Genetic Diversity of *Thielaviopsis basicola*

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The disease black root rot (BRR) is caused by the soil borne fungal pathogen *Thielaviopsis basicola*. This fungus has a broad host range, infecting 137 species, with a worldwide distribution (Honeess, 1994). The combination of a wide host range with the ability to produce persistent resting spores contributes to a high disease impact. BRR symptoms are readily identifiable in the field, with stunting of seedlings and characteristic black lesions on younger roots. While not killing the seedlings, except in extreme cases or in association with other seedling diseases, the stunting can carry through to maturity with significant yield reductions. In Australia BRR is a relatively new disease, having only been found in cotton fields in 1989 (Allen, 1990), yet it has rapidly become a widespread major problem particularly when season temperatures are below average.

Investigations are underway as a Cotton CRC project to assess the genetic diversity of the pathogen *T.basicola*. An understanding of the diversity is important for disease control measures to be instigated effectively; this includes plant breeding (even though resistance in cotton is yet to be found). The focus of this work is to examine the variation in Australia of strains of *T.basicola*, and from this information determine the pathogen's likely origins and effects on field outbreaks. To achieve this, molecular analysis of diversity will be combined with pathogenicity testing.

Pathogenicity, the ability to inflict damage on a host, can be used to measure variability in a pathogen population. However it is dependent on the host species/cultivars being assessed and on the environmental conditions prevalent and therefore has limitations in determining the underlying genetic variability. Knowledge of the underlying genetic variability is important in breeding for resistance; determining origins of a pathogen population and in predicting epidemics. Morphological analysis, can be used for studies on variability as it allows assessment of the pathogen independent of the host, but there are only a few limited characters that can be assessed (eg spore size, culture growth rate) and they are still highly influenced by the environment. Molecular markers, however, offer a direct means of assessing genetic variability independent of environmental influences and are limitless as the DNA itself is assessed. Pathogenicity is still very important and should be assessed in conjunction with molecular techniques to correlate genetic diversity to changes in host pathogen interaction.

There are numerous molecular markers available for analysis of genetic diversity; in initial studies in this project randomly amplified polymorphic DNA (RAPDs) will be used. RAPDs involves using the polymerase chain reaction (PCR) to randomly amplify fragments from the fungal DNA. If there are differences in the DNA, different sized fragments will be created which can be seen as differences in bands present (polymorphisms) when the fragments are viewed on an electrophoresis gel (see figure 1). The advantage of using RAPDs over other molecular marker methods is their low cost, high speed and that there is no need for prior sequence data. This method has been used successfully on *T.basicola* both in Australia (Pattemore, 1999) and overseas (Punja & Sun, 1999) to assess genetic diversity. Another method such as conserved gene analysis will be used to expand and confirm results from the RAPD analysis.

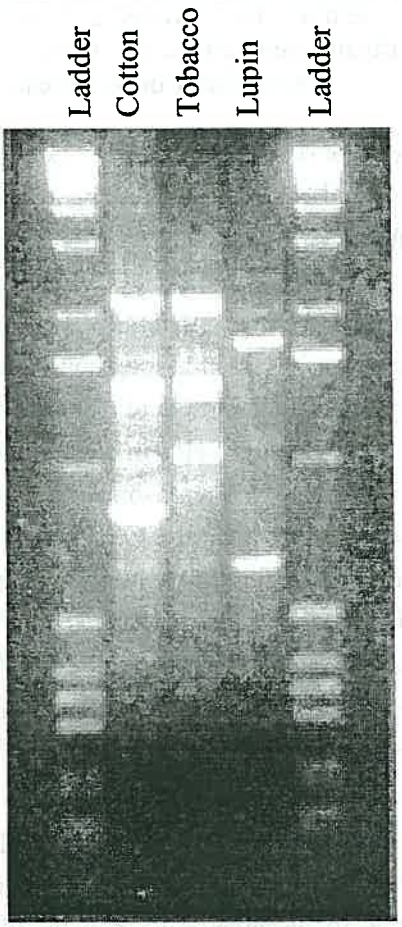

Figure 1: Agarose gel of RAPD products using DNA from Cotton, Tobacco and Lupin infecting strains of *T.basicola*. Showing variation in banding patterns (polymorphisms) between the three isolates. The ladder represents a standard marker to make comparisons by.

There are two possible sources of the *T.basicola* strains that infect cotton in Australia: 1) a recent introduction from overseas or 2) the adaptation of existing endemic strains to infect cotton. Both these possibilities can explain the increase in BRR incidence. Studies into the genetic clustering of the pathogen populations, taxonomy of the fungus and its lifecycle will help determine the source of these *T.basicola* strains in Australia.

The introduction of a novel virulent pathogen into a farming system can lead to a rapid spread of that pathogen, especially where the host crop lacks resistance and where competition from other microbes may be absent. From a single introduction it would be expected that genetic diversity would be low, even though the pathogen may be spread across a wide area. This was observed with *Fusarium oxysporum* f.sp. *apii* race 2 that causes Fusarium yellows in celery across North America, where all strains examined throughout a widely dispersed area were found to be genetically similar (Gordon & Martyn, 1997). Such low variance favours the tracing of the pathogen back to an original source or initial infection site.

Endemic strains, on the other hand would be expected to show geographical variations in populations, therefore isolates within one region would be very similar but dissimilar between regions. However in farming situations such a clear delineation would not occur as uncleaned vehicle and machinery movements can carry *T.basicola* from region to region and so disperse different genotypes. It is important to note that endemic does not necessarily mean native, just that the organism has been present for a long period of time making it widespread and genetically diverse.

Evidence from previous and current work suggests the adaptation of an endemic strain rather than a new introduction has occurred with *T.basicola* on cotton. It has been shown that exposure of a pathogen to a host will result in a more virulent pathogen, that will cause more disease (Simons & McDaniel, 1983), this has been suggested by Punja and Sun(Punja & Sun, 1999) with Canadian strains of *T.basicola*. In Australian cotton, it is likely the repeated cultivation of cotton has selected strains of *T.basicola* that were already present in soils, and has slowly favoured those which were most aggressive to cotton, until the point where symptoms of BRR began to appear. The work of Julie Pattemore (Pattemore, 1999) showed definite genetic similarities within regions, with the expected migration of some genetic material between some regions.

In the space of 12 years since the first occurrence of black root rot; it has grown to be an economically important disease for the Australian cotton industry. Without killing the plant it still manages to reduce yield at an increasing rate with every cotton crop. So far this project has optimised the chosen molecular marker technique, RAPDs, which have been previously proven to be able to show polymorphisms between *T.basicola* isolates. Due to the lack of information on the pathogen, background research confirming if *T.basicola* is endemic, and analysis of the relationship between genetic diversity and pathogenicity is central to managing the disease.

References:

Allen, S. J. (1990). Thielaviopsis basicola, a new record on cotton in Australia. *Australasian Plant Pathology, 19*(1), 24-25.

Gordon, T. R., & Martyn, R. D. (1997). The evolutionary biology of Fusarium oxysporum. *Annual Review of Phytopathology, 35*, 111-128.

Honeess, T. M. (1994). *Aetiological aspects of black root rot of cotton.* Unpublished Science Honours, University of New England, Armidale.

Pattemore, J. (1999). *Genetic diversity among population of Thielaviopsis basicola (syn Chalara elegans) from cotton and lettuce soils.* Unpublished Postgraduate Diploma in Science, The University of Queensland, Brisbane.

Punja, Z. K., & Sun, L. J. (1999). Morphological and molecular characterization of Chalara elegans (Thielaviopsis basicola), cause of black root rot on diverse plant species. *Canadian Journal of Botany-Revue Canadienne De Botanique, 77*(12), 1801-1812.

Simons, M. D., & McDaniel, M. E. (1983). Gradual evolution of virulence of Puccinia coronata on oats. *Phytopathology, 73*(8), 1203-1205.