C – EMERGING PULSE ROOT DISEASES

AUTHORS: Blake Gontar, Tara Garrard, Kelly Hill, Alan McKay, SARDI

TAKE HOME MESSAGES

- Pulse and canola crops are affected by root diseases.
- Next generation sequencing technology and PREDICTA®B tests have been used to survey SA, and now national, pulse samples from industry, revealing multiple potentially important soilborne pathogens.
- Field trials have been established with the aim of quantifying the benefit from controlling root diseases in pulses.

Background

International experience indicates that soilborne pathogens can be important constraints to production in pulse crops when cropping frequency increases (Gossen et al. 2016). In 2017, the loss of three chickpea crops to suspected Phytophthora root rot and a faba bean crop to Aphanomyces root rot, prompted the South Australian Grains Industry Trust (SAGIT) to fund a root disease survey of pulse and oilseed crops in South Australia (S218).

Phytophthora root rot, caused by Phytophthora medicaginis, is an important root disease of chickpeas in northern NSW. However, P. medicaginis was eliminated as the cause of loss of the three chickpea crops in South Australia (SA), using an existing PREDICTA®B (Northern Region) test.

New diagnostic research technology being developed by the GRDC-SARDI bilateral investments; DAS1907-001BLX and DAS1802-011BLX was used to test DNA extracted from the diseased chickpea roots and identified a different Phytophthora species, P. megasperma as the likely cause. A PREDICTA®B test was developed for this pathogen to support the survey.

In 2019, GRDC extended the survey nationally as part of DJP1907-002RMX. Department of Agriculture pathologists in each state coordinated sample collection and provided samples to SARDI for testing. A panel of 23 tests was assembled to survey the pulse and oilseed root systems collected from across Australia (Table 1 and Table 2). DNA extracted from these samples was also tested using next generation sequencing (NGS), to detect pathogens for which no PREDICTA®B-style test had been developed.

Although the presence of pathogens in symptomatic roots is concerning, it does not define any effect on yield. In 2020, SAGIT have funded a series of 39 pulse root disease field trials which have been sown at 20 GRDC-funded 'Southern Pulse Agronomy' sites around SA. Two crop species at each site were treated with a range of active fungicides/nematicides targeting various pathogens. Field trials will be evaluated to determine the effect of pathogen control on yield.

Methods

Survey

Pulse root samples were sent by agronomists and growers to Department of Agriculture pathologists in each state. Excess soil was washed from the roots and any plant material above the basal stem was removed. Roots were scored for disease, photographed and then dried and sent to SARDI. Samples were then processed through the PREDICTA®B laboratory and DNA was extracted. The Pulse Research test panel was run on the extracted DNA to quantify targeted pulse pathogens in the samples.

Table 1. Locations, sowing dates and crop types for each of the pulse root disease yield loss trials.

Region	Site	Sowing date	Crop type					
			Chick-pea	Faba bean	Field pea	Lentil	Lupin	Vetch
Mid North	Booleroo	12 May		X		X		
	Eudunda	4 May		X				X
	Farrell Flat	6 May		X		X		
	Hart	18 May				X		
	Maitland	25 May		X		X		
	Pinery	15 May	X			X		
	Port Broughton	13 May	X			X		
	Riverton	26 May	X	X				
	Tarlee	26 May		X		X		
	Turretfield	5 June	X		X			
	Warnertown	5 May		X		X		
South East	Bool Lagoon	18 May	X	X				
	Coomandook	11 May		X			X	
	Sherwood	11 May		X			X	
Eyre Peninsula	Kimba	26 May			X			X
	Stokes	27 May						
	Tooligie (1)	14 May	X		X			
	Toolige (2)	13 May				X	X	
	Wudinna	13 May				X		X
	Yeelanna	27 May		X		X		

DNA samples were also assessed using NGS to identify potentially important pathogens not detected by the Pulse Research test panel. Three Illumina® MiSeq® libraries were prepared for each sample using primer pairs that target the ITS1, ITS2 and elongation factor gene regions to aid identification of oomycetes (for example, Phytophthora) and fungal species (for example, Phoma and Fusarium species).

Where root samples showed distinctive/diagnostic symptoms, or where DNA tests indicated the presence of a potential pathogen, samples were plated on a variety of selective agar media in an attempt to culture the suspected pathogen(s). Selected isolates were tested for pathogenicity on host pulses, with more currently being tested.

Field Trials

At each of 20 southern pulse agronomy sites across SA (low, medium and high rainfall), replicated (n = 3) field trials have been sown for two crop types suited to the region. Sites, crop types & sowing dates are given in Table 1.

At each site, three soil samples were collected, with one for each bay of the combined two species trials. Soil samples were submitted to PREDICTA®B testing to determine which pathogens were present at levels likely to cause root disease.

Treatments were applied at seeding. Various pesticides, including fungicides, a fungicide/nematicide and a biological nematicide/bio-stimulant were applied as seed treatments and in-furrow liquid bands targeting fungi, oomycetes

and nematodes, as well as the combination of these. An untreated control was also sown.

Field trials were sown using standard plot trial narrow-point/press wheel seeders. Fertiliser (no fungicide) was applied at seeding per district practice. Trials have been managed since as per district practice including foliar fungicides. At each site, plots will be sampled by digging plants, washing and visually scoring root disease. Roots will then be tested using PREDICTA®B to determine the causal pathogens involved in any root disease and to confirm any treatments effects. Plots will be harvested for yield comparison.

Results and discussion

Survey

To date, 400 samples have been processed from across all cropping regions in Australia, including 97 collected in 2018 from SA and western Victoria (Vic). Crops tested include chickpea, lentil, faba bean, field pea, lupin, canola, vetch, clover and lucerne. Over 150 fungal and oomycete isolates have now been isolated, sequenced to confirm their identity and stored for future work.

Pulse research test panel

The results for the Pulse Research test panel for the 2019 national survey are summarised in Figure 1; results from SA in 2018 were very similar (data not shown). Rhizoctonia AG8, Pratylenchus spp., Pythium clade F and Phoma pinodella (this test also detects Didymella pinodes) were all common across crop types and regions.

P. neglectus (root lesion nematode) was detected at substantial levels in many crops including some that were considered to be poor hosts (for example, faba bean and field peas); these crops are known to limit multiplication (hence used as 'break crops') however the nematodes are clearly able to invade and cause damage. Their effect on yield of pulses has not been reported.

P. pinodella appears to have a broader host range amongst pulse crops and pasture legumes than generally expected. As part of a species complex involving other closely related fungi, P. pinodella is known to cause blackspot leaf and stem blight, as well as foot rot. However, its importance as a root rot pathogen has not been well-documented in pea, nor in any other pulses where it appears common.

Aphanomyces euteiches was found in 18% of samples in 2018 and 1% in 2019, all were from faba bean crops exhibiting moderate to severe root disease. In 2019, a test for Aphanomyces trifolii was added to the panel, with six samples (faba bean, lentil, and vetch) found to be infected. The pathogen was particularly prevalent in vetch (27% of vetch samples infected). This pathogen is typically associated with sub-clover (O'Rourke et al., 2010). The effect of A. trifolii on lentil and vetch has not been described, while the effect on faba bean has only been briefly described (O'Rourke et al., 2010).

Rhizoctonia solani AG8 and AG2.1, Pythium clade I and Macrophomina phaseolina (charcoal rot) were also present at substantial levels. Rhizoctonia AG8, the cause of Rhizoctonia root rot in cereal crops, also causes substantial root damage in pulses, despite pulses generally reducing paddock inoculum over the course of the season.

P. medicaginis was not detected in either year, probably due to drought in northern Australia. Conditions were conducive for Phytophthora in the GRDC Southern and Western Regions of Australia and P. megasperma and P. clandestina were detected in SA and Western Australia (WA) (lentil and lupin). Both species are known to have a wide host range, however their importance in southern Australian pulse crops is yet to be quantified.

Next Generation Sequencing (NGS) DNA from each root sample was analysed with NGS and a broad range of fungal organisms were detected (Figure 2); some of which have been reported to cause root disease of pulses.

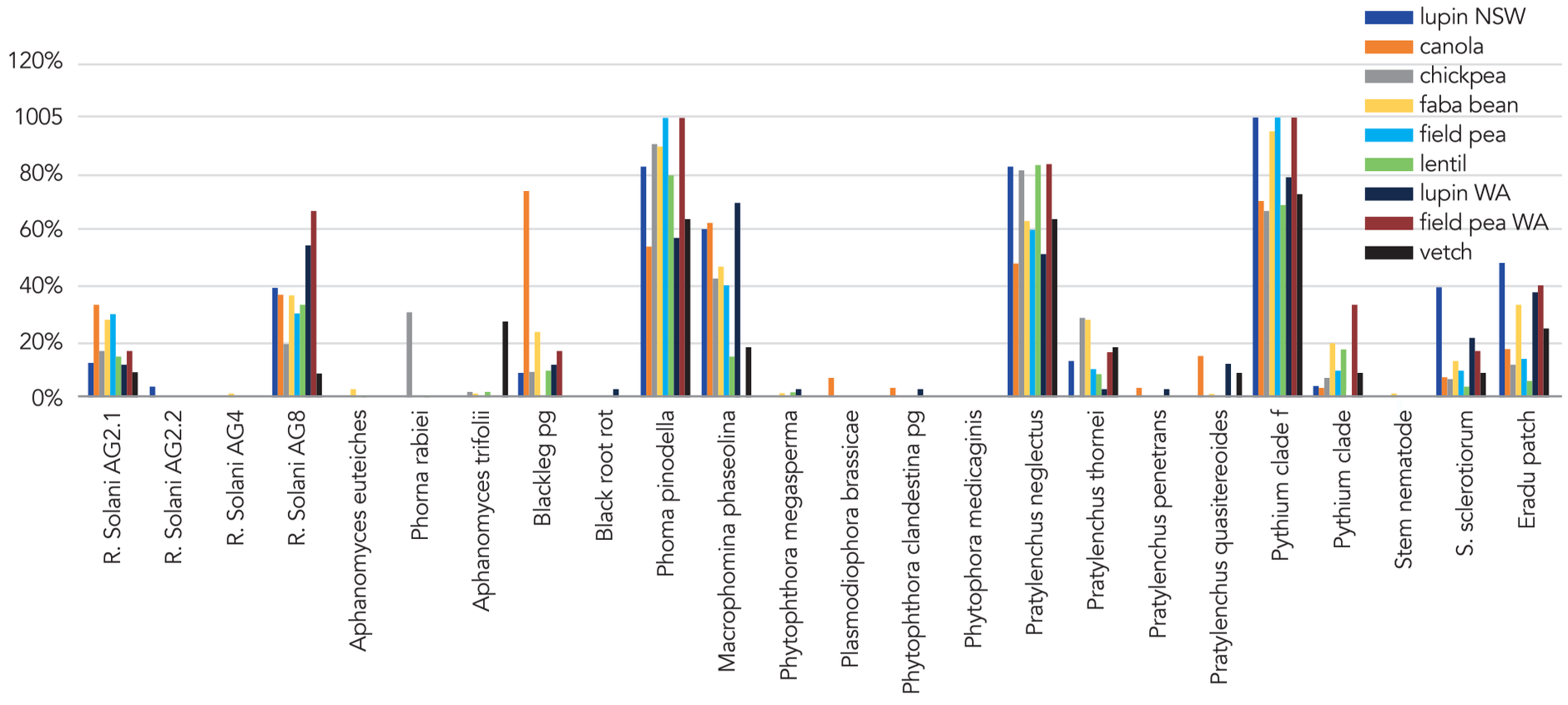

Figure 1. Frequency of detection of known pulse pathogens from national pulse and canola root samples in 2019.

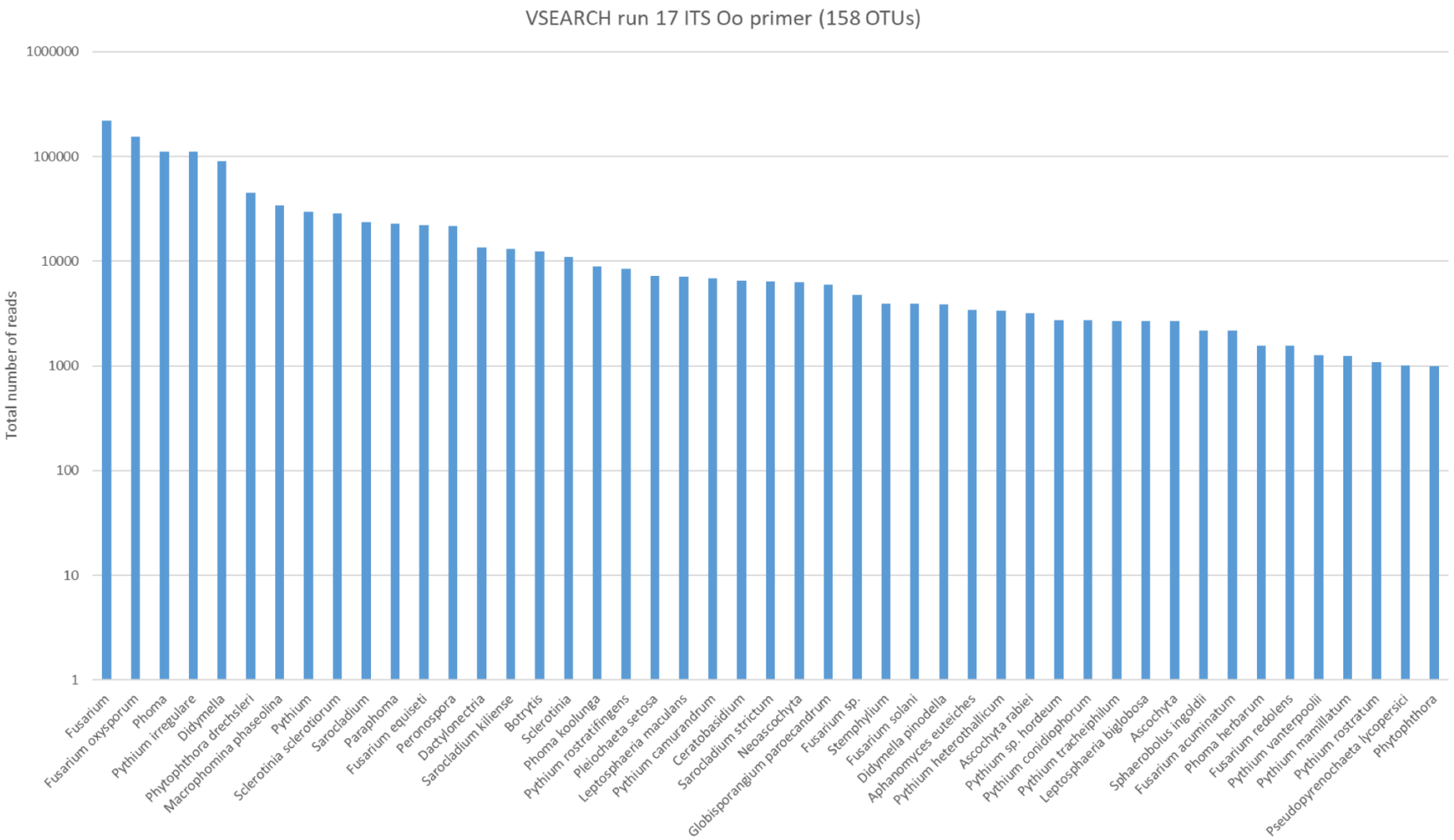

Figure 2. NGS detections of DNA of possible pathogen species in all 2019 pulse samples combined using primers aimed at oomycetes (Pythium, Phytophthora). Other primers are being used to better detect true fungi.

Organisms were identified as pathogens of interest based on international research and observed symptoms in plant samples. A number of pathogens of interest identified in this survey are summarised as follows:

Phytophthora spp. - sequence data identified several Phytophthora species present including P. megasperma, P. trifolii, and P. clandestina. All three species were detected in chickpea roots with symptoms of Phytophthora root rot in 2018. P. megasperma 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.

The potential of P. megasperma to also infect faba bean roots could have implications for the South East region and requires further investigation to confirm and quantify its extent and severity. Phytophthora root rot in the Northern Region is estimated to cost chickpea growers up to $8.2 million annually (Murray & Brennan, 2012).

Fusarium spp. - globally, Fusarium spp. feature frequently 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 and tentatively identified as detected by the survey, include F. solani, F. redolens, F. oxysporum, F. equiseti, F. avenaceum and F. acuminatum. Internationally, research groups are currently investigating the role of these species as potentially important components of disease complexes with A. eutiches and Phytophthora spp. (Banniza, 2016).

There are constraints on the resolution of the NGS, however Fusarium spp. are amongst the most common NGS detections in survey samples. The Illumina® MiSeq® sequences cannot differentiate Fusarium species to form a specialis. This limits our current ability to identify some of the most important root pathogens of chickpea (F. oxysporum f. sp. ciceris) and lentil (f. sp. lentis). Both however, are not currently known to occur in Australia (Cunnington et al., 2016, Pouralibaba et al., 2016).

Further investigation is needed to determine which, if any, of the above species play an important role in causing pulse root disease in Australia. Currently, Fusarium spp. are being isolated from the samples, their DNA sequenced using Sanger Sequencing to confirm identity, then tested in pot assays to determine pathogenicity (Figure 3).

Figure 3. Pot assays for pathogenicity of Fusarium spp. From left: F. avenaceum on faba bean, F. oxysporum + F. avenaceum on faba bean and faba bean control with no disease inoculum.

Field Trials

Pre-sowing PREDICTA®B results for all field trials are summarised in Table 2. Field trials have established well and are showing possible symptoms of root disease. However cold, dry conditions have limited yield potential at many sites, while the dry conditions may also have limited soil fungicide distribution and efficacy. Sites are currently being assessed for disease.

Conclusion

The survey has identified some important trends, although the National data is limited to only a single year. Phytophthora and Aphanomyces have been associated with crop failures in the high rainfall zones, but their distribution seems to be limited. Other potential root pathogens such as Fusarium spp., Rhizoctonia solani, Pythium spp., Pratylenchus spp. and Phoma spp. are much more common, within and across regions.

It is likely that pulse root diseases are contributing to poor water use efficiency and unexpected yield losses, and the risk is likely to increase with increased frequency of pulses in cropping sequences. Legume pastures may also be a significant source of infection. Their effect on yield is currently being evaluate through the SAGIT Pulse Yield Loss trials. These potential pathogens being widespread suggests they have greater potential for impact across the industry.

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Contact details:
Blake Gontar
SARDI Soil Biology and Molecular Diagnostics
Plant Research Centre, Gate 2B Hartley Grove, Urrbrae, SA, 5064
0430 597 811
blake.gontar@sa.gov.au

Table 2. Pre-sowing PREDICTA®B soil test results for Pulse Root Disease Yield Loss trial sites in 2020.

Notes	*R. solani AG2.1* pgDNA/g Sample*	*R. solani AG8* pgDNA/g Sample*	*Aphanomyces trifolii* kDNA copies/g Sample*	*Didymella pinodes/Phoma pinodella* pgDNA/g	*Macrophomina phaseolina* kDNA copies/g Sample*	*Phytophthora megasperma clade* kDNA copies/g Sample*	*Phytophthora clandestina* pg DNA / g soil	*Phytophthora medicaginis* kDNA copies/g Sample*	*Pratylenchus neglectus* nematodes /g soil	*Pratylenchus thornei* nematodes/g soil	*Pythium clade f* pgDNA/g Sample*	*Pythium clade l* pgDNA/g Sample*	*Stem nematode* nematodes/100 g soil	*S. sclerotiorum* kDNA copies/g Sample*	*Eradu* pgDNA/g Sample*
Booleroo	657	307	1	85	6	0	0	0	4	3	28	1	0	0	142
Eudunda	0	330	1	426	10	0	0	0	6	0	5	7	0	0	0
Farrell Flat	349	76	1	79	6	0	0	0	0	0	9	3	0	0	186
Hart	22	85	0	633	7	0	0	0	2	1	17	6	0	0	0
Maitland	0	13	5	18	6	0	0	0	233	0	129	20	0	1	1
Pinery	0	1284	0	0	0	0	0	0	37	0	54	3	0	0	0
Port Broughton	0	140	0	10	13	0	0	0	20	0	6	1	0	0	70
Riverton	0	887	4	158	8	0	0	0	12	1	6	4	0	0	13
Tarlee	0	361	0	306	3	0	0	0	6	24	24	2	0	0	0
Turretfield	86	0	0	106	43	0	0	0	6	0	52	1	16	0	12
Warnertown	0	150	0	152	9	0	0	0	3	2	39	3	0	0	0
Bool Lagoon	1	0	4	891	67	0	0	0	5	0	152	11	0	0	0
Coomandook	12	26	2	12	9	0	0	0	107	0	36	2	0	0	0
Sherwood	0	0	0	2134	27	0	0	0	0	0	36	0	0	0	23
Kimba	11	151	0	59	10	0	0	0	20	0	11	8	0	0	13
Stokes	3	177	2	279	1	0	0	0	7	0	40	0	0	0	13
Tooligie 1	0	0	0	323	7	0	0	0	27	0	23	0	0	0	0
Tooligie 2	6	150	0	53	12	0	0	0	2	0	15	1	0	0	0
Wudinna	399	24	0	65	16	0	0	0	30	3	13	7	0	270	0
Yeelanna	0	5	3	117	10	0	0	0	130	0	17	15	0	0	0

Pulse root disease trial plan

Row	Bay 1	Bay 2	Bay 3
	Buffer	Buffer	Buffer
1	Treatment 2	Treatment 4	Treatment 3
2	Treatment 2	Treatment 4	Treatment 3
3	Nil	Treatment 2	Treatment 2
4	Nil	Treatment 2	Treatment 2
5	Treatment 7	Treatment 7	Nil
6	Treatment 7	Treatment 7	Nil
7	Treatment 6	Treatment 6	Treatment 7
8	Treatment 6	Treatment 6	Treatment 7
9	Treatment 3	Nil	Treatment 4
10	Treatment 3	Nil	Treatment 4
11	Treatment 4	Treatment 5	Treatment 5
12	Treatment 4	Treatment 5	Treatment 5
13	Treatment 5	Treatment 3	Treatment 6
14	Treatment 5	Treatment 3	Treatment 6
	Buffer	Buffer	Buffer
	Rep 1	Rep 2	Rep 3

Key:

Treatment 1	Nil
Treatment 2	Fungi (Fusarium spp.) active
Treatment 3	Fungi + oomycete active (Rhizo spp. etc. + Pythium spp. etc.)
Treatment 4	Oomycete active (Pythium spp. etc.)
Treatment 5	Nematode bioactive + root stimulant
Treatment 6	2 + 3 + 5
Treatment 7	3 + 5

Inoculated at seeding with mixed Rhizoctonia solani AG8, Phoma pinodella and Fusarium avenaceum

Not inoculated - background disease inoculum only

N →

Seeding date: May 18, 2020
Fertiliser: MAP
Fertiliser rate: 80 kg/ha