1274 Origin and evolution of Fusarium oxysporum f. sp. vasinfectum: A case study in Australia

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ABSTRACT

In Australia, indigenous *Fusarium oxysporum* consist of at least five genetically distinct lineages and the two strains (VCG 11&12) of *F. oxysporum* f. sp. *vasinfectum* (*Fov*) are genetically related to lineage A, suggesting that they evolved locally. This is consistent with the observation that virulence of a lineage A isolate increased after 10 successive infections on susceptible cotton in the glasshouse. In 2004, 28 genotypes of *Fov* were identified, with 21 in widespread VCG 11 and 7 in restricted VCG 12. Geographic distribution patterns of the two VCGs may reflect different impacts of soil on their saprophytic ability and aggressiveness.

Fusarium wilt of cotton (*Gossypium hirsutum*) in Australia was first reported in southeastern Queensland during the 1993/94 growing season (Kockman, 1995). Stringent farm hygiene practices were quickly instituted, but despite this the disease has now spread rapidly to almost all major cotton growing regions, most likely through movement of field run-off water within irrigation catchments or via contaminated soil on dirty equipment and vehicles moving between districts. As a result, Fusarium wilt has become a limiting factor for Australian cotton production, causing severe crop losses (Kochman et al., 2002).

Fusarium wilt of cotton is caused by the soil-borne fungal pathogen, *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*). In Australia, it is attributable to two *Fov* strains, each of which belongs to a distinct vegetative compatibility group - VCG 01111 and VCG 01112 (Bentley et al., 2002). The simultaneous appearance of two *Fov* strains of different geographic origins (200 km apart) appears to be inconsistent with an overseas origin, suggesting that these strains may have evolved locally, a hypothesis supported by detailed genetic analyses. Thus, not only do the two Australian *Fov* strains differ from overseas *Fov* races 1 to 8 in DNA fingerprints and pathogenicity on differential hosts, but they have unique patterns of aesculin hydrolysis and volatile production.

Variation in genetic structure among local fungal populations can provide further clues to the origins of newly detected pathogens (Gordon and Okamoto, 1992; Appel and Gordon, 1994). In Australia, wild cottons (native *Gossypium* species) usually occur in remote, undisturbed areas where it is thought to be good sources of native *F. oxysporum*. Importantly, some wild cottons are highly resistant to Fusarium wilt, implying that native *Fov* may have been present in these ecosystems for extended periods of time. Surveys of native *F. oxysporum* from rhizosphere soils of wild cotton populations identified five genetically distinct lineages (designated A to E) based on AFLP fingerprints (**Fig. 1**), and lineage A is genetically related to the two Australian VCGs of *Fov* (**Fig. 2**). Furthermore, pathogenicity assays showed that while not all the lineage A isolates are pathogenic on cultivated cotton, the frequency of pathogenic isolates is significantly higher than in any of the other lineages (Wang et al, 2004). Pathogenic isolates are more virulent on wild cotton than they are on cultivated cotton, implying that native *Fov* may have coevolved with wild cottons. Lineage A strains were also found in cultivated and undisturbed soil collected from a cotton growing area believed to be the center of origin of this disease. Sequencing of EF-

1α and mtSSU genes showed that lineage A isolates are phylogenetically related to the two *Fov* strains occurring in Australian cotton fields (**Fig. 3**). Thus, AFLP analyses and gene genealogies strongly support local origins of the two Australian strains of *Fov*, which have evolved from lineage A of native *F. oxysporum* populations.

The occurrence of lineage A isolates in cotton field soil highlights the likelihood that new *Fov* strains may emerge in the future given that lineage A has already given rise to VCG 11 and VCG 12. The potential for the evolution of virulence in lineage A isolates was evaluated using serial passage assays on susceptible cotton plants in the glasshouse. Significantly increased virulence was observed in offspring isolates after 10 serial passages, suggesting that lineage A isolates do have the ability to evolve into more aggressive forms after continuous exposure to susceptible cotton. In addition, this work showed that cotton plays an important role during this evolutionary process as no clear increasing tendency of virulence was observed when serial passage assays were conducted on water agar.

The efficacy of disease management strategies depends, at least in part, on developing a better understanding of the genetic diversity and population structure of *Fov*. Twenty-eight genotypes of *Fov* were identified based on a study of 350 isolates collected from six major cotton growing regions in 2002 and 2004 (Wang et al, 2006; **Fig. 4**). They could be separated into two distinct groups (similarity 37%) that coincide with VCG differences. The VCG 11 group comprised 21 haplotypes and was further divided into two subgroups with one probably representing a new VCG as it is incompatible with either known Australian VCG. This is also consistent with the local origins of *Fov* in Australia. The VCG 12 group consisted of seven haplotypes that were all found in the same region. Geographically, VCG 11 is widespread, but VCG 12 remains restricted to the area where this *Fov* strain was initially found 13 years ago. No genetic structure was observed among *Fov* populations at broad geographic scales, although interestingly some local structure was observed. For example, the *Fov* populations in two fields in Boggabilla were distinct from all the others sampled from this region. Despite the general lack of population differentiation, significant variation in pathogenicity was found among different genotypes of *Fov*. Field surveys also showed that *Fov* populations may change over time. For example, in one field in dendrogram created from AFLP fingerprints of 28 haplotype representatives of *Fov*. Ref11 and Ref12 are two reference isolates of VCG 11 and 12. The numbers at the nodes of major clusters represent bootstrap values (%) generated by 1000 replicates.

Boggabilla, the dominant genotype was H-01 in 2002 but H-03 in 2004. Preliminary results from glasshouse trials showing that H-03 was more aggressive than H-01 on resistant cotton plants suggest that this change may be driven by the increasing resistance level of newly released cotton cultivars planted in the field. Therefore, changes in genotypic frequencies may represent the outcome of competitive interactions between different strains of *Fov*. Future studies will focus on soil and management impacts on the ecology and evolution of *Fov* since, as a soil-borne fungus, both aggressiveness and saprophytic abilities are inevitably influenced by soil biotic and abiotic factors. Insights into how *Fov* life history traits and soil factors interact to determine the evolution of virulence and persistence of *Fov* would aid in developing effective disease management strategies.

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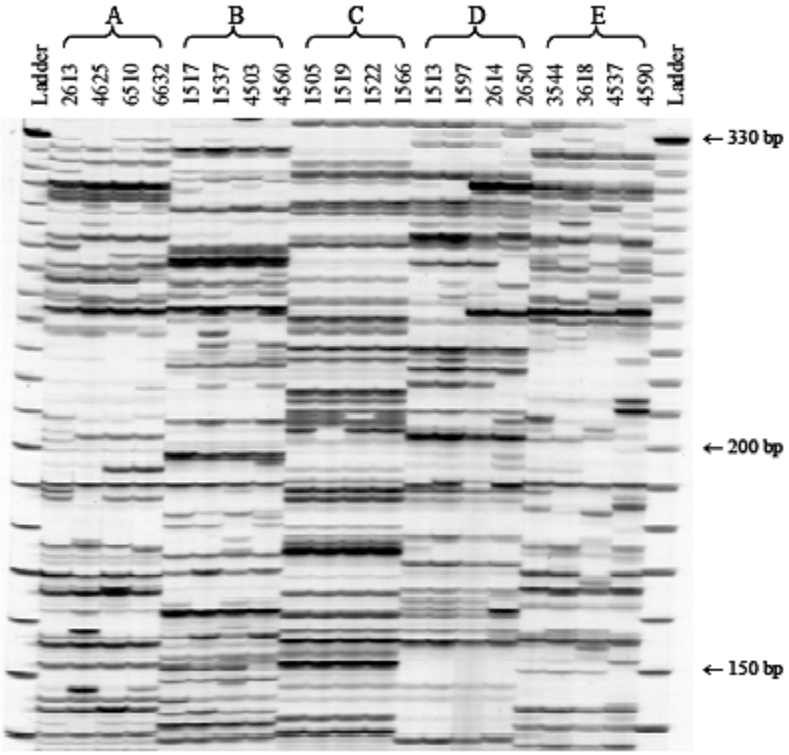


Fig. 1. A section of AFLP fingerprints of four representative *F. oxysporum* isolates of each of the five lineages (A, B, C, D, E) identified in this study.

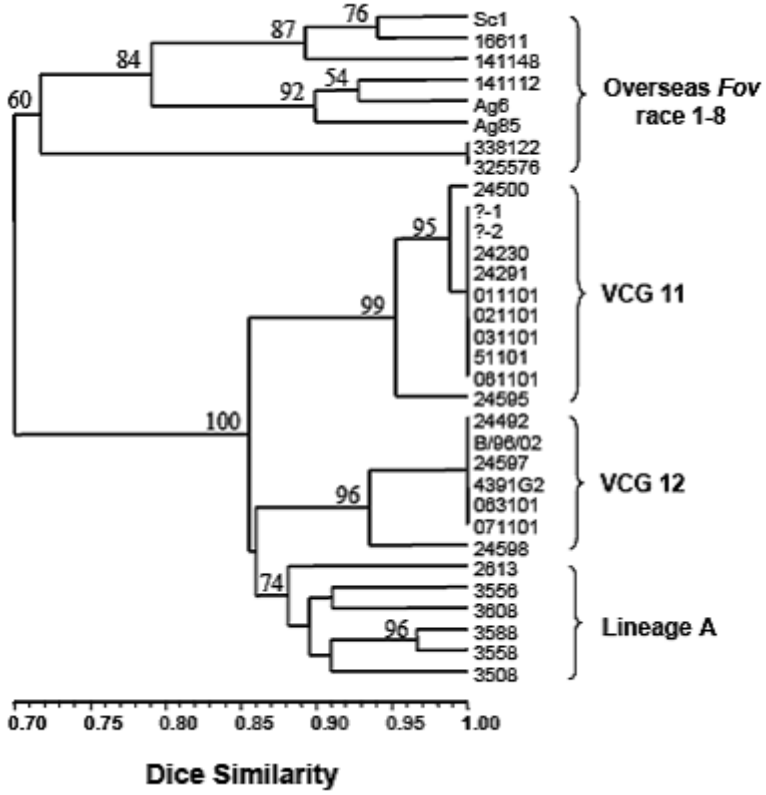


Fig. 2. UPGMA dendrogram constructed from AFLP fingerprints showing genetic relationships among overseas *Fov* races 1 to 8, VCG 11 and VCG 12, and lineage A of native *F. oxysporum*. The numbers at the nodes of major clusters represent bootstrap values (%) generated by 1000 replicates.

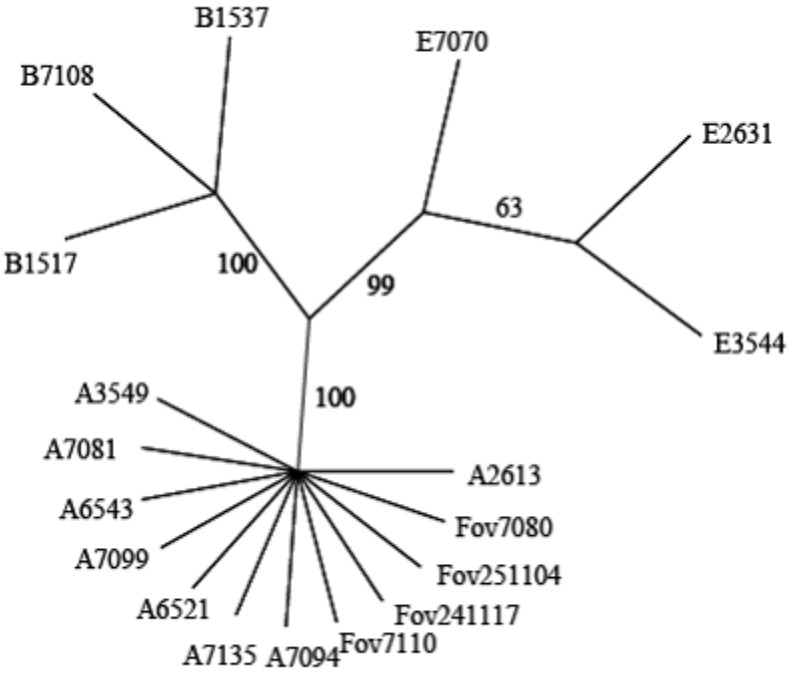
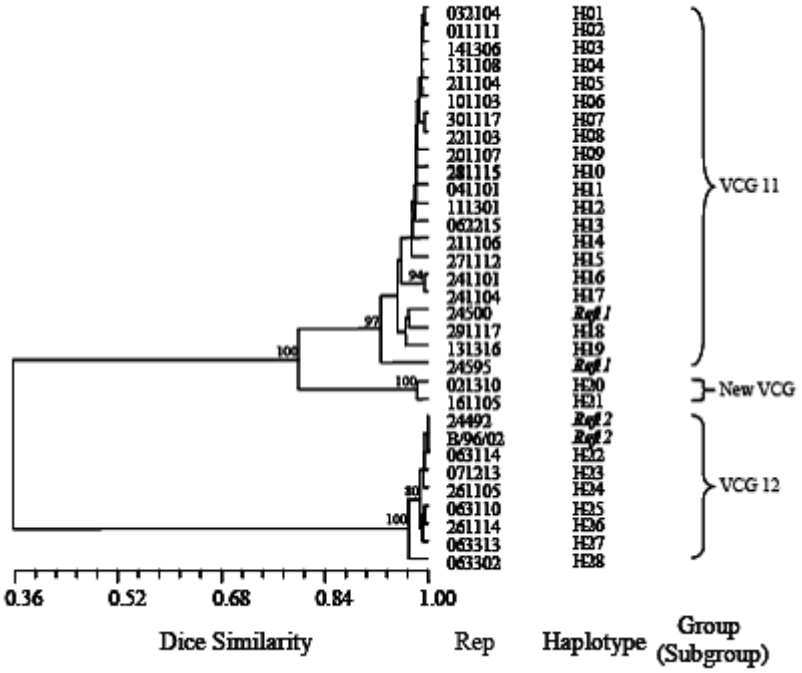


Fig. 3. An unrooted most parsimonious tree showing phylogenetic relationships among representatives of the Australian *Fov* and lineages A, B, and E of native *F. oxysporum*. The numbers on the major branch represent bootstrap values (%) generated by 10,000 replicates.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241101
241104
24500
291117
131316
24595
021310
161105
24492
B/96/02
063114
071213
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063302
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H03
H04
H05
H06
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H10
H11
H12
H13
H14
H15
H16
H17
Ref.1
H18
H19
Ref.1
H20
H21
Ref.2
Ref.2
H22
H23
H24
H25
H26
H27
H28
VCG 11
New VCG
VCG 12
100
97
100
94
100
0.36
0.52
0.68
0.84
1.00
Dice Similarity
Rep
Haplotype
Group
(Subgroup)

Fig. 4. UPGMA