Soil-borne disease suppression to *Rhizoctonia solani* AG8 in agricultural soils from a semi-arid region in South Australia

This thesis submitted in fulfilment of the degree of Doctor of Philosophy, Soil and Land Systems, School of Agriculture, Food and Wine, The University of Adelaide.

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Abstract

Soil-borne suppression to *Rhizoctonia solani* AG8 in agricultural soils from a semi-arid region in southern Australia

Rhizoctonia solani AG8 is a significant soil-borne pathogen of cereal roots in semi-arid Mediterranean regions of Australia and the Pacific North West region in the United States of America, causing severe productivity and economic losses to farmers. During the past twenty years the conversion of many farming systems to conservation tillage has meant that the mycelial network of the pathogen is no longer seasonally disturbed by cultivation which has subsequently increased the potential for greater incidence of Rhizoctonia root rot. There has been some success in reducing incidence by using modifications to direct drill seeding equipment enabling some disturbance to Rhizoctonia at sowing. However, a long term sustainable solution with both economic and environmental benefit, as concluded from a review of the literature (Chapter 1) is to harness the potential for biological control of the disease via natural or induced suppression in soils.

Biological suppression to specific disease organisms in soil has been reported worldwide from a range of environments. Further, the development of biological soil-borne suppression to Rhizoctonia root rot has been described for one specific agricultural location (Avon) in South Australia following a decade of stubble retention together with higher than average nutrient inputs (Chapter 1). The studies in this thesis investigate soil-borne suppression to Rhizoctonia in agricultural soils from a semi-arid region of South Australia called Eyre Peninsula (EP) that produces 40% of the State's grain. The context is that historically in Eyre Peninsula farming systems crop residue inputs to soil are inherently low, as are fertiliser N and P inputs. However, recent intensification of these systems with the implementation of continuous cereals and minimum or zero tillage has resulted in greater inputs of stubbles and fertilisers. Rhizoctonia root disease is prevalent in the mainly coarse textured alkaline soils of the region, and the reduction in cultural control associated with adoption of reduced till systems has highlighted a need for alternative control measures.

In a broad context, the key question addressed in this thesis is whether the soil ecology to suppress Rhizoctonia is present or can develop in these soils from a region considered an

extremely harsh environment climatically as well as edaphically. Specific key questions will be addressed in the discussion section of each chapter. The thesis, through a series of controlled environment studies, examines abiotic-biotic interactions between the soil, the *Rhizoctonia solani* pathogen and wheat seedlings. The work assesses how the soil organisms involved in disease suppression (both the pathogen *Rhizoctonia solani* AG8 organism and other antagonists or competitors) are influenced by cereal stubbles and fertilizer inputs to the system.

Through a series of preliminary experiments (Chapter 3) the important variables of soil moisture and amount of pathogen inoculum (e.g. number of pathogen infected agar plugs) suitable for a bioassay method were standardised, and used throughout the rest of the work described in this thesis.

Two controlled environment bioassay experiments (Chapter 4) were undertaken surveying soils from six sites across the region differing in physico-chemical and biological properties to elucidate the influence of abiotic and biotic factors on plant-soil-pathogen interactions and the potential for suppression of Rhizoctonia. A comparison was made with soil from the long term study site in SA (Avon) reported to be suppressive to Rhizoctonia. Studies growing wheat seedlings in sterilised soils demonstrated that the soils assessed were intrinsically different in terms of the growth supported by the abiotic matrix. Greatest shoot and root dry weight was observed in the soil from a region outside the EP (i.e. Avon) and the least was in an EP soil with extremely high calcium carbonate content (e.g. Streaky Bay) – a clear example of plant-soil abiotic interaction. Avon soil was confirmed as suppressive to Rhizoctonia root rot since the Avon soil inoculated with its own biotic component reduced root infection to 50% from more than 70% in the sterilised abiotic control. Whereas, for plants in the two EP soils with low calcium carbonate root infection was similar in the sterilised abiotic matrix to that in the soils inoculated with their biotic component, suggesting they were not biologically suppressive. Further evidence of the suppressive capability of the biotic component of Avon soil was obtained where it was inoculated into the two EP soils with higher carbonate and reduced root infection in plants grown in these two soils, although not in the lower carbonate content abiotic matrix of Minnipa, another EP soil. Surprisingly, considering the hostile edaphic conditions, root infection was reduced in the high calcium carbonate soil inoculated with its own biotic component, suggesting it was suppressive but not to the same extent as Avon. It was hypothesised this was possibly related to the organic C content in that soil being similar to Avon and higher than the other two EP soils. Shifts in soil organism community structure were observed when plants were grown in sterilised soils inoculated with the biotic component from another soil (i.e. rhizosphere soil from plants grown in another non-sterile matrix). Overall this work suggested there was some biotic potential for suppression in EP soils but low organic C was likely to be a constraint. EP soils were not as suppressive as Avon and abiotic constraints were highly likely, for example, the high carbonate reducing availability of P due to chemical fixation.

A long term glasshouse study (Chapter 5) was undertaken to measure the effect of carbon addition to two EP soils, as stubble or young root residues, on the potential to suppress Rhizoctonia. Other measurements in this experiment were microbial biomass carbon and quantitative PCR for DNA of pathogen and other specific micro-organisms implicated as contributing to disease suppression. C input to EP soils suppressed Rhizoctonia infection in wheat seedlings (despite abiotic constraints). C input as young roots increased DNA of *Rhizoctonia solani* and beneficial soil organisms *Microbacterium* spp*.* and *Pantoea agglomerans*. C input as stubble increased the populations of the beneficial soil organism, *Trichoderma* spp.

A further bioassay experiment (Chapter 6) investigated the effect of N and P fertiliser inputs on plant growth and Rhizoctonia suppression in two EP soils. The bioassay further investigated the interaction of these fertiliser nutrients with added available C in these two EP soils, one of which was highly calcareous. There was a positive plant growth response to added ammonium–N in both soils but no effect on Rhizoctonia infection. Addition of fertilizer P to the highly calcareous soil increased shoot and root growth and also Rhizoctonia infection without compromising effects on plant growth. Addition of available C (sucrose) with P fertiliser in the highly calcareous soil markedly suppressed Rhizoctonia infection.

Two final experiments focussed on measuring the changes in pathogen and other microbial communities in response to inputs of fertiliser and C in a highly calcareous EP soil, since Rhizoctonia root rot impacts are considered a particularly big issue in this soil type. In the first experiment (Chapter 7) it was hypothesised that fertiliser P may affect suppression of Rhizoctonia root rot not only via increasing plant growth but also by altering microbial community composition. Results showed that virulence of *Rhizoctonia solani* was unaltered by P addition although pathogen DNA in soil and plant root infection increased. The effect of P fertiliser on plant growth compensated for the effect of P on increased pathogen population and root infection. Whilst fertiliser P increased microbial activity no shifts were detected in communities so the effects of P on soil organisms involved in suppression of Rhizoctonia root rot were not conclusive. However, in the last experiment (Chapter 8) there were measured shifts in populations of organisms resulting from addition of fertiliser P in conjunction with stubble. The known suppressive soil organisms *Pantoea agglomerans* and *Microbacterium* spp. increased whereas *Rhizoctonia solani* (DNA) remained constant and hence Rhizoctonia infection decreased.

In summary, some soils from the EP region of South Australia expressed a degree of suppression to Rhizoctonia root rot via their biotic component in pot culture experiments. Furthermore, some of the soils, although not necessarily the same ones, contained soil micro-organisms implicated by other studies in suppression of Rhizoctonia root rot. The biotic component from some of the EP soils, whilst not suppressive in the soil matrix it was extracted from did demonstrate the potential to suppress Rhizoctonia root rot when transferred into another soil matrix, indicating an abiotic constraint to suppression. It is postulated that important abiotic properties in these EP soils were calcium carbonate content, with organic carbon and to a lesser extent mineral N and P also important since these latter properties bridge the abiotic to biotic divide. Important biotic properties are likely to be microbial activity, microbial community structure and the population of the pathogen, *Rhizoctonia solani* AG8.

Results from this thesis work suggest that suppression to Rhizoctonia root rot can occur in EP soils despite abiotic and biotic constraints of limited C and P. Improvement and maintenance of a high suppressive capacity in soils in this semi-arid environment will require integrated agronomy aimed at maintaining a healthy crop using fertilisers, particularly P. Available carbon appears to be the most limiting constraint to microbe based biological disease suppression of Rhizoctonia root rot in these soils. Therefore it is essential that adequate available C is supplied via stubble input to develop and maintain a highly functioning soil biota.

Although these results highlight that disease suppression to Rhizoctonia root rot is indeed possible in the constrained soils of the EP, the time required to develop this suppressive capacity in a field situation remains to be investigated.

Thesis Overview Chart

Chapter1 Introduction and Literature Review

Soil-borne disease suppression has been reported as an important component for controlling Rhizoctonia root rot in sustainable semi-arid farming systems, there is a limited understanding of factors affecting its development and expression.

General hypothesis: Eyre Peninsula South Australia is a hostile climatic and edaphic environment so may pose biotic and abiotic constraints to the development of soil-borne suppression to Rhizoctonia root rot

Chapter 2 Materials & Methods

Chapter 3 Preliminary Experiments to standardise bioassay

Standardisation of soil moisture content and number of pathogen plugs for bioassay.

Chapter 4 Survey of six EP soils and comparison to suppressive soil Avon

Hypothesis: EP soils may not be suppressive due to abiotic and/or biotic constraints

- Some Biotic potential for suppression in EP soils but low organic C possibly a constraint and may impose C limitation
- EP soils not as suppressive as Avon - abiotic constraints highly likely, especially high carbonate causing less available P due to fixation

Chapter 5 Stubble or root C input to three EP soils, one highly calcareous

Hypothesis: Input of C to EP soils will suppress Rhizoctonia root rot

- C input to EP soils suppressed *Rhizoctonia* infection in wheat seedlings (despite abiotic constraints)
- C input as young roots increased DNA of *Rhizoctonia* and beneficial soil organisms *Microbacterium* spp. and *Pantoea agglomerans*.
- C input as stubble increased the beneficial soil organism (*Trichoderma* spp.)

Chapter 6 Fertiliser and available C input to 2 EP soils, one highly calcareous

Hypothesis: Removing nutrient limitations in EP soils will directly or indirectly suppress Rhizoctonia root rot, but it may require additional C

- Positive plant growth response to added ammonium –N in both soils
- Addition of P to highly calcareous soil increased shoot & root growth and also *Rhizoctonia* infection without compromising effects on plant growth.
- Addition of available C (sucrose) with P fertiliser in a highly calcareous soil markedly suppressed *Rhizoctonia* infection

Chapter 7 P fertiliser rates in a highly calcareous EP soil

Hypothesis: Fertiliser P affects suppression of Rhizoctonia by increasing plant growth and altering microbial community composition

- Virulence of *Rhizoctonia* unaltered by P addition although pathogen DNA in soil and plant root infection increased
- P effect on plant growth overrides P effect on increased pathogen and infection
- Fertiliser P increased microbial activity but no shifts detected in communities so effects of P on soil organisms involved in suppression of *Rhizoctonia* inconclusive

Chapter 8 Stubble and P fertiliser input to a highly calcareous EP soil

Hypothesis: P fertiliser plus a complex C source will increase suppressive organisms

- *Pantoea agglomerans* and *Microbacterium* spp. increased and *Rhizoctonia solani* DNA did not
- Rhizoctonia infection decreased

Chapter 9 General Discussion

Thesis studies suggest soil-borne disease suppression to *Rhizoctonia* can occur in EP soils despite abiotic and biotic constraints of limited C and P

Requires integrated agronomy which aims to maintain a healthy crop and soil biota using fertilisers, particularly P to ensure adequate available C via crop residue inputs

Declaration

I certify that this work contains no material which has been accepted for the award of another degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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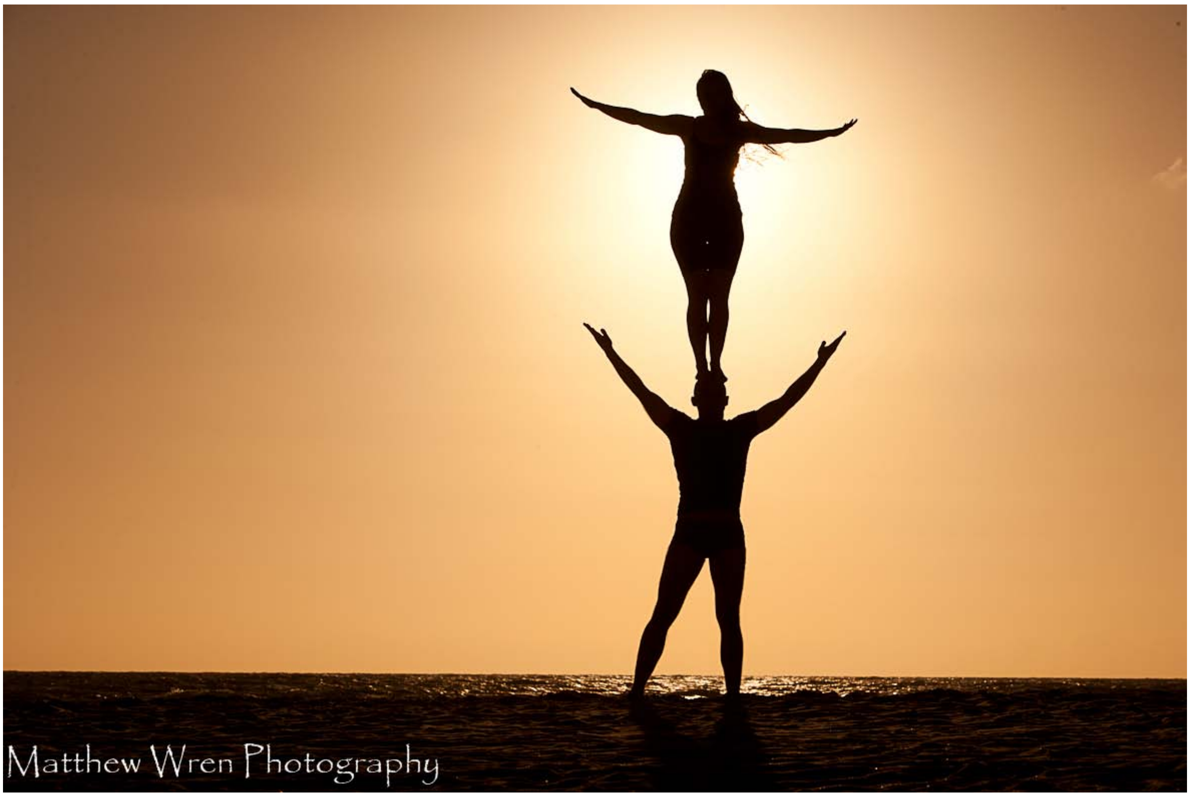

Standing on Chris' head, Glenelg, 2011. Photo taken by Matthew Wren.