FINAL REPORT

GRDC
Grains Research & Development Corporation

Biological Suppression of Root-lesion Nematodes in Grain-growing Soils

DAQ00164

Project Details

- **Project Code:** DAQ00164
- **Project Title:** Biological Suppression of Root-lesion Nematodes in Grain-growing Soils
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Summary

Root-lesion nematodes (RLNs) are found on 75% of grain farms in southern Queensland (QLD) and northern New South Wales (NSW) and are significant pests. This project confirmed that biological suppression of RLNs occurs in soils, examined what organisms are involved and how growers might enhance suppressiveness of soils. Field trials, and glasshouse and laboratory bioassays of soils from fields with contrasting management practices, showed suppressiveness is favoured with less tillage, more stubble and continuous intensive cropping, particularly in the top 15cm of soil. Through extensive surveys key organisms, *Pasteuria* bacteria, nematode-trapping fungi and predatory nematodes were isolated and identified as being present.

Conclusions

This project confirmed that suppressiveness to RLNs occurs in northern grain growing soils on a variety of sites and soil types. Research assays showed that the suppressiveness is biological and is both general (organic matter mediated) and specific in nature (specific organisms can be added to give a suppressive effect). Cropped soils in the northern region were just as suppressive as adjacent native or remnant scrub soils and having a pasture in the rotation did not necessarily improve suppressiveness.

Reducing or eliminating tillage and retaining stubble improved suppressiveness (especially in long term trials) and continuous or frequent cropping with resistant hosts, also reduce nematode multiplication, compared to just fallowing that ground.

A survey of soils from southern QLD and northern NSW showed about 25% of grain-growing soils with RLNs had *Pasteuria* sp. infected nematodes, but only 6% of the RLN population were infected. This bacteria has the potential to infest and kill RLNs, but populations appear too low to currently be having any great impact. Four different species of nematode-trapping fungi were found in 26 different soils from the region using traditional isolation techniques.

Collaboration with Dr Helen Hayden, Department of Environment and Primary Industries (DEPI) Victoria (VIC), led to the successful development of a method to detect these fungi, using DNA extraction from soil. More species within this group of fungi were able to be detected and subjectively quantified for a limited number of samples. With more development, this method could usefully detect the presence of these fungi and distinguish differences in their community structure, associated with different sites or management practices.

A fungal endophyte (*Fusariumnygamai*) isolated from a NSW wheat growing soil was found to reduce RLN multiplication by 40% compared to a control. This isolate, among others found, but not yet tested, could therefore have potential as a biological control agent, but more research would be required to confirm and develop this.

Recommendations

Assays on this soil confirmed that suppressiveness was greater in the top soil (0-15cm), where organic matter and soil biology levels are higher, however, it seems intensive cropping is more influential on soil suppressiveness, than addition of up to 20t/ha of organic matter (OM). Soil chemical and physical differences measured did not appear to influence suppressiveness significantly and fertiliser (nitrogen (N), phosphorous (P)) applications did not alter suppressiveness.

Growers using no-till, stubble retention practices and cropping, when soil moisture allows, are probably doing a great deal toward enhancing the suppressiveness in their top soil. Without these practices, it is estimated that RLN multiplication would be significantly greater, especially in top soils and therefore lead to much greater losses in productivity of susceptible crops. As RLN multiply right down to the soil profile, practical means of increasing soil biology, through improving carbon deposits, are needed to reduce multiplication further.

Research in this project has identified specific antagonists of the RLN (some for the first time) in northern grain growing soils. More targeted research on how to measure and enhance these organisms in soils is required.

Outcomes

This research has confirmed that suppressiveness can reduce RLN multiplication on wheat, particularly in surface soil layers. It has also identified many potential organisms that are present in these soils and capable of suppressing the pests.

Management practices such as reduced or no tillage (NT) and stubble retention all contribute to this suppressiveness of the nematode in soils, so growers employing these practices are aiding the biological capacity of soil to carry out key processes associated with suppressiveness. When used in conjunction with strategies of rotating hosts with resistant crops and using tolerant varieties, levels of RLNs should be reduced.

Due to the exponential nature of nematode multiplication, the lower the population of RLNs at the start of a crop, the lower the levels will be at the end of the season, thereby reducing numbers left in the soil, even when a susceptible crop, such as wheat is grown. Keeping populations below a damaging threshold level would be very beneficial. An economic assessment of this program's outcomes has been commissioned by GRDC.

Practices enhancing nematode suppression are likely to be in line with those considered generally softer on the environment, i.e. reduction in tillage and stubble retention,and those that improve soil carbon turnover. All these lead to more sustainable agricultural production Australia-wide.

Significant capacity and new knowledge, including discovery of potential biological control agents and method development (using both traditional manual and molecular techniques) have resulted from this project. A young scientist was trained in many aspects of microbiology and research relating to soil biology and crop management.

Achievement/Benefit

Root-lesion nematodes (*Pratylenchus thornei* and *Pratylenchus neglectus*) cost Australian growers in excess of $250 million per annum. Control of this pest relies on an integrated management program, which includes the use of tolerant or resistant varieties, crop rotation and good farm hygiene, but even then costs to production can be high. Enhancing the suppressiveness of soil to RLNs is a control option that deserves some consideration. Disease suppression is defined as the ability of a soil to suppress disease incidence or severity even in the presence of the pathogen, host plant and favourable environmental conditions.

The vast array of organisms in the soil can provide a degree of biological buffering against pathogens. Usually the level of suppressiveness in a soil will depend upon the combined effects of both general (organic matter -mediated) and specific (direct antagonism by a limited number of organisms) suppressiveness. This project aimed to better understand the suppressive nature of grain-growing soils and provide growers with methods to enhance suppressiveness of soils to RLNs.

Over four years, a total of 26 different paddocks trials involving more than 70 sampling sites were tested for suppressiveness. This included three long-term farm management trial sites where several fertiliser or tillage treatments were sampled. Also, seven of the sites were comparisons of cropped and pasture or native or scrub remnant soils that were in close vicinity, to gain an understanding of the impact cropping may have on suppressiveness to RLNs.

Repeated studies (2010-2014) of the different soils, through the use of glasshouse and laboratory bioassays, consistently showed general suppressiveness to RLNs does exist in a variety of soils. In the glasshouse assays, *P. thornei* increased only 2-5 times in unheated soil compared with 17 times when soil was heated prior to planting to eliminate the general soil biological community. Incorporating a small amount of unheated soil into heated soil (10% unheated) reduced multiplication rates by 60-89%, showing that specific organisms in those soils were also contributing to this suppression and that it was not a physical or chemical property of the soil per se affecting multiplication.

For 14 of the sites, the difference in suppressiveness, between soil from 0-15cm and the 30-45cm layers in the profile, was tested since *Pratylenchus* nematode populations tend to be highest to depths of 30-60cm. These tests showed that soil from 0-15cm is much more suppressive than soil from 30-45cm. Soil characteristics and crop and management histories were also analysed to understand the mechanisms driving this change. To date, Partial Least Squares Regression Analyses have not shown any specific correlation between particular soil chemical (macro and micro-nutrient levels) and physical (pH, texture, clay content) attributes and suppressiveness.

Over 130 soils from the northern region were surveyed for the presence of natural enemies (*Pasteuria* bacteria, nematode-trapping fungi and predatory nematodes) to RLNs. Glasshouse trials to increase populations of *Pasteuria* on hosts of *P. thornei* were conducted, but multiplication of the bacteria has been slow to negligible using traditional methods. More study is required to better understand the lifecycle and ecology of this bacterial parasite. Very little is known globally about this organism and no known research on the presence of *Pasteuria* on *P. thornei* has been conducted in Australia before this project. Collaboration with Dr Hayden (DEPI Victoria) on molecular methods of detection and quantification of *Pasteuria thornei* in soil indicated, that published primers showed mixed results and more research is required to refine this methodology. This would, however, be extremely useful as traditional methods of detection are extremely labour intensive and difficult. Also, traditional methods of isolating nematode trapping fungi (NTF) are being compared with new molecular methods being developed in collaboration with Dr Hayden. This work successfully showed NTF could be detected in soils using DNA extraction and terminal restriction fragment length polymorphism (TRFLP) techniques. Subjective quantification of levels in soil was also possible. Levels of these fungi were much higher in top soil layers, which correlates nicely with the findings of suppressiveness assays and indicates these organisms may be having some impact on RLN multiplication.

Preliminary studies to examine the influence of root endophytes on the multiplication of RLNs have been conducted. One exciting outcome came from a wheat pot assay, where the addition of a fungal endophyte (*Fusariumnygamai*) previously isolated from wheat roots from a property near North Star, NSW, significantly reduced *P. thornei* populations by approximately 40% compared to the control. More studies are required to confirm this as a potential biocontrol organism. Several other fungi were isolated from roots, but are yet to be tested for suppressive activity or be formally identified. The presence of arbuscular-mycorrhizal fungi (AMF) did not appear to reduce RLN multiplication. In fact, a pot experiment showed that AMF could increase RLN numbers most likely due to better plant and hence root growth of the mycorrhizal compared to the non-mycorrhizal unfertilised wheat.

Key farm practices that may enhance suppressiveness to RLNs were examined in this project. Assays showed little impact of factors such as, pastures in rotation on suppressiveness of the soil. In fact, cropped soils were generally just as suppressive as nearby native grassed or scrub areas. However, within cropped soils, frequent tillage did seem to have a significant impact on nematode multiplication with soils where no-till or stubble retained practices are employed, being more suppressive. A field trial at Hermitage Research Station, Warwick, was designed to study the impact of organic amendments (0, 5, 10 and 20t organic matter/ha) and various cropping regimes (continuous fallow, sorghum with residue retained, sorghum with no residue retained) on *P. thornei* multiplication in wheat grown after these treatments. While the organic amendments applied at 20t/ha improved soil biology (diversity and richness) and suppressiveness after 12 months, this effect was not obvious after 24 months and these treatments had no subsequent impact on *P. thornei* multiplication or yield of the susceptible wheat variety Strzelecki[A]. However, *P. thornei* multiplication on a host crop (wheat in 2013) was significantly less following two years of continuous cropping with resistant hosts (barley (2011), sorghum (2011/12), sorghum (2012/13)) when stubble was retained compared to two years of fallow or two years of the same crops, with stubble removed. This correlated with higher levels of free-living nematodes (indicating improved soil biology). Across all treatments, *P. thornei* multiplication was always lowest in the top 0-15cm compared to that in other soil intervals down to 60cm. Suppressiveness assays on this soil confirmed these findings of suppressiveness being greater in the top soil. However, it seems intensive cropping is more influential on soil suppressiveness than the addition of up to 20t/ha of organic matter. Soil chemical and physical differences measured did not appear to significantly influence suppressiveness.

Growers practising no-till, stubble retention practices and cropping, when soil moisture allows, are probably doing the most they can to enhance suppressiveness in the top soil. Without these practices though, it is estimated that RLN multiplication would be significantly greater especially in top soils and therefore lead to much greater losses in productivity of susceptible crops. As RLN multiply right down to the soil profile, practical means of increasing soil biology through improving carbon deposits are needed to reduce multiplication further. Research in this project has identified specific antagonists of the RLN (some for the first time) in northern grain growing soils. More targeted research on how to measure and enhance these organisms in these soils is required.