19. Emerging Pulse Root Diseases in the South East of South Australia

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KEY MESSAGES

- Pulse crops can suffer from root diseases.
- Next generation sequencing (NGS) technology has potentially identified multiple pathogens of economic significance on South Australian (SA) crops — further work needs to determine which are the most important.
- Intensification of pulses in cropping rotations can increase pulse soilborne diseases.
- If you suspect soilborne disease issues in your pulse crops, send samples to SARDI.

Background

International experience indicates soilborne pathogens can become important constraints to production in pulse crops when cropping frequency increases (Gossen et al. 2016). Several reports of crop failures in 2017, due to suspected root rot fungi, prompted further investigations of soilborne disease of pulses in the southern region.

The failure of three chickpea crops in the south east region of SA in 2017 was suspected to be Phytophthora root rot based on symptoms in crop and on the roots. The presence of Phytophthora was confirmed in diseased chickpea root samples using a genus specific enzyme linked immunosorbent assay (ELISA), however attempts to culture the pathogen failed and it was not detected by the PREDICTA®B test for *P. medicaginis*, the species causing Phytophthora root rot in chickpeas in the Northern Region. *P. megasperma* was suspected as the causal agent, but more work was required to confirm this.

The failure of a faba bean crop was caused by Aphanomyces root rot and was confirmed by the DNA test for *Aphanomyces euteiches* developed at SARDI.

These reports prompted the SA Grains Industry Trust (SAGIT) to fund a small project (S218) to survey root pathogens of pulses. The survey used existing DNA tests developed by PREDICTA®B and new tests and technology being developed by GRDC-SARDI Bilateral investments, DAS00137 and DAS1802-011BLX.

A pulse Research test panel was assembled, including available DNA tests for pathogens of pulses and was used to assess the pulse root survey samples. The tests included: *Pratylenchus neglectus, Pratylenchus thornei, R. solani AG8, R. solani AG2.1, R. solani AG2.2, R. solani AG4, Pythium clade f, Pythium clade I, Aphanomyces euteiches, Phytophthora medicaginis, Didymella pinodes/ Phoma pinodella and Macrophomina phaseolina.* These tests, many of which are used to deliver PREDICTA®B, are reliable for quantifying pathogens in samples. The main limitation is that no information is obtained on target pathogens for which there are no tests.

To solve this problem, NGS technology was used to identify a broader range of pathogens present in the diseased roots. The main limitations of this technology are that it is semi-quantitative, slow and generates large amounts of data which take time to analyse.

Activities

Pulse root samples were sent to SARDI by growers and agronomists predominantly from the South East region of SA. Excess soil was washed from the roots and any plant material above the basal stem was removed. The roots were then processed through the PREDICTA®B laboratory and the DNA was extracted and tested using the Pulse Research test panel to quantify targeted pulse pathogens in samples. The DNA samples was also assessed using NGS to identify potentially important pathogens not detected by PREDICTA B.

The survey has so far collected almost 100 samples of pulse roots from crops, most of which were showing signs of poor performance. The crops included chickpea, lentil, faba bean, field pea, lupin, canola, vetch, clover and lucerne.

PREDICTA®B analysis

From the tests currently available in the Pulse Research test panel (Table 1), *Pratylenchus neglectus, Pythium clade F and Didymella pinodes/Phoma pinodella* were the most prevalent pathogens in the samples. P. pinodella is known to have a broad host range and causes foot rot in field pea and sub clover. Its effect on other pulses is unknown. *Rhizoctonia solani* AG8 and 2.1, *Pythium clade* I and *Macrophomina phaseolina* (charcoal rot) were also present at significant levels. *R. solani* AG4, which can be a serious pathogen of pulses and other crops, was detected in one sample (Hwang et al. 2003). *A. euteiches* was found in 18% of samples, all from faba bean crops experiencing moderate to severe symptoms of the disease. *Pratylenchus neglectus* is common and known to be hosted by a range of pulses, its effect on pulse yields is not known.

There were no detections of *P. medicaginis* in the samples received despite some showing symptoms consistent with the disease and positive ELISA tests.

NGS analysis

DNA from each root sample was analysed with NGS and a broad range of pathogens were detected, some of which have been reported to cause root disease of pulses overseas. Pathogens identified of particular interest based on international research and symptoms from plant samples in this survey are summarised in Table 2.

Table 1. Percentage of samples containing pathogens quantified using existing DNA tests in a survey of pulse roots in the South East region of SA and the Wimmera region of Victoria in 2018.

Pathogen (Pulse test panel)	% Crop samples infected (Grop sampled)						
	Chickpea (34)	Faba bean (22)	Lentil (26)	Lupin (4)	Lucerne (4)	Field Pea (2)	Other* (5)
Pratylenchus neglectus	88%	63%	100%	100%	50%	100%	100%
Pratylenchus thornei	41%	32%	31%	0%	25%	0%	0%
Rhizoctonia solani AG8	32%	32%	50%	100%	25%	0%	0%
Rhizoctonia solani AG2.1	18%	27%	54%	0%	0%	0%	0%
Rhizoctonia solani AG2.2	0%	0%	0%	0%	0%	0%	0%
Rhizoctonia solani AG4	3%	0%	0%	0%	0%	0%	0%
Pythium clade F	85%	96%	77%	75%	100%	100%	80%
Pythium clade I	44%	68%	73%	25%	25%	50%	80%
Aphanomyces euteiches	0%	18%	0%	0%	0%	0%	0%
Phytophthora medicaginis	0%	0%	0%	0%	0%	0%	0%
Didymella pinodes/Phoma pinodella	88%	86%	100%	0%	50%	100%	40%
Macrophomina phaseolina	41%	0%	69%	N/A	N/A	N/A	N/A

**Other crop types include vetch, canola and clover.*

Table 2. Summary of NGS analysis of pulse root samples collected in the South East region of SA and Wimmera region 2018.

Pathogen	% Crop samples infected (Grop sampled)						
	Chickpea (17)	Faba bean (22)	Lentil (2)	Lupin (4)	Lucerne (4)	Field Pea (2)	Other* (5)
Aphanomyces euteiches	11%	22%	0%	0%	0%	0%	0%
Aphanomyces sp.	11%	13%	0%	0%	25%	0%	0%
Phytophthora megasperma/ crassamurra	24%	36%	0%	0%	50%	0%	20%
Phytophthora clandestina	5%	0%	0%	0%	0%	0%	0%
Phytophthora trifolii	5%	0%	0%	0%	0%	0%	0%
Phytophthora spp.	5%	32%	0%	0%	75%	0%	0%
Pythium irregulare	65%	64%	50%	75%	75%	50%	20%
Pythium oopapillum	29%	64%	0%	0%	50%	0%	0%
Fusarium solani	59%	32%	0%	75%	75%	50%	40%
Fusarium redolens	35%	0%	0%	0%	25%	0%	0%
Fusarium oxysporum	17%	0%	0%	0%	0%	0%	0%
Fusarium equiseti	71%	82%	50%	100%	100%	100%	80%
Fusarium acuminatum	88%	82%	50%	100%	100%	100%	60%
Macrophomina phaseolina	18%	18%	0%	25%	0%	0%	20%
Thielaviopsis basicola	6%	5%	0%	75%	0%	0%	20%

**Other crop types include vetch, canola and clover.*

Phytophthora spp.

Sequence data identified several *Phytophthora* species present including *P. megasperma/ crassamurra*, *P. trifolii* and *P. clandestina*. All three species were detected in chickpea roots with symptoms of Phytophthora root rot. *P. megasperma/ crassamurra* was also found on faba bean and lucerne roots. These *Phytophthora* species could have been the pathogens responsible for crop failures in the chickpea paddocks from 2017 and crop and root symptoms in 2018. These samples produced positive ELISA tests for Phytophthora species and were negative for P. *medicaginis* using the PREDICTA®B test. The potential of *P. megasperma/crassamurra* additionally infecting faba bean roots could have implications for the south east region and requires further investigation to confirm and quantify its extent and severity.

Australian research in the northern region on Phytophthora root rot (*P. medicaginis*) is currently the best reference point for the impacts of this disease in chickpeas. Further research in the Southern Region is required to determine how severe *P. megasperma/crassumurra* is and how it compares to Phytophthora root rot in the northern region were it was estimated to cost chickpea growers up to $8.2 million annually (Murray and Brennan 2012). The impacts are currently unknown for *P. megasperma/crassamurra* as there is limited research internationally.

The only chemical option available for Phytophthora root rot in the Northern Region is metalaxyl-based seed dressings, which can provide 6-8 weeks protection post seeding (Moore et al. 2011). Currently, the best options for growers to manage Phytophthora root rot are to use wide rotations and grow varieties that are moderately resistant (Amalraj et al. 2018).

Fusarium spp.

Globally, *Fusarium spp.* feature in research on pulse root diseases (Gossen et al. 2016, Li et al. 2017, Wong et al. 1985, Banniza et al. 2015). Species reported in the literature that have been tentatively identified by the NGS sequences as being present in root samples from SE of SA include *F. solani, F. redolens, F. oxysporum, F. equiseti* and *F. acuminatum*.

Further investigation is needed to determine which, if any of the above species present in the pulse roots play an important role in causing root disease in Australia. The most pathogenic, *F. oxysporums* include *F. oxysporum f. sp. ciceris* and *F. oxysporum f. sp. lentis*, these are not known to occur in Australia (Cunnington et al. 2016, Pouralibaba et al. 2016). We cannot identify isolates to formae speciales with the current NGS data.

The *Fusarium spp.* above are also being investigated internationally as potentially important components in disease complexes with *Aphanomyces sp.* and *Phytophthora spp.* (Banniza, 2016).

Macrophomina phaseolina

Detection of *M. phaseolina* is anticipated to be better using PREDICTA®B tests than NGS due to the specificity of the genetic region used in the PREDICTA®B test. The NGS data identified a large number of sequences to family and genus level that align with *M. phaseolina* but taxonomy could not be assigned to species level.

In Ethiopia, *M. phaseolina (Rhizoctonia bataticola)* causes up to 50% yield losses in chickpea and is also a serious disease on faba bean and lentil (Mitiku, 2017). In Australia, *Macrophomina* has caused losses in lupin in the Western Region when high temperatures occurred in early spring (Thomas et al. 2014). The significance of the pathogen in the Southern Region on pulses is currently unknown.

Thielaviopsis basicola

T. basicola sequences were detected on diseased chickpea roots grown in soil from near Naracoorte and from a diseased lupin root system from Coomandook. *T. basicola* causes black root rot and has a very broad host range including pulses, vegetables and cotton. Internationally, chickpea and lentil have been identified as susceptible to *T. basicola*, as well as causing disease in numerous cropping regions (Bowden et al. 1985, Abbas et al. 2007, Bhatti et al. 1992).

Didymella pinodes/Phoma pinodella

The NGS detected a large number of sequences in the genera *Didymella* and *Phoma*, however, there was limited classification to species level. This is an indication that the PREDICTA®B test for *Didymella pinodes/Phoma pinodella* is much better suited to this pathogen than the genomic region targeted in the NGS.

Tests for *P. megasperma* and *T. basicola* are under development and will be used to test stored DNA when ready.

Conclusions

The labelling of pulses as break crops has allowed pulse root diseases to be underestimated in the farming system. The risk is increasing with increased frequency of pulses in the cropping sequence. International research and reports of pulse crop failures has alerted researchers to the significance of these diseases.

This research is in the problem definition phase. SARDI will continue to survey pulse crops in South Australia in 2019 and 2020. Consultants and growers are encouraged to monitor their pulse crops and forward plant samples from poor performing areas that previously may have been attributed to waterlogging or other environmental stress.

If you are interested in assisting with the survey, contact Tara Garrard for sample kits.

CONTACT DETAILS

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