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Rhizosphere microbial communities associated with Rhizoctonia damage at the field and disease patch scale

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ABSTRACT

Rhizoctonia solani AG-8 is a major root pathogen in wheat (*Triticum aestivum* L.) systems worldwide and while natural disease suppression can develop under continuous cropping, this is not always the case. The main aim of our work was to elucidate the rhizosphere microbial community underlying a Rhizoctonia suppressive soil (Avon, South Australia) and to investigate how this community may develop in agricultural soils conducive to disease and of different soil type (Galong and Harden, New South Wales). The Avon suppressive soil community included *Asaia* spp. and *Paenibacillus borealis*, which were absent from a paired non-suppressive site. At Galong, soil taken from inside and outside disease patches showed no evidence of suppression, and disease suppression could not be transferred from the suppressive soil to the conducive soil from a different soil type and climatic area. 16S rRNA microarray analysis revealed *Pseudomonas* spp. were significantly more abundant inside than outside three disease patches at Galong. However, a survey of 32 patches across a range of stubble and tillage treatments at a nearby site showed no correlation between *Pseudomonas* and disease incidence. *R. solani* levels were significantly lower when stubble was retained rather than burnt or when nutrients (N, P and S) were incorporated with stubble during the non-crop period. Our results suggest soil type is an important factor for suppressive capability and that where specific disease suppression is absent, agronomic practice to increase soil carbon can encourage a non-specific microbial response that limits disease severity.

1. Introduction

Suppression of root pathogens by native soil biota in agro-ecosystems can provide a solution to crop diseases that are difficult to control (Almario et al., 2013a; Weller, 1988). The development of biological disease suppression in soil supporting monocultures or continuous cropping is a widespread natural phenomenon yet the microbial mechanisms are often poorly understood (Berendsen et al., 2012). A goal in learning more about the rhizosphere microbial community associated with suppressive and conducive soils is to adapt crop management to encourage development of more suppressive soil communities to reduce the impact of soil-borne diseases (Stone et al., 2004).

Rhizoctonia solani AG-8 is a major soil-borne root fungal disease in agricultural systems worldwide and has a wide host range, making it difficult to control through crop rotation. During fallow periods *R. solani* AG-8 survives as a saprophyte in the soil and infects roots of young seedlings, resulting in "bare patches" of dead or severely infected plants, especially in cereal crops (hereafter Rhizoctonia disease). Disease control has traditionally been through tillage, which breaks up the fungal hyphal network in the soil and controls volunteer plants which can act as a host-bridge (Roget et al., 1987). However, adoption of no-till farming as a soil conservation strategy (Llewellyn et al., 2012) has led to increased disease incidence and severity (Macnish, 1985b). Some wheat-growing soils in Australia (Macnish, 1988; Gupta et al., 2009; Roget, 1995) and in the Pacific north-west USA (Paulitz et al., 2012) have been identified as suppressive to Rhizoctonia root rot, a phenomenon in

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which the pathogen, though present, does not cause significant disease. However, at other experimental sites under extended cereal monoculture, such disease suppression has failed to develop. Under controlled conditions, suppression may develop in the presence of a susceptible host and a virulent pathogen (Lucas et al., 1993) but in the field, development of suppression is also affected by abiotic factors such as moisture and nutrition. For example, Mavrodi et al. (2012) demonstrated the inverse relationship between rainfall and rhizosphere colonization by bacteria capable of producing the secondary metabolite phenazine, which has been linked to reduction in soil-borne diseases.

At Avon, South Australia, Rhizoctonia damage increased in severity over 3–5 years of continuous cropping as pathogen inoculum levels increased, followed by a decline in disease symptoms under both conventional tillage and direct-drill, despite the continued presence of the pathogen (Roget, 1995). Following the decline, disease incidence remained low throughout 6 years of continuous cropping (Gupta et al., 2011). Wiseman et al. (1996) revealed the biological basis of disease suppression at Avon by mixing 10% soil from the suppressive and conducive soils with autoclaved field soil, inoculated with *R. solani*. Culturing bacteria from the suppressive Avon soil, Barnett et al. (2006) reported the synergistic effect of *Pantoea agglomerans*, *Exiguobacterium acetylicum* and Microbacteria in controlling disease. However, Gupta et al. (2011) suggested that the suppression at Avon is a function of composition and activity of a diverse microbial community (including bacteria, fungi and protozoa).

In non-suppressive soils, Rhizoctonia bare patches commonly recur between years but can also affect new areas, grow, shrink or disappear from one year to the next (Macnish, 1985a). Whether irregular distribution is caused by suppression at the edge of patches is unknown. Anees et al. (2010) found that soil from outside patches caused by *R. solani* AG 2-2 was conducive to disease but soil from inside the patches was less conducive to disease, suggesting suppression requires disease outbreak and develops inside affected areas.

Due to low culturability of most soil bacteria, molecular approaches provide new opportunities to study the nature of suppressive microbial communities. Sanguin et al. (2009) developed a 16S rRNA microarray to investigate bacteria associated with take-all decline (TAD) in wheat, revealing communities that were characteristic of highly diseased or suppressive stages. This array has also been applied to TAD in barley (Schreiner et al., 2010) and tobacco black root rot (Kyselková et al., 2009). Both studies found pathogen inoculation of conducive soils had little effect on microbial community structure in the rhizosphere. In contrast, Schreiner et al. (2010) found shifts in community structure with successive plantings and Kyselková et al. (2009) demonstrated clear community differences between soils known to be suppressive and conducive. This approach was later applied to Rhizoctonia disease of sugarbeet but was restricted to soil from a single field and growth chamber model systems (Mendes et al., 2011). Such efforts have been limited in the analysis of field-relevant issues such as soil type and farming management, despite the importance of Rhizoctonia as a disease of modern conservation cropping systems worldwide.

We investigated the rhizosphere community of a suppressive soil compared to a non-suppressive soil, and the association between rhizobacterial communities and Rhizoctonia disease incidence at the patch scale. The following hypotheses were tested: (i) rhizobacterial communities in soils taken from paired suppressive and non-suppressive fields differ; (ii) soils in Rhizoctonia bare patches have a rhizobacterial community distinct from neighboring soils supporting healthy plants; (iii) biological suppression can be transferred to a non-suppressive soil in a controlled environment, even if soil type differs, and this leads to changes in the rhizobacterial community; (iv) at a non-suppressive site, soil taken from inside disease patches is more suppressive than soil outside of patches; and (v) Rhizoctonia control by microbial communities is modulated by farming practices.

2. Materials and methods

2.1. Soils and sites

We sampled from three field sites with a history of Rhizoctonia (Table 1). The suppressive nature of the South Australian Avon soil towards *R. solani* was first described by Roget (1995). The site has been under direct drill continuous cereal for over 25 years. Non-suppressive soil was collected from an adjacent field that had been continuously cropped for at least 3 years and was under direct drill wheat. No disease patches were observed at the suppressive site at the time of sampling and at the non-suppressive site there were greater than 20 patches per 250 m^2 area. Disease ratings on plant roots (score 0–5) were <0.2 in the suppressive soil and 3.5 (diseased areas) in the non-suppressive field. Controlled environment soil transfer assays, indicated that the addition of soil from the suppressive field to the non-suppressive field soil significantly reduced disease incidence by up by 65% (Penton et al., 2014).

Two sites of similar soil type within 20 km in New South Wales were located at Galong and Harden. Galong was selected because of ongoing Rhizoctonia problems and clear evidence of disease incidence, which enabled collection of a large volume of soil for use in a pot experiment. Samples were taken in 2010 from inside and outside disease patches in direct drilled wheat. Harden is a long-term experimental site, which is not suppressive despite similar duration of cultivation and management as Avon. It has been in continuous cropping with a break-crop wheat sequence since 1990, but immediately prior to sampling wheat in 2010 the sequence was: C–W–W–W–C–W (where C: Canola and W: Wheat). Rhizoctonia has been monitored for over 20 years at the site after the disease was prevalent under no-till treatment in the establishment phase (Kirkegaard et al., 1995). No evidence for disease suppression has been found despite long-term no-till treatments, and soil transfer assays on these soils have shown very low disease suppressive potential (Gupta VVSR, unpublished). The field site contained four replicate blocks of plots under different tillage, stubble and nutrient input management regimes. A large number of patches were visible early in the 2012 season, enabling us to test our findings derived from analysis of a smaller number of patches at Galong.

2.2. Analysis of the rhizobacterial community defining the suppressive Avon soil

Soils were collected prior to sowing in 2010 across each of the suppressive and non-suppressive fields (a single field for each) and composited to provide a single sample representative of each field. Soils were air-dried.

To obtain rhizosphere samples, wheat seeds (var. Gregory) were surface sterilised by washing in 10% sodium hypochlorite, rinsed 12 times in sterile distilled water and pre-germinated on tissue paper overnight at 4 °C and then 24 h at room temperature. A single seedling was planted in 5 replicates of 50 ml tubes with a subsample of the suppressive or conducive soils wetted to 70% field capacity and grown for 2 weeks, watering to weight daily. At harvest, the roots were shaken lightly to remove bulk soil and each seedling individually transferred to 50 mL 0.02 mM $CaCl_2$. Roots and adhered soil were vortexed 3 × 30 s, the roots removed, the remaining soil suspension centrifuged at 5000 × *g* for 30 min and the supernatant decanted. The rhizosphere soil was frozen at −80 °C immediately, then freeze dried and stored at −20 °C.