Reducing the impact of the parasitic root lesion nematode on cereal crops

Summary
This project was a collaboration between the Bio-Protection Research Centre, Charles Sturt University (CSU) and SA Research and Development Institute (SARDI) to develop a bionematicide for root lesion nematodes (RLN) on cereals. Aims of the project included:
1. Develop a Trichoderma-based bionematicide for Pratylenchus thornei.
2. Identification and evaluation of existing commercial biopesticides with potential suitability for this crop/pathogen system.
3. Identification of indigenous strains of selected microbe groups that may have potential as bionematicides.
4. Development of seed coating formulations to adhere biopesticides to cereal seeds.
5. Field testing of formulated promising microbes against P. thornei.

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Conclusions
Nematodes are notoriously difficult to control in crops for a variety of reasons. Their rapid multiplication rates and subterranean habitat make them difficult to control by either chemical or biological means. This is exacerbated in the case of RLN, cyst and root-knot nematodes by the nematodes' tendency to enter the plant. While in some cases plant resistance or tolerance has alleviated problems with certain nematode species, the wide range of plant parasitic nematodes often means new species rise in pest status as plant resistance controls the original species.

In the current research program, several hundred microbial isolates were screened for their ability to control RLN in wheat. On many occasions, individual isolates appeared to perform well but this promising activity was not reproducible and none of the isolates tested was considered worthy of commercialisation. It should be noted that this variability also occurred with two commercial isolates that were also tested; particularly with Bacillus firmus (sold as Votivo®, usually in combination with the insecticide Poncho®). This inconsistency of efficacy of B. firmus against nematodes is also reflected in the peer reviewed literature.

Results achieved in this project, and references from the literature, suggest that microbial control of nematodes is possible, but efficacy is strongly influenced by as yet unknown biotic or abiotic factors. In the USA, millions of hectares are treated annually with B. firmus for nematode control in broadacre crops. It is likely this product will be introduced into other countries (including Australia) in the foreseeable future.

The success of using biological control against plant parasitic nematodes will most probably be increased by understanding the causes of variable efficacy rather than by screening more isolates.

Recommendations
If further research is to be undertaken, a program of research that aims to determine how biotic and abiotic factors may influence efficacy of biocontrol is recommended. This could comprise a series of hypothesis driven experiments using a small selection of the most promising isolates from the current program (including the isolate of T. spirale), and the two commercial products (the bacterium B. firmus and the fungus Purpureocillium lilacinus). Such a project could offer detailed insights into the ecology of biocontrol of below-ground organisms and potentially offer growers much more reliable biocontrol of nematode pests.

Outcomes
The main immediate benefit of the project has been building a strong collaborative research team and training a PhD student (Kylie Crampton). This has increased research capability in nematology, a field that has long been declining, and
strengthened capacity in soil microbiology, crop protection and field experimentation.

The project and its funders have received much publicity through conference presentations from all four of the collaborators on various aspects of the project. SARDI also publicised the project through grower awareness days.

These activities together have increased awareness of problems caused by *P. thornei* and highlighted the lack of effective options available to growers for nematode control.

The academic community has been alerted to the paucity of biocontrol options available to growers through one peer-reviewed journal publication (Wilson & Jackson, 2013). A manuscript describing another aspect of the project (formulations to coat microbes onto seeds) is currently under review for the Journal of Applied Microbiology.

While no organisms were identified early enough in the project to enable an approach to potential industrial investors, an isolate of *T. spirale* was shown to reproducibly reduce nematode infection in roots by up to 75%, which warrants further investigation.

**Achievements/Benefits**

The overall aim of the project was to develop a seed-applied biocontrol for RLN *P. thornei* in Australian cereal crops. The first step was a global desktop study to identify available biological control agents (BCAs) for nematode pests and evaluate their suitability for control of RLN in Australia. The conclusion of this desktop study was that the bacterium *B. firmus* and the fungus, *P. lilacinus*, both of which are marketed by Bayer CropScience, were worthy of testing for efficacy against RLN. Commercial strains of both organisms were obtained for use in bioassays and field experiments. The desktop study was comprehensive and of value to the global community of nematologists. A modified version of the study was published in the international peer-reviewed journal BioControl (Wilson & Jackson, 2013).

In addition to testing commercially available strains, the project also used two other sources of potential BCAs - the Lincoln University strain collection of *Trichoderma* spp. BCA and novel indigenous soil microbes identified in and isolated from three cereal cropping regions of Australia by Kylie Crampton, a PhD student at CSU.

Once selected, strains were used in two sets of complementary laboratory bioassays at Lincoln University and SARDI. Throughout the course of the project, more than 300 strains of microbes were screened and compared with the two commercial strains. All strains that performed well in individual bioassays were included in future assays to demonstrate reproducibility of protection. This work identified strains of *Trichoderma* spp., *Bacillus* spp., *Paecilomyces* spp. and *Verticillium* spp. that performed better than the commercial strains in at least some of the bioassays.

Strains that were effective in laboratory assays were also tested by SARDI in glasshouse assays in different cereal growing regions of Australia over two years. While in some field experiments, individual strains significantly enhanced plant growth, none consistently showed significant reductions in RLN populations in soil or plant roots.

Another aim of the project was to develop a robust formulation that would adhere individual BCA agents to cereal seeds and promote their survival during seed storage. Eight different potential seed-coating formulations were screened for their ability to adhere *T. atroviridae* spores to wheat seeds. The five best-performing formulations were then tested for their ability to support spore survival when seeds were stored at a range of temperatures and relative humidities. The most promising formulation was based on xanthan gum and was already the subject of an AgResearch patent. This formulation met the targeted specification of supporting 10 million colony-forming units (cfu)/seed with less than 1 log10 loss of viability after three months. A report on the excellent results obtained with this patented formulation has been accepted for publication in the international peer-reviewed journal, Journal of Applied Microbiology.

Because none of the isolated strains consistently reduced *P. thornei* under field conditions, the project team, in conjunction with GRDC staff, felt the data was not of sufficient value to justify an approach to commercial partners or secure any intellectual property (IP). However, a glasshouse trial conducted in early 2015 showed a strain of *T. spirale* caused a highly significant, 75% reduction in the number of *P. thornei* penetrating wheat roots. This strain also showed promising results in SARDI experiments.

The project has created a synergistic team comprising four groups: LU and AgResearch from New Zealand and CSU and SARDI from Australia. The team had regular video-conferences at approximately three monthly intervals and met face to face
annually at venues in Australia. The team has increased the skill capacity in crop protection and particularly in nematology by training a PhD student, Kylie Crampton, who intends to submit her PhD by March 2016. In her studentship, Kylie learned much about identifying microbes by morphological and molecular means, culturing nematodes and undertaking laboratory and field-based bioassay experiments.

Other research

There are numerous options to build on the current research project. One is to test the most promising isolates against other cereals diseases such as crown rot or other nematode pests such as cereal cyst nematode (CCN - *Heterodera avenae*). This research, while clearly having applied aims, would be predominantly fundamental in nature. Co-funding could be sought from a variety of sources including government agencies in Australia and New Zealand. Another possibility is to seek co-funding from Bayer CropScience who is the commercial producer of *B. firmus* and *P. lilacinus*. This company has a commercial interest in improving efficacy of its products and GRDC already has close ties with Bayer CropScience through the Herbicide Innovation Partnership. Syngenta is now selling *Pasteuria* products into the broadacre crops in the USA targeting soybean cyst nematode and may also be interested in potential Australian markets.

Intellectual property summary

No specific IP was identified.

Additional information
