

Novel management strategies for the control of fusarium root rot in lentil

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Key findings

- An experimental seed treatment was observed to control some negative effects, including seedling survival (%), caused by *Fusarium avenaceum* (fusarium root rot) in lentil.
- Rhizobia growth in lentil was not affected by the presence of an experimental seed treatment.
- Further research on lentil is required to understand the interaction between the experimental seed treatment, fusarium root rot and rhizobia strain.
- The seed treatment used in this experiment is not registered for use in lentils or for the control of fusarium root rot and was used for experimental purposes only.

Introduction

Lentils are a common break crop across the Mid North of South Australia (SA). Over the past two seasons (2023 and 2024) the Upper North, Mid North and Lower North of SA combined, grew approximately 39,000 hectares of lentils per year, equating to an estimated 57,060 metric tons. This makes lentils an important commodity to SA's agricultural industry (Department of Primary Industries and Regions, 2023).

Soil borne diseases affect a range of cereal and pulse crops, significantly impacting the Australian grains industry. In wheat alone, diseases such as root lesion nematode (*Pratylenchus* spp.), and crown rot (*Fusarium* spp.) are estimated to cause losses of \$134-\$404 million each year (Murray & Brennan, 2009). As pulses are relatively new to Australian farming systems, first planted in the 1990's, (Pulse Australia, 2015) soilborne diseases affecting these crops are poorly understood. Their impacts often go unnoticed, as they are less visual compared to foliar diseases (Gontar et.al. 2024).

Surveys across Australia demonstrated that root diseases are common in pulses, and further work has led to the identification of key soilborne pathogens likely reducing pulse yields (Gontar et.al. 2024). Pathogens such as *Fusarium avenaceum*, *Rhizoctonia solani* AG8 (rhizoctonia root rot), and *Didymella pinodella* (the major pathogen of the ascochyta blight foliar disease complex of field pea) are commonly found in soil and root DNA tests through the Mid North of South Australia, and across other Australian growing regions. The effect these pathogens have on lentil yield is not well known, however approximately 20% of poor performing pulse roots were found to have *Fusarium avenaceum* present (Gontar et.al. 2024).

A field trial was established at the Hart field site to quantify yield loss from fusarium root rot in lentil, and an experimental seed treatment was identified that can potentially reduce yield loss. A controlled environment experiment was established at SARDI at the South Australian Waite Research Institute to validate field observations and effects of the seed treatment control of *F. avenaceum*. The aim of this controlled environment experiment was to investigate the benefit lentil producers might observe from the use of a suitable seed treatment where *F. avenaceum* is known to be present.

Methodology

Trial design and treatments

In 2024, a lentil pot experiment was implemented at SARDI at the South Australian Waite Research Institute. This experiment was conducted on PBA Hallmark lentils and was designed as a two-way randomised complete block design with six replicates, using RStudio statistical software. The experiment had two main treatments: no seed treatment (nil), and an experimental seed treatment applied at 80 mL/100 kg seed. Three concentrations of *F. avenaceum* pathogen were applied as colonised millet grain inoculum, alongside a control treatment (sterile millet – no inoculum) (Table 1). The seed treatment used in this experiment is not registered for the control of fusarium root rot in lentil and was used for experimental purposes only.

Table 1. Treatment combinations used in the 2024 pot experiment at SARDI Waite Research Institute to assess the effects of an experimental seed treatment on fusarium root rot in lentils. The concentration rates of the pathogen are in relation to the total soil weight (% w/w).

Seed Treatment	Pathogen
Nil	Sterile millet (nil)
Seed treatment	Sterile millet (nil)
Nil	0.25% w/w <i>F. avenaceum</i>
Seed treatment	0.25% w/w <i>F. avenaceum</i>
Nil	0.5% w/w <i>F. avenaceum</i>
Seed treatment	0.5% w/w <i>F. avenaceum</i>

Methods and assessment

The pot experiment was established on August 21, 2024, using a fine sand and peat (UC) potting mix blend. Prior to sowing, the potting mix was autoclaved, a process involving steam treatment to remove bacteria and other organisms which may impact experiment results.

The pathogen inoculum, applied at seeding, was produced by adding 7-day old cultures of *F. avenaceum* growing on a petri dish made from Potato Dextrose Agar (PDA) to a sterile plastic bag containing 1.5 kg of sterile millet grain. The bags were gently mixed every two days to encourage full colonisation on millet grain. This pathogen inoculum was left to develop until all grains were colonised, before being dried at 40°C for seven days, and then passed through a 2 mm sieve to produce a homogeneous, flowable inoculum. To get two rates of the fusarium pathogen (0.25% and 0.5%), two separate bags of sterile millet were coated with each fusarium pathogen rate. The millet was left for the *Fusarium avenaceum* to adequately grow.

Colonised millet grain was added to soil at a rate of either 0.25% w/w (weight to weight ratio) or 0.5% w/w of UC soil mix. The inoculum was thoroughly mixed through the soil before being added to pots. Control treatments (nil inoculum) were prepared using non-colonised sterile millet grain applied at 0.5% w/w UC soil mix.

Lentil pots that received the seed treatment were coated on the day of sowing. Lentils in treatment pots which did not contain a seed treatment (nil) were not coated but all other methods remained the same. Following application, all seeds including nil treatments were sown dry at a rate of five seeds per pot.

Pots were placed in a glasshouse and maintained at a constant temperature of 20°C. The moisture level in each pot was maintained at 80% of field capacity. Field capacity was calculated by preparing a pot with soil for sowing as above (with no seeds) and watering the pot until the water drained heavily through the bottom of the pot. After leaving the pot to drain upright on a wire rack for 24-hours, it was watered to saturation using the same method. The soil, now at maximum saturation, and the pot the soil is contained in was weighed, and that weight was recorded. Next, the soil only was transferred into a silver aluminium foil tray, which was placed in a specialised drying oven at 100°C for twenty-four hours. After drying, the soil was weighed again, and 80% of field capacity was calculated. From the calculation results, 80% field capacity was achieved by watering every pot up to a weight of 448 g every two days.

To promote normal nodulation, rhizobia was added to every pot after seedling emergence as 1 mL of liquid rhizobia suspension containing 10^9 cfu/mL per seedling (cfu = colony-forming unit). The suspension was pipetted directly onto the seedling base.

Measurements conducted during this experiment included plant establishment as the number of germinated plants and number of surviving seedlings three weeks later, shoot weight (g), root weight (g) and root health scores (scale 0–10, where 0 = no disease and 10 = total root infection and lesioning).

Plant establishment counts were first conducted on September 2, and again three weeks later on September 19, with the aim to determine how the presence or absence of the seed treatment and varying pathogen rates (nil 0%, 0.25% and 0.5%) impacted seedling survival. On September 19, plants were removed from pots to conduct lentil shoot and root measurements. Using scissors, the root and shoot system were separated and placed into a drying oven at 60°C for 48 hours. Once removed from the oven, root and shoot weights were recorded. Root weight and root health data could not be analysed due to unforeseen contamination factors negatively influencing results.

A second experiment was established on September 20 at SARDI, Waite Research Institute, to test the effect of experimental seed treatment on rhizobia growth. This was conducted on a petri dish made from Potato Dextrose Agar (PDA) under controlled temperature conditions to investigate impacts of rhizobia growth resulting from the presence of the seed treatment.

Distances were measured from the centre of the rhizobia growth to the edge of the colony, and from the centre of the rhizobia growth to the centre of the cardboard disk onto which the seed treatment was pipetted.

Results and discussion

Plant establishment

Treatments with the fusarium pathogen present at both concentrations of 0.25% and 0.5% w/w, without a seed treatment, showed reduced plant numbers with only 33% survival rate (0–2 plants per plot surviving out of 5), compared to 87%, with the inclusion of the experimental seed treatment (Figure 1). Similar results were observed between treatments with no pathogen (nil treatment) and those where seed treatment + pathogen was present. This result shows the inclusion of the experimental seed treatment provided effective control of *Fusarium* root rot for seedling survival.

Shoot weight

Significant differences were observed for shoot weight, with the lowest weights recorded in treatments where *Fusarium avenaceum* was applied at 0.25 and 0.5% with no applied experimental seed treatment.

Reduced shoot weight was observed for seed treatment + 0.5% *Fusarium avenaceum* when compared to nil treatments (no pathogen as sterile millet +/- seed treatment). The findings indicate that although seed treatment had a positive influence on plant survival rate, a reduction in individual plant biomass was observed where higher levels of fusarium were present (Figure 2).

When compared to various treatments, the absence of the pathogen allows for optimal growth, indicating that the presence of fusarium root rot is a limiting factor to plant performance and seed treatment alone may not completely reduce the pathogen's effects (Figure 1).

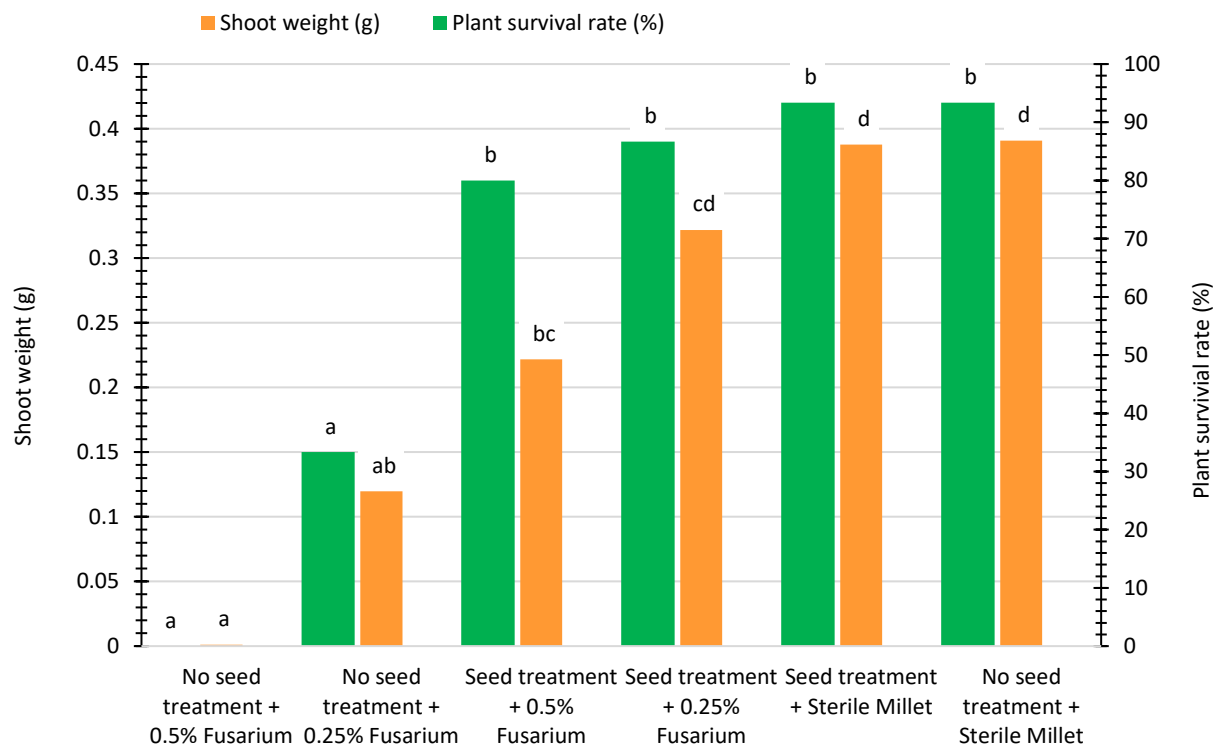


Figure 1. Comparison of the effect of a seed treatment and *Fusarium avenaceum* concentrations on plant counts and shoot weight in lentils. Bars for each measurement with the same letters are not significantly different.



Figure 2. Visual comparison of treatments in replication 1 of the pot trial, showing biomass differences across pathogen concentration +/- seed treatment. Top (L-R): No seed treatment + no fusarium, no seed treatment + 0.25% fusarium, no seed treatment + 0.5% fusarium. Bottom (L-R): Seed treatment + no fusarium, seed treatment + 0.25% fusarium, seed treatment + 0.5% fusarium.

Potato Dextrose Agar (PDA) results

Results from the Potato Dextrose Agar (PDA) plate experiment showed that the presence of experimental seed treatment did not affect rhizobia growth (Figure 3). Rhizobia was spread across the agar plate and small cardboard disks containing seed treatment concentrations of 0%, 0.3%, 1% and 3% were delicately placed on top. The result, as shown in Figure 3, demonstrates that in a controlled environment, the rhizobia growth is not affected by the presence of the experimental seed treatment. Results suggest that the addition of this seed treatment is beneficial, and rhizobia growth is not affected. Further research across various environmental conditions and soil types should be considered to validate 2024 results.

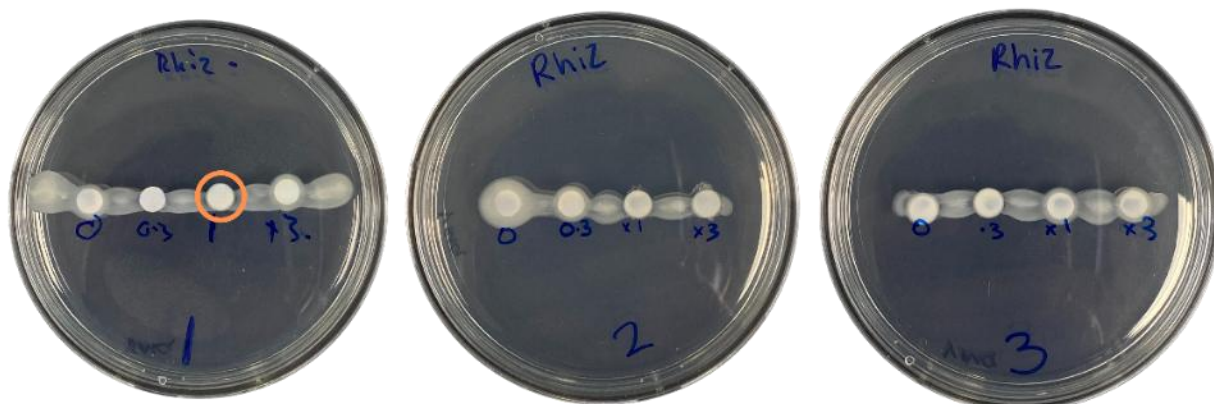


Figure 3. Rhizobia and seed treatment interaction on agar plates. Photos show replication 1-3 (L-R).

Summary

This report investigates novel management strategies for controlling fusarium root rot in lentils, focusing on an experimental seed treatment not registered for use on lentils. While lentils have been part of the Australian agricultural industry since the early 1990s, their role has evolved from a break crop to a significant export product. Soilborne diseases, particularly fusarium root rot, pose challenges for lentil growers, with no sufficient fungicide treatments available.

Results suggest that the experimental seed treatment used in this study, particularly where lower concentrations of fusarium root rot were present, show good control. Results from the Potato Dextrose Agar (PDA) plate experiments show no negative effects on rhizobia growth from the experimental seed treatment. Future research opportunities would be beneficial to explore the interactions between this seed treatment and rhizobia, as well as the control of fusarium root rot in additional field studies.

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