

Occurrence of the black root rot fungus in soils surrounding Australian cotton properties

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Typically the fungus *Thielaviopsis basicola*, the casual organism for black root rot, is generally considered to be widespread in both cultivated and uncultivated soils; surveys were conducted in the catchments surrounding cotton properties to determine if this was the case for Australia. Knowledge of the origin and distribution of soilborne diseases can make significant impacts on both the choice and effectiveness of control measures implemented. If *T. basicola* had been endemic as suggested by overseas studies, then the lack of commercially effective control measures would give little hope for control of the disease in cotton in the near future. As it stands this research has shown that *T. basicola* only occasionally occurs (in highly disturbed sites) outside of cultivated cotton land, meaning that there is potential to control the spread of disease to unaffected farms and fields through the implementation of appropriate farm hygiene measures.

About black root rot

Black root rot as caused by *Thielaviopsis basicola* (Berk. And Br.) Ferraris, is a soilborne fungus which attacks the root system of the plant (particularly at seedling stage) resulting in stunting of the plant. It is of economic significance to many agricultural and horticultural crops including tobacco, bean, cotton, pansy and lettuce (Nag Raj & Kendrick, 1975), in most cases the plants are not killed as a result of infection but yields and hence profits are greatly reduced due to the stunting.

For the cotton industry this is a relatively new problem, which was discovered in 1989 (Allen, 1990). In those 15 years since its discovery the disease has spread to all of the major cotton growing valleys in NSW and several in Queensland, with 30% of all plants surveyed in NSW cotton fields showing signs of the disease (Nehl, Allen, Mondal, & Lonergan, 2004).

The defining characteristic of this species is the production of thick walled chlamydospore chains (Figure 1) which can survive long periods of dormancy in soil. This ability to remain dormant in the soil allows the spores to survive transport in any soil movements and has aided widespread distribution such as in California where the disease was found as likely to be present in cultivated as uncultivated sites (Yarwood, 1981).

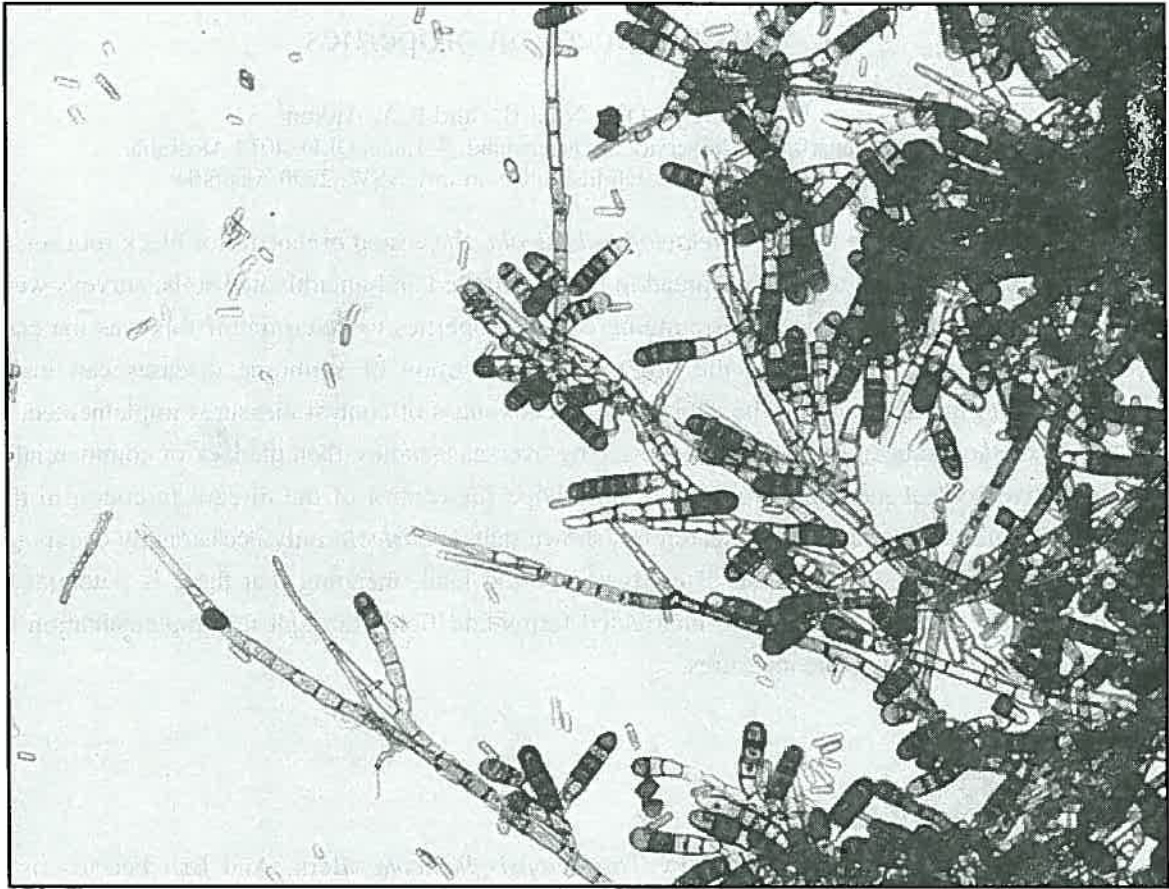


Figure 1: *Thielaviopsis basicola* showing both small endoconidia and larger chlamydospores

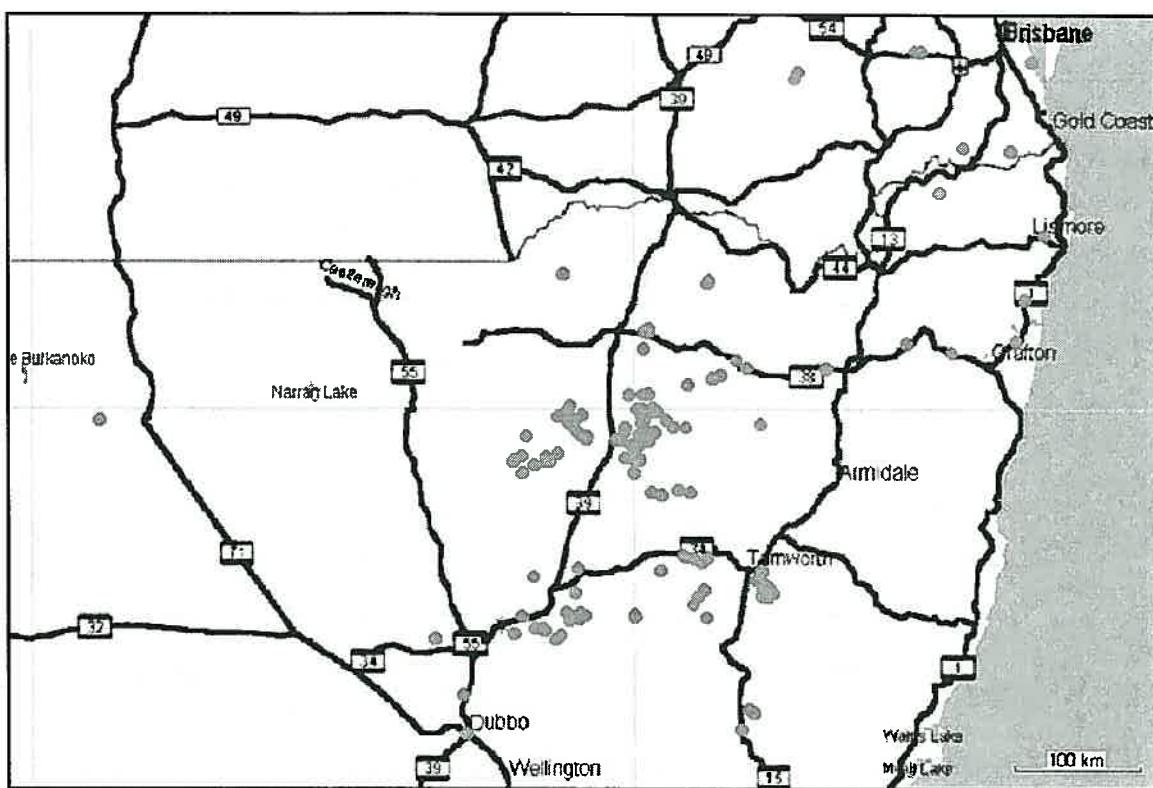
Sampling/Detection

Soil samples were collected from 190 sites, particularly concentrating on sites in the upper catchments of cotton growing valleys (Figure 2). The sites were selected to cover a range of vegetation types ranging from highly disturbed areas (such as roadsides) to low disturbance areas (such as native forest and grazing land). An additional focus of the sampling was to target sites near native cotton species, particularly *Gossypium sturtianum* var. *nandewarensense*, which is a known host for *Thielaviopsis basicola*.

Sampling and assessment techniques were based from previous work conducted by Yarwood (1981). At each site three soil samples were taken which were then bulked to provide a single aggregate sample, which was air-dried before assessment. Petri dishes containing five carrot disks on pre-moistened filter paper were inoculated with soil suspension (15 plates per sample) and incubated for 10 days before visual examination for the presence of chlamydospores was carried out.

Of the 200 sites sampled only two were confirmed to have *T. basicola* present at detectable levels. Both of the positive samples were taken from roadside sites that were highly disturbed and received runoff from the road surface. All undisturbed sites, including the samples near native cotton plants did not have any detectable levels of the pathogen. This low

presence in non-cultivated land is very different to overseas distribution studies, and suggests



that *T. basicola* is not endemic to cotton growing regions of NSW.

Figure 2: Map of showing sites where soil samples were taken to survey for presence of *T. basicola* in the catchments surrounding cotton properties.

Genetic diversity

Once pure cultures were established from the carrot disk baits, DNA was extracted and a variety of molecular techniques employed to estimate genetic similarity. A reference collection of *T. basicola* isolates from known sources was used to place the genetic similarity of the two new roadside isolates (JHB52 and JHB73) in context with existing culture collections.

Two molecular marker technologies were employed; Randomly Amplified Polymorphic DNA (RAPD) fingerprinting and intergenic spacer (IGS) sequencing. Primarily the RAPDs were used to generate a cluster diagram of the relative similarity of the new isolates to the reference collection (Figure 3), while the IGS data was used to confirm this cluster analysis (data not shown).

The new isolates were found to cluster in a grouping separate to those isolates from cotton properties. This clustering shows that the roadside isolates are genetically distinct to those which cause the problem of BRR in the Australian cotton industry. Most importantly, based from previous work by other researchers in this field (Pattimore, 1999), and some initial testing as part of this project the roadside strains would be unlikely to be able to cause field BRR symptoms in cotton.

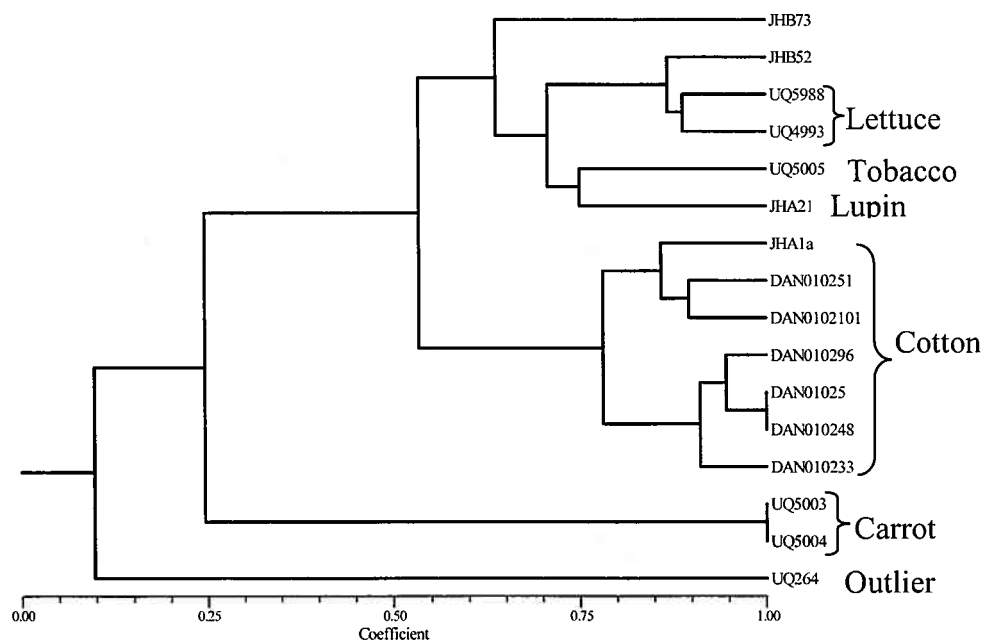


Figure 3 Genetic similarity of new isolates(JHB52 and JHB73) to reference collection from various hosts and one outlier.

Key findings and what this means

It is unlikely that BRR outbreaks are being sourced from non-cultivated land, this is based on the lack of *T. basicola* in the catchments surrounding cotton properties; combined with the genetic dissimilarity of the few strains that are present from ones that infect cotton. When compared to the work conducted in California (Yarwood, 1981) where the disease is widespread, this poses an obvious question; if the disease is not endemic where are new infections on Australian cotton properties coming from?

As a soilborne disease with resilient resting structures it is easy for *T. basicola* to move with adhering soil on vehicles, machinery and boots. This was shown in surveys of uncleaned vehicles visiting the Australian Cotton Research Institute (Nehl et al., 2004), where fungal spores were detected in samples from the vehicle underbodies. This movement of soil from infected properties is the most likely source of new infections on properties, making the best control measure the use of farm hygiene techniques to prevent the movement of soil onto farms, particularly from other cotton properties. This also applies to farms that do not have the disease present in all fields, by preventing the movement of soil to “clean” fields there is the opportunity to prevent further spread and hence further income loss.

Acknowledgements

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