes were used in the virulence comparison bioassays

Two-week-old cotton seedlings were inoculated and grown using the same method as described above. The tests were conducted using a completely randomized design. Fifteen plants of each cultivar, three per 10-cm pot, were used for each isolate and the trial was conducted twice. Fusarium wilt was identified and assessed six weeks after inoculation by the appearance of vascular discoloration. Disease incidence (percentage of symptomatic plants out of a total of 30 plants inoculated with the two isolates of the same *Fov* genotype) was used to compare the virulence of genotypes.

Competition among *Fov* genotypes on susceptible and resistant cotton cultivars

Competition among *Fov* genotypes was determined on cotton cultivars Siokra 1–4 and Sicot 189. Siokra 1–4 was representative of the fully susceptible cultivars grown in these fields before 2002. Sicot 189 was selected as the moderately resistant cultivar. While several resistant cultivars were grown in the fields over the experimental period, all were derived from the same germplasm and all carried the same resistance genes. Dual inoculation trials were conducted to determine the influence of cotton cultivars on the outcome of competitive interactions between the current dominant genotypes I-A (VCG 01111) and II-A (VCG 01112) and the emerging genotype I-B (VCG 01111) whose frequency in field #7 consistently increased across the period 2002 to 2006. Inoculation trials were carried out as described above except that seedlings were simultaneously inoculated with mixed inocula (5.0×10^6 spores ml^{-1}) consisting of two genotypes in equal proportions. Pair-wise competition (I-A vs. I-B and II-A vs. I-B) was examined with isolate pairs 02-061108 vs. 04-241101 and 04-261106 vs. 04-241101 (first trial) and isolate pairs 02-061122 vs. 04-241114 and 04-261117 vs. 04-241114 (second trial). For each

treatment, 50 plants were inoculated, from which four to five diseased plants chosen at random were sampled six weeks after inoculation, with 10–12 single spores finally being isolated from each sampled plant. Isolates derived from these spores were identified to genotype based on AFLP fingerprints as described previously. If all isolates from a plant were identical then disease was assumed to be the result of mono-infection, while multi-infection was concluded to have occurred if the isolates included both fungal genotypes.

Data analyses

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 (the presence or absence of a band in total isolates) were used to estimate pair-wise genetic relationships among isolates using NTSYSpc 2.02j (Exeter Software, Setauket, NY). The number of genotypes was determined following Saleh et al. (2003). One or two representatives from each genotype were used to generate a similarity matrix based on the Dice coefficient, from which a UPGMA dendrogram of genotypes was constructed. Bootstrap values (10,000 replicates) for each branch were calculated using Winboot (International Rice Research Institute, Manila, Philippines).

Temporal variation was determined by partitioning total genetic variation among and within collections in different years using a hierarchical analysis of molecular variance. Population comparison was conducted by estimating pair-wise differences, and population differentiation was determined by performing the exact test based on genotype frequencies (Arlequin 3.11; Excoffier et al. 2005). In addition, the extent to which *Fov* populations changed over the experimental period was evaluated using Nei's measure of genetic distance, while gene and genotypic diversity of *Fov* populations from each collection was estimated using Nei's gene diversity and the Shannon-Wiener index (Popgene 1.31; Yeh et al. 1999).

In addition to the analyses of AFLP data, temporal variation of *Fov* populations was also estimated based on changes in the frequency of dominant genotypes in collections from different years.

Percentage disease incidence data from genotype virulence comparison trials were arcsine-square root transformed. Data from repeated trials were combined for analysis after tests for equal variances. Analyses of variance were performed using the general linear model of GenStat Release 12.1 (VSN International, Hemel Hempstead, UK).

Frequency isolate recovery data from field surveys and genotype competition trials were analysed using Chi-square tests in GenStat. The impact of susceptible and resistant cotton cultivars on competitive interactions between *Fov* genotypes was determined by comparing the frequencies of genotypes on sampled diseased plants.

Results

AFLP fingerprinting

Twenty-nine polymorphic loci were generated, 13 of which were retained for analysis after rare loci (≤5% frequency) were removed. Twenty-five distinct AFLP genotypes were identified (Fig. 1) that separated into two main groups, each corresponding to a known VCG (VCG 01111 references, isolates 24500 and 24595, occurred in group I; VCG 01112 references, isolates 24492 and B/96/02, nested in group II). Group I contained 150 isolates of eight genotypes, while group II consisted of 138 isolates of 17 genotypes. No significant differentiation was observed within either group as the highest bootstrap values were only 43 and 72 for groups I and II, respectively. Each group was dominated by two genotypes, with genotypes I-A and I-B accounting for 67.3 and 28.0% of group I isolates, and genotypes II-A and II-B accounting for 63.0 and 15.9% of group II isolates, respectively. Together, these four dominant *Fov* genotypes accounted for 87.5% of the isolates; the remaining genotypes occurred at significantly lower frequencies with the majority represented only by a single isolate (Fig. 1).

Temporal variation

The genetic structure of the *Fov* populations in the two sampled fields differed significantly. When total genetic variation was partitioned among and within fields, 72.5% of variation was attributed to differences between fields ($P<0.0001$) (Table 2a), a result consistent with the observation that only three

Table 2 Analysis of molecular variance (AMOVA) for populations of *F. oxysporum* f. sp. *vasinfectum* collected from fields #7 and E2: (a) overall analysis including both sites but ignoring years; (b) field #7 in 2002, 2004, and 2006; and (c) field E2 in 2002, 2004, and 2006. All results are based on 13 polymorphic AFLP loci identified as part of this study

Analysis	Source of variation	d.f.	Sum of squares	Variance components	% of the total variance	*P*-value[1]
(a)	Among fields	1	500.7	3.5167	72.5	<0.0001
	Within fields	286	381.2	1.3330	27.5	
	Total	287	881.9	4.8497		
(b)	Among years	2	12.8	0.0986	6.8	0.0049
	Within years	158	212.9	1.3474	93.2	
	Total	160	225.7	1.4460		
(c)	Among years	2	13.0	0.1373	10.7	0.0010
	Within years	124	142.5	1.1496	89.3	
	Total	126	155.5	1.2869		

[a] Based on 1023 random permutations

genotypes were common to both fields. As a consequence, temporal variation in the two *Fov* populations was analysed separately.

Significant temporal variation was observed in both *Fov* populations with 6.8 and 10.7% of total genetic variation attributed to differences among collections from different years (P=0.0049 and 0.0010) (Table 2b and c). Significant differences were also found among isolates collected within a single year from the same field. Compared to the 2002 collections, noticeable population differentiation occurred by 2004 (pair-wise difference=0.0061 and 0.0436; P=0.4775 and 0.0631), a trend that extended to 2006 (pair-wise difference=0.1047 and 0.1401; both P<0.0001), resulting in an increase in genetic distance between collections over time (Table 3a and b). This was also verified by results of Fisher's exact test with the 2006 samples showing significant population differentiation from those made in 2002 (P<0.0001 for both field sites).

Table 3 Pair-wise difference (above diagonal) and genetic distance (below diagonal) among collections of *F. oxysporum* f. sp. *vasinfectum* in 2002, 2004, and 2006 from (a) field #7 and (b) field E2[a]

	2002	2004	2006
(a) Field #7			
2002	–	0.0061	0.1047*
2004	0.0232	–	0.0370
2006	0.0284	0.0026	–
(b) Field E2			
2002	–	0.0436	0.1401*
2004	0.0074	–	0.0453
2006	0.0326	0.0205	–

[a] Significance: *P<0.001

Genetic diversity of *Fov* populations, as measured by Nei's gene diversity and the Shannon-Wiener index, also increased over time in both fields (Table 4). The number of genotypes increased from five to eight between 2002 and 2006 in field #7 (Table 4a), and from seven to 13 over the same period of time in field E2 (Table 4b). Relatively fewer *Fov* genotypes were found in the 2004 collections, which probably reflects the smaller sample size (Table 4).

Changes in the frequency of dominant *Fov* genotypes among three collections from field #7 also suggested significant temporal variation and population differentiation. The frequency of genotype I-A declined from 84.8% in the 2002 collection to 40.0% in the 2006 collection (χ^2=6.61; P=0.010). In contrast, the frequency of genotype I-B increased from 7.6 to 35.4% during the same time (χ^2=9.87; P=0.002) (Fig. 2a). However, such a marked increase was not observed in field E2 where the frequency of genotype I-B remained low during the experimental period (Fig. 2b).

Virulence comparison among dominant *Fov* genotypes

No significant difference in virulence was observed among the three dominant *Fov* genotypes. Disease incidence ranged from 53.3 to 56.7% when tested on the susceptible cultivar Siokra 1–4. Similarly, no significant differences in virulence were seen on the

Table 4 Gene and genotypic diversity of *F. oxysporum* f. sp. *vasinfectum* populations collected in 2002, 2004, and 2006 from (a) field #7 and (b) field E2

Population	Year of collection	N[a]	M[b]	Ne[c]	H[d]	I[e]
(a) Field #7	2002	66	5	1.1325	0.1144	0.2238
	2004	30	3	1.3253	0.2325	0.3840
	2006	65	8	1.4320	0.2893	0.4564
(b) Field E2	2002	56	7	1.1092	0.0732	0.1227
	2004	19	4	1.1294	0.1134	0.2262
	2006	52	13	1.4482	0.3015	0.4753

[a] N=sample size

[b] M=number of genotypes identified by polymorphic AFLP loci

[c] Ne=effective number of alleles

[d] H=Nei's gene diversity

[e] I=Shannon-Wiener index.

resistant cultivar Sicot 189, although genotype II-A showed slightly higher virulence, causing disease incidence of 46.7%, while genotypes I-A and I-B only caused disease incidences of 20.0 and 23.3%, respectively.

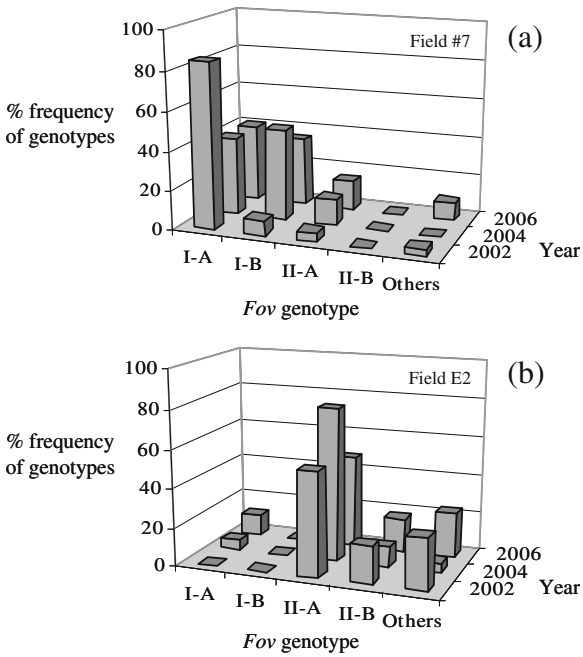

Fig. 2 Variation in frequency (%) of the four dominant genotypes among isolates of *F. oxysporum* f. sp. *vasinfectum* recovered from diseased plants collected in fields #7 (**a**) and E2 (**b**) in 2002, 2004, and 2006, respectively

Competitive interactions among *Fov* genotypes on different cotton cultivars

Competition among *Fov* genotypes was observed since infection of most plants in the dual inoculation trials was caused by mono-infections (Fig. 3). Differential impact of cotton cultivars on competition between genotypes I-A and I-B was also observed as their infectivity differed significantly on the two cotton cultivars (χ^2=4.47; P=0.034). Moreover, genotype I-B caused more infections on Sicot 189 than on Siokra 1–4 with its frequency on diseased plants increasing from 5% (1 of 20 plants) on Siokra 1–4 to 31.6% (6 of 19 plants) on Sicot 189 (Fig. 3a; χ^2= 8.84; P<0.003). Similarly, when dual-inoculated with genotype II-A, the ratio of genotype I-B infection also increased from 55% (11 of 20 plants) on Siokra 1–4 to 78.9% (15 of 19 plants) on Sicot 189 (Fig. 3b; χ^2= 5.77; P<0.016). These results are suggestive of a specific preference and/or adaptation of this *Fov* genotype to the quantitative resistance QTLs carried by Sicot 189.

Discussion

Fusarium oxysporum formae speciales have previously been characterised as having low evolutionary potential because of their exclusively asexual mode of reproduction, small population size, and limited genotype flow among populations (McDonald and

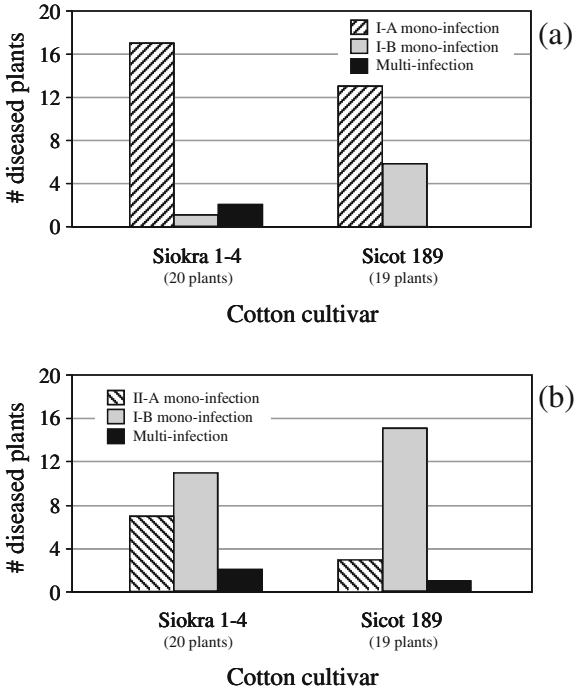

Fig. 3 Numbers of plants on which the disease was caused by mono-infection (all tested spores from a plant were in the same genotype) or multi-infection (spores were in different genotypes) in dual inoculation trials. Trials were conducted twice on two cotton cultivars. Inocula contained two genotypes of *F. oxysporum* f. sp. *vasinfectum*, either I-A and I-B (**a**) or II-A and I-B (**b**), that were mixed at the ratio of 1:1. Numbers of total plants sampled from two trials are indicated in parentheses below cotton cultivars

Linde 2002). However, significant temporal variation was found in this study, with 6.8–10.7% of total genetic variation attributed to within-population shifts in genetic structure among years (Table 2b and c), suggesting that *Fov* populations can evolve rapidly. This is consistent with the high genetic diversity of *Fov* populations observed elsewhere (Abo et al. 2005), but differs from other fungal pathogens, e.g., *Mycosphaerella graminicola* (Chen et al. 1994) and *Setosphaeria turcica* (Borchardt et al. 1998), both of which showed stable genetic structure over multiple years with <1% of total genetic variation attributed to temporal population differentiation. The more dynamic situation observed in this study is probably due to increasing selection pressure exerted by the deployment of more resistant cultivars in the fields where Fusarium resistance (F-rank) increased from 95 for Sicot 70 grown in 2002 to 101 for Sicot 71BR grown in 2006 (Salmond 2003). Similar patterns were also reported for *Setosphaeria turcica* in the U.S. with isolates collected over the period 1974 to 1994 showing a correlation between pathogen population differentiation and selection pressure imposed by resistance genes deployed in maize cultivars (Ferguson and Carson 2007).

Significant shifts in *Fov* population composition were observed in field #7 during the period 2002 to 2006, with the frequency of genotype I-A declining from 84.8% to 40.0%, while genotype I-B increased from 7.6% to 35.4% (Fig. 2a). It is conventional wisdom that pathogens have greater evolutionary potential than host plants due to their higher mutation rates and shorter generation times, leading to local adaptation, i.e., showing enhanced performance on sympatric or the most prevalent genotypes of the host (Ebert 1994; Montarry et al. 2008). Local adaptation has been recognised in various pathosystems, occurring at a scale from individual plants to metapopulations (Thrall et al. 2002; Capelle and Neema 2005; Laine 2005).

Pathogens attacking genetically homogeneous crops are expected to go through selective sweeps favouring particular genotypes due to the release of cultivars carrying either increased or novel resistance (Barrett et al. 2008). Adaptive evolutionary shifts of pathogen populations in response to variation in host resistance has been observed in many agricultural pathogen-host interactions, e.g. *F. oxysporum* f. sp. *lycopersici* on tomato (Gale et al. 2003), *Cronartium ribicola* on five-needled white pine (Richardson et al. 2008), and *Phytophthora infestans* on potato (Montarry et al. 2008). In the current study, adaptation of *Fov* genotype I-B to resistance was found in dual inoculation trials, where it occurred at a higher frequency on plants of the resistant cotton cultivar Sicot 189 than on susceptible cultivar Siokra 1–4 (Fig. 3).

Co-infection of host plants by several pathogens or more than one genotype of a single pathogen is common in nature (Kedera et al. 1994; Maltby and Mihail 1997; Schürch and Roy 2004). Thus, the potential for competition exists between these co-infecting pathogens or genotypes (Lebreton et al. 1999; Zhan et al. 2002; Abang et al. 2006). Here the basis for temporal variation in *Fov* genotype frequency was examined by measuring the relative proportion of current and potential dominant *Fov* genotypes successfully colonizing plants that were dual-inoculated under conditions promoting competition.

Inter-genotype competition appeared to be strongly mediated by the host because the competitive ability of *Fov* genotypes varied differentially among the two cotton cultivars. Genotype I-B always showed enhanced competitive advantage on resistant Sicot 189 compared to its performance on susceptible Siokra 1–4 (Fig. 3a). Similarly, in contrast to the decline of genotype II-A infection on Sicot 189, the proportion of genotype I-B infection increased from 65% on Siokra 1–4 to 84.2% on Sicot 189 in the second competition trial (Fig. 3b). Given that no difference was found in virulence among the three *Fov* genotypes tested in the dual inoculation trials, these results suggest that competition among *Fov* genotypes is mediated by the cotton host, particularly in relation to resistance genes deployed in cultivars. This is supported by previous work showing that host genotypes have a strong impact on the dynamics of pathogen populations and host-mediated competition often occurs between pathogen genotypes when they co-exist (Chin et al. 1984; Harvey et al. 2001; Zhan et al. 2002; Lannou et al. 2005).

Ninety-eight percent of the resistant Sicot 189 plants and 90% of the susceptible Siokra 1–4 plants sampled from the dual inoculation trials of this study were infected by single *Fov* genotypes (Figs. 3a and b). This suggests an antagonistic inter-genotypic interaction between pathogen genotypes such that successful infection of a cotton plant by one *Fov* genotype can result in exclusion of subsequent infection by others. A similar result has been reported in the *Epichloë bromicola-Bromus erectus* interaction with single infections identified on most plants treated with a mixed-genotype inoculum of the fungal endophyte (Wille et al. 2002). It is possible that such antagonism plays an important role in cross-protection and the induced resistance against Fusarium wilt diseases observed in previous studies (Martyn et al. 1991; Jorge et al. 1992).

Of particular note was the finding that the relative ability of genotype I-B to attack resistant hosts became significantly greater when co-inoculated with other genotypes (Fig. 3). Schürch and Roy (2004) reported that some *Mycosphaerella graminicola* genotypes showed lower virulence in mixed inoculum than expected from single infections. Overall, these observations suggest that the infection success of pathogen strains in situations where co-infection is likely may be difficult to predict from their performance in single infection experiments as competition may influence their virulence (Maltby and Mihail 1997; Weeds et al. 2000). Little is known about the mechanisms underlying this phenomenon or the extent to which such processes play a role in determining the evolutionary dynamics of pathogen populations in the field. Further investigations should lead to better insight into host-pathogen interactions as well as facilitate the development of sustainable disease control strategies.

Acknowledgements This research was supported by the Cotton Research and Development Cooperation of Australia. We thank Walter Tate and Matthew Woods for their technical support. We also thank Dr Stephen Allen (Cotton Seed Distributors), Dr David Nehl (New South Wales Department of Agriculture), Dr Augusto Becerra Lopez-Lavalle and Janelle Scown (CSIRO Plant Industry) for their assistance in sample collection.

References

Abang, M. M., Baum, M., Ceccarelli, S., Grando, S., Linde, C. C., Yahyaouli, A., et al. (2006). Differential selection on *Rhynchosporium secalis* during parasitic and saprophytic phases in the barley scald disease cycle. *Phytopathology, 96*, 1214–1222.

Abo, K., Klein, K. K., Edel-Hermann, V., Gautheron, N., Traore, D., & Steinberg, C. (2005). High genetic diversity among strains of *Fusarium oxysporum* f. sp. *vasinfectum* from cotton in Ivory Coast. *Phytopathology, 95*, 1391–1396.

Barrett, L. G., Thrall, P. H., Burdon, J. J., & Linde, C. C. (2008). Life history determines genetic structure and evolutionary potential of host-parasite interactions. *Trends in Ecology and Evolution, 23*, 678–685.

Borchardt, D. S., Welz, H. G., & Geiger, H. H. (1998). Spatial and temporal variation of genetic marker patterns in *Setosphaeria turcica* populations from Kenya. *Journal of Phytopathology, 146*, 451–457.

Burdon, J. J., & Jarosz, A. M. (1991). Host-pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini*: patterns of resistance and racial variation in a large host population. *Evolution, 45*, 205–217.

Burdon, J. J., & Thrall, P. H. (1999). Spatial and temporal patterns in coevolving plant and pathogen associations. *The American Naturalist, 153*, S15–33.

Burdon, J. J., & Thrall, P. H. (2001). The demography and genetics of host-pathogen interactions. In J. Silvertown & J. Antonovics (Eds.), *Integrating Ecology and Evolution in a Spatial Context* (pp. 197–217). Oxford: Blackwell.

Burgess, L. W., Summerell, B. A., Bullock, S., Gott, K. P., & Backhouse, D. (1994). *Laboratory manual for Fusarium research*. Sydney: University of Sydney.

Capelle, J., & Neema, C. (2005). Local adaptation and population structure at a micro-geographical scale of a fungal parasite on its host plant. *Journal of Evolutionary Biology, 18*, 1445–1454.

Chen, R. S., Boeger, J. M., & McDonald, B. A. (1994). Genetic stability in a population of a plant pathogenic fungus over time. *Molecular Ecology, 3*, 209–218.

Chin, K. M., Wolfe, M. S., & Minchin, P. N. (1984). Host-mediated interactions between pathogen genotypes. *Plant Pathology, 33*, 161–171.

Clay, K., & Kover, P. X. (1996). The Red Queen hypothesis and plant/pathogen interactions. *Annual Review of Phytopathology, 34*, 29–50.

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

Ebert, D. (1994). Virulence and local adaptation of a horizontally transmitted parasite. *Science, 265*, 1084–1086.

Excoffier, L. G., Laval, I., & Schneider, S. (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online, 1*, 47–50.

Ferguson, L. M., & Carson, M. L. (2007). Temporal variation in *Setosphaeria turcica* between 1974 and 1994 and origin of races 1, 23, and 23 N in the United States. *Phytopathology, 97*, 1501–1511.

Gale, L. R., Katan, T., & Kistler, H. C. (2003). The probable center of origin of *Fusarium oxysporum* f. sp. *lycopersici* VCG 0033. *Plant Disease, 87*, 1433–1438.

Harvey, P. R., Langridge, P., & Marshall, D. R. (2001). Genetic drift and host-mediated selection cause genetic differentiation among *Gaeumannomyces graminis* populations infecting cereals in southern Australia. *Mycological Research, 105*, 927–935.

Jorge, P. E., Green, R. J., & Chaney, W. R. (1992). Inoculation with Fusarium and Verticillium to increase resistance in Fusarium-resistant tomato. *Plant Disease, 76*, 340–343.

Kedera, C. J., Leslie, J. F., & Claflin, L. E. (1994). Genetic diversity of *Fusarium* Section *Liseola* (*Gibberella-fujikuroi*) in individual maize stalks. *Phytopathology, 84*, 603–607.

Kim, Y., Hutmacher, R. B., & Davis, R. M. (2005). Characterization of California isolates of *Fusarium oxysporum* f. sp. *vasinfectum*. *Plant Disease, 89*, 366–372.

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

Kolmer, J. A. (1989). Virulence and race dynamics of *Puccinia recondita* f. sp. *tritici* in Canada during 1956–1978. *Phytopathology, 79*, 349–356.

Laine, A.-L. (2005). Spatial scale of local adaptation in a plant-pathogen metapopulation. *Journal of Evolutionary Biology, 18*, 930–938.

Lannou, C., Hubert, P., & Gimeno, C. (2005). Competition and interaction among stripe rust pathotypes in wheat-cultivar mixtures. *Plant Pathology, 54*, 699–712.

Lebreton, L., Lucas, J.-M., & Andrivon, D. (1999). Aggressiveness and competitive fitness of *Phytophthora infestans* isolates collected from potato and tomato in France. *Phytopathology, 89*, 679–686.

Lipsitch, M., Siller, S., & Nowak, M. A. (1996). The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution, 50*, 1729–1741.

Maltby, A. D., & Mihail, J. D. (1997). Competition among *Sclerotinia sclerotiorum* genotypes within canola stems. *Canadian Journal of Botany, 75*, 462–468.

Martyn, R. D., Biles, C. L., & Dillard, E. A. (1991). Induced resistance to Fusarium-wilt of watermelon under simulated field conditions. *Plant Disease, 75*, 874–877.

McDonald, B. A., & Linde, C. (2002). Pathogen population genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology, 40*, 349–379.

Montarry, J., Corbiere, R., Lesueur, S., Glais, I., & Andrivon, D. (2006). Does selection by resistant hosts trigger local adaptation in plant-pathogen systems? *Journal of Evolutionary Biology, 19*, 522–531.

Montarry, J., Glais, I., Corbiere, R., & Andrivon, D. (2008). Adaptation to the most abundant host genotype in an agricultural plant-pathogen system—potato late blight. *Journal of Evolutionary Biology, 21*, 1397–1407.

Pietravalle, S., Lemarié, S., & van den Bosch, F. (2006). Durability of resistance and cost of virulence. *European Journal of Plant Pathology, 114*, 107–116.

Richardson, B. A., Klopfenstein, N. B., Zambino, P. J., McDonald, G. I., Geils, B. W., & Carris, L. M. (2008). Influence of host resistance on the genetic structure of the white pine blister rust fungus in the western United States. *Phytopathology, 98*, 413–420.

Rogoes, R. R., Nowak, M. A., & Bonhoeffer, S. (2000). Evolution of virulence in a heterogeneous host population. *Evolution, 54*, 64–71.

Saleh, A. A., Zeller, K. A., Ismael, A.-S. M., Fahmy, Z. M., El-Assiuty, E. M., & Leslie, J. F. (2003). Amplified fragment length polymorphism diversity in *Cephalosporium maydis* from Egypt. *Phytopathology, 93*, 853–859.

Salmond, G. (2003). Disease ratings: Another management tool for cotton growers. *The Australian Cottongrower, 24*(4), 9.

Schürch, S., & Roy, B. A. (2004). Comparing single- vs. mixed-genotype infection of *Mycosphaerella graminicola* on wheat: effects on pathogen virulence and host tolerance. *Evolutionary Ecology, 18*, 1–14.

Sullivan, M. J., Melton, T. A., & Shew, H. D. (2005). Managing the race structure of *Phytophthora parasitica* var. *nicotianae* with cultivar rotation. *Plant Disease, 89*, 1285–1294.

Thrall, P. H., Burdon, J. J., & Bever, J. D. (2002). Local adaptation in the *Linum marginale-Melampsora lini* host-pathogen interaction. *Evolution, 56*, 1340–1351.

Thrall, P. H., & Burdon, J. J. (2003). Evolution of virulence in a plant host-pathogen metapopulation. *Science, 299*, 1735–1737.

Ullstrup, A. J. (1972). The impacts of the southern corn leaf blight epidemics of 1970–1971. *Annual Review of Phytopathology, 10*, 37–50.

van den Bosch, F., & Gilligan, C. A. (2003). Measures of durability of resistance. *Phytopathology, 93*, 616–625.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., et al. (1995). AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research, 23*, 4407–4414.

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

Wang, B., Brubaker, C. L., Summerell, B. A., Thrall, P. H., & Burdon, J. J. (2010). Local origin of two vegetative compatibility groups of *Fusarium oxysporum* f. sp. *vasinfectum* in Australia. *Evolutionary Applications* (in press).

Wang, B., Brubaker, C. L., Tate, W., Woods, M. J., Matheson, B. A., & Burdon, J. J. (2006). Genetic variation and population structure of *Fusarium oxysporum* f. sp. *vasinfectum* in Australia. *Plant Pathology, 55*, 746–755.

Wang, B., Brubaker, C. L., & Burdon, J. J. (2007). Origin and evolution of *Fusarium oxysporum* f. sp. *vasinfectum*: A case study in Australia. World Cotton Research Conference-4 (Lubbock, TX, 10-14 September 2007) CD.

Weeds, P. L., Beever, R. E., & Long, P. G. (2000). Competition between aggressive and non-aggressive strains of *Botrytis cinerea* (*Botryotinia fuckeliana*) on French bean leaves. *Australasian Plant Pathology, 29*, 200–204.

Wille, P., Boller, T., & Kaltz, O. (2002). Mixed inoculation alters infection success of strains of the endophyte *Epichloë bromicola* on its grass host *Bromus erectus*. *Proceedings of the Royal Society B: Biological Sciences, 269*, 397–402.

Yeh, F. C., Rongcai, Y., & Boyle, T. (1999). *Popgene Version 1.31*. Edmonton, USA: University of Alberta.

Zhan, J., Mundt, C. C., & McDonald, B. A. (2001). Using restriction fragment length polymorphisms to assess temporal variation and estimate the number of ascospores that initiate epidemics in field populations of *Mycosphaerella graminicola*. *Phytopathology, 91*, 1011–1017.

Zhan, J., Mundt, C. C., Hoffer, M. E., & McDonald, B. A. (2002). Local adaptation and effect of host genotype on the rate of pathogen evolution: an experimental test in a plant pathosystem. *Journal of Evolutionary Biology, 15*, 634–647.