Research project. The interactions between arbuscular mycorrhizal fungi, rhizobia and the root-lesion nematode *Pratylenchus thornei* in mungbean

Authors E.C. Gough[1*], K.J. Owen[1], R.S. Zwart[1], J.P. Thompson[1]

[1]Centre for Crop Health, Institute for Life Sciences and the Environment, University of Southern Queensland, Toowoomba, Queensland, Australia

* Correspondence:
Corresponding Author
elaine.gough@usq.edu.au

Abstract

Mungbean hosts the beneficial nitrogen-fixing *Bradyrhizobium* bacteria, arbuscular mycorrhizal fungi (AMF) and the parasitic root-lesion nematode *Pratylenchus thornei*. Yield constraints in the subtropical grain region include nodulation failure and parasitism by *P. thornei*. We investigated the interactive effects of AMF, *Bradyrhizobium, P. thornei*, nitrogen (N), phosphorus (P) and zinc (Zn) in a vertisol in glasshouse pot experiments using a factorial design of treatments. Biomass, yield, nodulation, biological N fixation, *P. thornei* population densities, mycorrhizal colonisation and plant shoot nutrition were assessed after 6 and 11 weeks. Rhizobia and AMF acted synergistically to increase biomass, yield, nodulation, biological N fixation, and plant nutrition. Mungbean inoculated with rhizobia and AMF had increased biological N fixation compared to co-inoculations with rhizobia and fertiliser P, due to increased P concentration and uptake in mycorrhizal plants. Nitrogen uptake to the plant was greater following inoculations with rhizobia compared to inoculations with N. Inoculation with AMF increased the plant concentration and uptake of P and Zn, greater than the application of these fertilisers. AMF also increased the concentration of Cu. However, AMF colonisation increased *P. thornei* population densities, which did not correlate with increased root biomass, or increased shoot concentration of N, P or Zn. Mycorrhizal plants infested with low levels of *P. thornei* (1 nematode/g soil and roots) had reduced biomass, nutrient uptake and biological N fixation. These results highlight the crucial benefits that the symbiotic organisms AMF and *Bradyrhizobium* contribute to improve mungbean productivity and yields in soils of the subtropical grain region. Alternative management methods are required to limit *P. thornei* reproduction on mycorrhizal mungbean, such as breeding *P. thornei* resistant mungbean cultivars.

Executive Summary

Mungbean (*Vigna radiata*) is a short season high value legume grown in summer in the subtropical grain region of Eastern Australia. Mungbean has been integrated into the cereal cropping systems due to its role as a crop break for cereals, and its ability to fix nitrogen (N) via a symbiosis with *Bradyrhizobium* bacteria which converts atmospheric N_2 into the plant available ammonia (NH_3) in nodules in plant roots. Agronomic practice of inoculation of mungbean with the commercial *Bradyrhizobium* isolate CB 1015 should increase N fixation, fixing sufficient N to support the crops own growth. However, surveys undertaken in the region demonstrated that the majority of crops are poorly nodulated, and fixed only 30% of their N requirements. This can lead to yield losses of up to 50% where soil nitrate levels are already low (Herridge et al., 2005; Gentry 2010).

Arbuscular mycorrhizal fungi (AMF) are soil-borne beneficial fungi that are symbiotically associated with the roots of many crop species grown in the region including mungbean. Spores in the soil germinate in the presence of a suitable host and the fungal hyphae infiltrate the plant roots, exchanging the plants photosynthetic carbon for increased uptake of poorly mobile nutrients such as P and Zn from the soil and fertiliser sources (Smith & Read, 2010). Arbuscular mycorrhizal fungi have also been implicated in alleviating biotic stress caused by plant pathogens (Whipps 2004) and altering the population densities of *Pratylenchus* spp. (Hol & Cook 2004; Yang et al., 2014). However, the magnitude and direction of the nematode population changes may depend on the AMF species/genus and crop host (Gough et al., 2020). Crops such as sorghum, maize, sunflower, chickpea and mungbean have a high level of mycorrhizal dependency- that is, they require good colonization of their roots with mycorrhizal fungi to obtain adequate yields and a lack of AMF can result in a failure to yield well even under sufficient levels of soil nutrition (Thompson et al., 1994). Inoculation with AMF and rhizobia has been reported to increase yields, biomass and nodulation in other legumes such as chickpea and French bean (Chalk et al., 2006). We hypothesized that nodulation failure evident in mungbean crops may be related to a lack of mycorrhizal spores present in the soil in the region.

The root-lesion nematode *Pratylenchus thornei* is a plant parasitic nematode distributed in up to 67% of paddocks in the region (Thompson et al., 2010). On entering the root cortex, the nematode feeds and migrates through the root cells, damaging the tissues, which results in a reduction in nutrient and water uptake and a subsequent loss of yield in intolerant and susceptible host crops (Thompson et al., 2012; Whish et al., 2014; Owen et al., 2019). Mungbean as a susceptible host crop, allows the multiplication of *P. thornei* in its roots resulting in yield loss, especially under conditions of water stress. Increases in *P. thornei* populations can result in yield in subsequent intolerant crops in the cropping sequence (Owen et al., 2014). *Pratylenchus* spp. may also affect reduce nodulation and biological N fixation in other crops such as in chickpea and soybean (Castillo et al., 1998; Hussey and Barker 1976).

We hypothesised that (a) AMF may lead to improved nodulation, biological N fixation, yield and biomass in rhizobia-inoculated mungbean plants, (b) there is an interaction between AMF and *P. thornei* and AMF colonisation that will lead to changes in *P. thornei* populations densities in mungbean roots, (c) *P. thornei* may affect nodulation and biological N fixation in mungbean and, (d) inoculation of AMF will improve the availability of N, P and Zn to the plant despite constraints by *P. thornei* infestation.

Inoculation with AMF and rhizobia acted synergistically to increase plant biomass (1.7 to 4.0 times), seed yield (2.7 to 4.4 times), nodulation (2.0 to 4.5 times), biological N fixation of all parameters measured and to significantly increase the uptake and concentration of N, P and Zn.

Plants inoculated with *P. thornei* alone did not have a significant reduction in biomass or seed yield in either experiment. However, plants co-inoculated with AMF and *P. thornei* had reduced biomass and seed yield at low rates of 1 *P. thornei*/g soil. At higher rates of 10 *P. thornei*/g soil there was no reduction in biomass or seed yield. *Pratylenchus thornei* increased the concentration of N in the plant in both experiments. Nodulation was reduced by *P. thornei* at early times of assessment. The % N derived from the atmosphere (% Ndfa) - a measure of N fixation by legumes- was not affected by any level of *P. thornei* but low rates of *P. thornei* in the presence of AMF had a reduced amount of fixed N to the plant as compared to AMF plants alone. Low rates of *P. thornei* also reduced the efficiency of mycorrhizae to increase the uptake of nutrients to the plant.

Plants inoculated with AMF had increased population densities of *P. thornei* which was not correlated to root biomass, but may be related to either a reduction in defence chemicals, alterations in phytohormone response or another unknown mechanism. This remains to be determined. Inoculation with N, P or Zn reduced *P. thornei* population densities compared to inoculation with *P. thornei* alone.

This project highlights the synergistic effect of AMF and rhizobia in improving yield, in both the presence and absence of infestation with *P. thornei.* It contributes to the understanding of nodulation failure, emphasizing the role of AMF in improving nodulation rates and biological N fixation in mungbean. Modification of cropping systems to establish adequate mycorrhizal spore abundances in the soil by growing crops that proliferate mycorrhizal spore production, while reducing *P. thornei* populations in the soil followed by a soil analysis by PreDictaB® prior to planting, may greatly increase mungbean production closing the yield gap for this high value crop.

Contents

Background

Mungbean are a short season high value legume integrated into the cereal cropping systems of the subtropical grain region of eastern Australia. As a N-fixing legume, they have the potential to fix their requirements for N through a symbiotic association with the N fixing *Bradyrhizobium* bacteria which fix atmospheric N_2 into the plant available form of ammonia (NH_3) in specialised structures called nodules on the roots. However, previous surveys undertaken in the region demonstrate that the majority of crops are poorly nodulated, fixing only 30% of their N requirements which can result in yield losses of up to 50% (Herridge et al., 2005; Gentry 2010). Nodulation failure may be attributable to factors including incorrect inoculum or inoculation techniques, high temperatures at inoculation, high levels of soil nitrate, or deficiencies of nutrients essential to nodulation such as P, molybdenum (Mo), calcium (Ca), copper (Cu) and Zn (Drew et al., 2012; O'Hara et al., 2001). The soils of the subtropical grain region are known to be deficient in many of these essential nutrients including P, Zn and Cu (Bell et al., 2010).

Arbuscular mycorrhizal fungi (AMF) are a group of beneficial organisms that evolved over 450 million years ago and form important symbiotic associations with the roots of up to 80% of all terrestrial land plants, including the roots of many economically important crops (Parniske 2008). They increase the uptake of poorly mobile nutrients such as P and Zn into the crop in exchange for the plants photosynthetic carbon (Smith & Read 2010). They achieve this increased uptake using their hyphae which can extend much further past the root exclusion zone, and 1 cm^3 soil can contain up to 100m of mycorrhizal hyphae (Miller et al., 1995). AMF have also been implicated in reducing the effects of biotic and abiotic stressors such as pathogen attack, plant-parasitic nematode infestation, salinity and drought (Whipps 2004, Yang et al., 2014, Augé 2001). Many crops in the region are highly dependent upon AMF for uptake of nutrients and for increases in yield. Crops that have a high mycorrhizal dependency in the region include sorghum, maize, chickpea, faba bean and mungbean. Other crops such as wheat, oats and barley have a lower mycorrhizal dependency, though the cultivation of these crops can increase AMF spore levels in the soil (Thompson et al., 1997). Canola and other crops in the *Brassicaceae* are non-hosts and do not increase AMF spore levels. As an obligate biotroph, AMF depend on a living host to survive and land that has been in bare fallow will have a reduced level of AMF which can result in Long Fallow Disorder- the symptoms of which resemble P and Zn deficiency but is in fact related to a lack of AMF spores in the soil. Synergism between AMF and rhizobia can increase yields, plant nutrition, nodulation and N fixation in other legumes such as chickpea and soybean (Chalk et al., 2006; Javaid 2017). Our hypothesis is that nodulation failure may be caused by a lack of AMF spores in the soils under mungbean production.

Mungbean is also a susceptible host to the root-lesion nematode *Pratylenchus thornei.* This plant parasite feeds and migrates through root tissue, resulting in a loss of root function and a reduction of water uptake to the plant, resulting in subsequent yield losses in intolerant host crops (Owen et al., 2010). *Pratylenchus thornei* is the species of nematode commonly found in the subtropical grain region (Thompson et al., 2010). Cultivation of susceptible host crops allows the multiplication of the nematode, increasing the populations to damaging levels in the soil and roots. The economic damage threshold of 2 *P. thornei*/g soil can have detrimental effects on subsequent intolerant crops.

Pratylenchus, AMF and rhizobia occupy a very similar ecological niche in the plant roots. In the literature, interactions can occur between AMF and *Pratylenchus* resulting in suppressive, stimulatory or neutral effects of AMF on *Pratylenchus* reproduction depending on AMF species and/or crop hosts (Gough et al., 2020). Nematodes may also interact with rhizobia resulting in reduced nodulation levels and negative effects on N fixing capacity (Castillo et al., 2008; Hussey & Barker 1976). The interaction between host, parasitic nematode and beneficial symbionts is likely to be quite specific. As yet, there has been no research into the interaction between these organisms in the northern region in mungbean.

Project objectives

1) To determine how AMF, rhizobia and *P. thornei* alone and together affect the parameters seed yield, plant biomass, nodulation rates and the uptake of N, P and Zn.
2) To determine the effects of AMF, rhizobia and *P. thornei* alone and together on biological N fixation
3) To determine the effect of the interactions between AMF, rhizobia and *P. thornei* when the levels of N, P and Zn are increased.

4) To determine the effect of the interactions between AMF, rhizobia and *P. thornei* on biological N fixation when the levels of N, P and Zn are increased

5) To determine the effect of co-inoculation of AMF and *P. thornei* on nematode reproduction and AMF colonisation of the plant roots.

Methodology

The first factorial experiment (designated 'Experiment 1') was designed to assess the interaction between AMF*, P. thornei* and rhizobia. Treatments of AMF (two levels), *P. thornei* (three levels) and rhizobia (two levels) were randomised in a split plot design. Inoculations were made at planting with three rates of *P. thornei* (0/g, 1/g and 10/g oven dried (O.D) soil equivalent) and two rates of an isolate of AMF species *Funneliformis mosseae,* isolated from the subtropical grain region (0/spores g, 16/spores g O.D equivalent). The *Bradyrhizobium* isolate used was the commercial isolate CB1015. No additional fertiliser was added.

In the second fully factorial experiment (designated 'Experiment 2') to assess the interaction between AMF, *P. thornei,* rhizobia and the nutrients N, P and Zn. Treatments of plus and minus treatments of AMF, *P. thornei*, rhizobia, N, P and Zn were randomised in a split plot design. Inoculations were made at planting using 10 *P. thornei* /g O.D equivalent, 16 *F. mosseae* spores/ g O.D soil equivalent, the commercial *Bradyrhizobium* CB1015 and nutrient treatments of Zn at 15 mg/kg soil as $ZnCl_{2,}$ P at 50 mg/kg soil as NaH_2PO_4 and N at 200 mg/kg soil as $Ca(N0_3)_{2.}$ Soil chemical properties for both experiments were as follows; pH (1:5 (soil:water) 8.5, Nitrate- N (2M KCl) 24.5 mg/kg, Colwell Phosphorus 45 mg/kg, Zinc 1.45 mg/kg.

Plants were assessed at 6 and at 12 weeks (at the vegetative and reproductive stages) after inoculation in Experiment 1, and 6 and 11 weeks in Experiment 2. Variables that were quantitatively assessed included plant dry biomass, seed weight, nodulation biomass and counts and root biomass from a 150 g subsample of homogenised soil and roots, *P. thornei*/kg soil, AMF % colonisation, and leaf chemistry measurements including total $\delta^{15}N$. The data was analysed using a split plot ANOVA, followed by Bonferroni's post hoc test on significant values using Genstat. Data for nodulation was square root transformed and *P. thornei* numbers were log transformed prior to statistical analysis.

Results

AMF and rhizobia interacted synergistically to increase seed yield, biomass, nodulation and uptake of nutrients including N, P and Zn to the plant

At 12 weeks, inoculation of AMF and rhizobia resulted in a synergistic effect (Fig. 1) resulting in a 6-fold increase (P<0.001) in both shoot biomass and seed yield compared to rhizobia inoculated plants alone in Experiment 1 (Fig. 2A and B). Co-inoculation with AMF and rhizobia increased nodule counts per plant 5-fold and nodule weight per plant 23-fold (P<0.001) (Fig. 2C and D).

Fig. 1. Illustration of the synergistic effects of co-inoculation with arbuscular mycorrhizal fungi (AMF) and rhizobia on mungbean growth at 12 weeks in Experiment 1. Treatments are as follows; L to R, 1: nil AMF, nil rhizobia, 2: + rhizobia, nil AMF, 3: nil rhizobia, + AMF, 4: + rhizobia, + AMF. Photo source: Elaine Gough, USQ

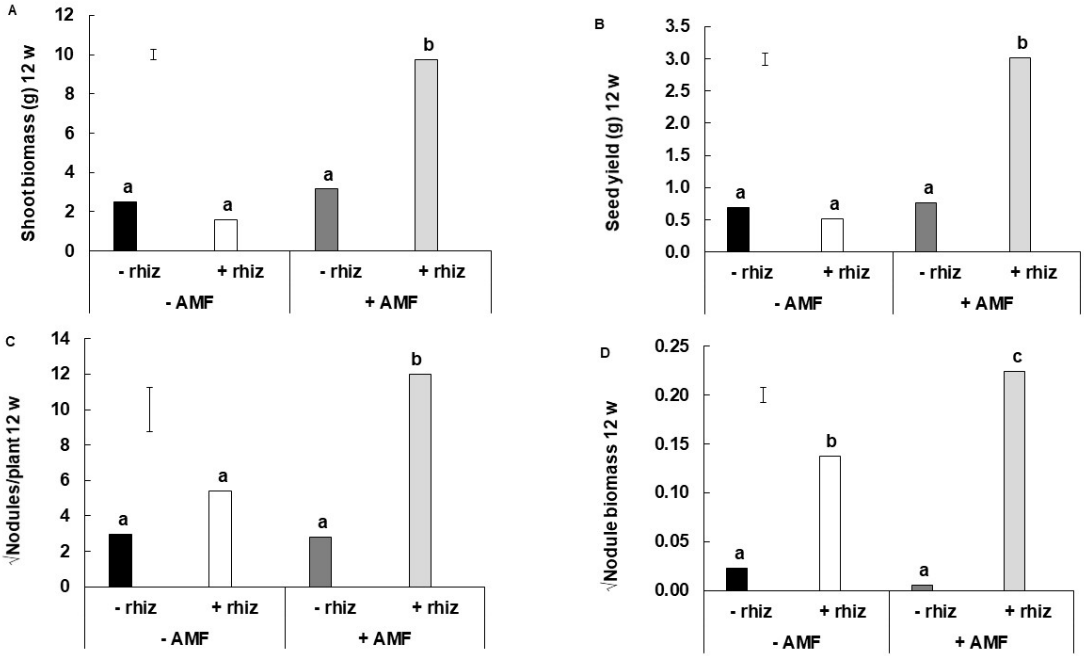

Fig. 2. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and rhizobia (rhiz) on **(A)** shoot biomass per plant, **(B)** seed yield per plant, **(C)** nodule counts per plant and, **(D)** nodule weight per plant in mungbean at 12 weeks after sowing in Experiment 1. Nodule counts and biomass are square root transformed. Different letters above each bar in each figure indicate significant differences according to the Bonferroni test for multiple comparisons at *P*=0.05 for the interaction of AMF x rhizobia. The vertical bar represents the standard error of difference (s.e.d).

Inoculation with AMF significantly (P<0.001) increased the uptake of P and Zn while co-inoculation of AMF and rhizobia significantly increased further the uptake of N, P, and Zn at 12 weeks in Experiment 1 (Fig. 3A-C).

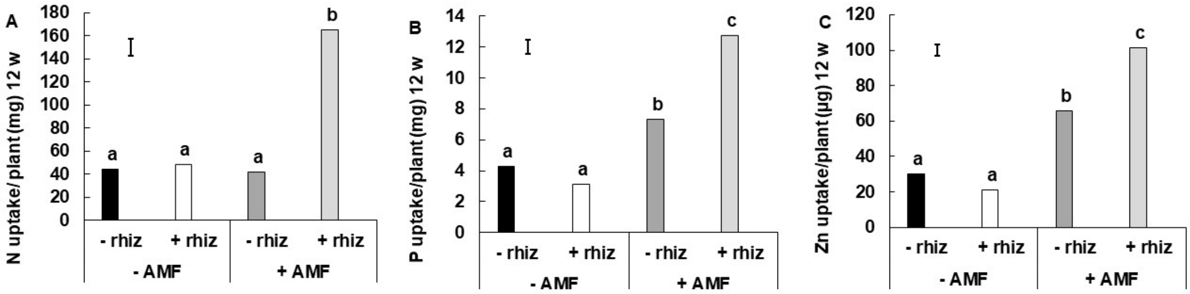

Fig. 3. The effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and rhizobia on the uptakes of **(A)** N (mg/plant), **(B)** P (mg/plant), and **(C)** Zn (µg/ plant) 12 weeks after sowing in Experiment 1. Different letters above each bar in each figure indicate significant differences according to the Bonferroni test for multiple comparisons at at *P*=0.05 for the interaction of AMF x rhizobia. The vertical bar represents the standard error of difference (s.e.d).

AMF and rhizobia increased biological N fixation to the plant versus rhizobia alone

Co-inoculation with AMF and rhizobia increased biological N fixation parameters of % N derived from the atmosphere (% Ndfa) (Fig. 4A), N uptake to the plant, (Fig. 4B), and fixed N (Fig. 4C) to the plant compared to plants inoculated with rhizobia alone in Experiment 1.

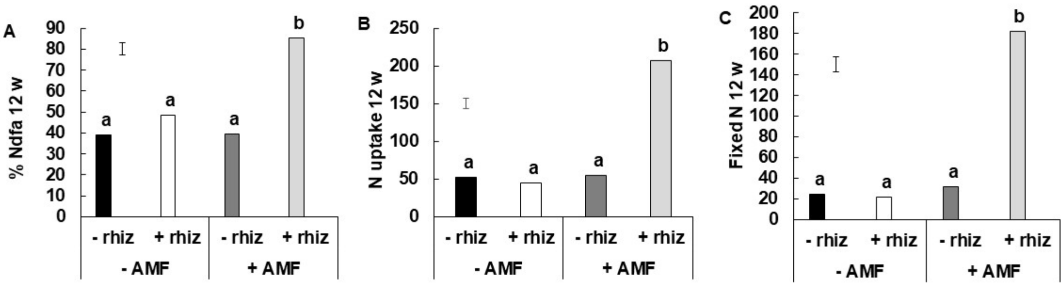

Fig. 4. The interactive effects of co-inoculation with arbuscular mycorrhizal fungi (AMF) and rhizobia on **(A)** percentage N derived from the atmosphere (% Ndfa), **(B)** N uptake (mg) and, **(C)** Fixed N from biological N fixation (mg) per plant in mungbean at 12 weeks in Experiment 1. Different letters above each bar in each figure indicate significant differences according to the Bonferroni test for multiple comparisons at at *P*=0.05 for the interaction of AMF x rhizobia. The vertical bar represents the standard error of difference (s.e.d).

Interactions between P. thornei*, AMF and rhizobia on biomass, seed yield, nodulation and plant nutrition*

Plants inoculated with *P. thornei* alone did not have a significant reduction in biomass or seed yield in either experiment. However, plants co-inoculated with AMF and *P. thornei* had a reduced biomass and seed yield in Experiment 1 but only at low rates of 1 *P. thornei*/g soil (Fig 5A, B) *Pratylenchus thornei* significantly reduced the number of nodules per plant in both experiments ($P<0.05$) (data not shown). The addition of *P. thornei* to plants co-inoculated with AMF and rhizobia had significantly ($P<0.05$) increased shoot N concentration (%) compared to plants in the absence of the nematode in both experiments (data not shown).

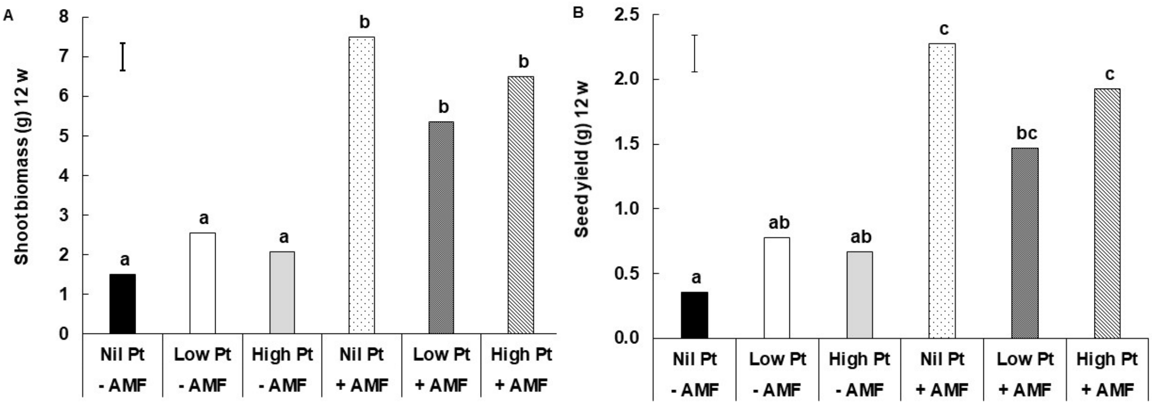

Fig. 5. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and *Pratylenchus thornei* (PT) on **(A)** shoot biomass per plant, **(B)** seed yield per plant at 12 weeks after sowing in Experiment 1. Different letters above each bar in each figure indicate significant differences at *P*=0.05 for the interaction of AMF x *P. thornei*.

Inoculation with AMF was comparable to fertilisation with P to improve N fixation, biomass, yield, nodulation and shoot P concentration in rhizobia inoculated plants

In Experiment 2, the addition of either P or AMF alone to rhizobia-inoculated plants significantly (*P*<0.05) increased the % Ndfa (6A), Fixed N (6B), shoot biomass and seed yield (6C), nodulation counts (6D) and nodule biomass (6E), and shoot P concentration (%) (6F) compared to inoculation with rhizobia alone. Inoculation with AMF significantly (*P*<0.05) increased % Ndfa (5A), nodule biomass (6E) and shoot P concentration (%) (6F) to a level greater than fertilisation with P. There was no further increase in the levels of these variates on adding both AMF and P together compared to inoculation with AMF alone.

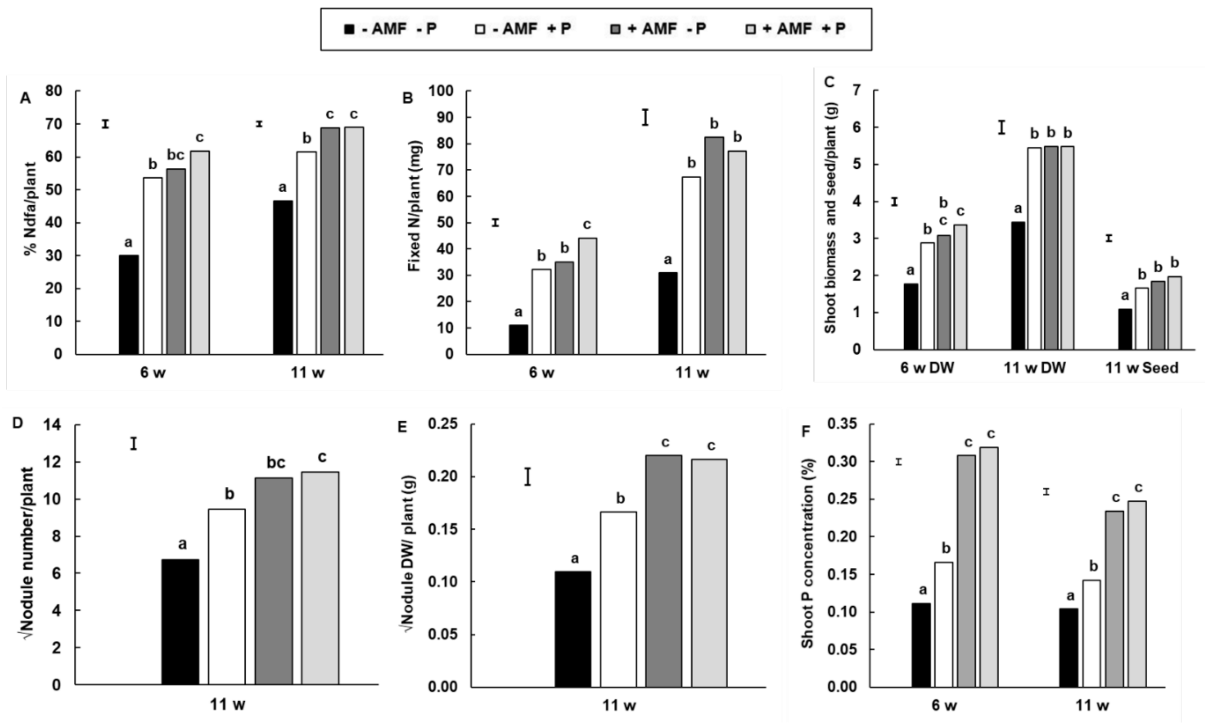

Fig. 6. The interactive effects of co-inoculation of arbuscular mycorrhizal fungi (AMF) and P in rhizobia-inoculated plants at 6 and/or 11 weeks (w) in Experiment 2 on **(A)** % nitrogen derived from the atmosphere (% Ndfa), **(B)**

fixed nitrogen (N) (mg/kg) in the plant, **(C)** shoot biomass/plant (DW) and seed weight/plant, **(D)** nodule number per plant, **(E)** nodule dry weight per plant and, **(F)** shoot P concentration (%). Nodule number per plant and nodule dry weight/ plant are means of square root transformations. Different letters above each bar graph at 6 or 11 weeks indicate significant differences for each variate separately according to the Bonferroni test for multiple comparisons at *P*=0.05 for the interaction of AMF x P. The vertical bar represents the standard error of difference (s.e.d.).

Inoculation with rhizobia increased biomass, yield, nodule biomass, and N concentration and uptake greater than fertilisation with N

In Experiment 2, inoculation with rhizobia significantly (*P*<0.05) increased the shoot biomass and seed yield (Fig. 7A), nodule biomass (Fig. 7B), shoot concentration of N (%) (Fig. 7C), N uptake to the plant (Fig. 7D) greater than fertilisation with N. There was a significant (*P*<0.05) reduction in yield, nodule biomass, and N concentration and uptake at 11 weeks in plants with added N and rhizobia together compared to rhizobia alone (Fig. 7A-D).

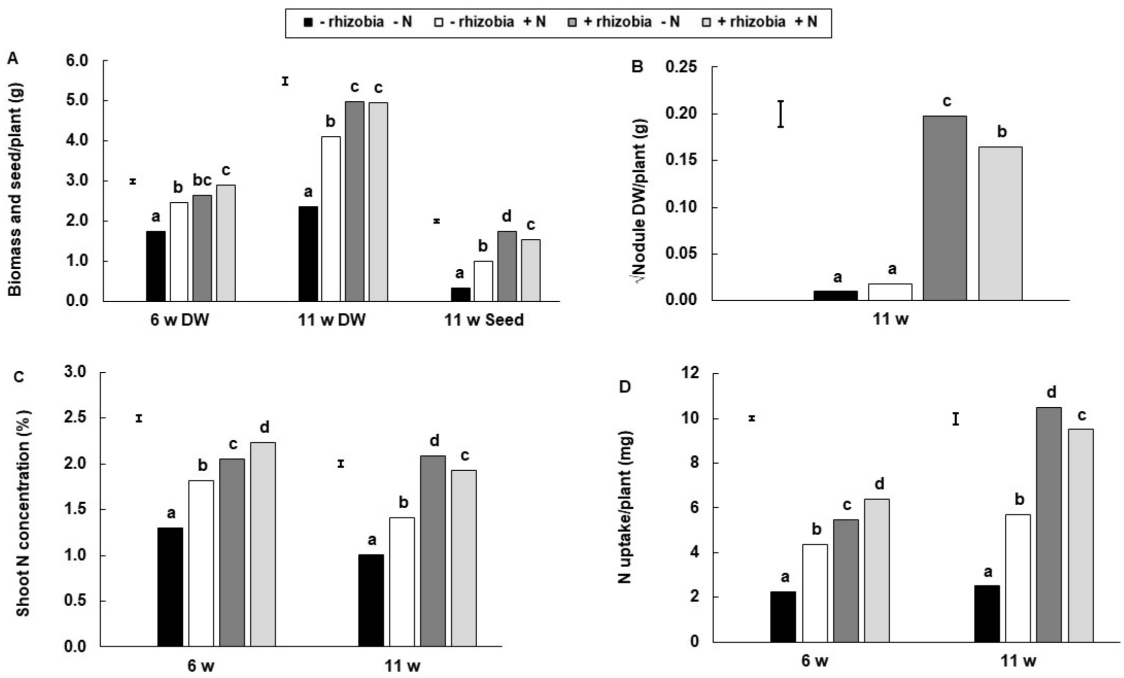

Fig. 7. The interactive effects of rhizobia and nitrogen (N) at 6 and/or 11 weeks (w) on **(A)** shoot biomass (DW) and seed yield, **(B)** nodule dry weight/plant, **(C)** shoot N concentration (%), **(D)** shoot P concentration (%), **(E)** shoot Zn concentration (mg/kg), **(F)** N uptake/plant (mg), **(G)** P uptake/plant (mg) and, **(H)** Zn uptake/plant (µg). Nodule dry weights are means of square root transformations. Different letters above each bar graph at 6 or 11 weeks indicate significant differences for each variate seperately according to the Bonferroni test for multiple comparisons at *P*=0.05 for the interaction of rhizobia x N. The vertical bar represents the standard error of difference (s.e.d.).

AMF increased P. thornei *populations while adding P, Zn or N reduced* P. thornei *populations*

In plants inoculated with AMF, *P. thornei* reproduction increased as compared to plants inoculated with *P. thornei* alone (Fig. 8), which was not correlated to root biomass (data not shown). Plants inoculated with *P. thornei* and either P, Zn or N had a significant reduction in *P. thornei* reproduction as compared to plants inoculated with *P. thornei* alone (Fig. 8).

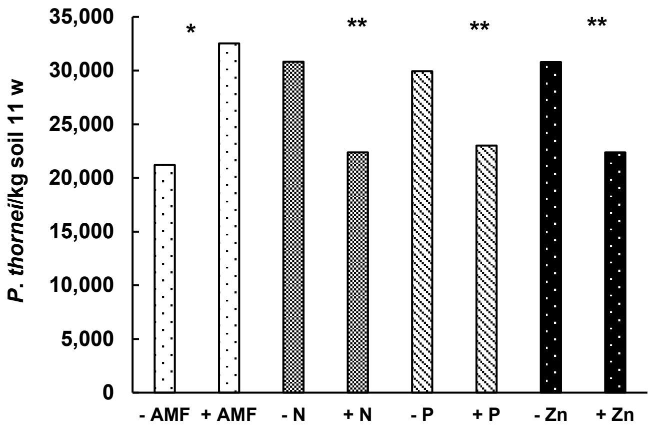

Fig. 8. The main effects of arbuscular mycorrhizal fungi (AMF) (-/+), nitrogen (N) (-/+), phosphorus (P) (-/+), and zinc (Zn) (-/+) on the population densities of *Pratylenchus thornei* in mungbean at 11 weeks after sowing in Experiment 2. *Pratylenchus thornei* values expressed are back transformed means from ANOVA. * denotes significance levels at $P<0.05$, ** denotes significance at $P<0.01$.

Mycorrhizal colonisation was reduced by rhizobia

In both experiments, the percentage root length colonised with AMF was high at early stages of assessment at 59% and remained high at 72% at 12 weeks. *Pratylenchus thornei* did not affect the percentage root length colonised by AMF but plants inoculated with both AMF and rhizobia had a significantly lower percentage root length colonisation by AMF (42%) as compared to roots inoculated with AMF alone (72%) ($P<0.05$). In Experiment 2, fertilisation with P did not reduce the percentage mycorrhizal colonisation at 6 or 11 weeks.

Discussion of Results

Co-inoculation with AMF and rhizobia resulted in a clear synergistic effect resulting in increased biomass, seed yield, nodulation, biological N fixation, and increased uptake of nutrients to the mungbean plant compared to plants inoculated with rhizobia alone (Gough et al., 2021). Co-inoculation also increased the plant uptake of nutrients N, P and Zn.

Legumes grown under P deficient conditions preferentially transfer their P supply into nodules, removing P from leaves and seeds to maintain the N fixing process (Schwember et al., 2019). Mycorrhizal fungi use their fine extraradical hyphae, which extends further into the soil past the root depletion zone, to increase the uptake of immobile P in the soil to the plant roots (Parniske 2008). In our experiments, inoculation with AMF increased the concentration and uptake of P and Zn in the plant to a level greater than the application of fertilizer P and Zn. Increased inflow of P and Zn has also been demonstrated in linseed and was related to high levels of AMF colonization (Thompson et al., 2013). The improved concentration and uptake of P in mycorrhizal mungbean significantly increased nodulation and the efficiency of biological N fixation as measured by % Ndfa to a greater level than fertilization with P in rhizobia inoculated plants. Inoculation with AMF also improved the supply of other nutrients like Zn and Cu to the plant which are essential to the N fixing process (O'Hara 2001). This research indicates the important role of AMF in improving biological N fixation efficiency, biomass and yield in mungbean in the subtropical grain region.

Mungbean is known to be responsive to high levels of P (GRDC 2017) and this demand for P was evident in this research for both biomass production and for seed yield. When AMF levels are low or undetectable, higher rates of fertilisers containing P or Zn should be applied to minimize yields loss. However, the huge beneficial effect AMF have on biomass, yields, nutrient uptake, nodulation and N fixation, along with other benefits such as improving soil stability (Rillig et al., 2015) and increasing water uptake (Auge, 2001) needs to be emphasized.

After a long bare fallow, or after cultivation of a crop that is not a host for AMF such as canola, spore levels in the soil are likely to be low as AMF depends on a living host for its survival. Spore levels in the soil can be assessed quantitatively using tools such as PreDictaB®, and detection levels below 10 kilo-copies of DNA/g soil can result in yield losses of up to 77% in mungbean when P levels in soil are low (7.5-17.5 mg P/kg Colwell P) (Thompson 1997). However, the use of PreDictaB® for assessment of AMF levels needs further research to calibrate the range of risk categories for AMF levels on yield loss to crops in soils with differing fertility levels. Growing a crop with a low mycorrhizal dependency, but retaining the capacity to multiply spores up in the soil such as wheat, oats and barley could be a strategy to increase AMF levels while not jeopardizing yield potentials, as these crops are less dependent on AMF for yields as compared to other highly dependent crops. Sunflower, sorghum, maize, chickpea and mungbean are crops with high mycorrhizal dependencies and therefore low levels of AMF spores in the soil may lead to yield penalties (Thompson et al., 1997). Further research to establish which crops and varieties maximize spore production in the cropping sequence is warranted.

Common soil deficiencies in the subtropical grain region include N, P, K, Zn and Cu (Bell et al., 2012), therefore the role AMF plays in increasing the concentration and uptake of these nutrients to mungbean needs to be highlighted. One of the major importers of mungbean from Australia is the Indian subcontinent and the Middle East, regions which suffer from Zn deficiencies in the general population (Cakmak et al., 2017). As AMF increases Zn concentration and uptake to the plant, it may be important in reducing these deficiencies in the grain and could be promoted for its role in biofortification (Pellegrino et al., 2014). Further research to investigate this is required.

Inoculation with *P. thornei* alone did not affect yield or biomass. However, co-inoculation of low levels of *P. thornei* and AMF resulted in a reduction of seed yield and biomass in mycorrhizal plants. Nodule counts were reduced by *P. thornei* infestation and analysis of plant nutrition demonstrated that *P. thornei* increased shoot concentration of N (%). As rhizobia nodulate and fix N more efficiently when N levels are low, this increased shoot N concentration could have had a knock-on effect on reducing nodule counts. However, this did not appear to significantly reduce the % Ndfa or fixed N to the plant. Control of *P. thornei* population densities by growing crops in rotations that are resistant or tolerant to the nematode is required to reduce levels below the economic damage threshold of 2/g soil (Owen et al., 2019).

Plants inoculated with AMF had increased population densities of *P. thornei.* This was not related to root biomass, or increased nutrition to the roots as inoculation with N, P or Zn reduced *P. thornei* populations. The role of changes in defence metabolites, phytohormones or other, as yet undetermined mechanism require elucidation. As interactions between AMF species, nematode, crop and cultivar are likely to be quite specific (Gough et al., 2020), further investigations are warranted to determine the interactions between AMF and *P. thornei* on different mungbean cultivars, and extend this research into investigations between AMF and *P. thornei* in different crops grown in the subtropical grain region.

Conclusion

Mungbean are a high value short season legume integrated into cropping rotations in the region. However, as nodulation failure and infestation with *P. thornei*, have been identified as a constraint to production resulting in yield gaps. Mungbean also has a high dependency on AMF for growth and yields, and low levels of AMF in soils require higher application of P and Zn fertilisers, further increasing costs to production.

Results from this research highlight the important synergistic effect between AMF and rhizobia and their combined effects on improving biomass, yield, nutrient uptake, and improving nodulation in mungbean in the subtropical grain region. The research indicates that the problem of nodulation failure in mungbean evident in the region which results in yield reduction, may be as a result of a lack of mycorrhizal spores in the soils. Furthermore, evidence from the research also indicates that AMF may aid in increasing biological N fixation potentially through supplying both P and other nutrients essential to the nodulation process. The role AMF play in increasing P and Zn concentration to the plant, improving plant nutrition is emphasized.

The interactive effects of AMF and *P. thornei* have been investigated in mungbean in this research. Both organisms are commonly found in soils of the region and the results show that *P. thornei* has a negative effect on biomass, yield and nutrition in mycorrhizal plants, while AMF increases the population of *P. thornei*. A reduction in rhizobial nodulation in mungbean may be a result of *P. thornei* infestation, potentially via increased N concentration to the plant.

Further work is required to investigate the interactive effects on more cultivars of mungbean in glasshouse and field trials. It would also be prudent to research which crops and varieties may maximize mycorrhizal spore production, and therefore improve yield and plant nutrition to subsequent mungbean crops in the sequence, while minimizing the multiplication of *P. thornei* to contribute to improved integrated pest management of *P. thornei*. It would also be of value to determine the rates of fertiliser P and Zn application in the presence and absence of AMF and therefore establish growth response curves which could advise fertilizer application efficiencies for long-term sustainability of mungbean production in the subtropical grain region.

Implications

The research aimed to investigate if nodulation failure in mungbean, and the subsequent negative impact on yield and productivity of this high value legume were affected by levels of AMF or *P. thornei* in the soil.

In this research we found that:

1) AMF interacted synergistically with rhizobia to improve biomass, yield, nutrient uptake to a much greater level than inoculation with rhizobia alone highlighting the value of this organism in improving crop production.
2) AMF improved biological N fixation by rhizobia to a greater level than the addition of P emphasizing its role in aiding rhizobia improve the N fixing process.
3) AMF increased the concentration of P and Zn in the plant- nutrients which are known to be deficient in soils of the subtropical grain region and are essential to the nodulation process.
4) AMF increased the multiplication of *P. thornei* in mungbean roots.
5) *P. thornei* reduced the biomass, yield and nutrient uptake of plants inoculated with AMF.
6) *P. thornei* may reduce nodule numbers in mungbean due to increasing shoot N concentration.

The results of the project emphasize the importance of AMF on improving yields and plant nutrition in conjunction with inoculation with rhizobia in mungbean. The importance of AMF on increasing the poorly mobile nutrients P and Zn to the plant potentially leading to efficient management of fertilizer regimes is also highlighted. The results of the project will inform growers of the benefits of management of AMF in soils in the region to improve yield stability, nodulation and close the yield gap in mungbean. The research should influence agronomic practices to manage both AMF and *P. thornei* in crop rotations, manipulating levels of both organisms which should result in increased mungbean yields.

Recommendations

The objectives of the research were to determine if AMF may aid in increasing biological N fixation by rhizobia in mungbean and if AMF may help increase yields in mungbean under constraints of *P. thornei* infestation. The results of the research indicate the significant role AMF play in improving biomass, nutrient uptake and yield stability in mungbean when co-inoculated with rhizobia in glasshouse experiments. Future research should validate the results of these experiments in field trials, using multiple cultivars of mungbean. The research should also extend to investigating the role AMF may play in improving yields of other crops grown in the subtropical grain region.

Further research is also required to establish which cultivars and crops would be most appropriate to use in rotations, to improve AMF spore levels in soils under dryland broad acre cultivation, while reducing the population of *P. thornei*. Soil analysis via quantitative tools such as PreDictaB® prior to planting is currently encouraged to assess the economic damage threshold for *P. thornei*. PreDictaB® testing is also available to assess the level of AMF in the soil. However, further research needs to be made in clarifying the risk ratings of low AMF levels to yields of various crops under differing soil nutrition especially P and Zn. When clarification of the risk ratings of low AMF levels on various crops is established, industry usage of this valuable resource by agronomists and growers will increase.

AMF levels may be increased through crop sequences of hosts that allow the multiplication of spores in the soil. As AMF require a living host to survive on, the use of cover crops or short fallows could be advantageous to improving AMF levels for subsequent crops. Further research is required to investigate which cover crops may improve spore levels while removing the least amount of nutrients and moisture from the soil profile for subsequent crops in the sequence. Commercial industries have promoted the use of AMF as a biofertilizer- improving crop nutrition and therefore improving yields. Further research is warranted to test these claims independently in glasshouse and field trials to determine if these applicants can be used as inoculants at planting similar to rhizobia to aid close the yield gap in mungbean and other crops.

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