# Rhizoctonia root rot suppression in an alkaline calcareous soil from a low rainfall farming system

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## Abstract
Biological suppression of soil-borne disease organisms occurs worldwide in soils from a range of environments, and is considered to occur where pathogenic organisms are present in the soil but do not cause measurable reductions in productivity of a crop due to disease. The development of soil-borne suppression to Rhizoctonia root rot over a ten year period with high inputs has been reported in a farming system trial at Avon in South Australia but the extent to which it occurs on farms in the Eyre Peninsula region in SA is not known. The work reported here investigated, under controlled environment conditions, the effect of fertiliser and stubble inputs on the potential for a highly calcareous soil from a paddock on upper Eyre Peninsula in SA to suppress Rhizoctonia root rot. The aim was to assess how the soil organisms involved in this suppression - both the disease organism *Rhizoctonia solani* -AG8 and antagonistic or competitive organisms, were influenced by inputs of N and P fertilisers and carbon, and ultimately how this affected plant growth and expression of rhizoctonia root rot in wheat seedlings.

**Key Words**
*Rhizoctonia solani*, soil-borne disease suppression, fertiliser, nitrogen, phosphorus, calcareous soil

## Introduction
Soil-borne diseases are one of the major constraints to cereal productivity in large areas of southern Australia causing economic losses to growers (Murray and Brennan 2009). The incidence and severity of Rhizoctonia bare patch caused by *R. solani* AG8, which is particularly prevalent in the mainly coarse textured alkaline soils on upper Eyre Peninsula (EP) in South Australia, depends on a wide range of abiotic and biotic factors. These include the physicochemical characteristics of the soil, amount of Rhizoctonia inoculum, community composition and activity of soil organisms and inputs of fertiliser and crop residues. On average, amounts of crop residues for EP farming systems are relatively 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 increased inputs and plant productivity, but has concurrently reduced options for cultural control of Rhizoctonia root root. One potential alternative control measure is biological soil-borne suppression to Rhizoctonia which occurs worldwide in a range of environments, and has been reported in a long term trial at Avon in South Australia following a decade of stubble retention together with higher than average nutrient inputs (Roget 1995). Appreciable levels of disease suppression exist in some EP soils (Davey et al. 2008). Also, labile C input can induce suppression in some EP soils (Davey et al 2010) which highlights that current inputs to these soils may be a limitation to the development of disease suppression. Since P deficiency, caused by fixation in soil due to high calcium carbonate contents, is common in the EP region, and fertiliser N inputs tend to be conservative, these inputs may also be limiting the development of disease suppression. The aim of the work reported here was to assess how disease suppression to Rhizoctonia root rot in a highly calcareous soil from EP in South Australia, was influenced by N and P fertiliser inputs, with or without addition of readily available carbon.

## Materials & Methods
*Bioassay to assess expression of soil-borne disease suppression*
A bioassay was used in all experiments to assess expression of soil borne disease suppression as described in Wiseman & Neate (1996). Briefly, soil in pots was inoculated with pathogen (agar plugs of *Rhizoctonia solani* AG-8) and incubated for 2 weeks. Wheat plants (5 per pot) were then grown in the soil from sterilised seeds for 4 weeks and soil maintained at 75% WHC. Shoots were cut off and roots extracted. Roots were visually scored for severity of Rhizoctonia root rot on a scale of 1(zero) to 5 (severe) as per MacDonald and Rovira (1983) and measured for % root infection using the method described by (Barnett et al 2006). Three experiments were undertaken using a highly calcareous soil from Streaky Bay. Experiment 1 tested the effect of addition of N (added as ammonium or nitrate at 100 mgN/kg soil) or P (at 50 mgP/kg soil) on plant

growth and soil-borne suppression of Rhizoctonia root rot. Experiment 2 measured changes in the *Rhizoctonia* pathogen and particular organisms associated with soil-borne disease suppression in response to P added at a range of rates (5,25 &50 mgP/kg soil) to the highly calcareous soil. DNA for *R. solani* AG8 and a few specific disease suppressive organisms was quantified using real time PCR (Ophel-Keller et al. 2008) provided as a commercial service by SARDI. Microbial activity and catabolic diversity profiles were also generated using a modified carbon substrate utilization method (Campbell et al. 2003). Similar measurements were made in Experiment 3 to investigate whether addition of an agronomic C input, wheat stubble at 2.8 or 5.6 mgDM /kg soil, altered the effects of P fertiliser addition on soil organisms. Soil amended with stubble and control soils (unamended) were incubated moist at 15$^{O}$C for six weeks prior to the P additions and the bioassay.

**Results and Discussion**

*Experiment 1 – N & P fertiliser effects on plant growth and rhizoctonia infection on roots*

There was no significant plant growth response to addition of N as either nitrate or ammonium when compared to the nil fertiliser treatment (Fig 1b). Addition of nitrate-N increased root infection caused by *Rhizoctonia* (Fig 1a) whereas ammonium-N did not, and possibly this contributed to the dry weight of plants fertilised with ammonium-N being greater than those fertilised with nitrate-N (Fig. 1).

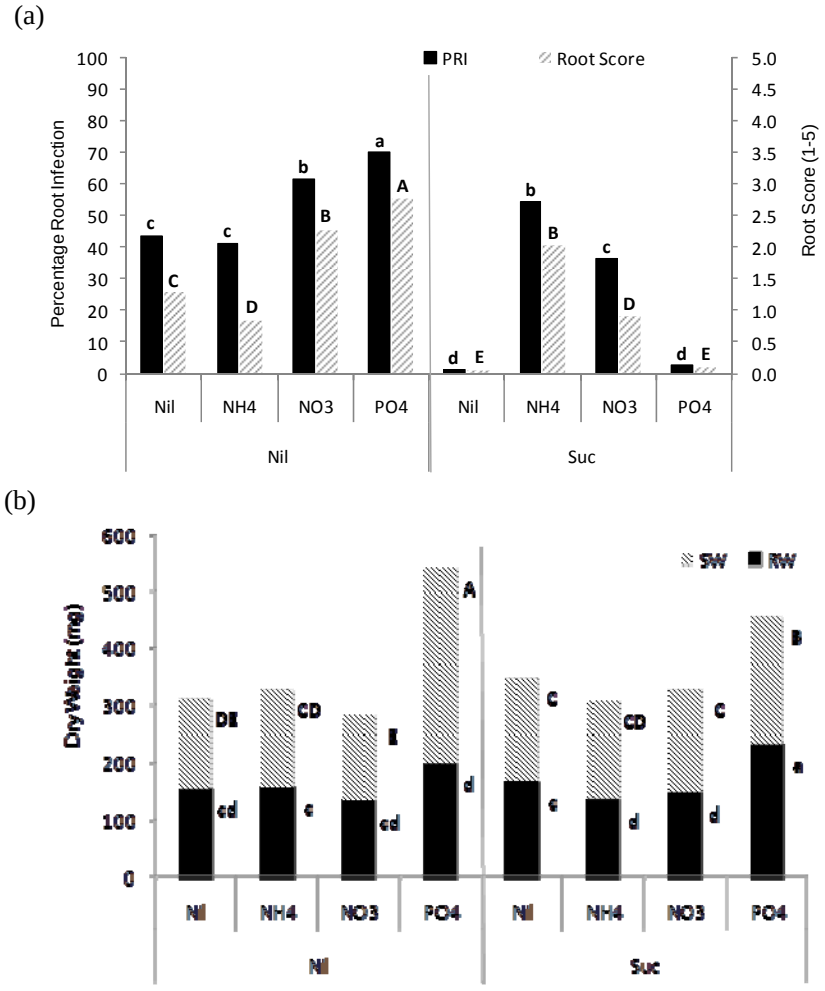


**Figure 1: (a) Percent root infection & root score and (b) root & shoot dry weights of 28 day old wheat plants grown in a highly calcareous soil with no additional nutrients (Nil), with N or P added (NH4, NO3 , PO4) and with or without addition of sucrose at 1% soil dry weight. All treatments were inoculated with the pathogen *Rhizoctonia solani* AG8. Different letters indicate significant differences for the lsd of treatment means at P=0.05.**

Addition of P caused a marked increase in shoot and root growth (Fig.1) despite also increasing root infection. Whereas, addition of available C (sucrose) with P fertiliser markedly suppressed root infection,

highlighting that C availability probably prevents this soil reaching full potential for disease suppression. The qualitative visual scoring of root disease symptoms generally resulted in comparable rankings of the treatment effects on root disease expression as those obtained using the quantitative technique of measuring percent root infection (Fig. 1).

*Experiment 2- P fertiliser effect on DNA for rhizoctonia and suppressive organisms*
Amount of *Rhizoctonia solani* AG8 DNA was significantly lower in the soil without added P and it increased with increasing P rate, as did the DNA of the known suppressive organism *Microbacterium* sp. (Table 1). However, DNA for other beneficial organisms *Pantoea* sp and *Trichoderma* group A remained constant across treatments, and *Trichoderma* group B was absent (Table 1). Microbial activity as measured from carbon utilisation profiles (data not shown) also significantly increased with increasing fertiliser P application.

**Table 1: Amounts of DNA ($log_{(10)}$ pg DNA per g soil) for specific beneficial organisms and *Rhizoctonia solani* AG8 in soil isolated from bioassay pots after 4 weeks growth of wheat plants. Soils inoculated with *R. solani* AG8 and with varying rates of P application (0, 5, 25 and 50 mg P / kg soil).**

| mg P added per kg soil | Pot | 0 | 5 | 25 | 50 | l.s.d$_{(0.05)}$ |
|---|---|---|---|---|---|---|
| | | | $log_{(10)}$ pg DNA per g soil | | | |
| Pantoea | | 0.80 | 0.76 | 0.95 | 0.80 | ns |
| *Microbacterium* | | 4.24 d | 4.34 c | 4.50 b | 4.63 a | 0.07 |
| *Trichoderma* group A | | 0.69 | 0 | 0.36 | 0.72 | |
| *Trichoderma* group B | | 0 | 0 | 0 | 0 | |
| *R. solani* AG8 DNA g soil $_{(4weeks)}$ | | 3.6 c | 3.7 c | 3.9 b | 4.1 a | 0.09 |

**Different letters show significant differences between treatment means at P= 0.05**

The positive effect of P fertiliser on plant growth in this highly calcareous soil seen in experiment 1 was confirmed and this appeared to compensate for the fact that P addition increased root infection. Hence the reduction in plant dry weight for diseased plants (compared to healthy controls) per % root infection was less at the higher rate of P (Fig. 2).

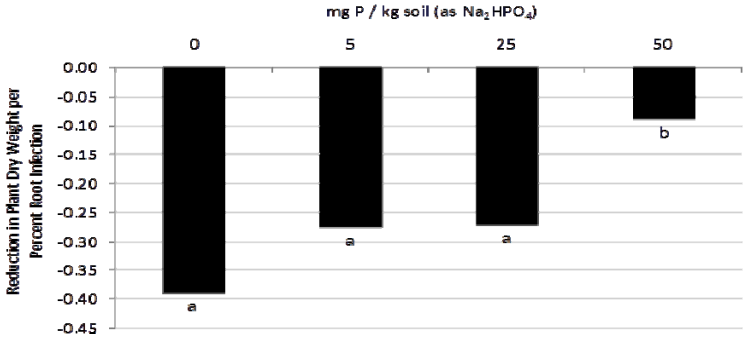


**Figure 2: Reduction in plant dry weight (Inoculated pots –healthy controls) per percent root infection for pots inoculated with Rhizoctonia solani. Different letters indicate significant differences at l.s.d.=0.05.**

*Experiment 3*
Similar to Experiment 2 the addition of P to the highly calcareous soil significantly increased *Pantoea agglomerans* and *Microbacterium sp*. Further, the effect was greater where stubble was added, with the greatest increase in the DNA of these organisms observed at the higher stubble addition (data not shown). Stubble addition alone did not increase *Rhizoctonia solani* AG8 DNA. Overall, the total effect of P addition to stubble amended soil was less Rhizoctonia infection on wheat seedling roots compared to that in soil without P and/or stubble addition.
The carbon source utilisation profile provided further indication that changes in the microbial community structure with P addition were more noticeable when stubble was also added. There was a strong similarity in the profiles for P amended soils that had not had any stubble added (see within the black ring in Figure 3). Whereas in soil amended with stubble at 2.8 g/kg there was a noticeable shift away from the profiles for soil without stubble, and also the 25P treatment shifted in a different direction to the 50P treatment (indicated by the pink and grey rings in Figure 3).

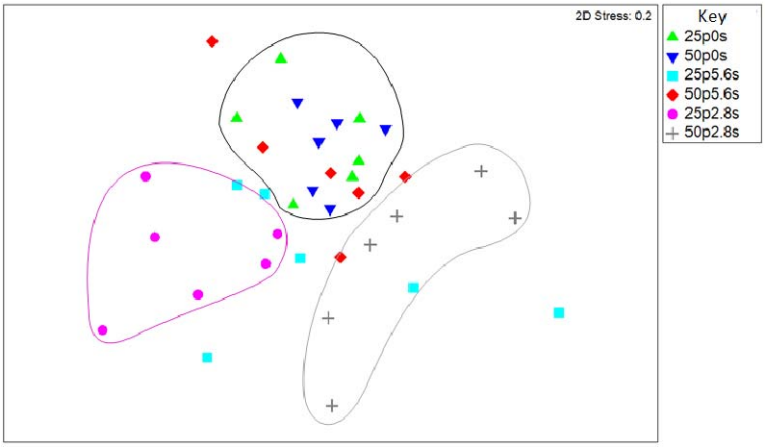


**Figure 3: Multi-dimensional scaling plot based on bray Curtis similarity for log(x+1) transformed carbon source utilization profiles after 6 weeks incubation with stubble (0, 2.8, 5.6 g/kg) and 2 weeks incubation with 25 and 50 mg P/kg soil. All treatments were inoculated with *Rhizoctonia solani* AG8 inoculum.**

## Conclusion

Inherent soil constraints on plant growth and the activity of suppressive soil organism, caused by low available C and low P availability, appear to be limiting suppression of Rhizoctonia root rot in this highly calcareous soil from upper Eyre Peninsula. Important agronomic strategies to induce and maintain a high suppressive capacity in this soil type are: (a) using adequate rates of fertilisers, especially P, to promote vigorous crop growth and support development of suppressive organisms, and (b) retention of stubbles to increase available C in the system and support organisms that suppress Rhizoctonia whilst also minimising any potential increase in *Rhizoctonia sp.* caused by P fertiliser addition. However, the timeframe required for these management strategies to be effective in developing strong suppressive capacity in the highly calcareous soils of the farming systems of Eyre Peninsula remains to be investigated.

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