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Evaluation of a new DNA tool to detect Phytophthora in paddocks

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Key findings

Knowledge of *Phytophthora medicaginis* (*Pm*) DNA concentration in soil can assist Phytophthora root rot (PRR) management.

In a field trial, treatments with 0 and 100 oospores/plant resulted in low *Pm* DNA concentrations a month after sowing and had significantly less disease development and significantly higher yields than Treatments with higher oospore concentrations at sowing in 2014.

Similar PRR disease levels and yield losses resulted from medium to high *Pm* soil inoculum concentrations at sowing in the *Pm* susceptible chickpea variety Sonali.

The *Pm* DNA test is capable of identifying *Pm* in soil samples collected from grower paddocks across the northern region.

Pm DNA results and *Pm* isolation results agreed for most paddock samples with 82% of positive and 97% of negative samples being consistent. However, results for three samples indicate that further work is required to address some issues including potential sub-sampling effects.

Introduction

Phytophthora medicaginis (*Pm*), the cause of Phytophthora root rot (PRR) disease of chickpea is endemic and widespread in southern QLD and northern NSW. The pathogen carries over from season to season on infected chickpea volunteers, lucerne, native medics and as resistant structures (oospores) in the soil.

A PreDicta B® soil DNA test has been developed by the South Australian Research and Development Institute (SARDI) under the GRDC project DAS00137. The test aims to quantify the amount of *Pm* DNA in soil samples and to provide a measure of the amount of *Pm* inoculum (infected root tissue and oospores) in paddocks from which those samples were collected.

It would be useful if the DNA levels detected by the *Pm* test prior to sowing could predict the likely level of PRR disease development during the growing season and potential associated losses. For example, would paddocks with nil, low and high *Pm* inoculum level respectively have nil, low and high PRR disease development and yield losses? It is also necessary to evaluate the ability of the *Pm* DNA test to detect *Pm* in soil samples from growers paddocks across the northern grains region.

We assessed the capability of this *Pm* DNA test to:

1. Predict the risk of PRR disease development and potential yield losses in chickpea
2. Quantify *Pm* inoculum in soil collected from commercial paddocks

Site and experimental details

Disease development and yield loss prediction	Inoculum detection
Location: Tamworth Sowing date: 3 July 2014 Variety: Sonali (PRR susceptible) Design: Plots 2 × 2.1 m with six replicates Sampling: *Pm* DNA in soil, *normalised difference vegetation index* (NVDI), disease symptoms, grain yield	Soil samples from paddocks in northern NSW and southern QLD, collected 2013 Glasshouse bioassay to bait *Pm* isolates from soil samples. Sonali seedlings grown in a soil-sand mixture, *Pm* isolated from stem cankers. Soil *Pm* DNA analyses of 400 g soil sample from each paddock

Treatments

Disease development and yield loss prediction	Inoculum detection
Six treatments of 0, 100, 500, 1000, 2000 or 4000 *Pm* oospores per plant applied at sowing	Soil samples from 47 paddocks and one *Pm* control sample (MET14)

Results

Pm inoculum level, PRR disease development and yield

- Soil *Pm* DNA results differed significantly among the oospore treatments one month after sowing but also indicated that some *Pm* was already present at the site (Table1). Background inoculum levels could have contributed to the higher than expected DNA value for the 100 oospores/plant treatment.
- The season in Tamworth was drier than usual but following 39 mm of rain from 18–20 August some PRR symptoms (wilting and chlorosis) were observed, then in mid-September during a period (15–22 September) of hot dry winds and high evaporative demand (≥ 5 mm/day) many plots showed severe PRR disease symptoms.
- In mid-September indirect biomass measurement by the reflectance based NVDI showed significant declines in NVDI values in treatments with increasing numbers of oospores/plant.
- By the end of September the nil oospores/plant had fewer diseased plants than the 100 oospores/plant treatment, which itself had less diseased plants than the Treatments with higher inoculum levels (500 to 4000 oospores/plant treatments). The percentage of diseased plants did not differ between the 500 to 4000 oospores/plant inoculum treatments.
- Disease assessments recorded on the 23 September reflected the final grain yields for this trial, with the 0 and 100 oospores/plant treatments each having the highest and second highest yields respectively, and the yields of treatments between 500–4000 oospores/plant not differing.
- There were relatively weak correlations between the post-sow soil *Pm* DNA concentrations and PRR disease (r = 0.45) and chickpea yields (r = -0.39), although the percentage of diseased plants was a strong predictor of grain yield (r = -0.96).

***Table 1**. Oospore treatment, soil Pm DNA concentration, biomass, PRR assessment and yield in 2014 Pm inoculum level trial (standard error of difference between means, SED; transformed, TS)*

Inoculum treatment (oospores/plant)	*Pm* DNA concentration, (DNA/g soil) 4 August	NVDI 12 September	log-TS % of PRR diseased plants (back TS) 23 September	Grain yield, (kg/ha) log-TS (back TS)
0	44	0.68	0 (8.0)	7.1 (1248.9)
100	1280	0.55	3.1 (65.7)	3.6 (38.1)
500	443	0.34	6.4 (98.1)	1.4 (4.0)
1000	2123	0.47	4.7 (90.8)	1.5 (4.4)
2000	1905	0.36	5.3 (94.5)	1.1 (2.9)
4000	3590	0.30	* (100)	0.5 (1.6)
L.S.D. (*P*=0.05)	1749	0.10	0.87 (SED)	1.3

**100% values excluded from analyses*

Pm DNA detection in soil from commercial paddocks

- Twenty six of the soil samples produced plants with some disease symptoms, but *Pm* like cultures were isolated from only ten samples, nine from grower paddocks and one from the MET14 control soil.

- Of the 48 soil samples (including the MET14 control soil), 11 had positive *Pm* DNA results. Overall, most samples (9/11, 82%) which had positive DNA results yielded *Pm* cultures and most samples (36/37, 97%) which had negative DNA results did not yield *Pm* cultures (Table 2).
- Three samples gave contradictory results. One sample which yielded a *Pm* culture was negative for *Pm* DNA. Sub-sampling error may explain this result as this sample was part of a 5 kg trowel collected sample; the 400 g subsample used for *Pm* DNA analysis may not have contained *Pm* DNA.
- Two other samples were positive for *Pm* DNA but did not yield *Pm* cultures. One of these had one seedling with a canker but *Pm* could not be isolated, in the other sample, seedlings in all five cups remained healthy. These two samples had lower *Pm* DNA values (1,467 and 2,507 *Pm* copies/g soil) than all other samples (range 3022–872,069 *Pm* copies/g soil) except one (1,219 *Pm* copies/g soil). Possible explanations for these results are: (i) more time may be required for symptoms to develop in low *Pm* DNA samples, or (ii) that the pathogen had died but some DNA was still detectable.

***Table 2**. Comparison of* Phytophthora medicaginis *(Pm) DNA detection in 48 soil samples and isolation success of Pm from Sonali chickpeas grown in these samples*

		48 samples analysed for *Pm* DNA	
		11/48 + *Pm* DNA	**37/48 nil *Pm* DNA**
48 soil samples baited with chickpeas for *Pm*	10/48 + *Pm* isolates	9/11 (positives)	1/37 (false negatives)
	38/48 nil *Pm* isolates	2/11 (false positives)	36/37 (negatives)

Summary

Pm inoculum level, PRR disease and yield

Phytophthora can reproduce rapidly and cause new infections over a relatively short period. This may explain how for *Pm* the 500 to 4000 oospores/plant treatments had very similar PRR disease symptom measurement values at 23 September, which then led to similar yield outcomes. These results indicate that for a susceptible variety like Sonali, PRR disease can build up to high levels under conducive conditions and cause considerable yield losses despite differences in initial *Pm* inoculum levels. Although the relationships between *Pm* DNA concentrations and PRR disease and chickpea yields were relatively weak, the trial showed low *Pm* inoculum levels (0 and 100 oospores/plant) had significantly less disease and significantly higher yields than treatments with higher starting oospore concentrations.

These initial results are encouraging as they suggest that significantly lower PRR disease and higher yields occur at low *Pm* DNA concentrations with a highly susceptible variety. The test may be suitable at identifying low *Pm* inoculum sites where chickpea varieties with better *Pm* resistance (such as Yorker[(b)]) may be grown with less impact of PRR on yield. The test may also be useful at identifying nil from low *Pm* sites, however, trial sites with nil *Pm* will need to be identified to fully assess this aspect.

Pm DNA detection in commercial in paddocks and disease risk determination

The initial results are promising with an overall good correlation between *Pm* DNA detection and *Pm* isolation. However, further work is required to address some issues including impact of sub-sampling.

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 which were sampled. Therefore, the spread and locations of sampling across a paddock will affect how representative DNA results are for that paddock. Because of the risk of rapid PRR disease build up following wet conditions it may 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 sampled and so still cause PRR and reduce yield.

To determine the risk of PRR disease it may be appropriate to target locations in a paddock where there is the best chance of *Pm* inoculum being present. The pathogen thrives in soil with high moisture contents and so is often concentrated in low lying regions of paddocks where pooling of water following rain may occur. Targeting low lying areas and weedy areas of paddocks during PreDicta B® soil sampling may provide the best strategy for predicting a paddocks risk of developing PRR in chickpea.

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