Impact of fungicide seed coating on rhizobia survival and nodulation of pea plants

RESEARCH

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Location
Minnipa - Bruce Heddle
Rainfall
Av. Annual: 325 mm
Av. GSR: 241 mm
2018 Total: 244 mm
2018 GSR: 178 mm
Soil Type
Red sandy loam, pH_{CaCl_2}: 7.8
Plot Size
12 m x 1.8 m with 25 cm row spacing (6 rows)
Trial Design
Experimental, complete randomised

Key messages

- **Nodulation was reduced when pea seeds were coated with P-Pickel T fungicide before inoculation.**
- **Reductions in rhizobia numbers on fungicide-coated seeds occurred very quickly, within two hours, but the toxic effect of the fungicide continued after sowing.**
- **Dry soil conditions are likely to have exacerbated fungicide toxicity to the rhizobia.**

Why do the trial?

Legumes are frequently inoculated with rhizobia at sowing, to improve nodulation and nitrogen fixation. Rhizobia can be supplied as peat, freeze-dried (liquid) or granular inoculant formulations. At sowing time, farmers often wish to apply different treatments together, to increase the efficiency of the sowing operation. In some cases, rhizobial inoculant is combined with the application of commonly used seed pesticides, but this may result in toxic effects on the rhizobia. Given the importance of rhizobial survival to crop production, there is a need for independent data and guidelines to inform farmers about the potential reduction in legume nodulation and nitrogen fixation arising from the combination of various treatments with inoculants.

Laboratory data has shown that under sterile conditions, P-Pickel T is toxic to *Rhizobium leguminosarum* which nodulates pea, bean, lentil and vetch. This work also showed that peat may offer protection to the rhizobia compared with freeze-dried inoculant. The objective of this work was to determine the potential toxicity of the fungicide P-Pickel T (PPT) to rhizobia applied as a commercial inoculant (peat and freeze-dried) on field pea (*R. leguminosarum*, group F) in field conditions in a soil with a low rhizobial background.

How was it done?

A field site with low background of field pea rhizobia was selected on Bruce Heddle's property, near Minnipa Agricultural Centre. The trial was a completely randomised design with three replications. Pea *cv*. Oura seeds were coated with the label recommendations for PPT, and then inoculated with either a commercial freeze-dried or peat formulation, again at commercial rates. Seed was sown immediately (0 h) or stored at room temperature in the dark for 24 h before sowing. Plots with no inoculation (Nil) were also sown as controls. The plot sizes were 12 m x 1.8 m with 25 cm row spacing (6 rows), and an estimated plant density of 54 plants per m^2.

Before sowing, rhizobial counts from the inoculated and PPT treated/untreated seeds were performed to determine if there were adequate numbers of rhizobia on seed. Seed samples (10 seeds) from all treatments (excluding Nil) were washed and diluted 10^{-1} to 10^{-5} in sterile water, and each dilution was plated drop-wise on sterile agar. After incubation, colonies were counted and rhizobia numbers per seed were calculated.

The trial was sown on 30 June and a nodulation assessment was conducted on 20 September (12 weeks after sowing). Plants and roots with soil were dug up in groups of three across the central four rows of the plot approximately 1 m apart, with a total of 12 plants collected from each plot. Soil was gently shaken from the roots which were then washed clean for nodule counts. Nodule fresh weight from each plot was also collected. On 19 October (16 weeks after sowing) shoot dry weight measurements were taken at peak biomass, and yield data was recorded after harvest. Nitrogen fixation measurements are currently being analysed using the N^{15} natural abundance method.

What happened?

Conditions were very dry (gravimetric water content 8% w/w) and sowing delayed (30 June) due to low soil moisture. After sowing there was about 5 mm of rainfall over the following week. At nodule sampling, it was visually easy to differentiate the well-nodulated plants from those with low nodulation, as the latter were stunted and yellow. Rainfall throughout the growing season was below average (2018 GSR 178 mm), which reduced yield and biomass production.

Table 1 shows that rhizobial counts taken from the inoculated seeds before sowing verified that there were no detectable rhizobia on the seeds with freeze-dried inoculant that was stored for 24 h before sowing, even in the absence of fungicide. However, there were adequate (4.8 x 10^5 cfu/seed) populations of bacteria associated with seed treated with the peat and freeze-dried inoculant at 0 h (Table 1). The peat and freeze-dried inoculant treatment that had been coated with PPT 24 h before sowing, showed a significant decline in rhizobia numbers.

Figure 1 shows that there was a much lower number of nodules per plant grown from seed inoculated with freeze-dried rhizobia compared to peat formulation. Figure 1 shows that there was a negative effect of PPT on plant nodulation. Although plants treated with the peat inoculant treatments without fungicide had 72% greater nodules/plant compared to the Nil treatment, the nodule number was still relatively low. There was very low nodulation in the freeze-dried inoculant treatments both with and without the fungicide seed dressing, but a larger decrease in nodule number can be seen in the seed treated with fungicide and stored for 24 h before sowing, compared with seed that was sown immediately after inoculation.

Nodule fresh weight per plant (Figure 2) was correlated with the average nodule number per plant (r=0.91, data not shown). Figure 2 shows a similar pattern to plant nodule number, with a decrease in nodule fresh weight in seed coated with PPT before inoculation. In particular, the plants had lower nodule fresh weight in the PPT treatments compared to the no fungicide treatments.

Table 1 Rhizobial counts from seeds (10 seeds) prior to sowing

Treatment	Fungicide	Time (0 h)	Log_{10} cfu/seed
Peat	-	0	5.5
Peat	+	0	5.0
Peat	-	24	4.9
Peat	+	24	4.1
FD	-	0	4.7
FD	+	0	below detection
FD	-	24	below detection
FD	+	24	below detection

Peat = peat slurry inoculum, FD = freeze-dried inoculum, +/- fungicide coating and inoculated before sowing (0 h) or stored for 24 h before sowing.

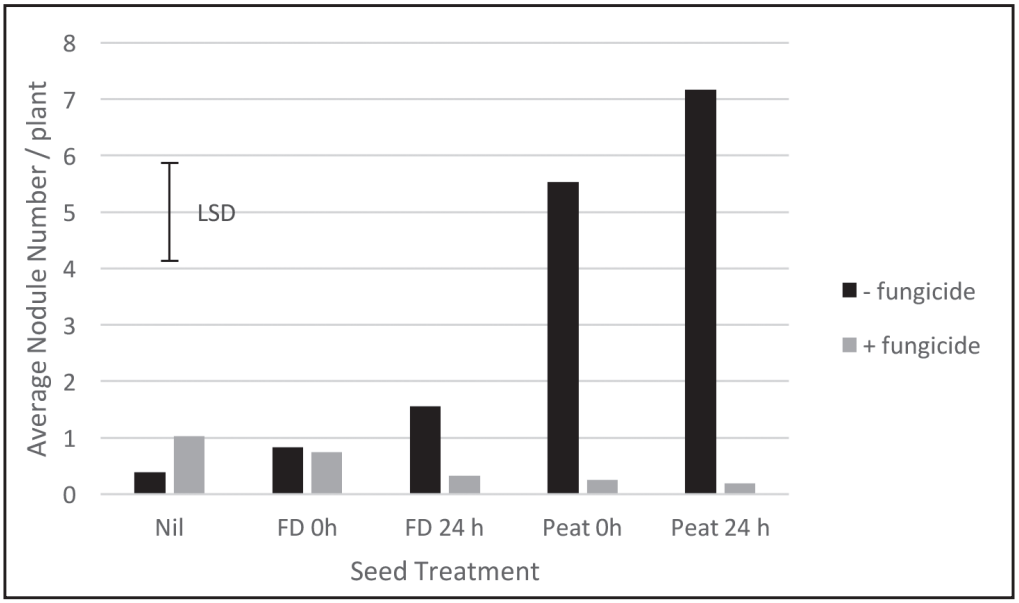

Figure 1 Effect of seed fungicide treatment PPT on average nodule number of plants from seed inoculated with freeze-dried or peat slurry at 0 h or 24 h before sowing

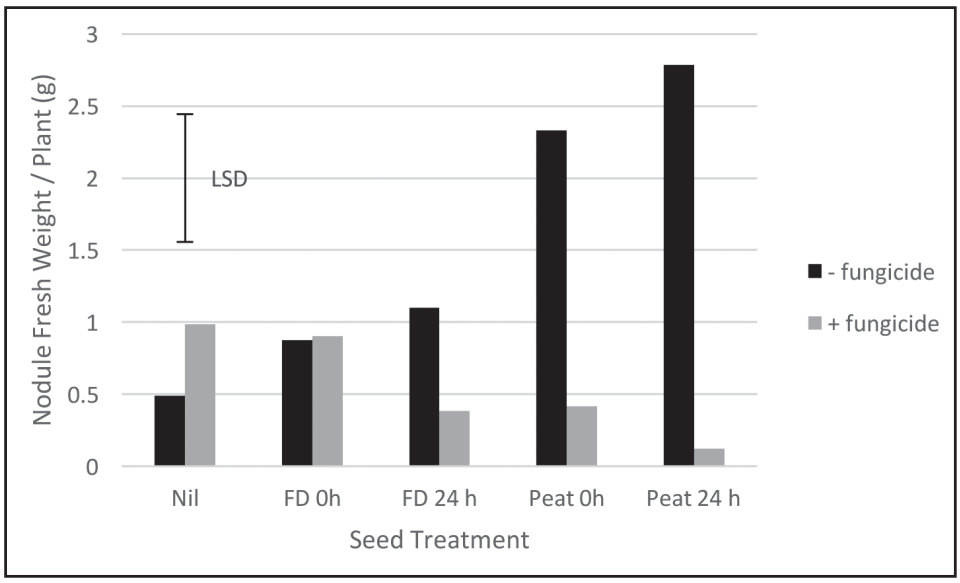

Figure 2 Effect of seed fungicide treatment PPT on nodule fresh weight of plants from seed inoculated with freeze-dried or peat slurry at 0 h or 24 h before sowing

The fresh nodule weight was lower in the freeze-dried treatments than the peat inoculant, so there was not such a dramatic decrease when combined with the PPT seed dressing. The Nil treatment had only a few nodules, but these nodules had a high fresh weight.

What does this mean?

In vitro studies in the laboratory and nodulation experiments conducted under sterile conditions have shown that the fungicide PPT is toxic to some commercial strains of rhizobia. In this experiment, we investigated if this effect was observed in a field situation. The low number of nodules detected in the Nil treatment confirms that the field site had very low nodulation resulting from background soil rhizobial populations (Figure 1). However, adequate nodulation for field pea on light soils is considered to be 20 nodules per plant, which was not achieved in this field trial (Drew *et al*. 2012).

For seed coated with the fungicide PPT and inoculated with a peat slurry, there was a decrease (91% 0 h and 84% 24 h) in rhizobial survival on the seed and subsequent nodulation. It has previously been recommended to sow coated and inoculated seed within 6 hours to avoid toxicity to the rhizobia (Drew *et al*. 2012, Table 5.4), however our results show that rhizobial survival on the seed decreased rapidly before sowing (less than 2 hours). With freeze-dried inoculant, nodulation was much reduced (78 to 85% less) compared with the peat formulation. Rhizobial survival on the seed was reduced, which resulted in fewer nodules without the presence of PPT.

Some of the plants with a low nodule number appeared to have much bigger nodules than the plants with more nodules, but this did not completely compensate for the loss of nodulation, as nodule fresh weight also declined in the presence of PPT. In general there was a decrease in plant nodulation and nodule weight, despite adequate rhizobial numbers on PPT treated seeds at sowing.

The dry conditions at this site may have contributed to the observed toxic effect of PPT, as the low soil moisture and rainfall means that the rhizobia are in contact with the fungicide longer than in a year of increased rainfall. Moisture stress during growth and development would also have contributed to inconsistent shoot weight and yield data, which was not correlated with nodulation measurements.

The data suggest that in a season where conditions are likely to be stressful (e.g. under moisture stress), then the added stress of exposure to toxic fungicide can be quite detrimental to nodulation and N fixation. The best results were obtained with peat formulation which appears to have a protective effect on rhizobial survival. Separating the fungicide and rhizobia, e.g. by applying inoculant as liquid in furrow or as a granular formulation, may lead to avoidance of toxic interactions and adequate nodulation. It would be useful to test these options in the field.

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Reference

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