Evaluation of a DNA tool to determine risk of chickpea Phytophthora root rot

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Introduction

Phytophthora medicaginis, which causes chickpea Phytophthora root rot (PRR), is endemic and widespread in southern QLD and northern NSW. Under conducive conditions, PRR can cause 100% yield loss. The pathogen survives from season to season on chickpea volunteers, lucerne, native medics, sulla and as resistant structures (oospores) in roots and soil.

A PreDicta B® soil DNA test has been developed by the South Australian Research and Development Institute (SARDI) to quantify the amount of *P. med* DNA in soil samples and so provide a measure of the amount of *P. med* inoculum (infected root tissue and oospores) in paddocks. In this second season of studies, we assessed the test's capability to:

1. predict the risk of PRR disease and potential yield losses in chickpea
2. detect *P. med* inoculum in soil from commercial paddocks.

Site and experimental details

Disease development and yield loss prediction

Location:	**Warwick, QLD**
Sowing date:	**10 June 2015**
Variety:	**Yorker(ᵇ) (moderate PRR resistance)**
Design:	**Plots 5 × 2.1 m with five replicates**
Sampling:	***P. med* DNA in soil, disease symptoms, grain yield**
In-crop rainfall:	**160 mm**

Inoculum detection

Soil samples from paddocks in southern NSW, VIC and southern QLD, collected 2014.
Glasshouse bioassay to bait *P. med* isolates from soil samples. Sonali(ᵇ) seedlings grown in a soil-sand mixture, *P. med* isolated from stem cankers.
Soil *P. med* DNA analyses of a 400 g soil sample from each paddock

Treatments

Disease development and yield loss prediction

Inoculum treatments: 0, 40, 130 and 660 *P. med* oospores per plant applied at sowing

Irrigation treatments: in-crop supplementary irrigation, dryland

Inoculum detection

Soil samples from 43 paddocks and one *P. med* control sample

Results

P. med inoculum level, PRR disease development and yield

- Post sowing soil *P. med* DNA results differed significantly among the oospore treatments, but also indicated that some *P. med* was already present at the site (Table 1).
- On 13 October (end of flowering), the irrigated 130 and 660 oospores/plant treatments had significantly more PRR than the dryland 130 and 660 oospores/plant treatments (Table 1). By 12 November (dryland treatments senescing), the irrigated 40, 130 and 660 oospores/plant treatments had significantly more PRR than the dryland 40, 130 and 660 oospores/plant treatments.
- The interaction of irrigation (to simulate a PRR conducive season) and oospore treatments on grain yield was complex as indicated in (Table 1 and Figure 1):

1. At low inoculum levels (zero and 40 oospores/plant), irrigation increased yield compared with dryland.
2. For medium inoculum (130 oospores/plant), irrigation had no significant effect on yield.

Key findings

Increasing levels of inoculum (oospores/plant) of *Phytophthora medicaginis* (*P. med*) was strongly correlated with the decreasing yield of Yorker(ᵇ), a moderately resistant chickpea variety.

An inoculum level of 660 oospores/plant (PreDicta B® >5000 *P. med* copies/g soil) at sowing significantly reduced yield compared with lower inoculum levels under both dryland and irrigated conditions.

Testing soil samples from grower paddocks in 2015 confirmed earlier results, that the PreDicta B® soil *P. med* test can identify *P. med* in commercial fields.

These findings provide further evidence that the PreDicta B® *P. med* test will be a useful tool for growers to determine their risk of Phytophthora root rot before sowing chickpeas.

Note: the SARDI PreDicta B® test for *Phytophthora medicaginis* is under development and is not yet available commercially.

3. For the highest inoculum level (660 oospores/plant), irrigation reduced yield compared with the dryland treatment.

These interactions suggest that at low PRR levels, the primary effect of irrigation is on yield, but at high PRR levels the primary effect is on disease development. However, these relationships are likely to vary from season to season due to differences in rainfall (Figure 1).

Table 1. *Oospore and irrigation (D dryland, I irrigated) treatment, soil DNA P. med concentration, PRR assessment and yield in 2015 P. med inoculum level trial*

Inoculum and irrigation treatment (oospores/plant)	*P. med* DNA concentration 11 June (DNA/g soil)	PRR rating 13 October	PRR stunted plants 12 November (cm row)	Grain yield (kg/ha)
D-0	342	1.1	16	3198
D-40	1986	1.7	18	2961
D-130	3051	2.0	88	3038
D-660	5357	3.1	203	2402
I-0	169	1.2	6	3914
I-40	1765	1.8	78	3631
I-130	2996	2.8	185	2966
I-660	5925	4.2	395	1764
LSD (P=0.05)	1092.6	0.58	46.4	480.7

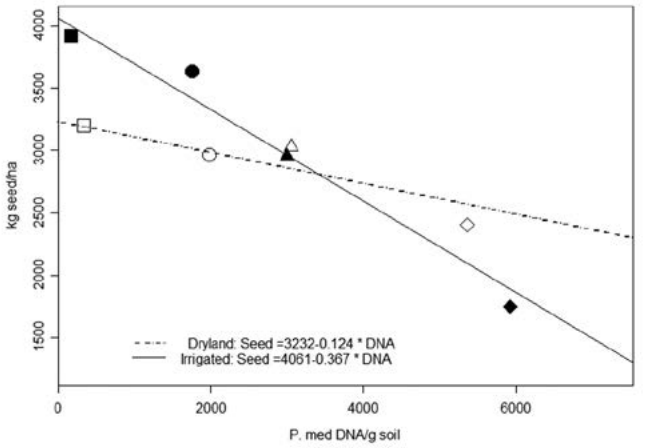

Figure 1. *Multiple regression for plot soil P. med concentrations at sowing vs. grain yield for dryland (white symbols, broken line) and irrigated (black symbols, solid line) treatments (model $R^2 = 0.745$), treatment means presented*

P. med DNA detection in soil from commercial paddocks

- Ten of the 43 paddock soil samples produced PRR-like cankers on plants, *P. med*-like cultures were isolated from eight samples from grower's paddocks; *P. med*-like cultures were also isolated from the control soil, giving a total of nine *P. med* isolates. One of the samples produced cankers that were not caused by *P. med*.
- Of the 43 paddock soil samples (including the control soil), nine had positive *P. med* DNA results. Comparing the DNA results with the isolation results showed that most (8/9, 89%) samples that had positive DNA results also recovered *P. med* cultures, and that most (33/34, 97%) samples that had negative DNA results also did not recover *P. med* cultures (Table 2).
- Notably, one sample (LOU2), which recovered a *P. med* culture, was negative for *P. med* DNA.
- One sample (A) was positive for *P. med* DNA, but seedlings in all five cups remained healthy. This sample had a lower *P. med* DNA value (1,234 *P. med* copies/g soil) than other samples (range 2,443–813,436 *P. med* copies/g soil). Possible explanations for this result are: (i) more time might be required for symptoms to develop, or (ii) that the pathogen had died, but some DNA remained in the soil sample, which is what the PreDicta B® *P. med* test detected.

Table 2. *Comparison of Phytophthora medicaginis (P. med) DNA detection in 43 soil samples and isolation success of P. med from Sonali chickpeas grown in these samples*

		43 samples analysed for *P. med* DNA	
		9/43 + *P. med* DNA	34/43 nil *P. med* DNA
43 soil samples baited with chickpeas for *P. med*	9/43 + *P. med* isolates	8/9 (positives)	1/34 (negatives)
	34/43 nil *P. med* isolates	1/9 (false positives)	33/34 (false negatives)

Summary

P. med inoculum level, PRR disease and yield

Can the *P. med* DNA soil test predict the risk of Phytophthora root rot? Based on the results of this trial with Yorker (MR) and the 2014 Tamworth trial with Sonali (S), the answer is YES.

For Yorker, significant yield loss can be expected with starting (pre-sow sampling) inoculum levels above ca 3000 *P. med* DNA sequences/g soil (ca 130 oospores/plant). However, these values might need to be interpreted with some caution as seasonal conditions will modify outcomes, for instance, in a dry season less disease could develop from the same amount of inoculum.

As Phytophthora can reproduce rapidly and cause new infections over a relatively short period, there was concern that under PRR-conducive conditions (a wet season), low initial levels of inoculum could catch up to high initial levels and cause similar disease severity and yield loss. The 2015 season was wet, but not very wet. Under these conditions separation remained in the disease and yields of the low and high inoculum treatments.

P. med DNA detection in commercial paddocks and disease risk determination

The second season of detection capability results for the soil *P. med* DNA test were again generally promising, with most samples with positive and negative *P. med* DNA results corresponding to expected *P. med* isolation results. However, results for some samples indicate that further work is required to i) identify what factors could contribute to false negative results and ii) determine if false positives are due to the presence of dead or inactive *P. med* DNA.

The DNA result for a soil sample collected from a paddock can only provide an indication of inoculum concentration and disease risk for the areas of the paddock that were sampled. Therefore, the spread and locations of sampling across a paddock will affect how representative DNA results are of an entire paddock. Because of the risk of rapid PRR disease build-up following wet conditions, it might be appropriate to treat a negative PreDicta B® test result as indicating a low risk rather than a nil risk, as the pathogen could still be in areas of the paddock that were not adequately sampled and so could still cause PRR and reduce chickpea yield.

Work in 2016 will evaluate maximising the probability of detecting *P. med* by targeting those areas of the paddock where *P. med* is more likely to occur. The pathogen thrives in soil with a high moisture content and so often occurs in low lying regions of paddocks where pooling after rain can occur. The pathogen also carries over from season to season on infected chickpea volunteers, lucerne and native medics. Including low lying areas and weedy areas of paddocks during PreDicta B® soil sampling could provide the best strategy for detecting *P. med* and so identify a paddock's risk of developing PRR if a chickpea crop is sown.

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