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PII: S1049-9644(18)30732-1
DOI: https://doi.org/10.1016/j.biocontrol.2019.02.019
Reference: YBCON 3941

To appear in: *Biological Control*

Received Date: 29 October 2018
Revised Date: 22 February 2019
Accepted Date: 25 February 2019

Please cite this article as: Barnett, S.J., Ballard, R.A., Franco, C.M.M., Field Assessment of Microbial Inoculants to Control Rhizoctonia Root Rot on Wheat, *Biological Control* (2019), doi: https://doi.org/10.1016/j.biocontrol.2019.02.019

Field Assessment of Microbial Inoculants to Control Rhizoctonia Root Rot on Wheat.

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One sentence summary: Microbial strains applied as seed coatings were assessed in the field for control of Rhizoctonia root rot on wheat.

Key words: Biocontrol, *Streptomyces*, *Paenibacillus*, *Rhizoctonia*, wheat, field trials

Running title: Microbes for Rhizoctonia control on wheat

Abstract

Rhizoctonia root rot caused by *Rhizoctonia solani* AG8 is a major disease in dryland cereal crops. Previous research identified a suite of microbes using *in planta* bioassay screening that are effective as seed-coated inoculants for control of Rhizoctonia root rot on wheat. This paper assessed 23 strains in fields in Australia with a history of naturally occurring *R. solani* AG8. Due to the patchy nature of Rhizoctonia root rot in the field, a 2-phase split-plot field trial system was used to allow comparison for disease control efficacy in the same disease space. Seed applied strains were first assessed for their ability to reduce Rhizoctonia using 'microplots' which compare adjacent treated and untreated one metre rows. Up to 10% increases in plant growth and a 32% reduction in root disease was measured at eight weeks after sowing. Selected strains were then assessed in 20 m six row (3+3) split plots for their effects on early season wheat growth and root damage and for grain yield. A *Paenibacillus* and a *Streptomyces* strain were identified which were able to reduce root damage by 20% and 32% and increase grain yield by 4.2% and 2.8%, respectively, compared to untreated controls. The current best registered chemical control for Rhizoctonia root rot reduced root disease by 35% and increased yield by 3.0% in the same trial.

1. Introduction

The soilborne fungus *Rhizoctonia solani* Kühn is an important pathogen of many crops worldwide (Anees et al*.,* 2010). *R. solani* AG8 is the most economically important root disease in southern Australia's dryland cropping systems, causing an annual loss of up to $77 million in yield in wheat and barley (Murray and Brennan, 2009a; 2009b) and is also important in the Pacific northwest of USA (Jaaffar et al., 2016; Paulitz et al., 2002; Weller et al., 1986). *R. solani* causes root rot, reducing the ability of plants to access water and nutrients resulting in the stunting of seedlings (Paulitz et al., 2002). The severity of *Rhizoctonia* disease is uneven across the landscape, with areas of high disease levels forming distinctive "bare-patches", areas of substantially reduced plant growth up to several metres in diameter and can cover 20% of the crop area (Anees et al., 2010, Schillinger and Paulitz, 2006). Rhizoctonia is difficult to control because it has a wide host range (Cook et al., 2002a; Rovira, 1986) and no resistant cultivars are currently available to growers although synthetic

wheat lines are being developed (Mahoney et al., 2016; Okubara et al., 2009). New seed coated fungicides, Rancona® Dimension (Chemtura), EverGol® Prime (Bayer) and Vibrance® (Syngenta), and an in-furrow treatment, Uniform (Syngenta) have recently been registered for use in Australia (Almasudi et al., 2015; Bogacki et al., 2014; Hüberli et al., 2015), however, they still only provide partial control. Rhizoctonia root rot is also increased in direct-drill or minimal tillage and stubble retention farming systems and is a significant constraint to the uptake of these practices (Rovira, 1986; Pumphrey et al., 1987; Schroeder and Paulitz, 2006).

The development of *Rhizoctonia* root rot is influenced by other soil microorganisms, and examples of microbial disease suppression have been reported for cereals in Australia (Roget, 1995), USA (Schillinger and Paulitz, 2006) and in sugar beet (Mendes et al., 2011). Microbes have also been isolated and shown to be able to reduce Rhizoctonia disease on wheat (Barnett et al., 2006; Barnett et al., 2017; Broadbent et al., 1971; Dua and Sindhu, 2012; Mavrodi et al., 2012; Yin et al., 2013), and the potential for using biocontrol inoculants has been well documented (Berg, 2009; Dutta and Podile, 2010) with increasing social and economic drivers for the use of these agents (Bailey et al., 2010).

Previously, we selected microbes with potential to control Rhizoctonia root rot in a controlled environment room bioassay containing field soil from a Rhizoctonia infested site, wheat seedlings and an aggressive *R. solani* AG8 strain, with test microbes applied as seed coatings (Barnett et al., 2017). From an initial 2,310 strains assessed, 43 strains were better at reducing disease or increasing plant growth compared to our current best performing microbial strains for Rhizoctonia control. These strains were characterised for properties required for a commercial inoculant, e.g. survival on seeds and in storage, growth, stability, compatibility with agrochemicals, etc., with 23 strains selected for further evaluation in field trials.

Field trials are time consuming and expensive to run and need to be carried out in growers' fields which have the target disease problem so as to accurately reflect real life performance. An added problem is the patchy nature of Rhizoctonia induced disease (Anees et al., 2010; Schillinger and Paulitz, 2006) which results in high variability between replicate plots. Some of these problems can be addressed by (1) doing an initial assessment in short term small plot trials to provide information on disease control capability, (2) selecting fields sites with moderate to high levels of the target pathogen based on pathogen DNA analysis in areas known to be conducive to disease expression, and (3) using a split-plot design with paired microbial treated and untreated seeding rows next to each other so that the comparisons are valid as they are from samples taken from the same disease space.

This paper reports the field trial efficacy of 23 strains selected from our high throughput screening (Barnett et al., 2017) by first assessing strains in microplots, then larger scale field plots using a split-plot design in fields in South Australia with a history of naturally occurring *R. solani* AG8.

2. Materials and Methods

2.1. Strains assessed in field trials and culture conditions

The 23 microbial strains assessed in field trials in 2012 to 2014 were selected using the high throughput screening pathosystem described in Barnett et al. (2017). Strain designation, genus, isolation source, location and year of field trial assessment is given in Table 1. *Streptomyces* strain EN16 which was reported to provide soilborne disease control (Coombs and Franco, 2003; Franco et al., 2007), was also included as a benchmark strain. Strains were stored as glycerol stocks at -80^{o}C. For use, bacteria and fungi were cultured on bakers' yeast agar (BYA) and actinobacteria on mannitol soy agar (Barnett et al., 2017). Bacteria were grown for four days and fungi and actinobacteria grown for seven to 14 days to ensure adequate sporulation. All cultures were grown at 27^{o}C in the dark.

2.2. Seed inoculation for field trials

Seed inoculation with strains is described in detail in Barnett et al. (2017). In brief, cells or spores were scraped from agar plates into ¼ strength phosphate buffered saline (PBS) to form a concentrated suspension, absorbance at 550 nm was measured and the suspension diluted to the required cell density in a xanthan gum-alginate sticker solution (3 g L^{-1} xanthan gum, Sigma; 0.5 g L^{-1} Na-alginate, Sigma). For inoculation of 1 kg of wheat seed, 10 ml inoculum suspension was added to 21 ml sticker solution plus 0.3 ml pillar box red food dye (Queens) and the suspension added to seed and mixed until uniform coverage was achieved as indicated by the dye. The cell density that was applied was based on the most effective concentration determined for each strain in previous pot bioassays (Barnett et al., 2017) and to be in the range suitable for commercial application (10^4 to 10^6 cfu $seed^{-1}$). Seeds were planted within one day of application. For untreated controls, xanthan gum-alginate sticker was applied to seeds without the addition of microbes. The final concentration of microbes on

seeds was determined the day after planting by placing five seeds in one ml PBS, shaking vigorously, preparing a dilution series and plating onto agar medium as described in Barnett et al. (2017), with two replicate extractions per treatment.

2.3. Location of trial sites

Trial sites were selected in areas with a history of Rhizoctonia infestation, confirmed by assessment of pre-sowing soilborne fungal pathogen levels, with *Rhizoctonia solani* AG8 levels greater than 100 pg DNA g^{-1} soil and other soilborne pathogens below detection limits or at low concentration. Pathogen DNA levels were assessed by taking 50 x ~10 g samples across prospective trial sites from the top 10 cm of the soil profile for analysis by the Root Disease Testing Service at the South Australian Research and Development Institute. This service is provided commercially as PreDictaB™ (http://www.pir.sa.gov.au/research/services/molecular_diagnostics/predicta_b) (Ophel-Keller et al., 2008; Poole et al., 2015). Trial site location, and pre-sowing *R. solani* AG8 levels and in season rainfall are given in Table 2. All soils had a sandy loam texture. The previous crop at all trial sites was wheat.

2.4 Trial planting details

Trials were cultivated or planted with a 6 row seeder with narrow points and tynes at 250 mm row centres, cultivating to a depth of 10 cm. Fertiliser was added as either liquid NP fertiliser or a mix of granular DAP plus liquid UAN, all deep banded at 10 cm. Wheat cv. Kord CL (2012, 2013) or Grenade CL (2014) (imidazolinone resistance, Australian Grain Technologies) were used in field trials. Seed placement for 3+3 trials was 3 cm depth at a rate of 73 kg ha^{-1} at Wynarka in 2013 or 70 kg ha^{-1} at Lameroo in 2013 and 2014. Strains assessed in each trial as seed coatings are given in Table 1.

2.5. Microplot trials

All treatments were sown as one metre rows, arranged in a split-plot randomised complete block design with 6 replicates. Microbial strains were assigned to the main plots and treated or untreated seed sown as paired rows comprising the sub-plots. Six row plots were cultivated as described above with fertiliser applied but without seeds. Seeds were pre-sorted prior to inoculation to remove oversized, small or damaged seeds. Seeds were hand planted using a seeding template pressed into the cultivated rows to give a four cm seed spacing and two cm deep holes to ensure consistent spacing and depth to eliminate as much

variation in plant growth as possible. Paired rows were used to ensure comparisons between treated and untreated plants were in the same disease space.

Plant growth and root disease were assessed at eight weeks after sowing. Ten plants were removed together with roots and adhering soil. Roots were washed free of soil and assessed for *Rhizoctonia* disease on seminal and crown roots using a 0 to 5 scale (Disease Score, DS, 0=no disease, 5=maximum disease where all roots were truncated close to crown and black, Rovira, 1986). Roots and shoots were separated and dried at 60^{o}C for four days and weighed. Percent change of plants from microbe treated seeds compared to untreated seeds is calculated by % change = [(treated/untreated) x 100]-100.

In 2012, ten microbial strains were assessed at Karoonda and Pt. Julia. Nine strains were common to both sites with the tenth strain differing between sites.

In 2013, 11 microbial strains were assessed at Wynarka and Lameroo and included for comparison were *Streptomyces* strain EN16, our previous best *Rhizoctonia* biocontrol agent, and two current seed coated fungicides registered in Australia for *Rhizoctonia* control on wheat (Bogacki et al., 2014; Hüberli et al., 2015). Fungicides were applied at the recommended label rate, EverGol® Prime 0.8 ml kg^{-1} seed (Bayer, active ingredients penflufen 240 g L-1), Vibrance® 3.6 ml kg^{-1} seed (Syngenta, active ingredients metalaxyl-M 16.5 g L^{-1} difenoconazole 66.2 g L^{-1}, sedaxane 13.8 g L^{-1}).

2.6. Field trial 20 m, 3+3 plots

The larger 20 m long six row plots were used to assess selected strains for early season growth and root disease and for grain yield. Six row plots were sown as three rows with microbial treated seeds and three rows untreated (3+3) to allow comparison of treatment versus untreated in the same disease space. The experiment was arranged in a split-plot (treated-untreated) randomised complete block design with six replicates. For assessment of growth and root disease at eight weeks (2013) or 11 weeks after sowing (2014), twenty one plants were removed per half plot comprising three plants taken at seven equally spaced locations from the middle row of the three rows. The three plant samples for treated and untreated rows were taken adjacent to each other. Plants were assessed for plant growth and root disease as with microplots. Grain was harvested at the end of the season with a plot harvester to assess grain yield.

In 2013, six microbial strains selected from the 2012 microplots were assessed at Wynarka and Lameroo. In 2014, eight microbial strains were selected based on both the 2013 microplots and the larger 3+3 trials, for assessment at Lameroo. Also included was

Streptomyces strain EN16 and Uniform® (Syngenta, active ingredients 322 g L^{-1} azoxystrobin and 124 g L^{-1} metalaxyl-M), an in-furrow fungicide treatment. Uniform® was applied as split bands: three to four cm below the seed and on the soil surface at a rate of 200 ml ha^{-1} in 80 L ha^{-1} water.

2.7. Statistical analysis

All statistical analysis was performed using GenStat version 16 (VSN International Ltd.) or later. All trials (microplot and 3+3) were set up and analysed as a split-plot randomised complete block design with six replicates. Microbial strain or fungicide treatments were fitted as whole-plots (six row plot), paired treated and untreated rows were fitted as sub-plots. Fisher's protracted least significant difference (LSD) was used to compare means between treated and untreated rows. For comparison of treated compared to untreated over all microbial treatments over both sites for microplots in 2012 and 2013, data was analysed as a split-split-plot design, with site fitted as the higher level split-plot, with n=120 (2012, 2 sites x 10 treatments x 6 replicates) or n=144 (2013, 2 sites x 10 treatments x 6 replicates). Data were checked for normal distribution and homogeneity of variance before analysis and found to be normally distributed with no significant difference in variance between treatments.

3. Results

3.1. Microplot trials 2012

Ten microbial strains were assessed in microplots at two sites in 2012. Comparison of treated and untreated plants at eight weeks after sowing over both sites indicated a highly significant (P<0.001) effect of microbial treatment. Rhizoctonia damage of seminal and nodal roots decreased by 32 and 28 %, respectively. Shoot (P<0.001) and root (P=0.003) dry weight increased by 10 and seven percent, respectively (Table 3). There was no significant interaction between site and microbial treatment (P>0.05), however, mean shoot and root growth was significantly higher at Pt. Julia compared to Karoonda (P<0.001, Table 4). There was no significant difference (P>0.05) in root disease between the two sites.

Variation between untreated plots in shoot and root growth and root disease level is shown in Table 4, with shoot dry weight of the untreated ranging from 76 to 124 g $plant^{-1}$ at Karoonda and from 369 to 485 g $plant^{-1}$ at Pt Julia. In general, mean shoot and root weights

were increased with microbial inoculation but this was only significantly with strain F10 for shoot weight at Pt. Julia and strain S16 for root weight at Karoonda (Table 4). Mean seminal root disease score was lower for all microbial treatments at both sites with three strains (S17, S20 and F17) reducing disease at Karoonda between 44 and 47% compared to untreated plants (Table 4). Four other strains (S9, F9, F10 and F18) reduced seminal root disease at Pt. Julia between 34 and 41% compared to untreated plants (Table 4). Differences in reduction of Rhizoctonia disease score was more varied on nodal roots, with one strain (S4) reducing disease at Karoonda by 37% and five strains (S9, S16, S21, F10 and F17) reducing nodal root disease score at Pt. Julia between 32 to 53% compared to untreated plants (Table 4).

3.2. Microplot trials 2013

In 2013, 11 strains were assessed in microplots at two sites, along with benchmark control strain, EN16, and two seed applied fungicides; the results are shown in Tables 5 and 6. Comparison of microbe treated and untreated plants at eight weeks after sowing over both sites indicated a highly significant effect ($P<0.001$) of microbial treatment decreasing Rhizoctonia damage of seminal and nodal roots by 19 and 9 %, respectively, and increasing shoot and root growth by nine and eight percent, respectively (Table 3). There was no interaction between site and treatment ($P>0.05$), and no significant difference between sites for shoot weight or root disease score ($P>0.05$). Mean root dry weight was significantly higher ($P<0.001$) at the Lameroo site (126 mg plant^{-1}) compared to Wynarka (102 mg plant^{-1}). As with 2012 trials, there was considerable variation in untreated controls, with shoot dry weight varying between 698 to 948 mg plant^{-1} and seminal root disease score varying between 1.1 and 2.0 at Lameroo (Table 5). At Wynarka, shoot dry weight of untreated plants varied between 660 to 1086 mg plant^{-1} and seminal root disease score varied between 1.4 and 2.0 (Table 6).

Comparing individual treatments, two strains (S1 and F5) increased plant growth and reduced disease at both Lameroo and Wynarka (Tables 5 and 6). Strains S1 and F6, reduced both seminal and nodal root disease score at both sites with three other strains (F5, F11 and EN16) reducing disease score only at Lameroo (Table 5 and 6). In general, at both sites, more strains showed a reduction in seminal root disease compared to a reduction in nodal root disease (Table 5 and 6).

There was no significant effect of the seed coated fungicides in plant growth or root disease at Lameroo ($P>0.05$, Table 5). However, at Wynarka, EverGol® Prime significantly increased root dry weight by 19% and Vibrance® significantly increased shoot dry weight by

26% and root dry weight by 30%, even though there was no significant effect on root disease ratings (Table 6).

3.3. Field trials 20 m, 3+3 plots 2013

In 2013, six strains were assessed at two sites in larger 20 m plots (Table 7). At eight weeks after sowing, there was no change in shoot or root dry weight with microbial inoculation at either site, except for strain F17 which increased root dry weight by 16 and 22% at Wynarka and Lameroo, respectively (Table 7). Disease reduction was greater at the Lameroo site, with an 18 to 40% reduction in seminal root infection, compared to a 6 to 25% reduction at Wynarka (Table 7). All six microorganisms reduced seminal root disease at one site and F10 reduced seminal root infection at both sites (Table 7). Five strains (S4, S16, F10, S8 and F9) reduced seminal root infection at Lameroo between 22 and 40% compared to untreated controls (Table 7) while two strains (F10 and F17) reduced seminal root infection at Wynarka by 21 and 25%, respectively. Three different strains (F10 and F17 at Wynarka; F9 at Lameroo) reduced nodal root disease by 21, 24 and 29%, respectively (Table 7).

Mean grain yield over all treatments was significantly higher (P=0.007) at Lameroo (2.8 t ha^{-1}) compared to Wynarka (2.3 t ha^{-1}). Yield from treated plots (2.83 t ha^{-1}) was significantly higher than untreated plots (2.78 t ha^{-1}) at Lameroo. There was no significant difference (P=0.714) in yield between untreated and treated plots at Wynarka. For individual treatments, grain yield was not significantly different (P>0.05) at either site (Table 7). Strains S16 and F9 produced a 5.4 and 4.4% yield reduction, respectively at Wynarka compared to a small increase in yield at Lameroo, but these differences between treated and untreated plots were not significant. In contrast, F10 produced a non-significant increase of 4.6% at Wynarka and a slight negative impact on yield (-0.7%) at Lameroo. Strain S4 produced a 3.9% increase, P<0.05) when the data from both sites were pooled. Strain F17 produced a mean increase of 3.0% at both sites, but differences were not significantly different (P>0.05) between treated and untreated plots (Table 7).

3.4. Field trial 20 m, 3+3 plots 2014

In 2014, seven strains were assessed at one site, along with the benchmark control strain *Streptomyces* strain EN16, and Uniform® fungicide applied in-furrow for comparison. Strains were selected based on performance in 2013 20 m 3+3 trials (S4 and F17) or 2013 microplot trials (S1, S3, S17, F5 and F11).

At 11 weeks after seeding, comparing all microbe treated and untreated plots, there was a significant increase in shoot dry weight, 809 compared to 734 mg plant^{-1}, respectively (P<0.001). There was a significant decrease in seminal root disease score, 2.0 compared to 2.5 (P<0.001), and nodal root disease score, 2.3 compared to 2.6 (P=0.002), in microbial treated and untreated plots, respectively. There was a significant (P=0.029) increase (1.5%) in grain yield at the end of the season comparing all microbial treated plots (2.58 t ha^{-1}) with untreated plots (2.54 t ha^{-1}).

For individual treatments, there was no significant difference (P>0.05) between treated and untreated plots in shoot or root dry weight for any of the treatments (Table 8). Strains S4, F5 and Uniform® treatment decreased both seminal and nodal root disease score by 17 to 35% (Table 8), whereas Strain S3 decreased seminal root disease score by 20% and strain EN16 decreased nodal root score by 21% (Table 8). Strain S4 was the only treatment which significantly increased grain yield (4.2%) compared to untreated control plots. F5, EN16 and Uniform® produced a non-significant mean increase in grain yield by 2.8, 2.5 and 3.0% (Table 8).

4. Discussion

Two stains, *Paenibacillus* S4 and *Streptomyces* F5, provided relatively consistent reduction of Rhizoctonia root rot in the field. Strain S5 reduced disease on seminal roots by 12 to 38% over five trials with grain yield increases of 3.8 to 4.2 % over the three trials where yield was assessed. Strain F5 reduced disease on seminal roots by 21 to 32% over three trials and a non-significant yield increase of 2.8%. These results were comparable to the in-furrow Uniform® fungicide treatment and superior to the seed coated fungicide treatments Vibrance® and EverGol®. Uniform is currently the most effective registered fungicide treatment in Australia for control of *Rhizoctonia* root rot on wheat (Bogacki et al., 2014; Hüberli et al., 2015).

Published data for biocontrol of Rhizoctonia root rot on wheat in the field is limited and comes from the Pacific Northwest of the USA; results for our strains assessed in Australia performed to a comparable degree to that reported for the American strains. Kim et al. (1997), assessed *Bacillus* strain L324-96 and *Pseudomonas* strain Q69c-80 for Rhizoctonia control on spring wheat at two sites over two years as seed coatings, with variable results. Strain L324-96 produced a 23% grain yield increase one year and a 20% yield reduction the next year at the same site. Strain Q69c-80 produced a 5.3% yield reduction in the one year

measured. These strains produced a 2.6 to 23% reduction in Rhizoctonia root rot (Kim et al., 1997). Cook et al., (2002b) also assessed these strains for Rhizoctonia control over five trial sites and four years, where *Bacillus* strain L324-96 produced a non-significant increase of 5.4 and 2.0% grain yield increase in spring and winter wheat, respectively, and Q69c-80 a 3.2 and 1.2 % grain yield increase in spring and winter wheat, respectively. *Pseudomonas* strain Q8r1-96 was also assessed in these trials and produced a non-significant 5.6 and 6.2% grain yield increase in spring and winter wheat, respectively. The single year that root disease data was given showed that there was no significant reduction in Rhizoctonia root rot with microbial inoculation (Cook et al., 2002b). In contrast, our strains (S4, F5 and others) produced significant decreases in root rot in the field when inoculated at much lower levels (usually under 10^6 cfu seed^{-1} and often less than 10^5 cfu seed^{-1}) than was used for the American strains which were inoculated at over 10^6 cfu seed^{-1} for L324-96 and 10^7 cfu seed^{-1} for Q69c-80 (Kim et al., 1997; Cook et al., 2002b).

Assessing strains for control of Rhizoctonia root rot on-farm presents a number of challenges, including selection of sites where disease is expected and the uneven distribution of Rhizoctonia patches across a field (Anees et al., 2010; Schillinger and Paulitz, 2006). The sites used in this present study provide a rigid evaluation of the microbes because they were fields which had greater than 100 pg *R. solani* AG8 DNA per gram of soil, which is considered high risk for disease expression (Ophel-Keller et al., 2008; Poole et al., 2015). Sites were also in low rainfall areas (between 176 and 266 mm of in season rainfall) (Table 2) which is known to be conducive to expression of Rhizoctonia root rot (Gill et al., 2001; Okubara et al., 2014; Poole et al., 2015). Sites also had low to non-detectable levels of other root pathogens. This approach avoided the need to artificially inoculate trial sites with the pathogen, as in Smith et al., (2003). Individual plants had a root disease score between zero (no disease) and five, the maximum disease with roots being short black stumps. The mean disease score for plots ranged from a minimum of 0.7 to a maximum of 3.6 (Tables 4, 5, 6, 7 and 8).

Rhizoctonia produces patches that have substantially reduced plant growth and can be unevenly distributed across the field (Anees et al.; 2010, Schillinger and Paulitz, 2006). One way to overcome this problem was to use paired plots in a split-plot design so as to make comparisons between untreated and treated samples in the same disease space as was used by Bogacki et al. (2014). This idea was modified in the development of a microplot system to allow a greater number of strains to be assessed for their ability to reduce disease in a low cost manner prior to full scale field trials. The use of a planting template ensured plants were

evenly spaced to reduce variability. This system was first tested at two sites in 2012 to evaluate the system and used again in 2013. When treated and untreated plots were compared for both years, the results were highly significant for increases in shoot and root growth at eight weeks after sowing by 7 to 10% and for reducing root disease on seminal and nodal roots (Table 3). Reduction in root disease was 28 and 32% for nodal and seminal roots respectively in 2012. This was greater than the 9 and 19 % reductions in disease on nodal and seminal roots, respectively, in 2013 and probably due to the 2012 strains having the greater disease control in pots, which was based on assessment of root disease on seminal roots on four week old wheat seedlings. This result affirms the methodology used for our screening process in Barnett et al., (2017) using plant pathosystems with field soil is suitable for selecting strains that can reduce disease in the field.

When individual treatments were considered, high variability was noted between the mean values of untreated rows in the microplots (Tables 4 and 5) and in the 20 m 3+3 plots (Tables 6, 7 and 8) in both dry weight of plants and root disease. Untreated rows and plots had identical seed treatments with only the sticker solution without microbes and highlights the variability inherent in fields with Rhizoctonia induced disease. Nonetheless, the experimental design employed has enabled evaluation of disease control by seed coated microbes. For example, at the microplots at Karoonda (Table 4) there was a 63% difference from lowest (76 mg plant^{-1}) to highest (124 mg plant^{-1}) values in shoot dry weight in untreated plots so that even with a 45% increase in shoot dry weight with strain S16, this was not significantly different (P=0.091). Compared to the Pt. Julia site with 31% variation between highest and lowest values in shoot dry weight, an increase of 18% (strain F10) was significant (P=0.016). The variability between plots is also noticeable in the root disease scores, resulting in only relatively large decreases (>30%) in disease being significantly different, with an inconsistent degree of change being significantly different at P=0.05 for individual treatments, for example, at Karoonda in 2012, a 37% decrease in nodal root disease with strain S4 seed coating was significantly different from untreated (P=0.044), whereas a 43% reduction with strain F17 was not (P=0.064, Table 4).

Published data on field efficacy of fungicides for control of Rhizoctonia root rot is very limited. In 1990, Smiley et al. (1990) assessed 13 fungicide seed treatments at three sites in the USA Pacific north-west and concluded all treatments were ineffective or unreliable for controlling Rhizoctonia root rot. More recently, Almasady et al. (2015) assessed two fungicides, Dividend® (Syngenta, active ingredients difenoconazole and metalaxyl-M at 92 and 23%, respectively) and Rancona® Dimension (Chemtura, active ingredients ipconazole,

metalaxyl and N-Methyl-2-pyrrolidone at 2.38, 1.95 and 35-45%, respectively) on wheat seedlings in pots using river sand and loam, with and without pasteurisation, using artificial Rhizoctonia inoculum. Dividend® was found to have little effect in reducing root rot and Rancona® Dimension to have significantly improved disease control over Dividend®. Root disease severity was reduced by 13 to 23% with low and high rates, respectively, of Dividend and reduced by 48 and 61% with low and high rates, respectively, of Rancona® Dimension, but only one table of pooled results was presented making it difficult to determine the significance of these changes. There does not appear to be any published field data on the efficacy of these treatments for Rhizoctonia control on wheat.

More recently, two fungicide seed treatments (EverGol® Prime and Vibrance®) and an in-furrow fungicide treatment (Uniform®) have been registered for Rhizoctonia control on wheat and barley in Australia and field efficacy data reported, with the in-furrow treatment providing greater disease control and yield increase compared to the seed treatments (Bogacki et al., 2014; Hüberli et al., 2015). In our trials, neither fungicide seed treatment provided significant reduction in seminal or nodal root disease at either site, with mean differences of 23% increase to 20% decrease compared to untreated controls (Tables 5 and 6), which was comparable to the mean reductions reported by Bogacki et al. (2014) over multiple sites. This is in contrast to the microbial seed treatments where seven (S1, F1, F4, F5, F6, F11 and EN16) out of 11 strains significantly (P<0.05) reduced seminal root score at both sites between 11% and 44% compared to untreated controls (Tables 5 and 6). This indicates that, at least at the sites assessed, that seed coated microbial inoculation can provide better Rhizoctonia root rot control than the seed coated fungicides.

Despite the large degree of variability between replicate plots it was still possible to identify a number of strains that increased plant growth and reduced root disease in the microplots over both trial sites, enabling the selection of strains for the larger 20 m 3+3 trials. In 2013, six strains were assessed at two sites, S4, S16, F10, F17 and F9 were selected based on 2012 microplots (Table 4). Inclusion of S8 was based on results from pot bioassays (Barnett et al., 2017) that had not been included in the microplots. Some strains which performed well in the microplots were not included in the 20 m trials due to relatively poor survival on seed (S20, *Trichoderma*) or potential registration difficulties (S21, *Aspergillus*) (Barnett et al., 2017). As with the microplots, there was considerable variability between replicate plots, with only strain F17 significantly increasing root dry weight at Lameroo and Wynarka, while the other strains had little impact on early season plant growth (Table 7). There was a greater response to reduction of root disease, with strains reducing seminal root

disease score to a greater degree at Lameroo (18 to 40% reduction) compared to 6 to 25% reduction at Wynarka. There was no significant difference (P>0.05) in grain yield with microbial inoculation of seed at either site, however, mean grain yield for strain S4 was 4.1 and 3.8% at Lameroo and Wynarka, respectively, and strain F17 increased mean yield by 3.0% at both sites.

Strains S4 and F17 were assessed again in 2014 in the 20 m plots along with six strains (S1, S3, S17, F5, F11 and EN16) selected based on the 2013 microplots and the in furrow chemical treatment Uniform®. Strain S4 was the only treatment to significantly (P<0.05) increase yield (4.2% increase). Strain S4 at 10^5 cfu seed^{-1}, a *Paenibacillus* sp., also significantly (P<0.05) reduced seminal and nodal root disease scores by 17 and 20% respectively. Strain F5 at 8 x 10^3 cfu seed^{-1} (*Streptomyces* sp.) significantly reduced root disease to a comparable level to the Uniform® fungicide treatment, but did not result in a significant yield increase (P>0.05). The yield increase of 3.0% in our trial for the same in-furrow Uniform® treatment (split application of 200 ml ha^{-1} above and below the seed) was less than the mean yield increase of 11% over 3 trials reported by Bogacki et al. (2014). For the chemical seed treatments, two out six and three out of 11 trials gave a significant yield increase with EverGol® Prime and Vibrance®, respectively (Bogacki et al., 2014; Hüberli et al., 2015), comparable to a significant increase in one out of three trials for strain S4.

Rhizoctonia root rot is difficult to control, and it seems that biological, chemical and management practices, individually, will only provide partial control. Thus, the microbes identified in this study will need to be integrated with other management options to minimise losses to this disease. Current options for Rhizoctonia control are the management of weeds (Roget et al., 1987; Smiley et al., 1992), strategic tillage below the seed and crop rotations (Roget et al., 1996; Rovira, 1986), paired rows and placement of fertiliser below the seed (Cook et al., 2000). Microbes might also be combined with chemical fungicides, but this will have to be investigated on a case by case basis as combining microbes with fungicides has been reported to both increase and decrease yield (Cook et al., 2002b). For example, combining *Bacillus* strain L324-96 with Raxil-Thiram reduced yield but combining *Pseudomonas* strain Q8r1-96 with Dividend significantly increased grain yield of wheat (Cook et al., 2002b). Strains S4 and F5 are relatively tolerant to seed applied fungicides on agar plates (Barnett pers. comm.) but further research is needed to assess the impact of combining these strains with fungicides in the field.

5. Conclusions

Paenibacillus strain S4 was identified as being able to increase yield by around 4% in each of the three trials tested, and resulted in a significant reduction of Rhizoctonia root rot at eight to eleven weeks after planting in the five trials it was assessed. As well, *Streptomyces* strain F5 was identified as producing significant reductions in Rhizoctonia root rot in all three trials it was assessed in, and producing a non-significant mean yield increase of 2.8% in the one trial where yield was assessed. Both these strains reduced disease in the field when inoculated onto seed at a level which would be amenable to commercial use of an inoculant and performed as well as or better than the latest chemical technology registered for use for Rhizoctonia control on wheat. Both these strains warrant further assessment in the field to establish the reliability of disease control and yield increasing performance.

Funding

This work was supported by the Australian Grains Research and Development Corporation, grant numbers UF00007 and UF00008.

Acknowledgements

P. Bogacki and A. McKay, SARDI Soil Biology and Diagnostics for pathogen DNA analysis and trial site selection. Sophia Zhao for technical assistance. J. Desboilles, University of South Australia Agricultural Machinery Research and Design Centre for planting of trials. SARDI New Variety Agronomy group for managing weed control and harvesting the trials. All the grain growers who kindly collaborated with conducting the trials on their land from 2012 to 2014.

Conflict of interest. The authors have no conflict of interest.

References

Almasudy, A.M., You, M.P. Barbetti, M.J., 2015. Influence of fungicidal seed treatments and soil type on severity of root disease caused by *Rhizoctonia solani* AG-8 on wheat. Crop Prot. 75, 40-45.

Anees. M., Edel-Hermann, V., Steinberg, C., 2010. Build up of patches caused by *Rhizoctonia solani*. Soil Biol. Biochem. 44, 1661-1672.

Bailey, K.L., Boyetchko, S.M., Längle, T., 2010. Social and economic drivers shaping the future of biological control: A Canadian perspective on the factors affecting the development and use of microbial biopesticides. Biol. Control 52, 221-229.

Barnett, S.J., Roget, D.K., Ryder, M.H., 2006. Suppression of *Rhizoctonia solani* AG-8 induced disease on wheat by the interaction between *Pantoea*, *Exiguobacterium* and Microbacteria. Australian J. Soil Res. 44, 331-342.

Barnett, S., Zhao, S., Ballard, R., Franco, C. 2017. Selection of microbes for control of Rhizoctonia root rot on wheat using a high throughput pathosystem. Biolog. Control 113, 45-57. http://dx.doi.org/10.1016/j.biocontrol.2017.07.003.

Berg, G., 2009. Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl. Microbiol. Biotech. 84, 11-18.

Bogacki, P., Desbiolles, J., Correll, R., Hüberli, D., MacLeod, B. and McKay, A., 2014. Targeted liquid delivery of fungicides: a new tool for Rhizoctonia root rot control, in: Eyre Peninsula Farming Systems Summary. pp. 89-92. https://eparf.com.au/research-project/ep-farming-systems-summary-2014/ (accessed 23.05.17).

Broadbent, P., Baker, K.F., Waterworth, Y., 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. Aust. J. Biol. Sci. 24, 925-944.

Cook, R.J., Ownley, B.H., Zhang, H., Vakoch, D., 2000. Influence of paired-row spacing and fertilizer placement on yield and root diseases of direct-seeded wheat. Crop Sci. 40, 1079-1087.

Cook, R.J. Schillinger, W.F., Christensen, N.W., 2002a. Rhizoctonia root rot and take-all of wheat in diverse direct-seed spring cropping systems. Can. J. Plant Pathol. 24, 349-358.

Cook, R.J., Weller, D.M., El-Banna, A.Y., Vakoch, D., Zhang, H. 2002b Yield responses of direct-seed wheat to fungicide and rhizobacteria treatments. Plant Disease 87, 780–784.

Coombs, J. Franco, C.M.M., 2003. Isolation and identification of actinobacteria from surface-sterilized wheat roots. Appl. Environ. Microbiol. 69, 5603–5608.

Dua, S. Sindhu, S.S., 2012. Effectiveness of rhizosphere bacteria for control of root rot disease in improving plant growth of wheat (*Triticum aestivum* L.). J. Microbiol. Res. 2, 26-35.

Dutta, S. Podile, A.R., 2010. Plant growth promoting rhizobacteria (PGPR): the bugs to debug the root zone. Crit. Rev. Microbiol. 36, 232-244.

Franco, C., Michelsen, P., Percy, N., Conn, V., Listiana, E. Moll, S., Loria, R., Coombs, J., 2007. Actinobacterial endophytes for improved crop performance. Australas. Plant

Microbes for Rhizoctonia control on wheat

Pathol. 36, 524–531

Gill, J.S., Sivasithamparam, K., Smettem, K.R.J., 2001. Effect of soil moisture at different temperatures on Rhizoctonia root rot of wheat seedlings. Plant and Soil 231, 91-96.

Hüberli D., Conner, M. MacLeod, B., Battaglia, R., Forsyth, L. Parkin, B., Klein, T., Clarke, M., Robertson, G., Correll, R. Desbiolles, J., Bogacki, P., McKay, A., 2015. *Rhizoctonia solani* AG8: New breakthroughs in control and management. In: 2015 Agribusiness Crop Updates, 24 - 25 February, Perth, Western Australia. URL: http://researchrepository.murdoch.edu.au/id/eprint/25655/1/Rhizoctonia_solani_AG8.pdf (accessed 23.05.17).

Jaffar, A.K.M., Paulitz, T.C., Schroeder, K.L., Thomashow, L.S., Weller, D.M., 2016. Molecular characterization, morphological characteristics, virulence, and geographic distribution of *Rhizoctonia* spp. in Washington State. Phytopath. 106, 459-473.

Kim, D.S., Cook, R.J, Weller, D.M., 1997. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown with reduced tillage. Phytopath. 87, 551-558.

Köhl, J., Postma, J., Nicot, P., Ruocco, M., Blum, B., 2011. Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. Biol. Control 57, 1-12.

Mahoney, A.K., Babiker, E.M., Paulitz, T.C., See, D., Okubara, P.A., Hulbert, S.H., 2016. Characterizing and mapping resistance in synthetic-derived wheat to Rhizoctonia root rot in a green bridge environment. Phytopath. 106, 1170-1176. doi.org/10.1094/PHYTO-02-16-0055-FI.

Mavrodi, O.V., Walter, N., Elateek, S., Taylor, C.G., Okubara, P.A., 2012. Suppression of Rhizoctonia and Pythium root rot of wheat by new strains of *Pseudomonas*. Biol. Control 62, 93-102.

Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, M., Piceno, Y.M., DeSantis, T.Z., Andersen, G.L., Bakker, P.A.H.M., Raaijmakers, J.M., 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332, 1097-1100.

Murray, G.M., Brennan, J.P., 2009a. The Current and Potential Costs from Diseases of Wheat in Australia. Grains Research and Development Corporation, Kingston, Australian Capital Territory.

Murray, G.M., Brennan, J.P., 2009b. The Current and Potential Costs From Diseases of Barley in Australia. Grains Research and Development Corporation, Kingston, Australian Capital Territory.

Okubara, P.A., Steber, C.M., DeMacon, V.L., Walter, N.L., Paulitz, T.C., Kidwell, K.K., 2009. Scarlet-Rz1, an EMS-generated hexaploid wheat with tolerance to the soilborne necrotrophic pathogens *Rhizoctonia solani* AG-8 and *R. oryzae*. Theor. Appl. Genet. 119, 293-303. doi: 10.1007/s00122-009-1038-x.

Okubara, P.A., Schroeder, K.L., Abatzoglou, J.T. Paulitz, T.C., 2014. Agroecological factors correlated to soil DNA concentrations of Rhizoctonia in dryland wheat production zones of Washington state, USA. Phytopath. 104, 683-691.

Ophel-Keller, K., McKay, A., Hartley, D., Herdina, Curren, J., 2008. Development of a routine DNA-based testing service for soilborne diseases in Australia. Australas. Plant Pathol. 37, 243-253.

Paulitz, T.C., Smiley, R., Cook, R.J., 2002. Insights into the prevalence and management of soilborne cereal pathogens under direct seeding in the Pacific Northwest, U.S.A. Can. J. Plant Pathol. 24, 416–428.

Poole, G.J., Harries, M., Hüberli, D. Miyan, S., MacLeod W. J., Lawes, R., McKay, A., 2015. Predicting cereal root disease in Western Australia using soil DNA and environmental parameters. Phytopath. 105, 1069-1079.

Pumphrey, F.V., Wilkins, D.E., Hane, D.C., Smiley, R.W., 1987. Influence of tillage and nitrogen fertiliser on Rhizoctonia root rot (bare patch) of winter wheat. Plant Dis. 71, 125-127.

Roget, D.K., 1995. Decline in root rot (*Rhizoctonia solani* AG-8) in wheat in a tillage and rotation experiment at Avon, South Australia. Aust. J. Exp. Agric. 35, 1009-1013.

Roget, D.K., Venn, N.R., Rovira, A. D. 1987. Reduction in Rhizoctonia root rot of direct drilled wheat by short-term chemical fallow. Aust. J. Exp. Agric. 27, 425-430.

Roget, D.K., Neate, S.M., Rovira, A.D., 1996. Effect of sowing point design and tillage practice on the incidence of Rhizoctonia root rot, take-all and cereal cyst nematode in wheat and barley. Aust. J. Exp. Agric. 36, 683–693.

Rovira, A.D., 1986. Influence of crop rotation and tillage on Rhizoctonia bare patch of wheat. Phytopath. 76, 669-673.

Schillinger, W.F., Paulitz, T.C., 2006. Reduction of Rhizoctonia bare patch in wheat with barley rotations. Plant Dis. 90, 302-306.

Schisler, D.A., Slininger, P.J., 1997. Microbial selection strategies that enhance the likelihood of developing commercial biological control products. J. Ind. Microbiol. Technol. 19, 172-179.

Schroeder, K.L., Paulitz, T.C., 2006. Root diseases of wheat and barley during the transition from conventional tillage to direct seeding. Plant Dis. 90, 1247-1253.

Smiley, R.W., Wilkins, D.E., Klepper, E.L., 1990. Impact of fungicide seed treatments on Rhizoctonia root rot, take-all, eyespot and growth of winter wheat. Plant Dis. 74, 782-787.

Smiley, R.W., Ogg, A.G., Cook, R.J., 1992. Influence of glyphosate on Rhizoctonia root rot, growth, and yield of barley. Plant Dis. 76, 937–942.

Smith, J.D., Kidwell, K.K., Evans, M.A., Cook, R.J., Smiley, R.W., 2003. Assessment of spring wheat genotypes for disease reaction to *Rhizoctonia solani* AG-8 in controlled environment and direct-seeded field evaluations. Crop Sci. 43, 694-700.

Weller, D.M., Cook, R.J., MacNish, G., Bassett, E.N., Powelson, R.L., Petersen, R.R., 1986. Rhizoctonia root rot of small grains favored by reduced tillage in the Pacific Northwest. Plant Dis. 70, 70–73.

Yin, C., Hulbert, S.H., Schroeder, K.L., Mavrodi, O., Mavrodi, D., Dhingra, A., Schillinger, W.F., Paulitz, T.C., 2013. The role of bacterial communities in the natural suppression of Rhizoctonia bare patch of wheat (*Triticum aestivum* L.). Appl. Environ. Microbiol. 79, 7428-7438.

Table 1. Strains, genera, source of microbes and location of trials in the 2012, 2013 and 2014 growing seasons. Strains were first assessed in one metre microplots then in 20 m six row plots with three microbe treated and three untreated (3+3) rows. Site locations were: Kar, Karoonda; Pt Jul, Port Julia; Wyn, Wynarka; Lam, Lameroo.

Strain	Genera	Source	Microplot trials	20 m 3+3 trials
S1	*Bacillus*	wheat root	2013 Wyn & Lam	2014 Lam
S3	*Bacillus*	wheat root	2013 Wyn & Lam	2014 Lam
S4	*Paenibacillus*	wheat root	2012 Kar & Pt Jul	2013 Wyn & Lam 2014 Lam
S8	*Bacillus*	Triticale rhizosphere		2013 Wyn & Lam
S9	*Bacillus*	Triticale rhizosphere	2012 Kar & Pt Jul	
S10	*Microbacterium*	Triticale root	2012 Kar	
S11	*Paenibacillus*	wheat root	2013 Wyn & Lam	
S12	*Streptomyces*	Triticale rhizosphere	2013 Wyn & Lam	
S16	*Bacillus*	wheat root	2012 Kar & Pt Jul	2013 Wyn & Lam
S17	*Bacillus*	wheat rhizosphere	2012 Kar & Pt Jul	2014 Lam
S20	*Trichoderma*	wheat rhizosphere	2012 Kar & Pt Jul	
S21	*Aspergillus*	wheat rhizosphere	2012 Kar & Pt Jul	
F1	*Streptomyces*	potato tuber	2013 Wyn & Lam	
F2	*Streptomyces*	*Callitris preissii* root	2013 Wyn & Lam	
F4	*Streptomyces*	*Eucalyptus camaldulensis* root	2013 Wyn & Lam	
F5	*Streptomyces*	bulk soil	2013 Wyn & Lam	2014 Lam
F6	*Streptomyces*	bulk soil	2013 Wyn & Lam	

F8	*Streptomyces*	bulk soil	2013 Wyn & Lam	
F9	*Streptomyces*	*Pittosporum phyliraeoides* root	2012 Kar & Pt Jul	2013 Wyn & Lam
F10	*Streptomyces*	bulk soil	2012 Kar & Pt Jul	2013 Wyn & Lam
F11	*Streptomyces*	*Callitris preissii* root	2013 Wyn & Lam	2014 Lam
F17	*Streptomyces*	wheat root	2012 Kar & Pt Jul	2013 Wyn & Lam 2014 Lam
F18	*Streptomyces*	*Callitris preissii* root	2012 Pt Jul	
EN16	*Streptomyces*	wheat root	2013 Wyn & Lam	2014 Lam

Table 2. Field site locations, pre-season *Rhizoctonia solani* AG8 DNA levels and April to December in season rainfall.

Site	Year	Location	*R. solani* AG8 pg g^{-1} soil	April-Nov Rainfall (mm)
Port Julia	2012	S34.635362°, E137.851145°	102	266
Karoonda	2012	S35.068801°, E140.032790°	138	213
Wynarka	2013	S35.14535°, E139.68714°	257	243
Lameroo	2013	S35.28839°, E139.68714°	106	176
Lameroo	2014	S35.287969°, E140.475642°	427	183

Table 3. Combined analysis of microplot trials in 2012 and 2013 showing shoot and root dry weight (mg DW) per plant and seminal and nodal root disease score (DS, 0-5) at 8 weeks after sowing for microbial seed treated and untreated plots. % change = [(Microbe treated/untreated)x100]-100. In 2012, N=120: 2 sites x 10 microbial treatments x 6 replicates. In 2013, N=144: 2 sites x 12 microbial treatments x 6 replicates.

	Treated	Untreated	Fprob	% change
2012.				
Shoot DW mg plant^{-1}	283	256	<0.001	10
Root DW mg plant^{-1}	55	52	0.003	7
Seminal root DS	0.8	1.2	<0.001	-32
Nodal root DS	1.1	1.5	<0.001	-28
2013.				
Shoot DW mg plant^{-1}	871	799	<0.001	9
Root DW mg plant^{-1}	119	110	<0.001	8
Seminal root DS	1.3	1.6	<0.001	-19
Nodal root DS	2.5	2.7	<0.001	-9

Table 4. Results from 2012 microplot trials at Karoonda and Port Julia, eight weeks after sowing. Data is the mean of 10 plants sampled per row, showing shoot and root dry weight (DW) per plant and seminal and nodal root disease score (DS, 0-5), six replicates. Seeds were coated with microbes (Treated) or coated with sticker solution without microbes (Untreated). Initial microbial populations on seeds at planting is given as log_{10}(cfu seed^{-1}), n=2. Percentage change (% change) = [(Microbe treated/untreated)x100]-100.

		Shoot DW mg plant^{-1}			Root DW mg plant^{-1}			Seminal Root DS		
Strain	Log_{10} (cfu seed^{-1})	Treated	Untreated	%change	Treated	Untreated	%change	Treated	Untreated	%change
Karoonda										
S4	5.7	105	103	3	35	33	5	0.8	1.3	-38
S9	6.2	87	91	-5	33	30	7	0.8	1.0	-15
S10	7.3	122	124	-1	40	37	8	1.1	1.5	-25
S16	6.0	129	89	45	37	28	30*	0.5	0.8	-38
S17	6.2	129	102	26	38	35	11	0.6	1.2	-47*
S20	4.1	105	76	39	36	32	14	1.0	1.9	-46*
S21	5.0	98	80	23	31	29	6	0.6	0.8	-26
F9	5.1	106	93	14	37	35	6	0.9	1.3	-33
F10	6.0	89	100	-12	32	32	-1	1.2	1.5	-22
F17	5.4	101	92	10	33	34	-1	0.8	1.5	-44*
Pt Julia										
S4	5.6	438	386	14	75	70	7	1.0	1.3	-24
S9	6.1	449	450	0	76	73	4	0.8	1.2	-34*
S16	5.7	441	399	10	69	70	-1	1.0	1.4	-26
S17	5.9	433	393	10	76	68	12	0.9	1.3	-34
S20	4.3	465	446	4	77	72	7	0.9	1.1	-21
S21	4.6	510	485	5	80	81	-1	0.7	1.0	-30
F9	5.9	425	369	15	67	65	4	0.8	1.3	-41*
F10	5.4	484	408	18*	79	70	13	0.7	1.1	-37*
F17	5.5	473	419	13	79	72	10	0.8	1.0	-15
F18	7.1	470	421	12	78	73	7	0.8	1.3	-35*

*Treated significantly different from untreated at $P \leq 0.05$ by Fisher's LSD

**Treated significantly different from untreated at $P \leq 0.01$ by Fisher's LSD

Table 5. Results from 2013 microplot trials at Lameroo eight weeks after sowing. Data is the mean of 10 plants sampled per row, showing shoot and root dry weight (DW) per plant and seminal and nodal root disease score (DS, 0-5), six replicates. Seeds were coated with microbes (Treated) or coated with sticker solution without microbes (Untreated). Initial microbial populations on seeds at planting is given as log_{10}(cfu seed^{-1}), n=2. Percentage change (% change) = [(Microbe treated/untreated)x100]-100.

		Shoot DW mg plant^{-1}			Root DW mg plant^{-1}			Seminal root DS		
Strain	Log_{10} (cfu seed^{-1})	Treated	Untreated	% change	Treated	Untreated	% change	Treated	Untreated	
S1	5.6	885	698	27*	127	96	32*	1.2	1.8	-3
S3	4.8	1243	948	31*	178	145	23*	1.3	1.5	-
S11	4.9	814	769	6	129	120	7	1.2	1.7	-3
S12	5.2	789	806	-2	115	113	2	1.5	1.3	
F1	6.0	842	747	13	117	111	6	1.4	1.8	-2
F2	6.2	988	859	15	136	122	11	1.4	1.5	-
F4	4.8	905	879	3	120	133	-9	1.2	1.6	-2
F5	5.3	947	789	20*	138	115	21*	1.4	1.8	-2
F6	4.1	910	890	2	128	124	2	0.9	1.6	-4
F8	4.8	868	943	-8	121	133	-10	1.3	1.6	-
F11	4.5	892	762	17	126	117	8	1.2	2.0	-4
EN16	4.9	899	748	20	141	111	27*	0.9	1.7	-4
EverGol® Prime		926	912	2	138	131	5	1.2	1.3	
Vibrance®		871	831	5	127	118	7	0.9	1.1	-2

*Treated significantly different from untreated at $P \leq 0.05$ by Fisher's LSD

Table 6. Results from 2013 microplot trials at Wynarka after eight weeks after sowing. Data is the mean of 10 plants sampled per row, showing shoot and root dry weight (DW) per plant and seminal and nodal root disease score (DS, 0-5), six replicates. Seeds were coated with microbes (Treated) or coated with sticker solution without microbes (Untreated). Initial microbial populations on seeds at planting is given as log_{10}(cfu seed^{-1}), n=2. Percentage change (% change) = [(Microbe treated/untreated)x100]-100.

		Shoot DW mg plant^{-1}			Root DW mg plant^{-1}			Seminal root DS		
Strain	Log_{10} (cfu seed^{-1})	Treated	Untreated	% change	Treated	Untreated	% change	Treated	Untreated	
S1	5.5	880	704	25*	124	95	31*	1.2	1.6	-2
S3	4.5	800	701	14	104	92	13	1.3	1.7	-2
S11	4.8	802	790	1	110	101	9	1.4	1.6	-
S12	5.6	892	1086	-18	99	121	-18	1.7	1.6	
F1	5.7	960	878	9	99	111	-11	1.3	1.5	-1
F2	5.6	900	797	13	111	97	14	1.4	1.7	-
F4	4.4	749	755	-1	92	96	-4	1.4	1.9	-2
F5	5.5	813	660	23*	102	88	16	1.3	2.0	-3
F6	4.5	648	749	-14	79	91	-13	1.6	2.0	-2
F8	4.9	1034	779	33*	123	105	17*	1.4	1.5	-
F11	5.5	711	678	5	99	96	4	1.3	1.7	-2
EN16	5.6	852	837	2	104	99	5	1.5	2.0	-2
EverGol® Prime		732	674	9	114	96	19*	1.4	1.6	-
Vibrance®		838	663	26*	121	93	30*	1.8	1.4	2

*Treated significantly different from untreated at $P \leq 0.05$ by Fisher's LSD

Table 7. Results from 2013 trials: 20 m 3+3 split-plot trials at Lameroo and Wynarka. Data is the mean of 21 plants sampled per plot, showing shoot and root dry weight (DW) per plant and seminal and nodal root disease score (DS, 0-5) at eight weeks after sowing and grain yield, six replicates. Seeds were coated with microbes (Treated) or coated with sticker solution without microbes (Untreated). Initial microbial populations on seeds at planting is given as $\log_{10}$(cfu seed^{-1}), n=2. Percentage change (% change) = [(Microbe treated/untreated)x100]-100.

		Shoot DW mg plant^{-1}			Root DW mg plant^{-1}			Seminal root DS			Nodal
Strain	$\log_{10}$ (cfu seed^{-1})	Treated	Untreated	% change	Treated	Untreated	% change	Treated	Untreated	% change	Treated
Lameroo											
S4	5.0	455	431	6	52	51	2	1.0	1.4	-27*	2.1
S8	5.2	448	433	3	53	48	10	1.0	1.4	-26*	1.9
S16	5.4	457	438	4	56	50	11	1.0	1.6	-34*	1.8
F9	4.4	477	469	2	54	55	-1	1.1	1.4	-22*	1.8
F10	5.5	446	476	-6	48	54	-10	0.9	1.6	-40*	2.0
F17	4.5	412	372	11	52	43	22*	0.8	1.0	-18	1.5
Site mean		449	437	3	53	50	5	1.0	1.4	-28**	1.9
Wynarka											
S4	5.0	242	213	13	39	35	12	1.2	1.3	-12	2.4
S8	5.0	226	252	-10	41	39	4	1.1	1.2	-8	2.5
F9	4.3	234	225	4	39	36	6	1.2	1.3	-6	2.2
S16	5.1	224	224	0	40	42	-4	1.1	1.3	-10	2.3
F10	4.9	250	265	-6	40	41	-3	1.1	1.3	-21*	2.1
F17	4.8	243	245	-1	44	38	16*	1.1	1.5	-25*	2.4
Site mean		236	237	0	40	39	5	1.1	1.3	-14**	2.3

*Treated significantly different from untreated at $P \leq 0.05$ by Fisher's LSD
**Treated significantly different from untreated at $P \leq 0.01$ by Fisher's LSD

Table 8. Results from 2014 20 m 3+3 plot trials at Lameroo. Data is the mean of 21 plants sampled per plot, showing shoot and root dry weight (DW) per plant and seminal and nodal root disease score (DS, 0-5) at eight weeks after sowing and at final grain yield, six replicates. Seeds were coated with microbes (Treated) or coated with sticker solution without microbes (Untreated). Initial microbial populations on seeds at planting is given as log_{10}(cfu seed^{-1}), n=2. Percentage change (% change) = [(Microbe treated/untreated)x100]-100.

		Shoot DW mg plant^{-1}			Root DW mg plant^{-1}			Seminal root DS			Nodal	
Strain	Log_{10} (cfu seed^{-1})	Treated	Untreated	%change	Treated	Untreated	%change	Treated	Untreated	%change	Treated	
S1	5.2	789	731	8	101	104	-3	1.8	2.1	-11	2.3	2
S3	4.4	790	727	9	102	96	6	2.3	2.9	-20*	2.8	2
S4	4.9	719	751	-4	94	102	-8	2.1	2.5	-17*	2.1	2
S17	6.0	845	722	17	111	103	8	1.8	2.2	-17	2.3	2
F5	3.9	847	746	14	105	97	8	1.9	2.8	-32*	2.5	3
F11	5.1	826	720	15	106	99	7	2.2	2.6	-15	2.6	2
F17	4.7	890	820	9	116	108	7	1.9	2.3	-15	2.3	2
EN16	5.6	765	652	17	100	92	9	2.0	2.4	-15	1.9	2
Mean of microbes		809	734	10**	104	100	4	2.0	2.5	-18**	2.3	2
Uniform®		730	747	-2	104	96	9	1.4	2.2	-35*	1.7	2

*Treated significantly different from untreated at $P \leq 0.05$ by Fisher's LSD
**Treated significantly different from untreated at $P \leq 0.01$ by Fisher's LSD

Barnett et al CRedit Author Statement

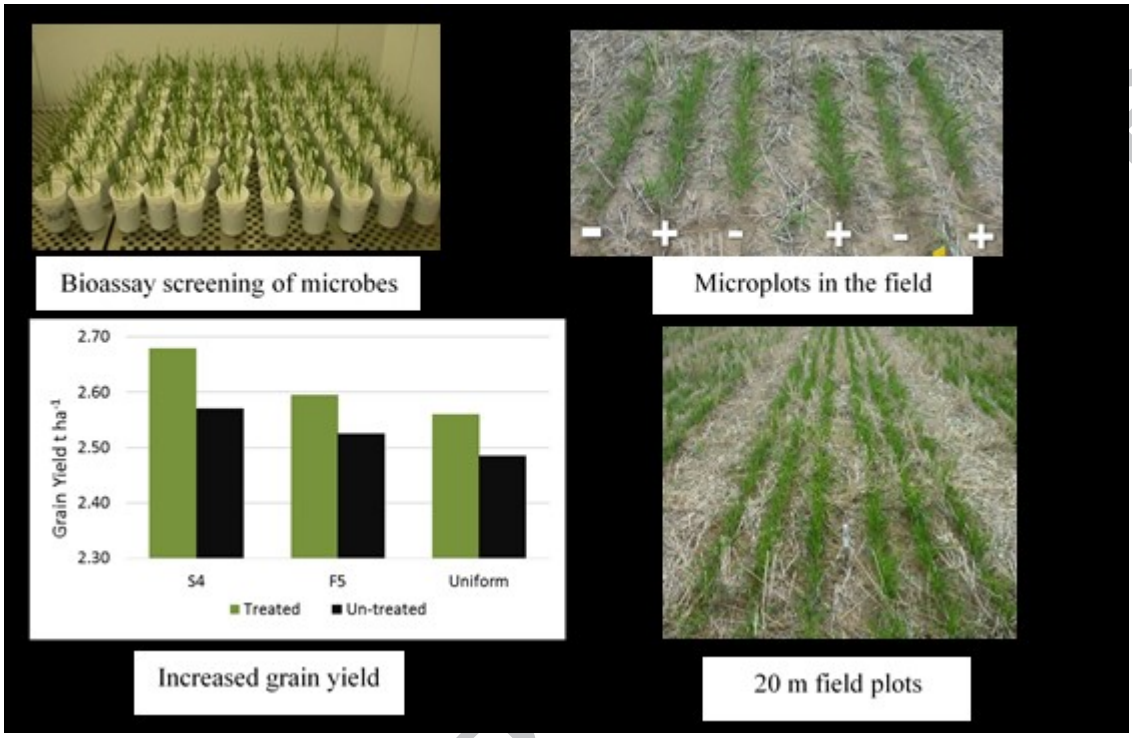
The following contains the roles for each author

S. Barnett: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Roles/Writing – original draft; Writing – review & editing

R. Ballard: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Roles/Writing– review & editing.

Microbes for Rhizoctonia control on wheat

C. Franco: Conceptualization; Funding acquisition; Investigation; Project administration; Resources; Supervision; Roles/Writing– review & editing.



Highlights

- Seed applied microbes assessed for Rhizoctonia root rot control in field.
- An alternate row microplot system developed for initial field assessment.
- Effective strains then tested in 20 m split-plots for disease control and yield.
- *Paenibacillus* S4 and *Streptomyces* F5 were equal or better than chemical controls.
- Best performing strain reduced root disease 30% and increased yield up to 4.2%.