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Crop protection

Disease risk prediction evaluations for phytophthora root rot of chickpeas

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Key findings

- *Phytophthora medicaginis* (Pm) inoculum concentrations decline to low levels (within 6–12 months) of a diseased crop and the distribution becomes more uneven.
- Within 6–12 months, survival populations of Pm (oospores) can be below detectable levels based on both soil DNA and isolate baiting methods.
- These inoculum decline factors limit the ability of PreDicta B to identify paddocks that have a significant disease risk.
- The Pm test is useful for in-crop disease diagnosis when the pathogen is active and inoculum decline has not taken place.

Introduction

Phytophthora medicaginis (Pm), the cause of chickpea phytophthora root rot (PRR), is endemic and widespread in the northern grains region of NSW. Under conducive conditions, PRR can cause 100% loss in chickpea fields. The pathogen survives from season to season on chickpea volunteers, lucerne, native medics, sulla and as resting structures (oospores) in roots and soil. It is known that Pm inoculum concentrations are difficult to detect and quantify in paddocks when a susceptible host such as chickpea is not present.

The South Australian Research and Development Institute (SARDI) has developed a PreDicta™ B soil DNA test to quantify the amount of Pm DNA in soil samples and so provide a measure of the amount of Pm inoculum (infected root tissue and oospores) in paddocks. This study reports on the 2016 season assessment of the capability of this test to:

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

Field experiment — Disease and yield loss prediction trial

Location	Hermitage Research Station, Queensland (QLD)
Rainfall and irrigation	There was above average rainfall in June (110 mm) which delayed sowing. July (22 mm) and November (68 mm) rainfall was similar to long-term average values, but for both August (109 mm) and September (91 mm), rainfall totals were well above average – a total of 280 mm of in-crop rainfall.

Trial design	Trial design randomised complete block design with four replicates. Each plot (15 m^2) consisted of four in-furrow inoculated rows; four-row buffer plots were sown around each plot to limit inoculum spread between plots. At sowing and harvest, soil cores (0–150 mm) were collected from the two middle rows of each plot, pooled and analysed for soil Pm concentration by SARDI. PRR disease assessments (% infected plants, or row length of severely infected plants) and grain yields were measured from those same rows.
Sowing date	22 July 2016
Plant population	32 plants/m^2, variety Yorker[b]
Harvest date	15 Dec 2016
Treatments	• Oospore treatment: a range of Pm levels were established by applying four concentrations (0, 27, 140 and 494 oospores/plant) of a mixture of oospores (10 isolates) in-furrow at seeding. • Irrigation treatment: irrigated plots were watered (27 mm from 27–29 Nov) with dripper tape delivering between 0.6 to 0.7 mm/hour; the remaining plots had no irrigation

Field survey

Ability to monitor Pm concentrations in commercial paddocks.

Locations	Coonamble in central-western NSW, Moree in northern NSW and Goondiwindi in southern Qld.
Rainfall	Coonamble rainfall June–Oct in 2015 was 112 mm and June–Oct in 2016 was 388 mm. Goondiwindi rainfall June–Oct in 2015 was 20 9 mm and June–Oct in 2016 was 321 mm. Moree rainfall June–Oct in 2015 was 122 mm and June–Oct in 2016 was 323 mm.

Survey methods

Three farms where PRR had been an ongoing problem, and Pm had been isolated from diseased plants, were sampled in April 2016 and again in Nov–Dec 2016. Where possible at each farm, growers or agronomists identified paddocks with ongoing PRR problems, including the area in each paddock where PRR was often first observed or where Pm had been isolated from samples. These areas were designated as hotspots and their GPS position marked.

For hotspot areas, four separate samples were collected following a 'W' collection pattern (32 points along the entire pattern, each point ~6 m apart, using a 150 mm depth corer) in each paddock. At each collection point, the four cores for each separate sample were taken from a single stubble row, with each core taken 2–3 cm apart.

Low lying areas of paddocks where there was pooling following rainfall (below contour banks, low areas of paddocks, dips) were sampled and raised or uniform areas were also collected. These areas provided three bulked samples (32 cores each) for "low areas" and three bulked samples (32 cores each) high areas" from each field; their GPS positions were also marked. Using this method, 12 paddocks were sampled in April 2016, another four paddocks were also sampled with either only hotspot or only low vs high samples collected.

For the April samples, three soil samples from each hotspot and all low vs high samples were sent to SARDI for analysis. The fourth hotspot sample was assessed for Pm in a glasshouse baiting experiment (five reps, cv. Sonali grown in a soil–sand mix). At the end of the baiting experiment, the soil–sand media in each cup was sent to SARDI for analysis.

In the November–December sampling period, all hotspot sites were resampled, all low vs high sample sites were revisited and samples collected from chickpea paddocks in 2016 showing any disease problems.

Results

Field experiment: disease and yield loss predictions

Trial results provided a poor relationship between Pm DNA values and both disease ($R = 0.17$) and yield ($R = -0.22$). However, high R values for PRR measurements and yield ($R = -0.70$) in the trial supported the assumption that the yield loss was predominately due to PRR.

The 2016 trial results consisted of a large number of nil DNA plots post-sowing, that later showed PRR symptoms (Table 1). Pm control samples included for analyses with these samples gave expected DNA values. It is not known why so many 2016 samples gave negative Pm DNA results yet PRR symptoms occurred in the plots.

Table 1. Number of plots (of 40) that had nil Pm DNA results after inoculation and the number of these plots that had PRR symptoms or not.

Post-sowing Pm DNA values		
Total nil DNA values	**nil DNA values & nil PRR symptoms**	**nil DNA values & PRR symptoms**
33/40	2/40	31/40

It could be expected that the probability of a false negative for paddock samples might be higher than those for small-plot replicated field trials as:

1. a single sample–single result from a paddock will be used to assess PRR disease risk
2. the sampling intensity per unit area of paddocks will be much lower than those of plots in field trials.

Field survey: April 2016 paddock inoculum results, detection variability

Six of the 13 paddocks with hotspot soil samples had positive Pm DNA results; all but one of these paddocks grew chickpeas in 2015 (Table 2). Of the six paddocks with positive DNA results, only two paddocks had all three samples test positive.

Given the close proximity (2–3 cm apart) of the cores sampled at each of the 32 points in a hotspot area, the variability in positive DNA results among the three samples warrants consideration. The results for paddocks 10 and 11, and in particular, for paddocks 3 and 13, indicate an uneven distribution of inoculum giving differing results, even for closely-collected soil samples.

Table 2. April 2016 hotspot sample location, paddock code, prior crop (wh:wheat, cp:chickpea), average hotspot sample Pm DNA and number of positive hotspot samples, April 2016 hotspot sample isolate baiting results (no. cankers, no. of putative Pm cultures) and post-experiment DNA results of baiting media.

Location	Code	2015 crop	Av. hotspot P. med DNA sequences/ g soil	Hotspot no. + samples	Av. no. cankers/cup	Total no. putative cultures	Av. P. med DNA sequences/g media
Coonamble	1	wh	0	0/3	0	0	0
Coonamble	2	wh	0	0/3	0	0	0
Coonamble	3	cp	209	1/3	0	0	205
Coonamble	4	cp	0	0/3	0	0	0
Coonamble	5	cp	0	0/3	0	0	0
Coonamble	6	cp	0	0/3	0	0	0
Coonamble	7	cp	0	0/3	0	0	544
Coonamble	8	cp	0	0/3	0	0	0
Goondiwindi	9	cp	1389	3/3	3	6	334767
Goondiwindi	10	cp	1205	2/3	2.8	9	348014
Goondiwindi	11	cp	690	2/3	0.75	2	618706
Goondiwindi	12	cp	2881	3/3	3	7	186981
Moree	13	wh	339	1/3	0	0	0

The baiting experiment results supported the soil DNA results, including that Pm inoculum was unevenly distributed in these samples.

PRR is often first seen to occur in low-lying areas of paddocks where water pooling occurs after heavy rainfall. However, results for hotspot detection success showed that using local knowledge to target sampling to areas where PRR had been observed, gave a slightly better success (4/11 cases) than just targeting low areas of paddocks (3/11 cases) (data not presented).

April vs November 2016 inoculum results, unexpected increases

Results for four Coonamble paddocks with nil Pm DNA results in April 2016 (Table 3) show increases from nil inoculum in a:

- break crop to substantial inoculum in chickpeas including areas with PRR-like symptoms (paddocks 1 and 2)
- prior chickpea crop to substantial inoculum in chickpeas including areas with PRR-like symptoms (paddocks 3 and 5).

Similar results were observed for two Goondiwindi paddocks that were in wheat in winter 2015 and contained no hotspots. The April low and high areas all returned 0 Pm DNA results. However, in November after a chickpea crop, the low sites were positive in one paddock, and low and high sites were also positive in a second paddock (results not presented). This second paddock, which was planted to a PRR-susceptible kabuli variety, had large areas of PRR losses. That high sites returned positive values suggests that the inoculum was resident at these sites, rather than due to inoculum arriving after April via the flow of storm water containing inoculum.

These results were unexpected (although from a small number of paddocks in a single season with high rainfall), as they indicate that testing before sowing a crop might not indicate future disease risk and associated inoculum concentrations.

Table 3. November 2016 average Pm DNA values for four Coonamble paddocks.

Paddock code	2015-2016 crop	November 2016		
		Hot av.	Low Av.	High Av.
1	wh-cp	0*	13,110[c]	4,107[d]
2	wh-cp	1,242[a]	6,447[d]	2,936[d]
4	cp-cp	0	6,662[c]	13,248[d]
6	cp-cp	3,417[b]	–[#]	–[#]

Number of positive Pm DNA samples (in superscript) for soil samples from a single hotspot area, low areas and high areas of paddocks. * only dead seedlings present in hotspot area, possible death from waterlogging; [a] 1 of 3 samples positive; [b] 2 of 3 samples positive; [c] 3 of 3 samples positive; [d] less than 3 samples collected as not all sites had disease symptoms; [#] no disease symptoms observed, no soil samples collected.

Conclusions

This work has not been able to develop disease risk categories for PRR in chickpeas using pre-sowing soil inoculum concentrations. Disease predictions can be affected by:

- the ability in wet seasons for low concentrations of Pm to multiply rapidly to cause PRR
- as Pm inoculum can also spread to neighbouring crops in run-off water.

Detection issues post-harvest and in break crops are the result of:

- Pm declining to low levels during break crops within 6–12 months
- low resting spore concentrations
- uneven distribution of inoculum across paddocks.

However, the Pm DNA test is a useful in-crop tool for growers and agronomists to confirm PRR diagnosis. For example, in 2016 some chickpea paddocks in north-western NSW were saturated causing some areas of the paddocks to die. Pm DNA analysis of soil samples from some of these areas has allowed agronomists and growers to identify if waterlogging or PRR caused the death.

Where PRR is suspected in chickpea crops, confirmation through isolating the pathogen from diseased tissue can be unsuccessful if the symptoms are advanced or the plants have died. Analysing soil samples for Pm DNA provided confirmation of a suspected case of PRR in QLD in 2015.

The key point to using this diagnostic tool will be the need to collect in-crop soil samples when the pathogen is active and inoculum concentrations are high.

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