VARIATION IN THE STYLOSANTHES ANTHRACNOSE PATHOGEN: IMPLICATIONS FOR AUSTRALIAN CULTIVARS

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ABSTRACT
Isolates of *Colletotrichum gloeosporioides* which cause anthracnose disease in the tropical pasture legume *Stylosanthes* were collected from its centre of diversity in South America. These and other isolates from Asia, Africa and Australia were studied using Random Amplified Polymorphic DNA (RAPD) markers and virulence on host differentials to assess the threat to Australian Stylosanthes cultivars from exotic races of this pathogen. A phenetic analysis of 90 isolates using RAPD markers showed a wide genetic diversity in the overall pathogen population. Compared to this, genetic diversity in the Australian population was very limited. A similar situation was noted for pathogenic variation where 43 of the 69 South American isolates could not be classified using linear discriminant functions developed using isolates of the current Australian races. Some isolates from Brazil caused serious anthracnose on Australian cultivars and accessions which are moderate to highly resistant to the Australian races. If accidentally introduced, these isolates may pose a potential threat to the Australian *Stylosanthes* cultivars.

KEYWORDS
Stylosanthes, anthracnose, pathogenic diversity, genetic diversity, RAPD markers

INTRODUCTION
In Australia, anthracnose disease caused by *Colletotrichum gloeosporioides* is a major constraint to the production, persistence and utilisation of *Stylosanthes* pastures. It has caused the demise of nine cultivars from commercial utilisation including 2 million ha of highly susceptible *S. humilis* pastures in the 1970's. Anthracnose continues to cause low to moderate damage to all existing cultivars. The situation is similar in Colombia where *S. guianensis* suffers losses of 65 to 100% (Lenné, 1986), and in Brazil where the utilisation of *S. capitata* and *S. guianensis* is severely impeded by anthracnose.

Biotype A of *C. gloeosporioides* is pathogenic on all *Stylosanthes* species, including *S. scabra* and *S. hamata*, which are economically important in Australia. Three races have been recorded on *S. scabra* in Australia (Chakraborty *et al*., 1996). Although *C. gloeosporioides* has developed new virulent forms following the release of resistant cultivars, the range of diversity in the Australian population is limited. Populations at the centres of origin in South America are more diverse (Miles and Lenné, 1984). The presence of complex races outside Australia poses a potential threat to our cultivars; if accidentally introduced, these can potentially devastate the stylo-based pastoral industry. To better define this threat, this study documents genetic and pathogenic variation in *C. gloeosporioides* isolates collected mainly from South America from hosts other than *S. guianensis*.

METHODS
Genetic variation in 90 isolates mainly from Brazil and Colombia with a small number from Asia, Africa and Australia was studied using Random Amplified Polymorphic DNA (RAPD) markers. Duplicate DNA samples of each isolate were amplified by polymerase chain reaction for 10 arbitrary decanucleotide primers (Operon Technologies Inc. Alameda, CA94501). Data on the presence and absence of bands for DNA fragments, run on 1.5% agarose gel, were used in a cluster analysis using the McQuitty algorithm.

Pathogenic variation of 73 isolates including four Australian reference isolates was assessed in Brazil using the Australian biotype A differentials, Seca, Fitzroy, 36260, 55860, Q10042, 93116 and 110354. A 10^6 conidia/ml suspension of each isolate was inoculated on to 3-5, 6-week old seedlings of each differential and assessed for severity 10 days after inoculation using a 0-9 point scale where 0, no disease and 9, plant death. Linear discriminant functions, developed using a training data set of Australian isolates with known race membership (Chakraborty *et al*., 1996), were applied to classify the overseas isolates using $\log_e$(severity + 1) transformed data on anthracnose severity for the host differentials.

RESULTS AND DISCUSSION
Some isolates from the same geographical location and/or host species were grouped together using the 10 RAPD primers. Other isolates from the same geographical location and/or host species were genetically diverse. All Australian isolates from *S. scabra* and *S. viscosa* were classified in a single group with three African isolates from *S. hamata*, an isolate from *S. fruticosa* from Thailand, and an isolate from *S. scabra* from the Philippines. There was less than 5% dissimilarity between isolates in this group compared with over 80% dissimilarity between the 6-8 major genetic groups.

Using linear discriminant functions developed with isolates of the three Australian races, 43 of the 69 South American isolates could not be classified into any of the existing races. These unclassified isolates are represented by a zero in Fig. 1. Isolates which could be classified into one of the Australian races have been labelled with that race number; 21 were classified as race 3 (virulent on Seca) and 5 as race 4/4a (virulent on Q10042, labelled as 2 in Fig. 1). Two isolates from *S. capitata* and 11 from *S. scabra* produced severity rating >4 on Seca. Five isolates produced severity rating >4 on 93116 which is highly resistant to all Australian isolates.

Results show that there is only a small component of the overall genetic and pathogenic diversity in Australia compared to that at the centre of diversity in South America and elsewhere. Isolates used in this work come from regions where *S. guianensis* and *S. capitata* are naturally distributed. Further testing of isolate from *S. scabra* and *S. hamata* from their native range is necessary for a more comprehensive understanding of this diversity.

If accidentally introduced, some isolates may pose a potential threat to Australian cultivars with a 93116 component and Seca. This reinforces the need for strong quarantine measures to protect Australian pastoral industries. At the same time, threat to commercial cultivars needs to be better assessed through screening of germplasm at centres of diversity and by predicting how new alien strains of the pathogen may establish in competition with existing races.

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Figure 1
Classification of 69 South American isolates using linear discriminant functions developed using anthracnose severity scores of 182 Australian isolates from three races on type A host differentials. Arrow represents increasing severity on a given differential. Only the reference isolates are labelled. Race assignment of other Australian isolates are designated by small numerals. Large numerals designate race assignment of the South American isolates with zero representing an unclassified isolate.