Office Use Only

Project Code	
Project Type	

FINAL REPORT 2021

PROJECT CODE	:	SUA920

PROJECT TITLE
Quantifying pulse root disease yield loss in SA

PROJECT DURATION

Project Start date	1/7/2020
Project End date	30/6/2021

PROJECT SUPERVISOR CONTACT DETAILS

Title:	**First Name:**	**Surname:**
Mr	Blake	Gontar
Organisation:		
The University of Adelaide/ South Australian Research and Development Institute		

ADMINISTRATION CONTACT DETAILS

Title:	**First Name:**	**Surname:**
Ms	Louise	Moore
Organisation:		
The University of Adelaide		

PROJECT REPORT

Executive Summary

In 2020, 35 replicated field trials were conducted at 20 sites across SA to investigate yield loss caused by root disease in pulse crops including faba bean (12), lentil (11), chickpea (6), vetch (3), lupin (4) and field pea (3).

A selection of seed and in-furrow chemical/biological products were selected in consultation with crop protection experts to control fungi, oomycetes and nematodes.

Root disease was common across all sites, averaging 2.2 on 0-5 scale, were 0 = no disease and 5 = no yield potential. Scores ranged from <1 in lentil and faba bean trials at Farrell Flat, to >3 in lentil trial at Warnertown. Moderate disease (>2) was observed at 26 sites; no trials had high levels of root disease (>4).

The treatments were relatively ineffective, reducing disease in only 10 sites. The combination of 4 actives was the best treatment at 7 sites; the Nil treatment had the most disease at 3 sites. At Maitland, the combination treatment reduced the disease score from 2.9 to 1.9 ($p < 0.001$) and produced a significant yield response.

We recommend different options be considered in future projects to investigate yield losses caused by root diseases in pulses, see recommendations section.

Project Objectives

This project had two main objectives:

1. Collaborate with GRDC Southern Pulse Agronomy project to assess yield impacts of root disease on pulses across South Australia
2. Evaluate management options for soilborne diseases of lentil, faba bean and chickpea grown in South Australia.

Overall Performance

The SARDI Pulse Agronomy Group incorporated the projects field trials into their core sites across SA. Root disease assessments were performed by Blake Gontar and Ioane Vakaci, and nodulation and nitrogen fixation assessments by Liz Farquharson. DNA testing was undertaken by the SARDI Molecular Diagnostics Group.

1. Chemical treatments were relatively ineffective overall, reducing root disease only modestly at most sites. Certain pathogens were unaffected by chemical controls. The small improvements in root health may not be sufficient to increase yield.
2. Pathogens detected may be present but may not have been the most-limiting constraint. At many sites in 2020, yield potential was limited by a relatively dry post-sowing period. Whilst disease did develop at all sites, disease was moderate only and may not have been yield-limiting.

Yield responses were detected at only 4 sites, and the largest increases were 0.35 and 0.14 t/ha at Bool Lagoon and Maitland, respectively.

Reductions in root disease were detected in 10 trials, however, these were generally <1 on a 0-5 scale.

Had the treatments worked, this project could have provided a quick assessment of the cost of root diseases to the pulse and grain legume industries. With more research on rates and methods of application it may be possible to improve the disease control. However, given that we know there is a broad range of potential pathogens involved it is worth considering fumigating sites and using artificial inoculum to evaluate pathogens individually and in mixtures.

Key Performance Indicators (KPI)

KPI	***Achieved (Y/N)***	***If not achieved, please state reason.***
Trial sites are tested and a fungicide plan is developed for the pathogens present, trials are sown with treatments and managed effectively throughout the growing season. Initial soil DNA tests were conducted for each site before sowing to determine inoculum presence of potentially important pathogens. Further soil DNA tests were conducted at, or immediately after, sowing with a sample taken across each bay of the two adjacent trials at each site. Treatments targeting fungal, nematode and oomycete pathogen groups were selected through discussion with crop protection experts of agri-chemical companies (NuFarm, Syngenta) as well as through previous research. All but one site (Stokes faba bean) was sown, managed effectively, sampled, assessed and harvested, contributing to a data set of 38 trials. The site at Stokes germinated poorly and became overrun with weeds.	Y	
Presentation at Hart Field Day as required. Hart Field Day was not run due to COVID. Blake Gontar presented the research at the Tarlee site to the mid-north HRZ field group (22/9/21), the Independent Consultants group (28/1/21) as well as to MacKillop Farm Management Group (28/3/21). Presentations were made by SARDI staff Amy Gutsche, Dylan Bruce and Amanda Pearce to EP, MN and SE grower groups in 2020.	Y	
Trial measurement including disease score, root pathogen DNA and yield are recorded, data analysed and interpreted. All 38 trials were sampled and visually assessed for root disease. Pathogen DNA was tested in all replicates of the Nil and 'complete' treatments. Yield was recorded for all trials. Data were analysed by SAGI biometricians and the analysis and interpretation are presented herein.	Y	

Technical Information

Background

Pulse root disease surveys conducted by SAGIT project S218 and extended nationally by GRDC-project DJP1907-002RMX revealed root disease is common and identified a range of potential pathogens. Growth room experiments demonstrated that isolates of *Fusarium*, *Phoma*, *Pythium*, *Phytophthora* were pathogenic on a broad range of crops. *Rhizoctonia* and *Pratylenchus* species commonly associated with cereals were also commonly found in diseased pulse roots, this does not mean they are good hosts.

S218 findings stimulated strong interest amongst growers and advisors. SUA920 was subsequently funded as a one year proof of concept project to evaluate use of registered and experimental products as research tools to measure the magnitude of yield loss in pulse and grain legume crops across SA.

Methods

Four products were selected in consultation with agrichemical crop protection experts, to control different components of the disease complex, these were applied in different combinations to produce 6 treatments including untreated (Table 1). Metalaxyl-m is the only product registered to control root disease in pulses.

The treatments were applied to 35 field trials at 21 sites across SA, using a randomised replicated block design with 3 replicates. To save costs, the trials were established adjoining the GRDC 'Southern Pulse Agronomy' program sites that were selected to represent the range of growing environments across SA, and without prior knowledge of soilborne disease load (Appendix A, Table 1).

The key traits (response variables) measured and analysed for this report were

- Yield (tonnes/hectare)
- Disease score (0-5 scale)
- Nodulation score (0-5 scale)
- Biomass (tonnes/hectare)
- Nitrogen fixation (kilograms fixed per hectare)

There were 11 faba bean, 10 lentil, 6 chickpea, 4 lupin, 2 chickpea and 2 vetch trials. Effects on disease and nodulation were assessed at 35 trials, yield at 34, biomass at 13 and nitrogen fixation at 11 trials.

A panel of 24 qPCR assays was used to assess soil pathogen loads at seeding by sampling soil across each bay (Appendix A, Table 2 and 3).

During spring, plants were sampled from four locations along each plot. Roots were washed and scored for disease using a 0-5 scale, where 0 = no disease and 5 = no yield potential, and nodulation was also scored on a 0-5 scale, where 0 = no nodules and 5 = excellent nodulation.

Pathogen loads in roots samples from the Nil and TTU treatments were assessed using same suite of qPCR assays. Trials were harvested and yields recorded.

Statistical analysis was undertaken by SAGI staff. Linear mixed models using restricted maximum likelihood were used to analyse the data using ASReml-R.

Table 1. Treatments applied to field trials.

Treatment	Product	Actives	Target organisms
A	Apron XL	Metalaxyl-m	Oomycetes i.e. Pythium, Phytophthora
U	Uniform	Metalaxyl-m + Azoxystrobin	Oomycetes + fungi (including Rhizoctonia solani AG8)
Tr	Trunemco	Bacillus amyloliquefaciens + cis-Jasmone	Nematodes
TrU	Trunemco + Uniform	As above	Oomycetes + fungi + nematodes
TTU	Above + Tymirium	As above + cyclobutrifluram	Oomycetes + fungi + nematodes + *Fusarium* spp.
Nil	NA	NA	Control

Results

Pathogen load at seeding

Results for existing qPCR tests indicated that *Rhizoctonia solani* AG8, *Phoma pinodella*, *Pythium* clade F and *P. neglectus* were frequently present at trial sites (Appendix A, Table 3). No information was obtained on *Fusarium* species found by S218, due to lack of qPCR tests at this time.

Yield Response

There were no significant differences between treatments for the multisite analysis of any of the six crop types.

However, there were yield responses at individual sites with the largest response with TTU treatment in chickpeas at Bool Lagoon, 0.35 t/ha (p = 0.031) (Figure 1) and Tooligie 0.13 t/ha (p = 0.01) (Appendix A, Figure 1). There were small significant yield increases in lentils at Maitland, 0.14 t/ha and in faba bean at Sherwood (Appendix A, Figures 2-3).

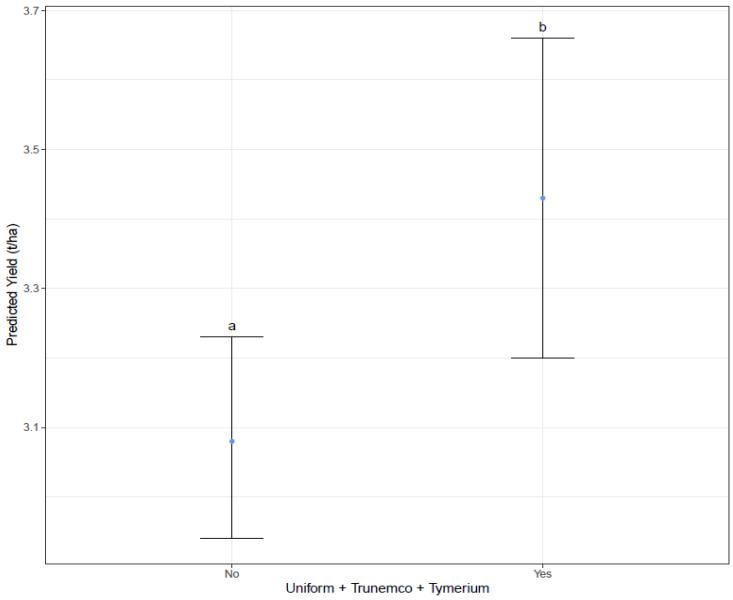

Figure 1. Predicted Yield for the main effect of TTU. Error bars represent the 95% confidence interval.

Mean trial yields ranged from 0.41t/ha in vetch at Wudinna to 5.36 t/ha in faba bean at Yeelanna. Mean treatment yields in each trial are summarised in Appendix A, Table 4.

Root disease scores

In general treatment effects on root disease scores were small.

Root disease was common, averaging 2.16 across all sites ranging from <1 in lentil and faba bean at Farrell Flat, to >3 in lentil at Warnertown. Moderate (>2) disease was observed at 26 sites, however no trials had high (>4) disease levels.

There were significant treatment effects on disease score in 10 trials. In 8, the TTU treatment reduced disease with the greatest reductions of 1.01 and 0.61 at Maitland lentil ($p < 0.001$) and Tooligie chickpea ($p < 0.001$), respectively. Yield improvements were observed at both sites.

Pathogen levels in roots

Root pathogen levels in the Untreated and TTU treatments were assessed using qPCR (Appendix A, Figures 4-9). A multisite analysis is progressing.

The TTU treatment appeared to reduce *P. pinodella* levels in:

- Faba bean at Bool Lagoon, Coomandook, Farrell Flat, Riverton, Sherwood and Yeelanna
- Lentil at Booleroo, Hart, Tarlee, Warnertown and Yeelanna, but had limited effect on *Pythium* clade F.

And also appeared to reduce Pythium clade F levels in:

- Faba bean at Bool Lagoon and Booleroo.

Nodulation

Nodulation treatment effects were observed in 8 trials; however, the results were inconsistent. TTU mostly improved nodulation, but in faba beans at Riverton and Booleroo, the untreated had significantly better nodulation.

Conclusions Reached &/or Discoveries Made

Despite limited yield responses, this one year proof of concept project has contributed to this emerging area of research. Key conclusions include:

1. Confirming root disease is common in the pulse and grain legume crops across SA, however treatment effects were modest at best, and no treatment or treatment combination provided complete root disease control.
2. Root disease scores indicate the products selected and/or methods of application used were inadequate to provide the level of disease control required to be useful as research tools to selectively control different components of the root disease complex.
3. *Phoma pinodella* was the most common pathogen measured with the current set of qPCR tests. All pulses appeared susceptible with little difference between crop types. The TTU treatment reduced infection at many sites.
4. Modest yield responses were measured at 4 sites, all associated with the TTU treatment, the largest increase, 0.34 t/ha was at Bool Lagoon and largest percentage increase, 14%, was measured under low rainfall and low yield potential at Toologie in chickpea.

Intellectual Property

NA

Application / Communication of Results

- Root disease was present at all 35 trials in 2020, ranging from <1 in lentil and faba bean at Farrell Flat, to >3 in lentil at Warnertown (0-5 scale). Moderate (>2) disease was observed at 26 trials, however there were no trials with high (>4) disease levels.
- Chemical/biological treatments were mostly ineffective. Treatment with a combination of 4 actives resulted in modest disease control, generally <1 unit (0-5 scale), in 10 trials.
- Yield effects were measured in 4 trials, the largest being 0.34 t/ha in chickpea at Bool Lagoon.
- The project demonstrates the need for better control methods for root disease in pulses, for both growers and as a research tool.

POSSIBLE FUTURE WORK

- Further studies are required to determine which pathogens cause sufficient yield losses under appropriate conditions to warrant development of management strategies.
- A change in strategy for conducting field trials is recommended. Consideration should be given to use of:
  - Fumigation to eliminate background pathogen loads.
  - Artificial inoculum used to manage pathogen loads and complexes.
  - Irrigation treatments used to produce a range of moisture conditions.
- While this strategy would limit the number of field sites to research centres, it would enable the focus on *Phoma pinodella* to be broadened to include *Fusarium*, *Pythium*, *Phytophthora* and *Aphanomyces* spp.
- Unexplained fumigation responses may help identify other important pathogens.
- qPCR tests should be developed for pathogens of interest to support research including selecting trials sites and evaluating treatments and potentially assist growers plan cropping sequences. In the interim, research should have access to sequencing technologies to help characterise the pathogen complexes.

APPENDIX A

Detailed Results: S-UA920 'Quantifying pulse root disease yield loss in SA'

Table 2. Site characteristics of pulse root disease trials in 2020.

Site	Crop 1	Crop 2	GSR	Annual Rainfall	Soil OC%	Soil pH (CaCl2)
Bool Lagoon	Faba bean	Chickpea	452	572	3.2	5.9
Booleroo	Faba bean	Lentil	344	472	1.08	5.1
Coomandook	Faba bean	Lupin	329	387	0.86	6.6
Eudunda	Faba bean	Vetch	360	431	1.65	7.6
Farrell Flat	Faba bean	Lentil	372	482	1.43	6.6
Hart	Lentil	NA	324	499	1.37	7.7
Kimba	Field pea	Vetch	253	333	0.96	7.65
Maitland	Lentil	Faba bean	418	513	2.34	7.5
Pinery	Lentil	Chickpea	253	322	1.37	7.9
Port Broughton	Lentil	Chickpea	263	353	1.54	7.9
Riverton	Faba bean	Chickpea	388	473	2.18	7
Sherwood	Faba bean	Lupin	338	449	0.95	5.2
Stokes	Faba bean	Lupin	411	486	1.3	6.38
Tarlee	Faba bean	Lentil	353	426	2.17	6.1
Tooligie1	Chickpea	Field pea	252	318	NA	NA
Tooligie2	Lentil	Lupin	252	318	0.85	7.78
Turretfield	Chickpea	Field pea	354	409	2.06	6.8
Warnertown	Faba bean	Lentil	344	489	0.76	7.7
Wudinna	Faba bean	Lentil	178	231	1.35	7.94
Yeelanna	Faba bean	Lentil	349	402	1.32	7.69

Table 3. Summary of qPCR assays used to test survey samples.

No.	Target species	No.	Target species
1	*Aphanomyces euteiches,*	13	*Phytophthora clandestina*
2	*A. trifolii*	14	*P. medicaginis*
3	*Didymella pinodes/Phoma*	15	*P. megasperma*
4	*Ditylenchus dipsaci*	16	*Plasmodiophora brassicae*
5	Eradu patch	17	*Pratylenchus neglectus*
6	*Rhizoctonia solani* AG2.1	18	*P. thornei*
7	*R. solani* AG2.2	19	*P. quasitereoides*
8	*R. solani* AG4	20	*P. penetrans*
9	*R. solani* AG8	21	*Pythium* clade F*
10	*Leptosphaeria maculans*	22	*Pythium* clade I*
11	*Macrophomina phaseolina*	23	*Sclerotinia sclerotiorum*

Table 3. Level of pathogens detected at seeding at the different trial sites (2020). Only the pathogens detected at any of the sites, out of the 24 tested are indicated

Region	Site	*R. solani* AG2.1	*R. solani* AG8	*A. trifolii*	*P. pinodella*	*M. phaseolina*	*P. neglectus*	*P. thornei*	*Pythium* clade f	*Pythium* clade I	Eradu patch
MN	Booleroo	21	62	0	2	3	0	1	36	0	11
	Eudunda	1	43	0	279	2	1	0	3	5	0
	Farrell Flat	248	48	1	9	15	1	1	16	2	191
	Hart	0	0	0	342	1	1	0	19	5	0
	Pinery	0	101	0	0	1	3	0	28	4	0
	Riverton	0	20	2	186	2	2	0	28	13	0
	Tarlee	2	141	0	104	1	1	4	36	4	0
	Turretfield	36	4	0	54	75	2	0	71	1	0
	Warnertown	4	6	0	89	15	0	0	46	7	0
YP	Port Broughton	0	0	0	11	89	1	3	13	5	0
	Maitland	18	0	0	3	1	35	0	21	57	0
EP	Kimba	8	60	0	35	10	2	0	12	5	4
	Stokes	29	49	2	103	1	2	0	239	0	36
	Tooligie 1	0	0	0	167	6	9	0	17	0	0
	Tooligie 2	56	75	0	75	12	1	0	28	2	0
	Wudinna	0	128	0	150	1	12	0	10	3	0
	Yeelanna	0	0	3	84	20	20	0	50	19	0
SE	Bool Lagoon	0	0	5	1775	222	2	0	271	28	0
	Coomandoook	0	10	1	25	131	7	0	36	2	0
	Sherwood	29	4	0	2373	71	0	0	67	0	39

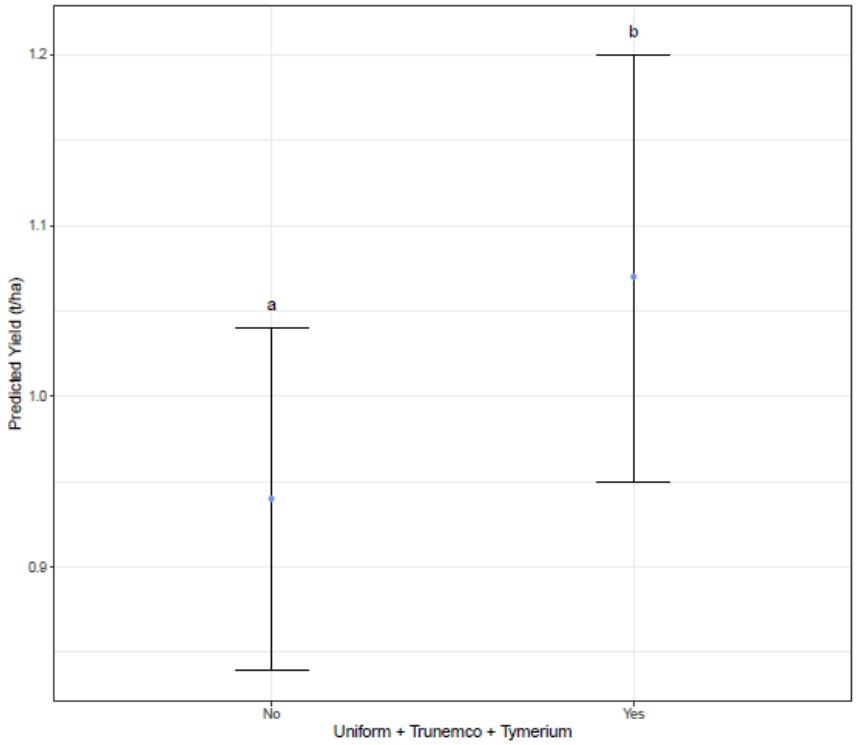

Figure 2. Chickpea yield with and without TTU treatment at Tooligie, 2020.

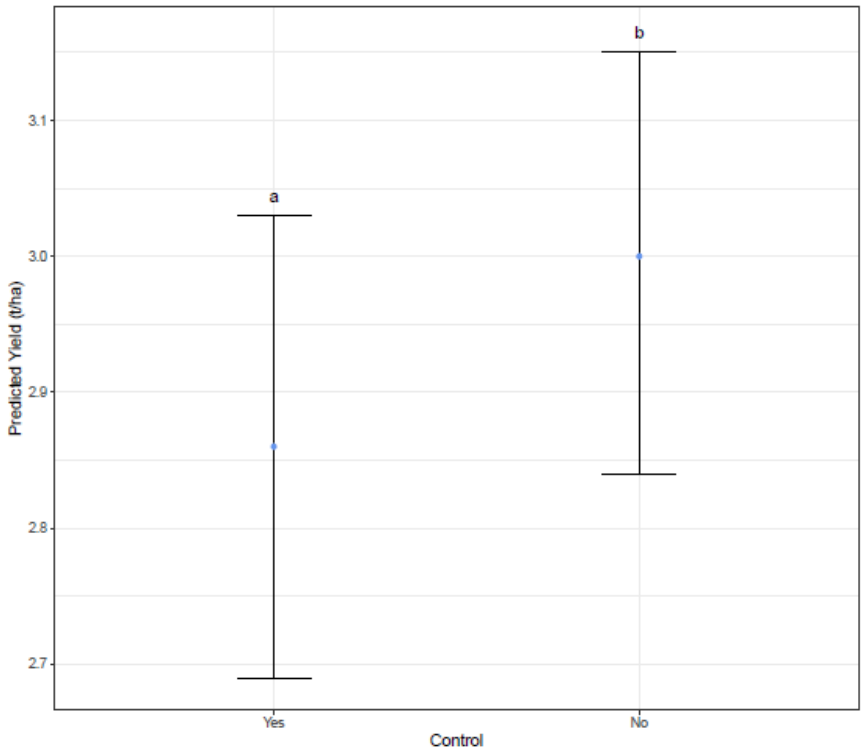

Figure 3. Lentil yield in control plots compared with any other treatment at Maitland in 2020.

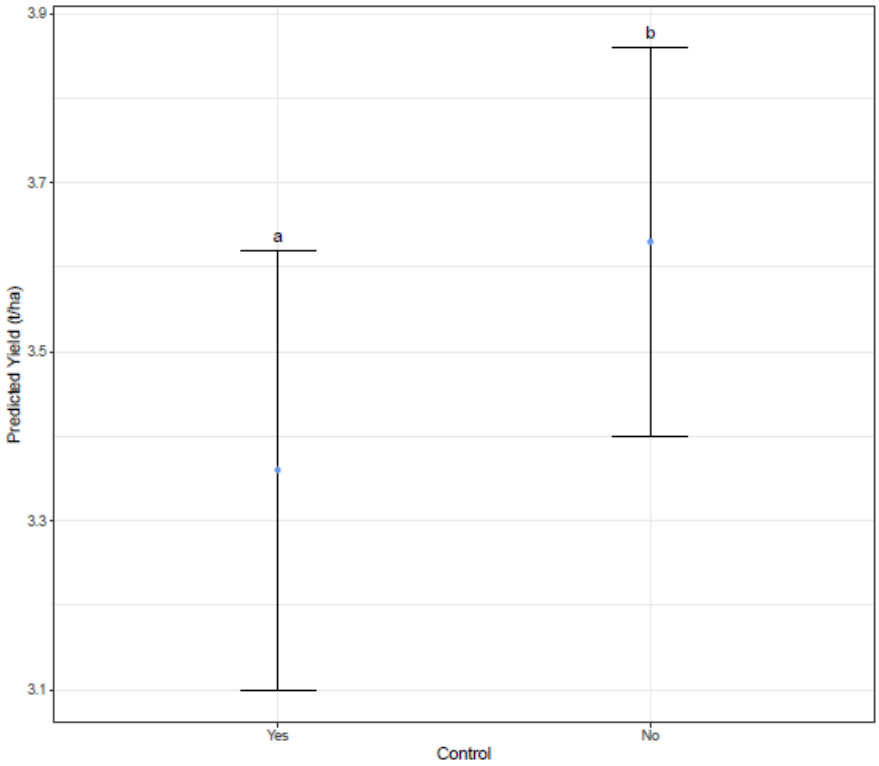

Figure 4. Faba bean yield in control plots compared with any other treatment at Sherwood in 2020.

Table 4. Mean yield of treatments applied to pulse crops in SA, 2020. Sites highlighted had significant effects and the significant treatment is indicated with an asterix.

Site	Crop	Nil	Tymirium + Trunemco + Uniform	Apron	Uniform	Trunemco + Uniform	Trunemco
Bool Lagoon	Chickpea	3.04	3.43*	3.15	3.03	3.08	3.13
Bool Lagoon	Faba bean	4.70	5.04	4.89	4.94	5.15	5.17
Booleroo	Faba bean	2.59	2.32	2.21	2.32	2.16	2.37
Booleroo	Lentil	1.94	1.71	1.75	1.66	1.92	1.73
Coomandook	Faba bean	3.83	3.76	3.66	3.74	3.96	3.90
Coomandook	Lupin	1.87	1.95	2.09	1.85	2.10	1.75
Eudunda	Faba bean	3.97	3.83	3.89	3.75	3.62	3.77
Eudunda	Vetch	4.01	4.15	4.12	4.17	4.07	4.12
Farrell Flat	Faba bean	4.86	4.84	4.94	4.82	5.01	5.07
Farrell Flat	Lentil	1.99	1.86	2.04	1.79	2.05	2.02
Maitland	Faba bean	4.52	4.85	4.47	4.57	4.68	4.63
Maitland	Lentil	2.86*	2.99	3.01	3.07	2.93	2.99
Pinery	Chickpea	3.25	3.30	3.23	2.87	3.23	3.25
Pinery	Lentil	2.72	2.67	2.83	2.71	2.73	2.81
Port Broughton	Chickpea	0.86	0.88	0.89	0.87	0.85	0.90
Port Broughton	Lentil	0.87	0.81	0.79	0.86	0.80	0.85
Riverton	Chickpea	2.19	2.03	2.03	2.07	2.00	2.08
Riverton	Faba bean	4.38	4.43	4.48	4.08	4.50	4.36
Sherwood	Faba bean	3.36*	3.70	3.59	3.63	3.73	3.51
Sherwood	Lupin	2.73	2.94	2.47	2.67	2.92	2.96
Stokes	Lupin	2.21	2.33	2.26	2.40	2.28	2.12
Tarlee	Faba bean	3.77	3.74	3.76	3.62	3.52	3.63
Tarlee	Lentil	3.06	3.16	3.44	3.11	3.18	2.97
Tooligie1	Chickpea	0.97	1.07*	0.95	0.95	0.93	0.90
Tooligie1	Field pea	0.71	0.77	0.77	0.87	0.64	0.78
Tooligie2	Lupin	0.93	0.88	0.82	0.83	0.94	0.88
Turretfield	Chickpea	2.42	2.65	2.59	2.57	2.48	2.61
Turretfield	Field pea	2.43	2.50	2.37	2.36	2.56	2.49
Warnertown	Faba bean	2.25	2.25	2.30	2.26	2.33	2.36
Warnertown	Lentil	2.19	2.18	2.27	2.17	2.19	2.17
Wudinna	Lentil	2.66	1.98	2.34	2.09	2.29	2.28
Wudinna	Vetch	0.44	0.36	0.38	0.45	0.45	0.37
Yeelanna	Faba bean	5.33	5.41	5.25	5.58	5.32	5.28
Yeelanna	Lentil	4.52	4.71	4.11	4.14	4.60	4.74

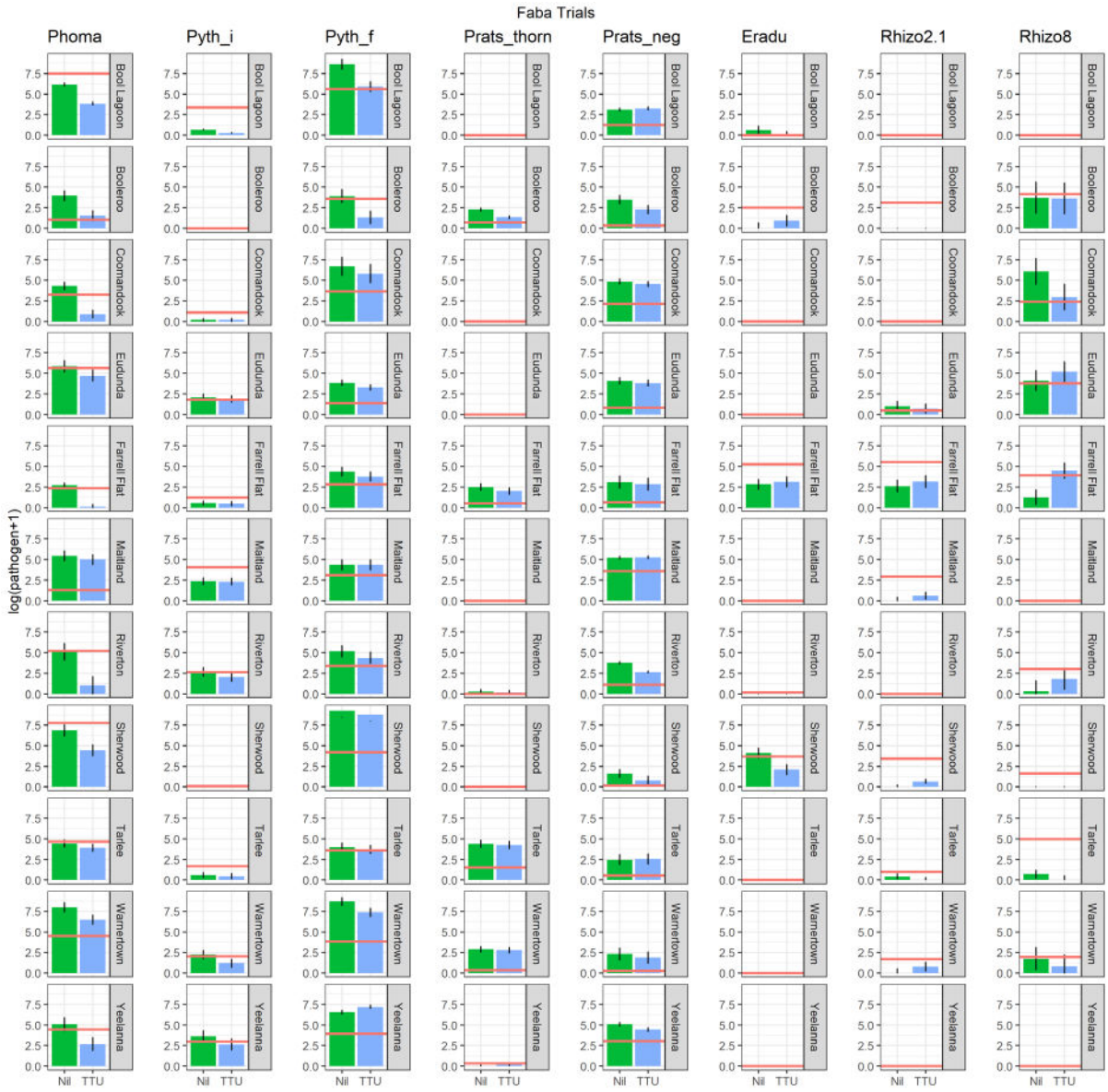

Figure 5. Comparison of pathogen levels using qPCR in roots of faba bean either treated with Tymirium + Trunemco + Uniform (TTU) or not (Nil) at 11 sites. The red line in each graph indicates starting soil pathogen DNA level.

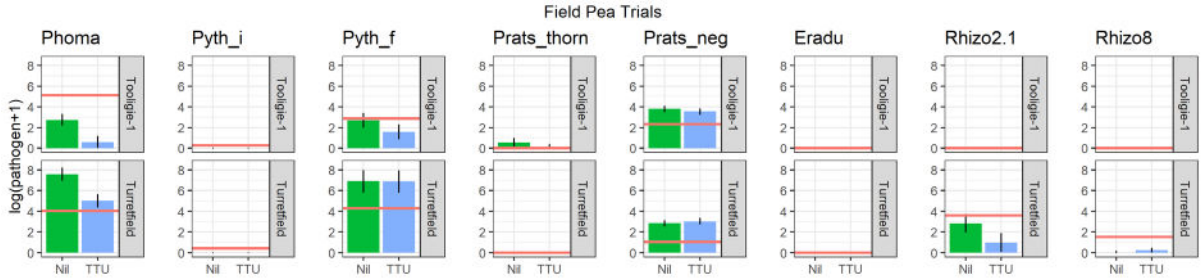

Figure 6. Comparison of pathogen levels using qPCR in roots of field pea either treated with Tymirium + Trunemco + Uniform (TTU) or not (Nil) at two sites. The red line in each graph indicates starting soil pathogen DNA level.

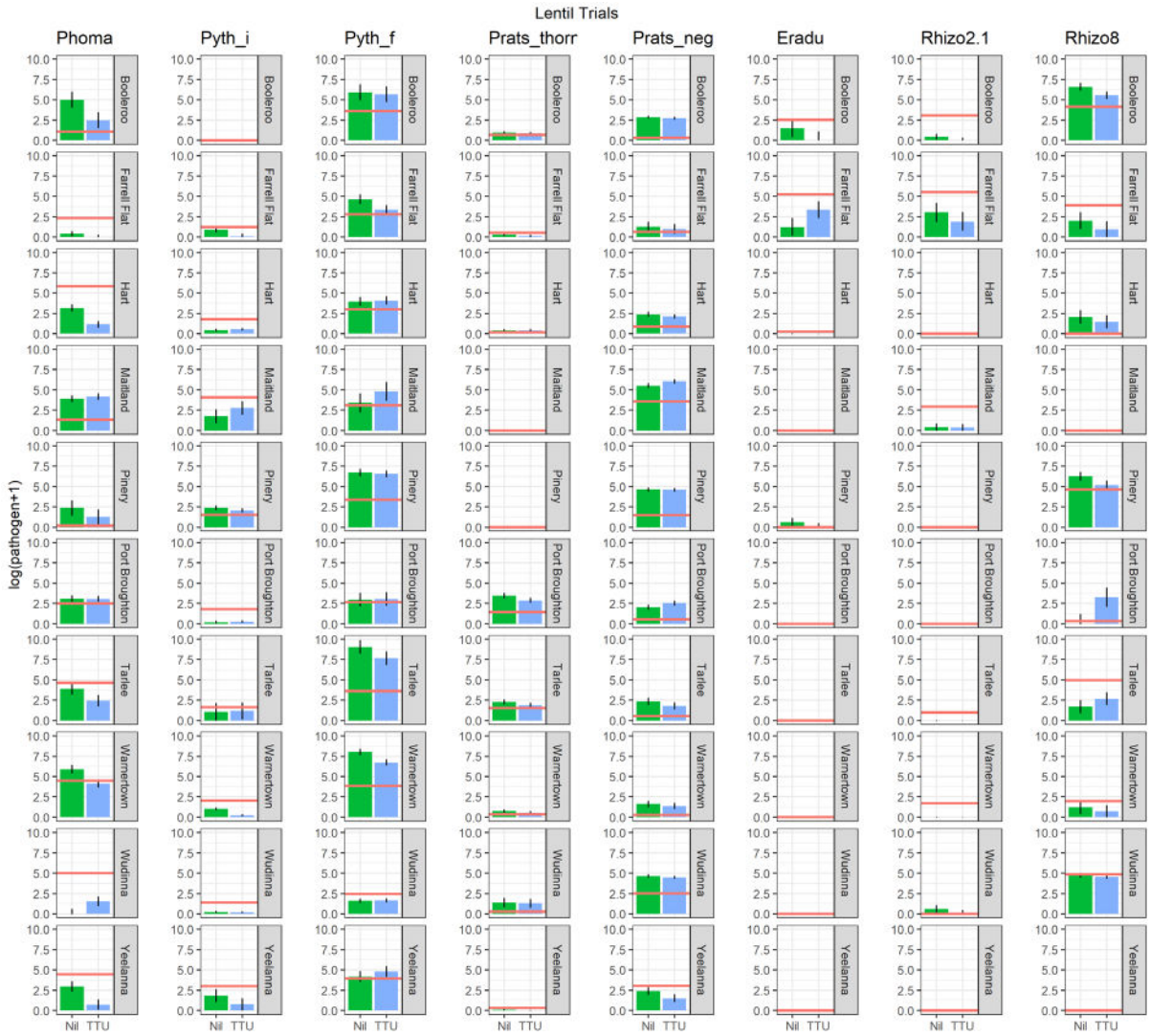

Figure 7. Comparison of pathogen levels using qPCR in roots of lentil either treated with Tymirium + Trunemco + Uniform (TTU) or not (Nil) at ten sites. The red line in each graph indicates starting soil pathogen DNA level.

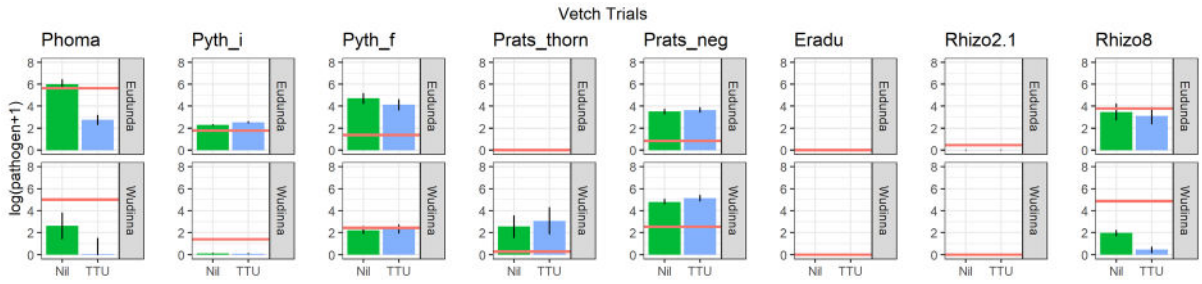

Figure 8. Comparison of pathogen levels using qPCR in roots of vetch either treated with Tymirium + Trunemco + Uniform (TTU) or not (Nil) at two sites. The red line in each graph indicates starting soil pathogen DNA level.

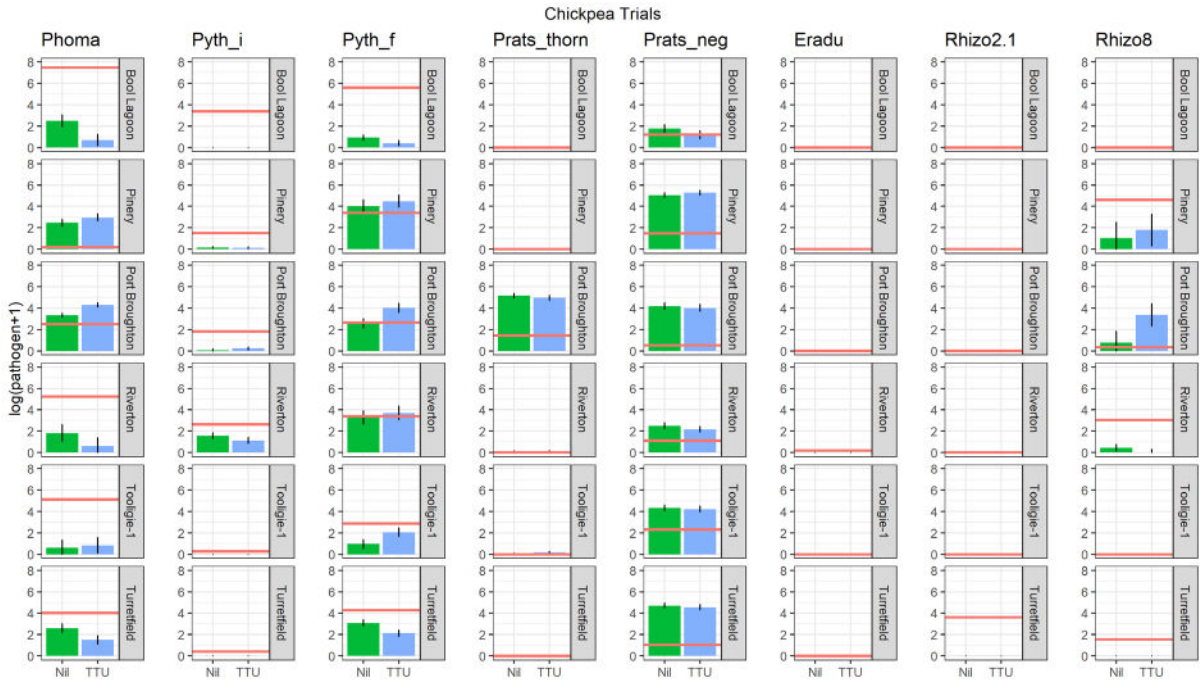

Figure 9. Comparison of pathogen levels using qPCR in roots of chickpea either treated with Tymirium + Trunemco + Uniform (TTU) or not (Nil) at six sites. The red line in each graph indicates starting soil pathogen DNA level.

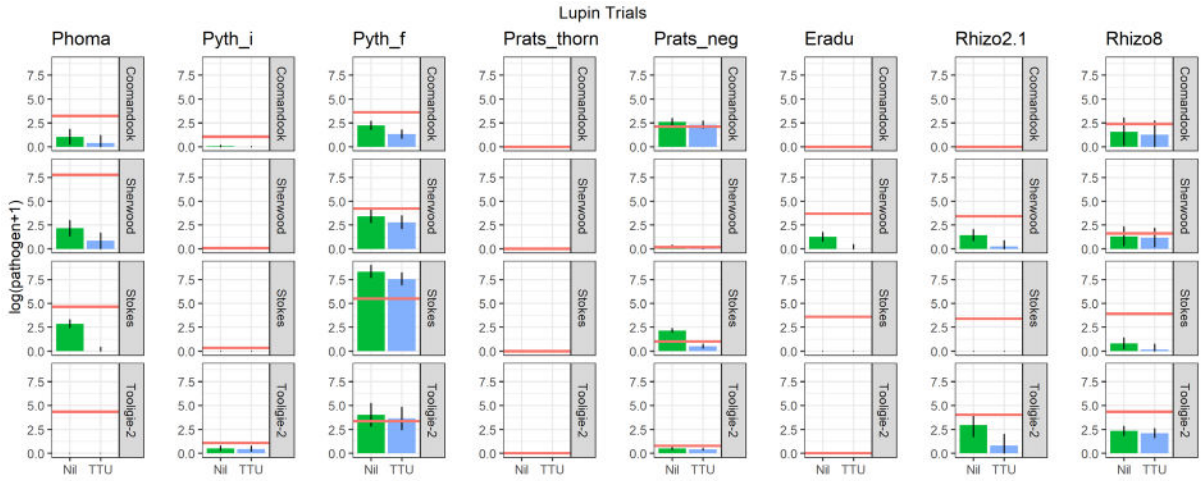

Figure 10. Comparison of pathogen levels using qPCR in roots of lupin either treated with Tymirium + Trunemco + Uniform (TTU) or not (Nil) at four sites. The red line in each graph indicates starting soil pathogen DNA level.