Bio-control Agents for Managing Black Root Rot in Australian Cotton

By

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Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help in preparing this thesis and all sources used have been duly acknowledged.

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Warmest regards

Jason Moulynox

Abstract

Cotton production is an important rural industry in Australia. Disease pressure from emerging pathogens such as *Thielaviopsis basicola* threatens to reduce production. Wide-spread application of Best Management Practices, a desire to have a reduced environmental impact and community demand for more sustainable produce means alternatives methods to treat disease are becoming more important. This study has investigated the use of three disease reduction strategies; soil bacteria, organic soil amendments and plant derived proteins to treat black root rot in Australian cotton.

Strategy 1: *Pantoea agglommerans*, *Exiguobacterium acetylicum* and *Microbacterium* sp. (PEM) have previously been shown to inhibit infection of wheat by *Rhizoctonia solani* AG8 in soil tests. *Azospirillum brasilense* has been shown to promote growth and suppress disease in a range of crops. In invitro directional growth tests, the PEM species suppress growth of *T. basicola* in the presence of cotton plants. Although the plant was shown to be a factor in the suppression, the effect was not due to an active host plant response and was still present when a cotton root extract was used as a fungal attractant instead of intact plants. PEM and *A. brasilense* were also found to reduce disease symptoms in pot trials but not in the field. Further study to determine any effect on yield is recommended.

Strategy 2: Various soil amendments have been used to suppress fungal pathogens. Three readily available soil amendments, yeast extract, zein and soy protein were tested in a pot trial to determine their ability to reduce survival of *Thielaviopsis basicola* spores. Disease symptoms in cotton seedlings planted in soil infested with *T. basicola* spores and amended with zein or soy protein three weeks prior to planting were not significantly reduced. Yeast extract reduced disease symptoms to zero when soil was sterilised prior to addition of *T. basicola* endoconidia and amendment, but not when live soil was used. None of these amendments are recommended for application in field trials.

Strategy 3: Rs-AFP2 is a 5kDa defensin protein naturally found in radish seeds and shown to suppress a range of plant pathogenic fungi. Invitro testing in this study implicated that the heat stable Rs-AFP2 protein has a role in suppressing *T. basicola*. To confirm the role of Rs-AFP2, the gene encoding Rs-AFP2 was cloned into a commercial strain of the yeast *Kluyveromyces lactis*. Growth inhibitions assays confirmed that Rs-AFP2 inhibits *T. basicola* in-vitro, however *K. lactis* was determined to be an unsuitable host strain for production of this protein. Further work on this protein is recommended using either *Saccharomyces cerevisae* or a glucosylceramide-negative *K. lactis* mutant.

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