Final Technical Report
CAS101: Using DNA diagnostics to monitor disease suppressive cotton farming systems

Executive Summary

Over the past 20 years, high yielding irrigated cotton crops have required increased inputs such as nitrogen and water to achieve their genetic potential. Coupled with the widespread adoption of the genetically modified Bollgard 3 varieties to reduce Helicoverpa damage, these high demand crops draw heavily on plant and soil resources during the boll fill period.

Throughout that same time-frame, soil borne diseases such as Verticillium and Black Root Rot have increasingly caused serious yield losses and lessened the number of cotton crops that can be grown in a sustainable sequence.

Verticillium and Black Root Rot are serious diseases of cotton. Their impact is quite regionally based. Black Root Rot is favoured by cool temperatures, so is more problematic in southern NSW. Verticillium is more concentrated in the central valleys such as the Macquarie, Lower and Upper Namoi, Gwydir and Macintyre. The dataset from this project showed Black Root Rot inoculum levels increased often by 2-5 times per cotton crop depending on the region and season. Verticillium inoculum levels increase much more rapidly, often by 20-50 times after cotton depending on the region and season.

Using DNA diagnostics is a reliable, repeatable and scalable system to measure and monitor inoculum population dynamics of Verticillium and Black Root Rot in cotton. Sampling fields on a grid basis generates valuable insights into the spatial distribution and population density of the disease. The spatial data layer (heatmap) of disease inoculum can then be used to compare with other factors that may influence the infection, severity and yield of cotton.

This project demonstrated that measuring inoculum population densities will give a strong indication of the subsequent Verticillium infection % and Black Root Rot severity. The resultant yield impact of both these diseases was also demonstrated, but the magnitude is quite dependent on weather conditions through the growing season. Verticillium strain virulence and agronomic influences such as nutrition and irrigation management will also influence final crop yield.

The advantage of using spatial disease inoculum heatmaps is that certain fields and zones within the field can be prioritized and managed to mitigate the impact of the disease detected.

A distinct relationship is evident between Black Root Rot with soil pH and Verticillium with CEC. This association may be direct or a function of other features of these soils such as water holding capacity. There is a less distinct association between soil inoculum and cations such as Magnesium and Sodium. Magnesic and Sodic soils had higher levels of BRR inoculum.

These inoculum interactions highlight predictive indicators to identify disease suppressive soils. Further analysis of interactions with biological components will only improve this understanding.

Web based platforms such as PCT AgCloud provide excellent capacity to facilitate the hosting of spatial data and to enable basic data analytics of those identified factors that drive crop yield.

Our collaboration with CSD, CRDC and CSIRO delivered the development of a new and exciting qPCR test to differentiate between the Defoliating and Non-Defoliating strains of Verticillium. This will be a

valuable tool in the future to identify trial sites, for in-field diagnosis and for field selection, once varieties are released based on their resistance to the relevant strains.

The cotton industry needs access to a diagnostic test for *Fusarium oxysporum (F.ov)*. Fusarium is the dominant pathogen in southern Queensland and parts of the Macintyre and Gwydir Valleys. Not knowing the levels of *F.ov* thwarts the accuracy and scientific benefit of the Vert/BRR test in those valleys. This should be an imperative for future funding and research.

Objectives:

- To establish a steering committee for disease survey protocols
- Reduce the impact of disease on cotton production through objective measurement of disease inoculum populations.
- Develop a user-friendly DNA molecular diagnostics tool and sampling methodology to provide reliable disease inoculum maps.
- Identify the spatial distribution and levels of inoculum across cotton fields and monitor their change over time.
- Provide geo-referenced soil and plant stem samples to be used in the development of a diagnostic test that differentiates between defoliating and non-defoliating strains of Verticillium.
- Produce findings such as data and maps that will allow the opportunity for associated soil health, nutrition and agronomy projects to build-on in the future.
- Identify sites and soils for future research projects, including Dr Gupta Vadakattu's (CSIRO) research into the functional capacity and resilience of soil biota.
- Contribute to the development of best practice disease management advice which will be actively communicated to the cotton industry.
- Compare inoculum levels with satellite NDVI, soil nutrients, stem cuts and yield maps to determine any correlations.
- Provide evidence to develop predictive indicators of disease risk and cotton yield loss.
- Compare the pathogenicity of site-specific Verticillium and its relation to the population of pre-plant multi gene inoculum and the associated single gene strain.
- Collaborate with CSD, CRDC and CSIRO to assist with the development of the D x ND single gene test.

Background:

The genetic yield potential of Cotton in Australia is improving every year, largely due to the advances made by the Cotton Breeding Australia breeding team. Yield gains from their breeding programme have exceeded 4% per annum over the last 20 years. Higher yields however require agronomic practices to keep pace with genetic potential, requiring crops to be grown longer, using more nitrogen, potentially more water, with extra boll loads, requiring greater demand on the soil and plants resources.

As the cotton growing area in Australia expands geographically, there is a need to understand the extent, distribution and population densities of soil borne diseases including Verticillium and Black Root Rot. Cotton Breeding Australia indicate that varietal resistance for high pressure scenarios is still 8-10 years away. In that intervening decade or so, judicious farming systems and agronomic practices will need to provide the platform for successful farm disease management.

Cotton growers in southern NSW are often restricted to only grow cotton every third year due to the significant detrimental impact Black Root Rot has on yield. Verticillium is requiring similar restrictive sequences in the central and northern valleys of NSW. According to CottonInfo, anecdotal reports from growers in recent years estimated yield losses from Verticillium ranging from 10-62%.

Since 2012, Crown Analytical Services has used qPCR technology (PredictaB) to measure and manage a number of soil borne pathogens, particularly Crown Rot and Root Lesion Nematodes in broadacre crops, especially winter cereals. Using that experience, in 2018, Crown Analytical Services ran a pilot project to test for soil-borne disease in Cotton using DNA, with the South Australia Research and Development Institute (SARDI) as their service provider. That pilot project showed merit as a rapid and reliable objective tool to measure and monitor soil pathogen inoculum population densities.

DNA is used for numerous scientific applications.

- qPCR testing was widely used as the most reliable diagnostic testing system during the COVID pandemic.
- DNA is used to confirm human genetic heritage.
- DNA was used to identify Varroa Mite sources.
- CRISPR/Recombinant DNA Technology

The significant advantages of using DNA for this scientific purpose are its reliability, its repeatability and its scalability. So long as the genome sequences are in place, numerous diseases can be diagnosed from a single sample.

Introduction

Prior to the initiation of this project, it was impractical for growers to understand the levels of soil disease inoculum in their fields, and growers were forced to base their decision whether to grow cotton that year, on previous experiences and “gut feel”. Developing a predictive tool using DNA molecular diagnostics that reliably measures disease inoculum is now allowing growers to make informed decisions regarding crop planning based on population densities of specific fields.

The ultimate defence against crop disease is plant host resistance. In 2013/14 a defoliating strain of Verticillium (VCG 1A) was detected in Australian cotton. Prior to that, only the non-defoliating strains VCG 2A and VCG 4B were known to be present. General observations in Australia have shown the defoliating strain VCG 1A is consistently highly virulent, whereas the non-defoliating strains VCG 2A and VCG 4B have more variability in their virulence. Cotton Breeding Australia have prioritised the development of verticillium resistant cotton. It is possible two V Ranks will be established in the near future for Defoliating and Non-Defoliating strains. Using DNA testing to determine which verticillium strain is present at a specific location will be fundamental to targeted management.

Disease has been the missing data layer. Precision Ag has evolved and uses geospatial data including Lint Yield, satellite imagery of NDVI or PCD to indicate crop biomass, chemical and physical soil properties including nutrients, ground elevation and EM to name a few. However, despite disease being such a key driver of crop performance, until now there has been no capacity to generate a geospatial map for each specific pathogen. This breakthrough is indeed an industry first.

Materials and Methods

Four to Six fields were chosen each year to be tested. Fields were chosen on farms known to have background levels of either Verticillium in the northern NSW or Black Root Rot in southern NSW.

Year 1 2020-21:

Valley	Farm	Field
Macintyre	Farm 1	GG CP4
Lower Namoi	Farm 2	T8
Upper Namoi	Farm 3	F3 N&S
Hillston	Farm 4	P12
Whitton	Farm 5	AV1

Year 2: 2021-22

Valley	Farm	Field
Macintyre	Farm 1	GG CP2
Lower Namoi	Farm 6	M5
Upper Namoi	Farm 7	W12
Hillston	Farm 8	H2 North
Hillston	Farm 4	R07
Whitton	Farm 5	B4

Year 3: 2022-23

Valley	Farm	Field
Macintyre	Farm 1	GG CP4
Upper Namoi	Farm 9	F7
Hillston	Farm 4	P12
Whitton	Farm 5	AV1

Pre plant soil sampling

Disease Sampling

GPS boundary and spatial 2 hectare grid map were created using QGIS and/or PCT Agcloud.

The spatial grid map was downloaded to a hand held device.

Fields were accessed using a Side by Side ATV.

GPS reference point were located.

Soil cores in two rows adjacent to the GPS site were sampled using a 12mm hand corer, 2 rows x 8 samples x 10cm depth. Samples taken from on top of the bed, where seed is to be planted.

All soil from the 16 cores placed into bar-coded and labelled sample bags.

Samples kept cool until dispatch was arranged to SARDI.

Pre plant Nutrient samples

At the same location, using a standard soil testing corer, sample soil were taken to 10cm (all sites).

Deep soil tests to 60cm taken using a hand held pneumatic soil sampler particularly for deep Nitrate.

All Soil nutrition samples placed into barcoded APAL bags and kept cool until dispatch was arranged.

Post picking soil sampling

Disease samples

Revisit the GPS reference points using the hand held device.

Fields were accessed using a Side by Side ATV or by foot where necessary.

Soil cores in two rows adjacent to the GPS site were sampled using a 12mm hand corer, 2 rows x 8 samples x 10cm depth. Samples taken from on top of the bed, adjacent to cotton stalks.

All soil from the 16 cores placed into bar-coded and labelled sample bags.

Samples kept cool until dispatch was arranged to SARDI.

Objective disease assessments

Black Root Rot (6-8 weeks post emergence)

NSW DPI and QDAF as part of their initial involvement in the project, had agreed to assist with Black Root Rot assessments at these sites. Where possible they included the fields in their disease surveys, albeit using different site location systems (random v targeted). The time of sampling was determined by the availability of the government pathologists. In most cases sampling was conducted 4-6 weeks post emergence.

Sampling protocol was to sample 20 plants at each site for incidence and severity rating/10.

% incidence and severity was calculated.

Verticillium stem cuts (Post defoliation or picking)

CAS staff conducted the stem cut program. Logistically the stem cut timing was determined by the grower's root cutting plans. If they were to root cut within a short period of time, before CAS could physically get to the field, arrangements were made to stem cut prior to picking.

Locate the GPS reference point.

Sample 10 plants in 4 adjacent rows (40 plants) on a presence / absence basis.

% incidence calculated

Capture and storage of digital data

Delta Ag arranged for the capture of time specific NDVI and PCD imagery for each field.

Delta Ag arranged for Yield maps to be captured and downloaded from USB or from MyJohnDeere. In Year 1, one farm (Farm 3) did not have yield mapping capacity, so a contractor was arranged to pick that field – resulting in a Variation to the project and CRDC contributing to that extra cost.

Yield and biomass imagery data, once processed is stored on PCT Agcloud. This data is available for the grower to access.

The spatial dataset was sent to Dr Patrick Filippi, a postdoctoral fellow at the University of Sydney, assisted by Dr Jie Wang to analyse the various spatial layers for correlations with disease inoculum under the CRDC project "Using multi-layered, multi-farm datasets to forecast yield gaps, and understand causes of variability in cotton." Dr Filippi accessed the datasets prepared by various precision ag service providers such as CAS, Delta Ag and PCT.

Analyses include:

- Correlation analysis (CAS) inoculum density x disease, showing clusters.
- Correlation plots for 7 variables (U Syd),
    - BRR Pre
    - BRR Post
    - BRR Incidence
    - BRR Severity
    - Verticillium Pre
    - Verticillium Post
    - Verticillium % incidence (Stem cuts)
- Scale
    - Per year
    - Per field
    - Whole dataset (all fields per year)
    - Clustering (3 clusters).
        - BRR 0-10, 10-25, >25 kcopies DNA/g soil
        - Vert 0-20, 20-60, >60 pg DNA/g soil
    - 3 years together
- Correlation plot
    - Pearson's r for each year, each field
- Boxplot analysis (same y scale and 3 clusters)
- Interpretive Learning SHAP plot analysis (U Syd)

Results

Disease Steering Committee

In accordance with Milestone 1, CAS established a cotton disease steering committee:

CRDC devised a Terms of Reference and Confidentiality Agreement, which participants signed.

The Terms of Reference is included in the Appendix.

Personnel included were:

- Elle Storrier (R&D Manager, CRDC)
- Warwick Stiller (Cotton Breeder, CSIRO)
- Ross Pomroy (Consultant, Nutrien, Darling Downs),
- Will Coulton (Grower, Macintyre),
- Brendon Warnock (Grower, Upper Namoi),
- Nic Clapham (Grower, Darling Downs),
- Allan Jones (Consultant, Griffith, Hillston)

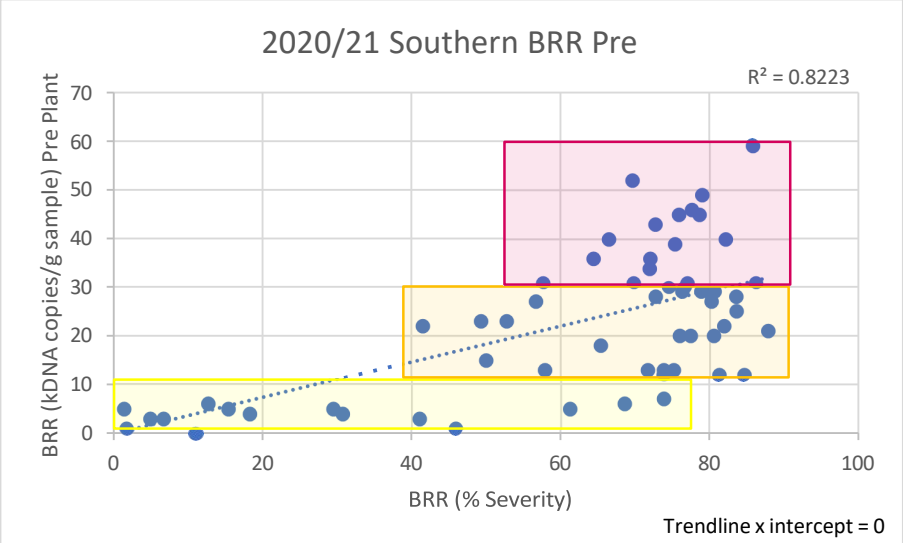
- Richard Malone (Grower, CFM, Griffith)
- Bob Ford & Hannah Hartnett (CSD, Narrabri)
- Beth Shakeshaft (Pathology extension, NSW DPI, Yenda)
- Duy Le (Plant Pathologist, NSW DPI, Narrabri)
- Linda Smith (Plant Pathologist QDAF)
- Rob Long & Jenny Brooks (Crown Analytical Services/Delta Agribusiness)

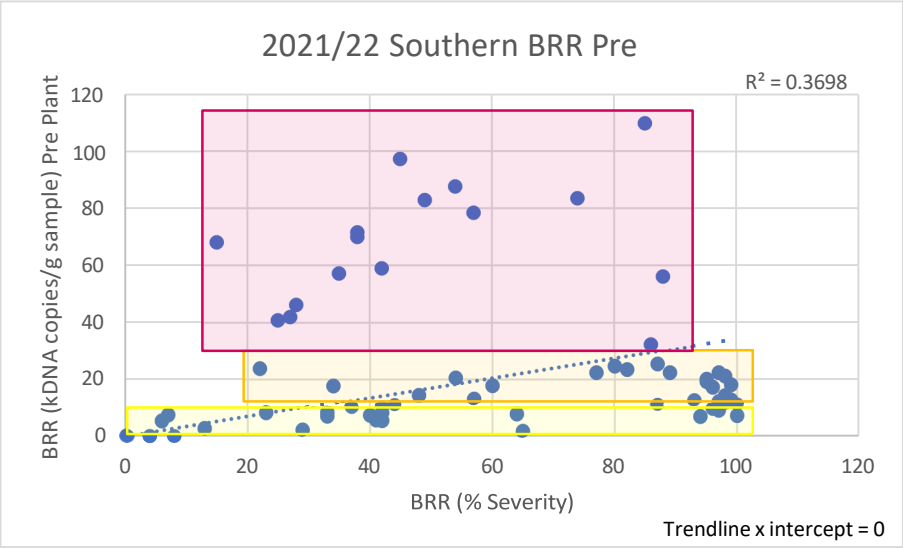
Two meetings were held giving updates on research and industry initiatives, 17th December 2020 and 8th July 2021, but there has been no further activity since then. A number of members of that committee are no longer involved in the cotton industry.

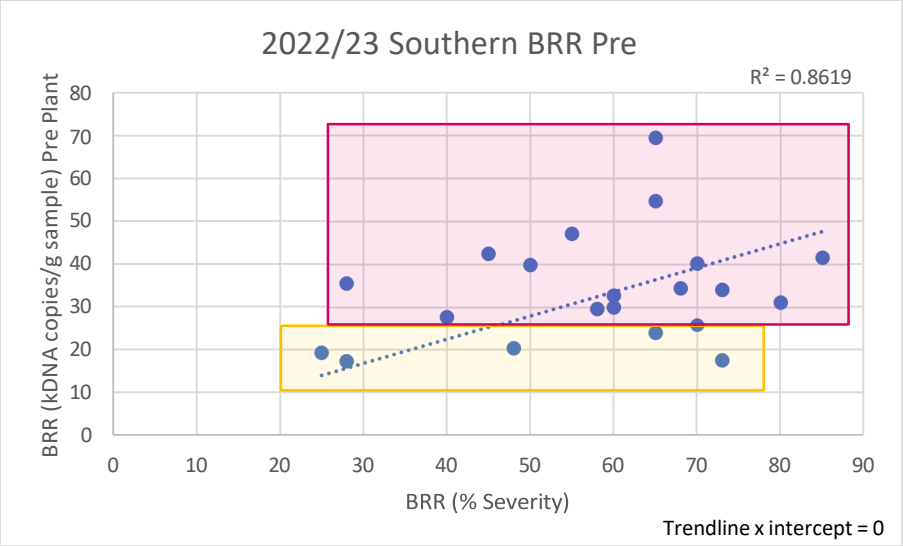
Inoculum DNA x Disease

Black Root Rot

All years showed a positive trend for inoculum DNA (kcopies DNA/g soil) x BRR severity Southern NSW, indicating that % BRR severity increases in response to increased BRR inoculum population densities pre plant. In the fields assessed, BRR inoculum (pre plant) did not provide a reliable indicator of early season BRR incidence. For instance, in 2020/21 all sites in the southern valleys showed 100% BRR incidence, irrespective of the initial inoculum level whereas stronger correlations were observed between BRR inoculum (kDNA/g soil) and disease severity.



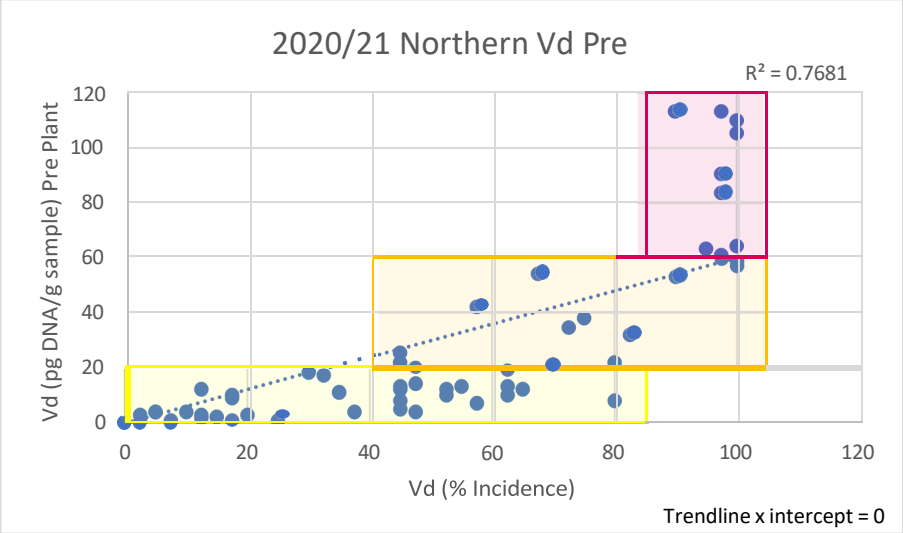


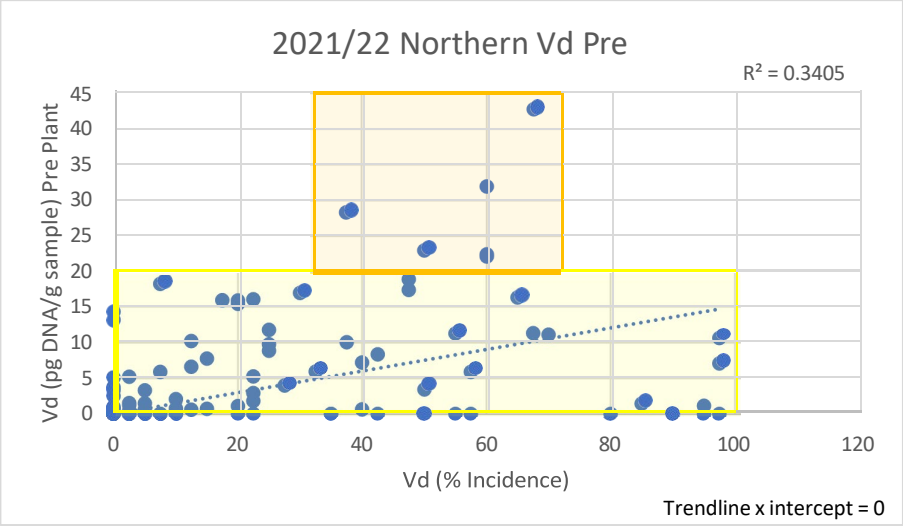


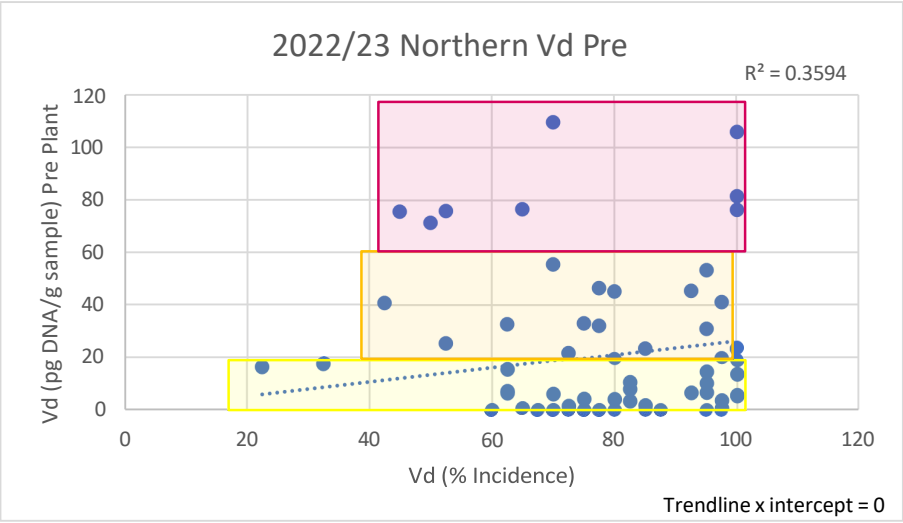
Figures 1 – 3: 3 years of correlation graphs analysing disease population density x BRR severity (prepared by CAS)

Verticillium

All years showed a positive trend for inoculum DNA (pg DNA/g soil) x Verticillium % incidence (stem cuts) in Northern NSW, indicating that % Verticillium incidence increased in response to increasing inoculum population densities. In 2020/21 the correlation between pre plant Verticillium was strong (R^2 0.77) over a starting inoculum levels. The fields selected in 2021/22 had lower starting Vd pre plant levels, whilst 2022/23 were higher. The trends in the second and third years were positive but the correlation was not as strong R^2 0.34 and 0.36 respectively.







Figures 4 – 6: 3 years of correlation graphs analysing disease population density x Verticillium % incidence (prepared by CAS)

Inoculum DNA x Yield

Yield							
2020/21	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
AV1	-0.67	-0.32	N/A	-0.77	N/A	N/A	N/A
CP4	-0.37	-0.25	-0.14	-0.03	-0.3	-0.34	-0.83
Glen 3N	0.22	0.08	0.09	0.46	-0.5	0	-0.47
Glen 3S	-0.13	-0.02	-0.4	-0.11	-0.31	-0.24	-0.58
Riv P12	-0.09	0.37	N/A	-0.05	N/A	-0.3	N/A
T8	0.54	0.45	0.76	0.59	-0.77	-0.37	-0.86
2021/22	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
B4	0.03	0.18	0.46	0.32	-0.22	N/A	N/A
CP2	-0.06	-0.16	N/A	0.16	0.09	-0.28	-0.42
W12	-0.26	-0.44	N/A	N/A	-0.63	-0.42	-0.68
H2N	0.05	0.07	-0.04	-0.05	-0.07	0.15	N/A
M5	N/A	-0.08	N/A	N/A	-0.16	0.15	-0.07
R07	-0.18	-0.1	0.16	-0.08	N/A	N/A	N/A
2022/23	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
CP4	-0.26	-0.26	N/A	N/A	-0.68	-0.56	-0.37
P12	0.01	-0.05	N/A	0.04	N/A	-0.17	N/A
W7	-0.18	-0.48	N/A	N/A	-0.4	0.24	-0.7
AV1							

Table 1: Summary table of correlation plot analysis for each field.

For Black Root Rot, 9 out of the 13 fields assessed showed a negative correlation between pre plant inoculum and yield, meaning yields declined in the presence of higher inoculum readings. When tested again at the same sites post-pick all those same sites, except one, also showed a negative yield trend.

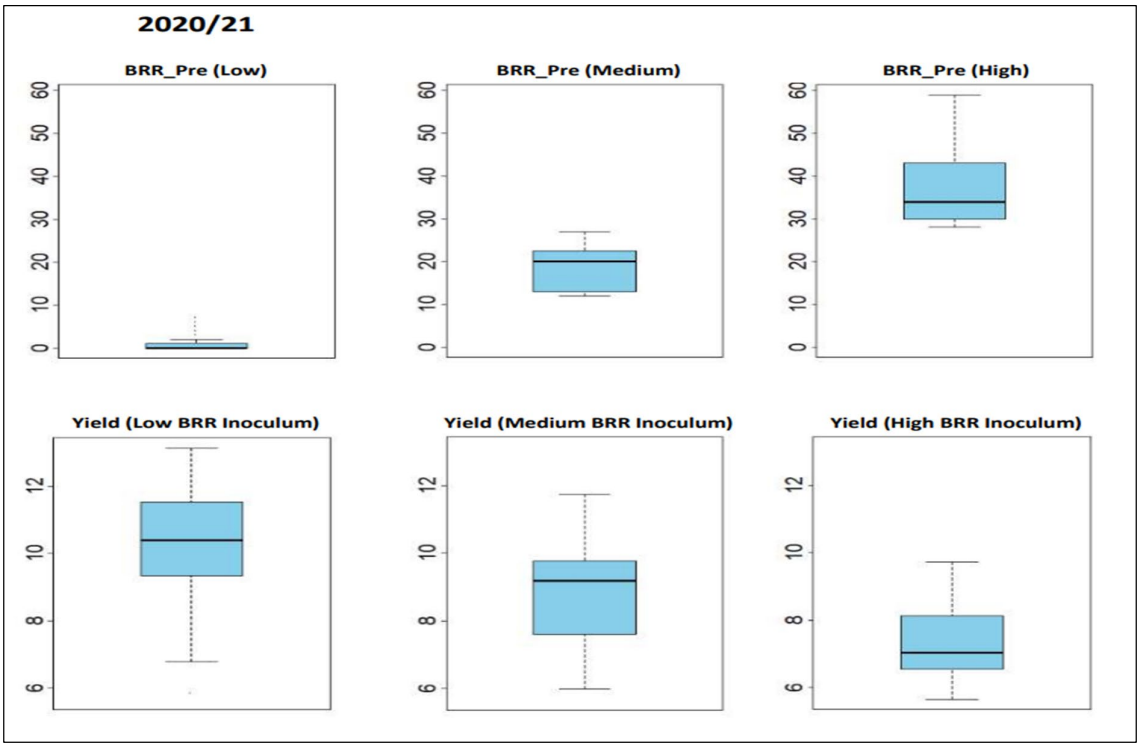
The trend between BRR Incidence and Yield was not as conclusive with only 3 out of 7 showing a negative correlation. The negative yield trend with Disease Severity was recorded at 6 out of 11 fields.

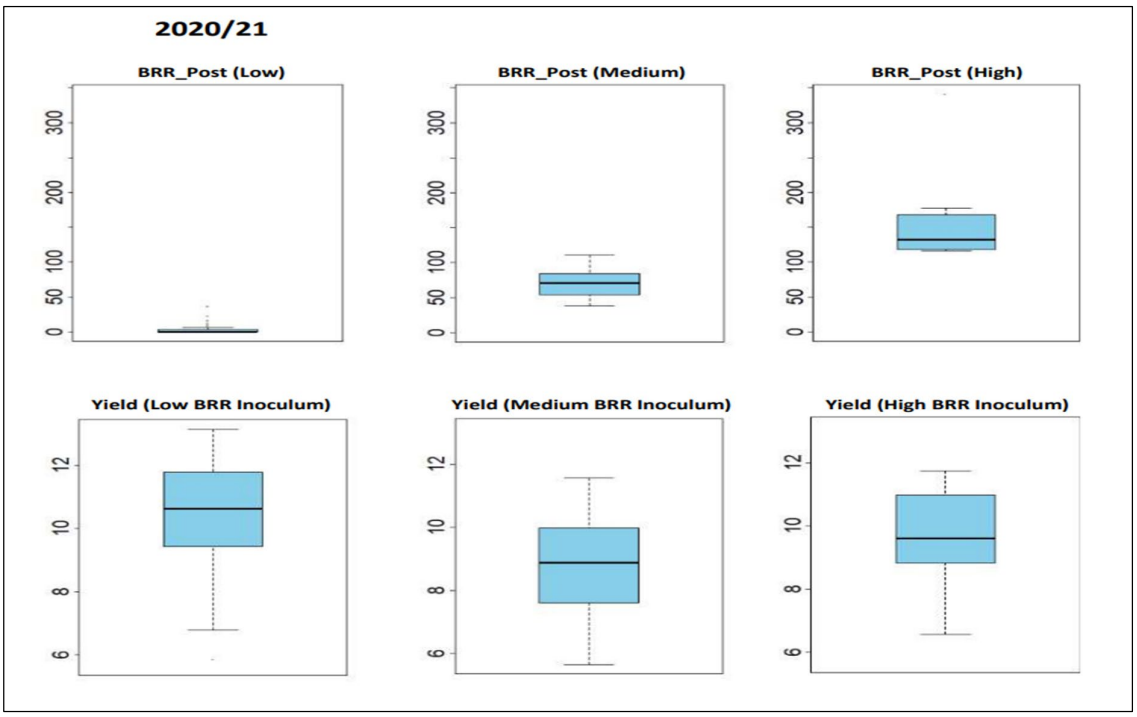
For Verticillium, 10 out of 11 fields showed a negative correlation between pre plant inoculum and yield. When tested again at the same sites post-pick, 8 out 11 fields also showed a negative yield trend and every field showed that a negative correlation between % incidence and yield.

Boxplot Analysis (all sites)

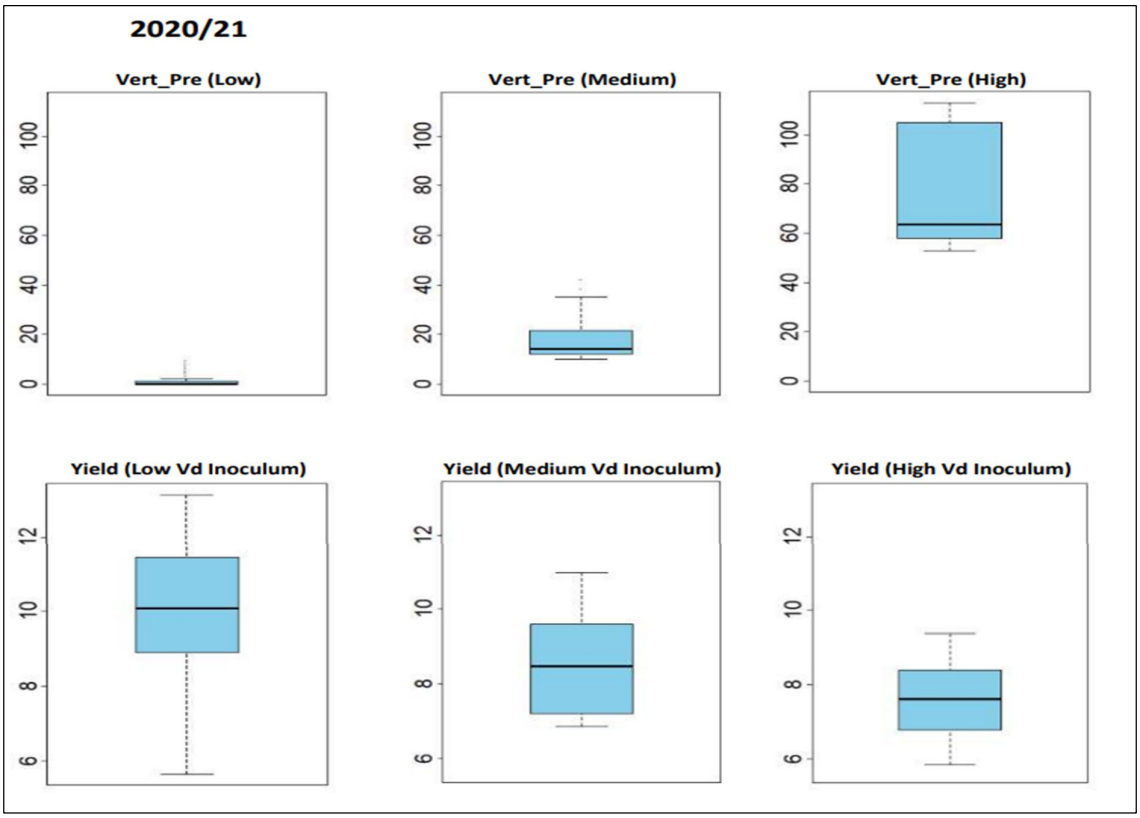
- Clustering (3 clusters).
  - BRR 0-10 (low), 10-25 (medium), >25 (high) kcopies DNA/g soil
  - Vert 0-20 (low), 20-60 (medium), >60 (high) pg DNA/g soil

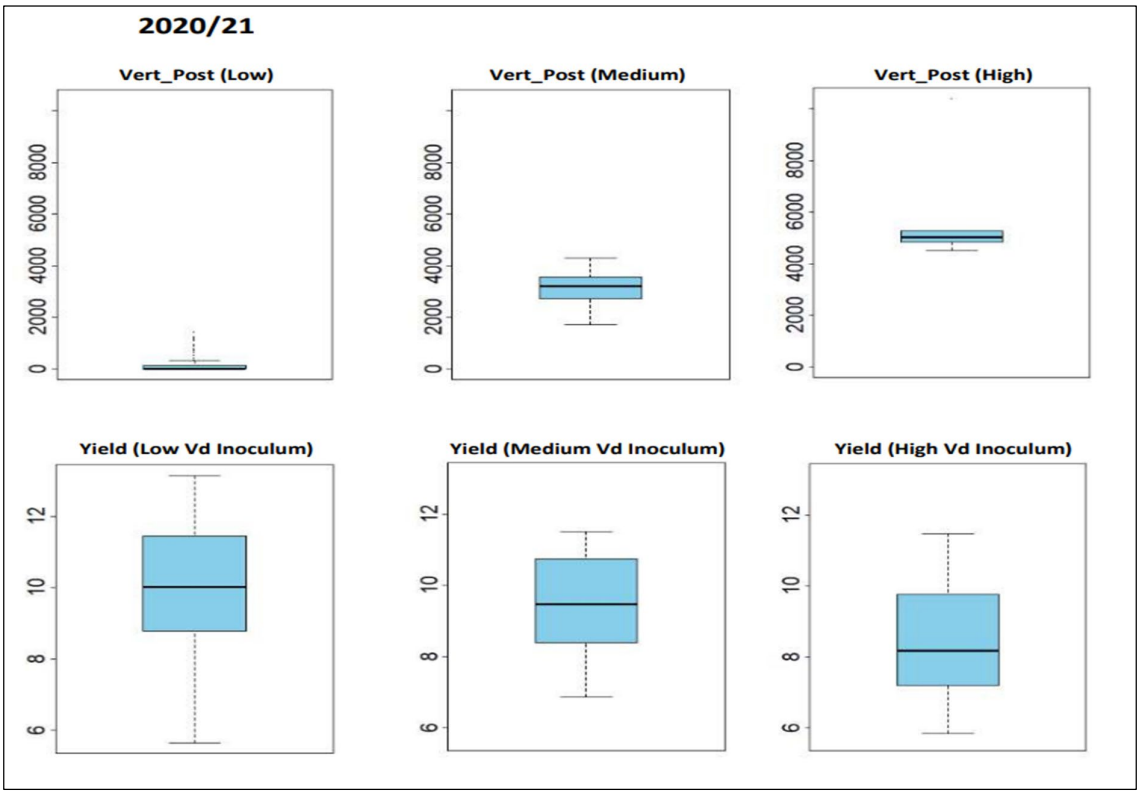
Yield losses can be visualised and measured. In 2020/21 yield losses from the clusters of “High” pre plant inoculum for both BRR and Vericillium were in the range of 2 – 3 bales per hectare.



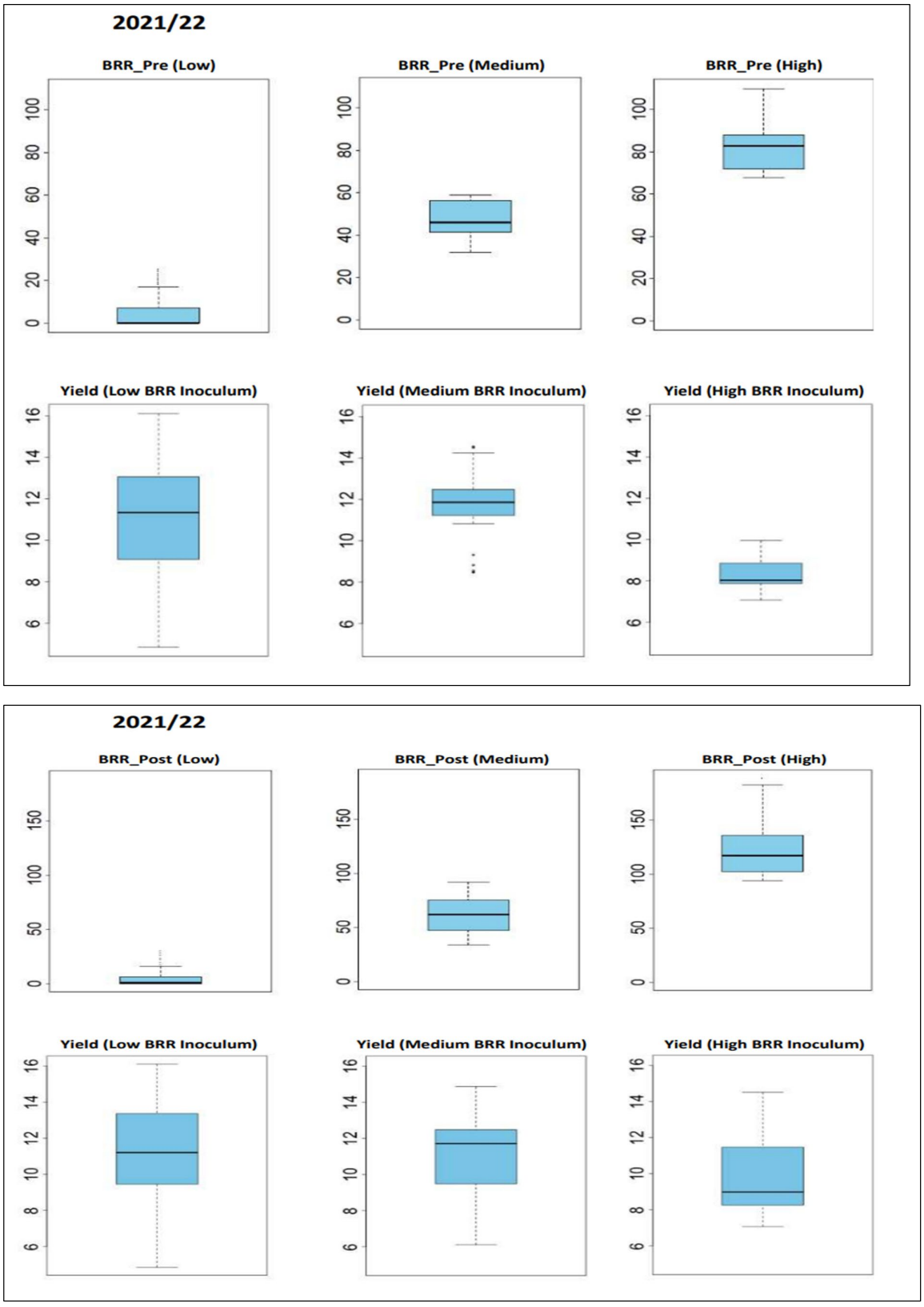


Figures 7,8: Boxplots analysis of Yield x inoculum for Black Root Rot 2020/21

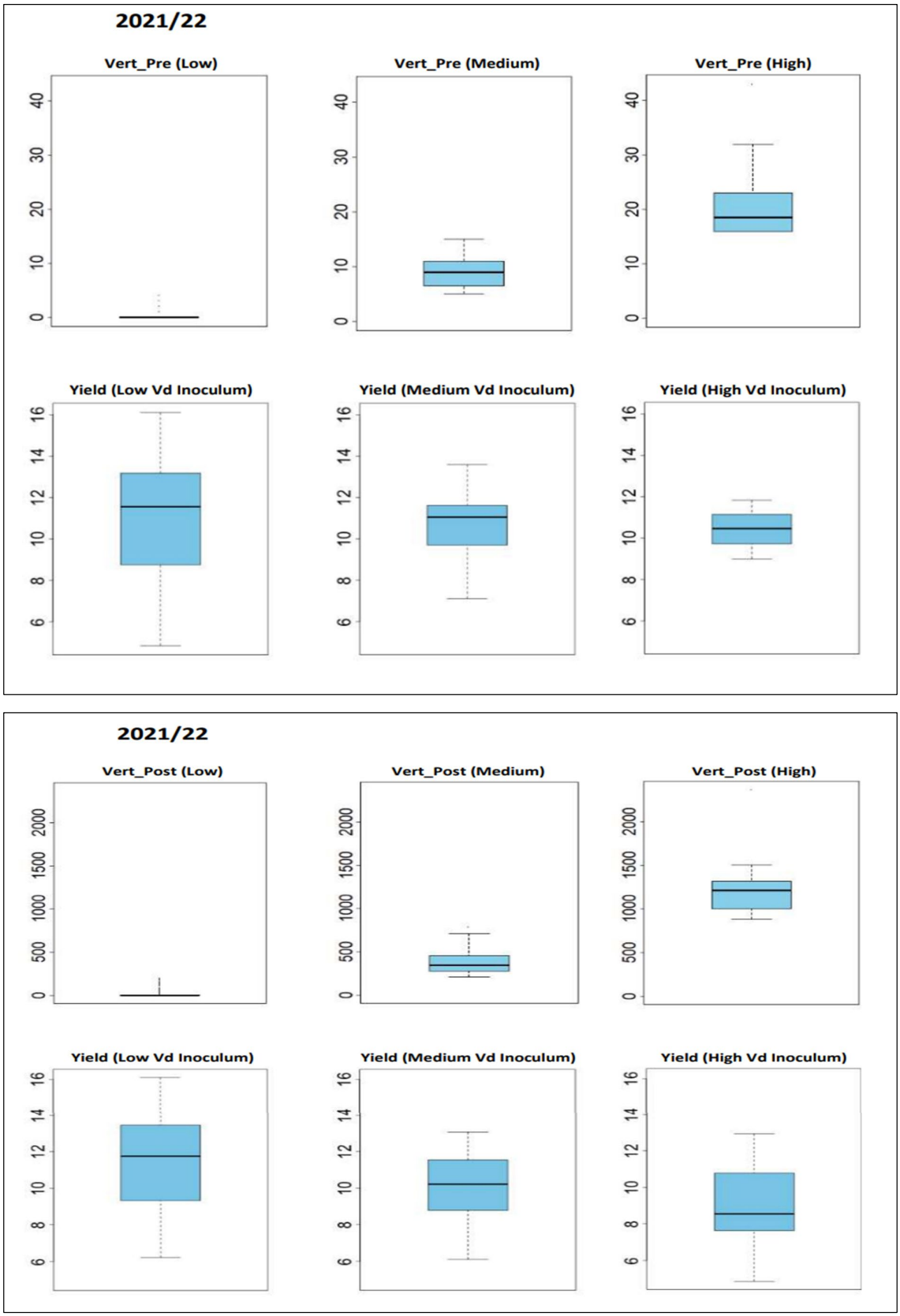




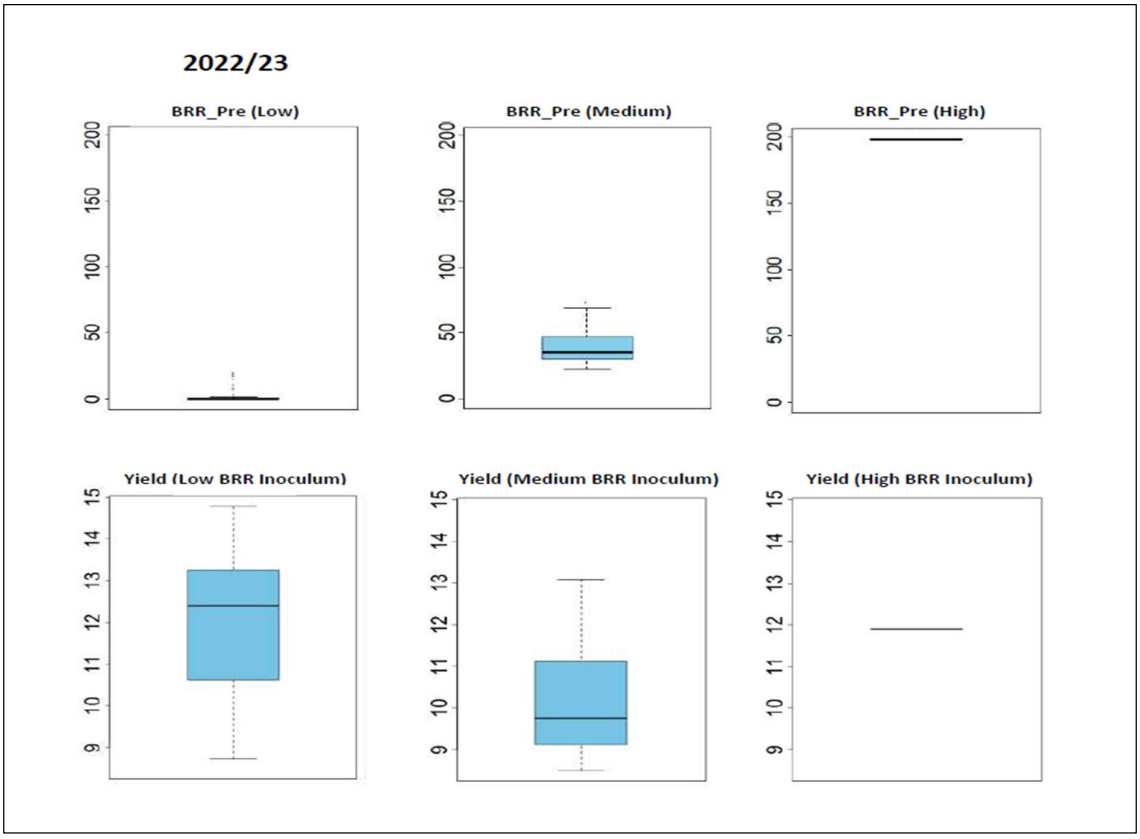
Figures 9,10: Boxplots analysis of Yield x inoculum for Verticillium 2020/21

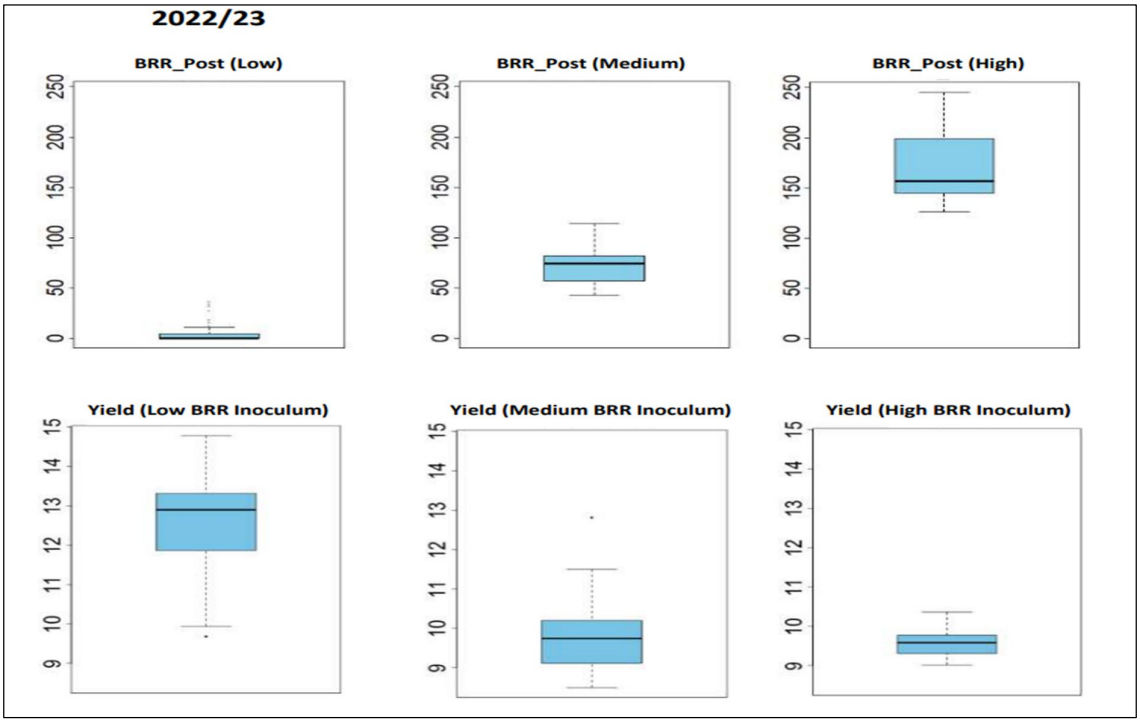


Figures 11,12: Boxplots analysis of Yield x inoculum for Black Root Rot 2021/22

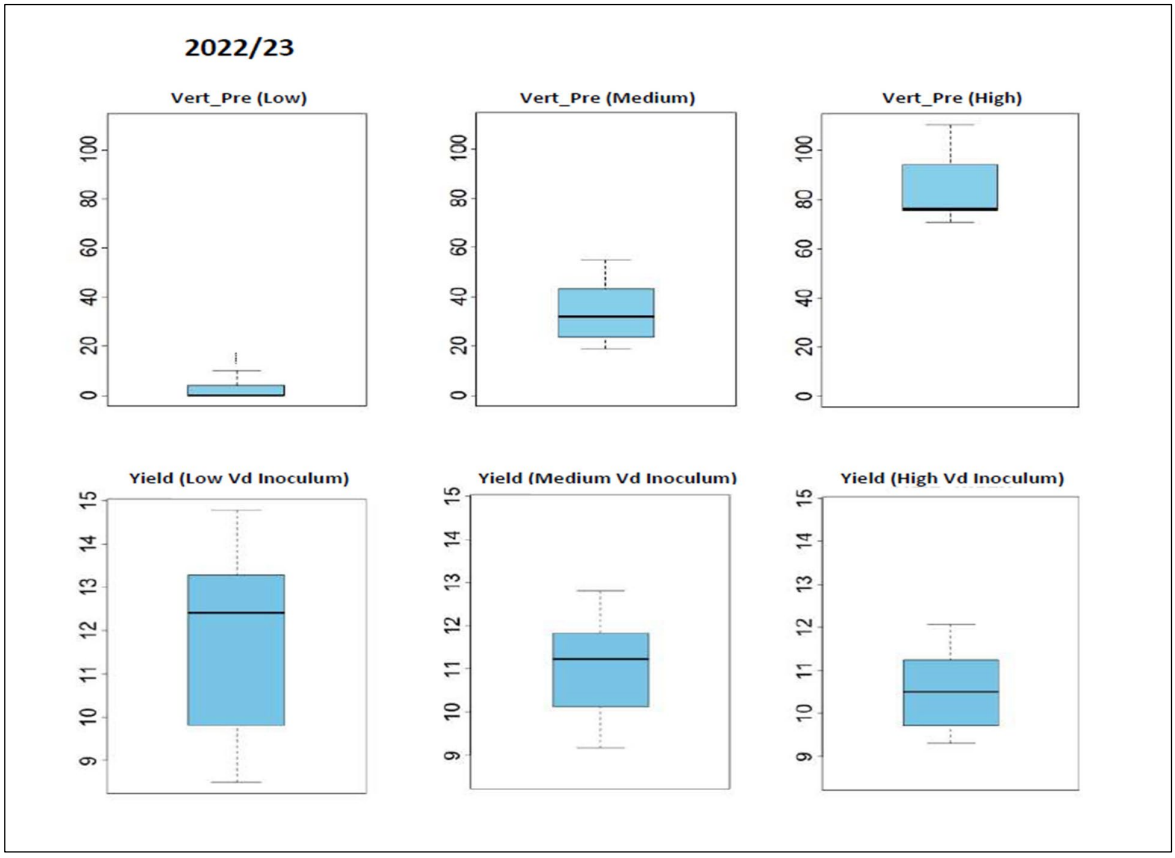


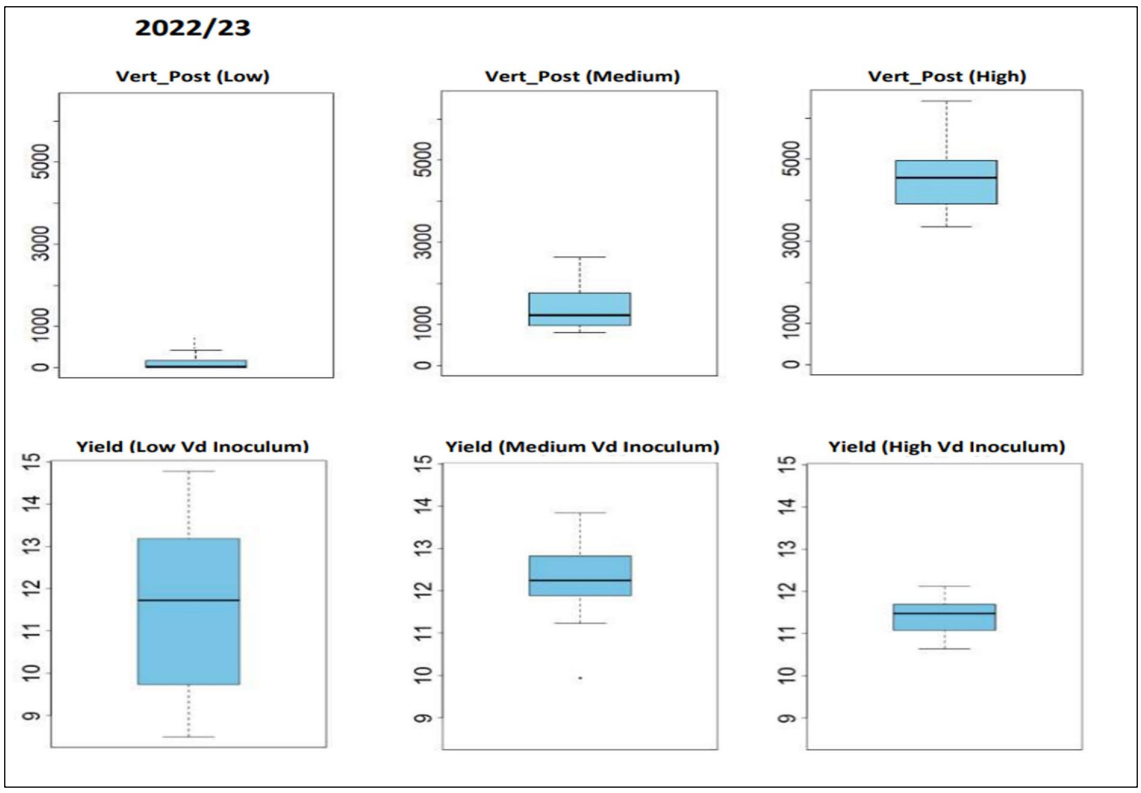
Figures 13,14: Boxplots analysis of Yield x inoculum for Verticillium 2021/22





Figures 15,16: Boxplots analysis of Yield x inoculum for Black Root Rot 2022/23





Figures 17,18: Boxplots analysis of Yield x inoculum for Verticillium 2022/23

Yield

Nitrate had the highest impact on yield, as indicated by the highest importance value and the highest feature posiioning. It showed that higher Nitrate values resulted in higher yield generally, with low values having a negaive impact on yield.

Soil pH had the second highest impact on yield, as shown by the importance value and feature posiioning. The high feature value (blue), in this case high pH (alkaline), had a posiive impact on yield with more acidic soils resuling in lower yields.

High levels of soil ESP are known to negaively impact yield, and this was clearly observed in this study, with high ESP values (blue points), showing a large negaive impact on yield up to 1.5 b ha^{-1}.

Caion Exchange Capacity and Colwell Phosphorus had very little impact on yield. They have the lowest importance value, lowest feature posiioning and least horizontal spread.

The soil borne diseases had a relatively clear impact on yield. While these diseases were not always the overall most important drivers of yield, it was clear that at some locations they were very strong drivers. This is expected as variables such as soil pH and nitrate are ubiquitous, whereas diseases are not always present at each site, so would therefore not always be a driver of yield. However, at those sites where diseases were present, it generally had a clear impact on yield.

For both BRR pre-plant and post-pick, high diseases values resulted in a lower yield, with observations of no diseases having very limited impact on yield. For Verticillium post-pick, very similar results were found to those for BRR. Perhaps the clearest interpretation could be found in the Verticillium pre-pant, where very high Verticillium values had a very clear negative impact on yield at select sites, whereas the majority of sites with no observation of Verticillium shows no impact on yield.

Overall, these results show that while key soil properties have an important role to play on driving cotton yield, plant diseases measurements are also crucial. The pattern of high disease measurement for both BRR and Verticillium and lower yields was very clear and indicates the importance of including this information when trying to understand the spatial and temporal drivers of yield. (P. Filippi, USYD)

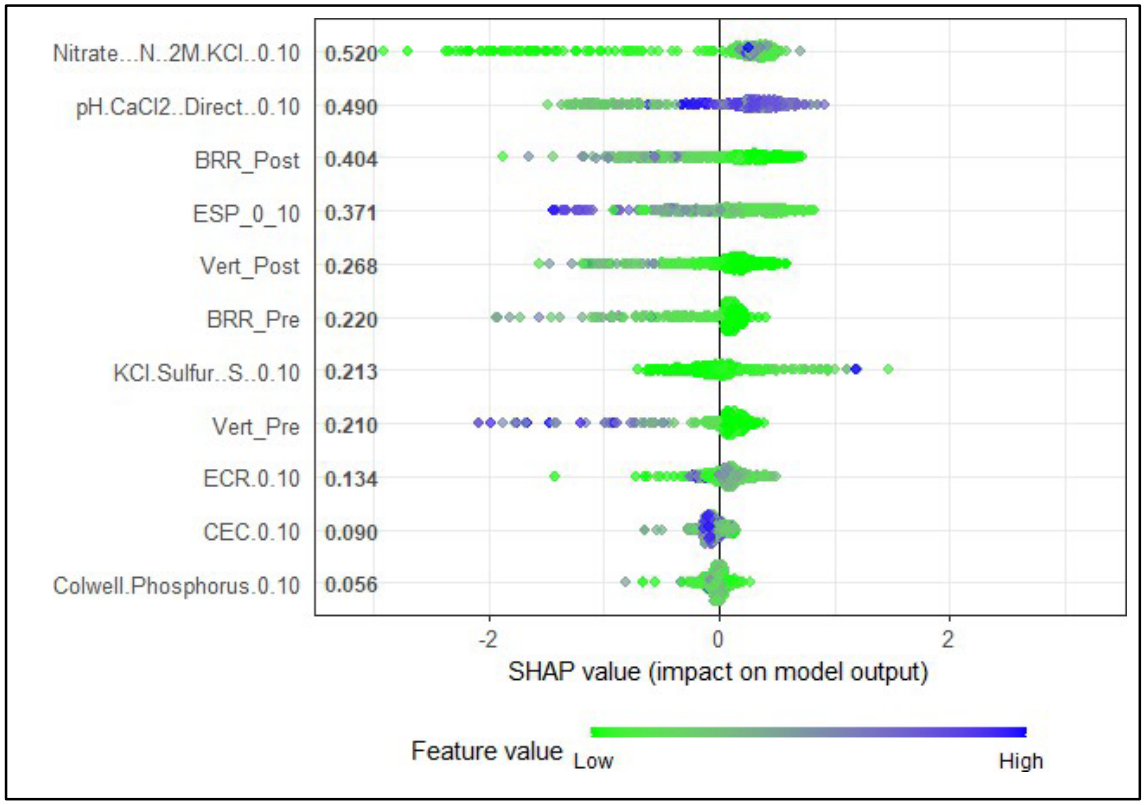


Figure 19: SHAP plot analysis of Yield – All Years, All sites

SHAP summary plots from the XGBoost model to understand drivers of cotton yield (b ha^{-1}) across all sites and seasons. The position on the x-axis is determined by the SHAP value, which represents the feature effect on yield for each yield observation. The colour indicates the feature value from low to high. Position on the y-axis is ordered by decreasing mean absolute SHAP value for each feature. (P. Filippi, USYD)

BRR Pre

Soil pH and yield had the clearest relationship with Black Root Rot pre-plant. Soil pH had the most impact on the inoculum of Black Root Rot, as indicated by the highest importance value and highest positioning of the feature. The high feature value has a negative SHAP value, which means that a high pH (alkaline soils) has had a negative impact on BRR inoculum. This is shown by blue points on the left of the y-axis. Lower pH (acidic soils) = Higher BRR pre. There is a strong correlation between the presence of BRR pre and yield. The high feature value of yield has a negative SHAP value, which shows that higher BRR measured pre plant decreases yield. The impact of the other included variables on yield were less clear. (P. Filippi, USYD)

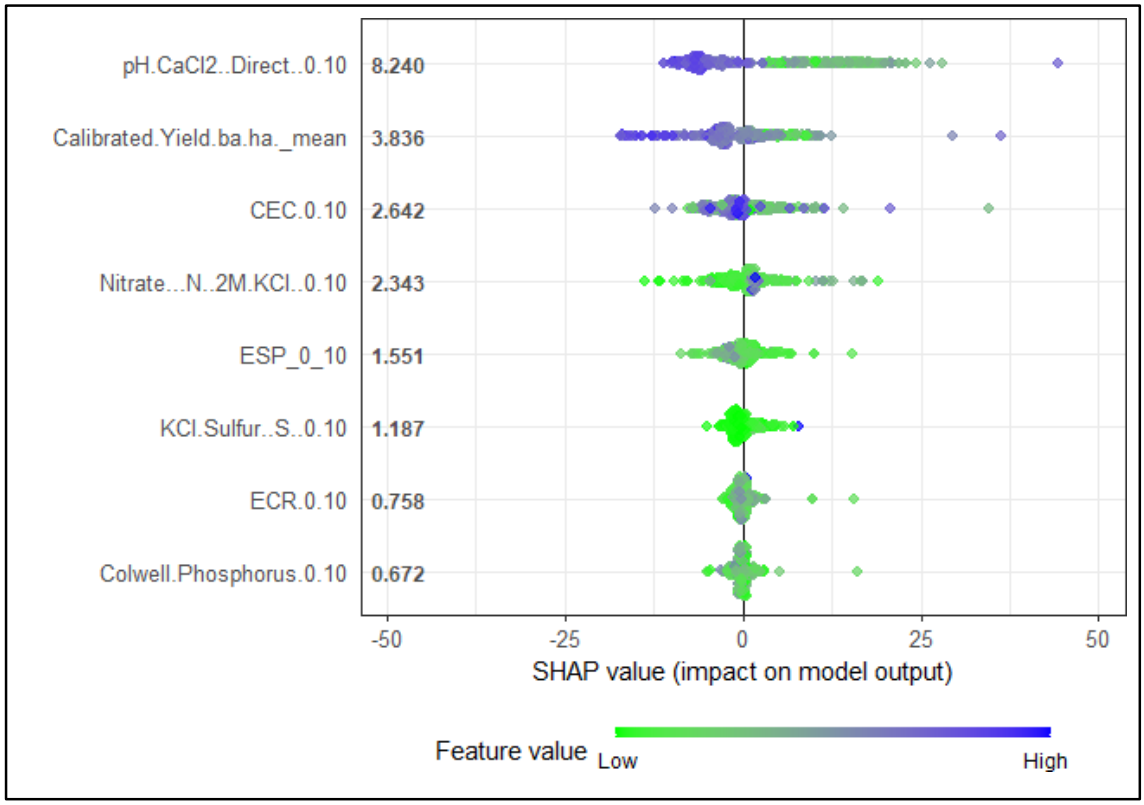


Figure 20: SHAP plot analysis of Black Root Rot inoculum pre plant – All Years, All sites

SHAP summary plots from the XGBoost model to understand drivers of black root rot (BRR) pre-plant (kcopies DNA/g) across all sites and seasons. The position on the x-axis is determined by the SHAP value, which represents the feature effect on yield for each yield observation. The colour indicates the feature value from low to high. Position on the y-axis is ordered by decreasing mean absolute SHAP value for each feature. (P. Filippi, USYD)

BRR Post

Soil pH had the most impact on the inoculum of Black Root Rot post-pick, as with the preplant of BRR. pH had the highest importance value, highest position and the high feature values have negative SHAP values. Lower pH (acidic) = Higher BRR post. As with preplant BRR, there is a strong correlation between the presence of the disease and decreased yield. Although not particularly clear, lower nitrate had a negative effect on BRR, with higher nitrate levels increasing the amount of BRR measured. This is likely because of residual N being left over in the soil where there is a high level of disease, and therefore lower yields which are not being taken up by the crop. The impact of the other included variables on yield were less clear, and Colwell Phosphorus, ESP and ECR had little impact on BRR inoculum. They have small importance values, low feature positioning and small horizontal spread with limited high feature value. (P. Filippi, USYD)

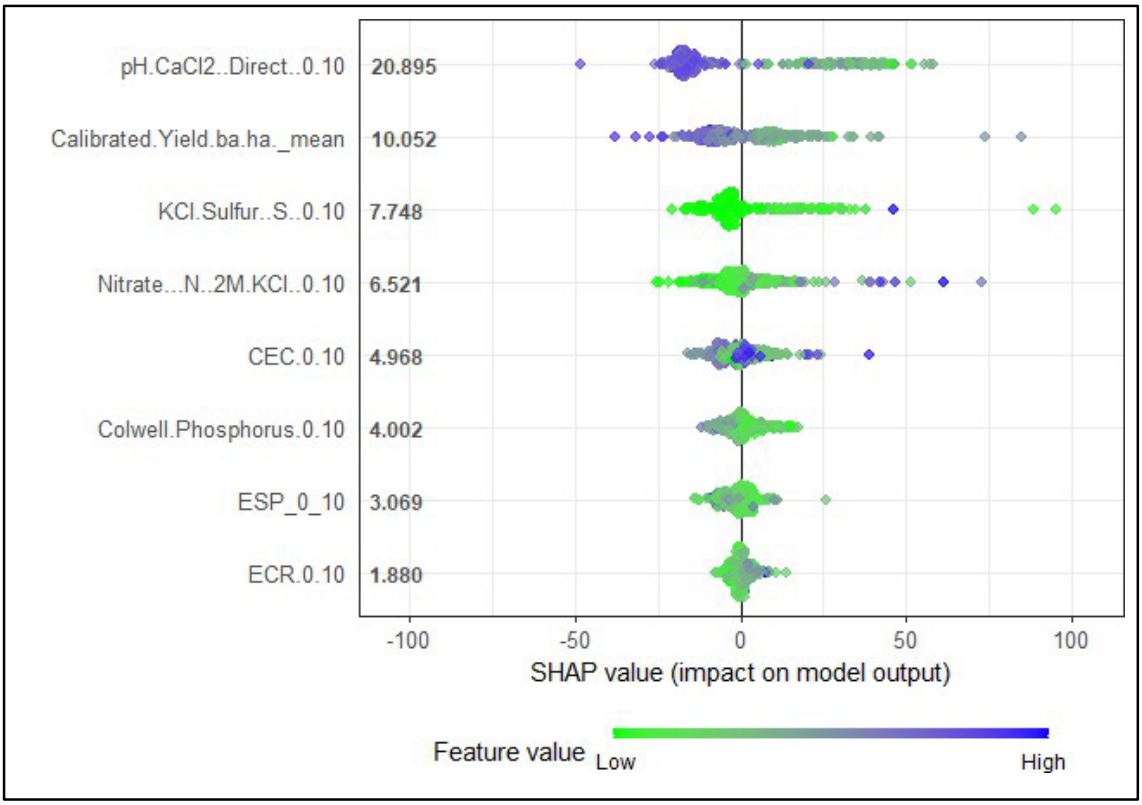


Figure 21: SHAP plot analysis of Black Root Rot inoculum post pick – All Years, All sites

SHAP summary plots from the XGBoost model to understand drivers of black root rot (BRR) post-pick (kcopies DNA/g) across all sites and seasons. The position on the x-axis is determined by the SHAP value, which represents the feature effect on yield for each yield observation. The colour indicates the feature value from low to high. Position on the y-axis is ordered by decreasing mean absolute SHAP value for each feature. (P. Filippi, USYD)

Vert Pre

Cation exchange capacity had the largest impact on the inoculum of Vert, indicated by the highest importance value and highest feature positioning. CEC had a positive impact on Vert, as shown by the high feature values with positive SHAP values. High CEC = High Vert pre. Although yield was an important variable, the relationship with Verticillium levels pre-plant was not particularly clear. This is likely because of the interactions with other soil diseases such as BRR that are not considered in the model, and the differences between regional yield differences and disease distribution. Low soil ESP values seem to increase levels of Vert. This is shown by the high feature values having negative SHAP values and the majority of the low feature values having positive SHAP values. High soil pH resulted in higher Vert levels, which is indicated by the majority of the high feature values having positive SHAP values. Soil nitrate had a minor positive impact, as shown by the few high feature values with positive SHAP values, meaning that very high nitrate values were correlated with high Vert levels. The same concept of this being related to residual N as previously explained for BRR post-pick is likely relevant

here as well. Soil ECR, KCl Sulfur and Colwell Phosphorus had little impact on vert with no clear relationships. (P. Filippi, USYD)

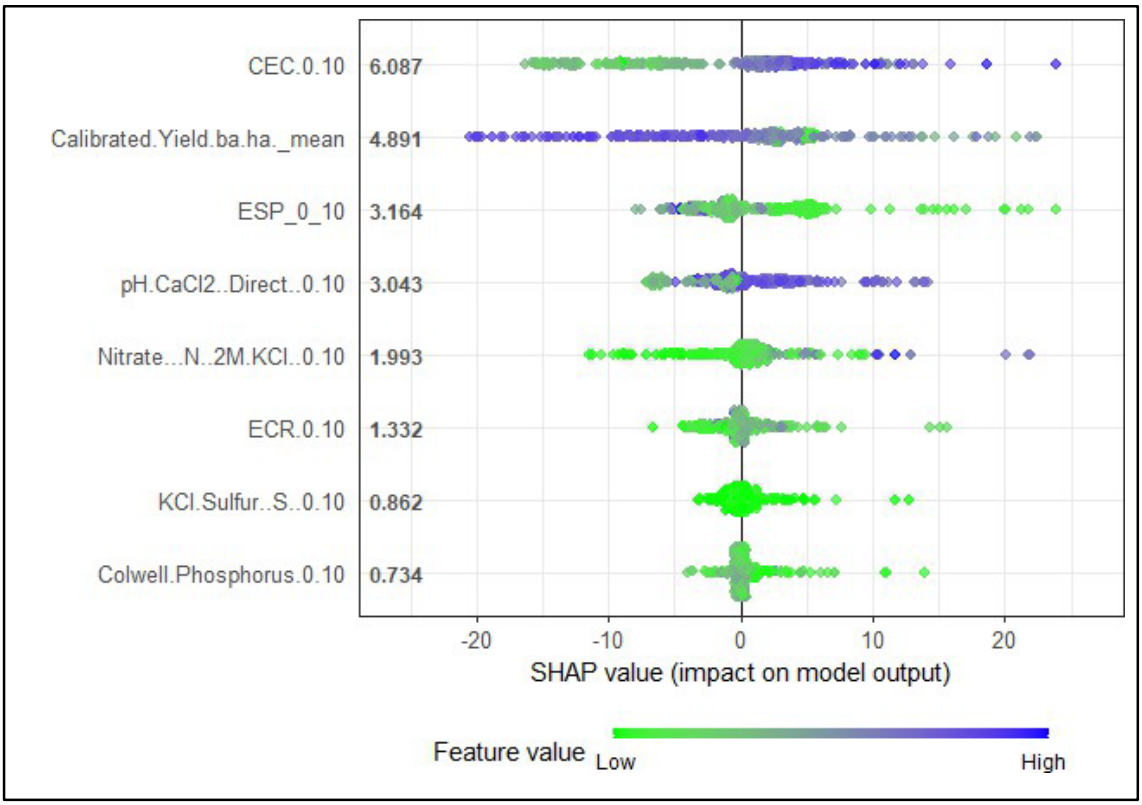


Figure 22: SHAP plot analysis of Verticillium inoculum pre plant – All Years, All sites

SHAP summary plots from the XGBoost model to understand drivers of Verticillium pre-plant (pgDNA/g) across all sites and seasons. The position on the x-axis is determined by the SHAP value, which represents the feature effect on yield for each yield observation. The colour indicates the feature value from low to high. Position on the y-axis is ordered by decreasing mean absolute SHAP value for each feature. (P. Filippi, USYD)

Vert Post

As with preplant Vert, CEC had the largest impact, shown by the highest importance value and highest positioning. The impact was positive, indicated by the high feature values having positive SHAP values. Higher CEC = Higher Vert post. Similar to Vert pre-plant, yield was deemed important, but was difficult to interpret which is likely related the interactions with other soil diseases, and regional yield differences. High soil ECR showed that it reduced Vert post-pick values, shown by the high feature

values having negative SHAP values and the majority of low feature values having positive SHAP values. The impact of the other included variables on yield were less clear. (P. Filippi, USYD)

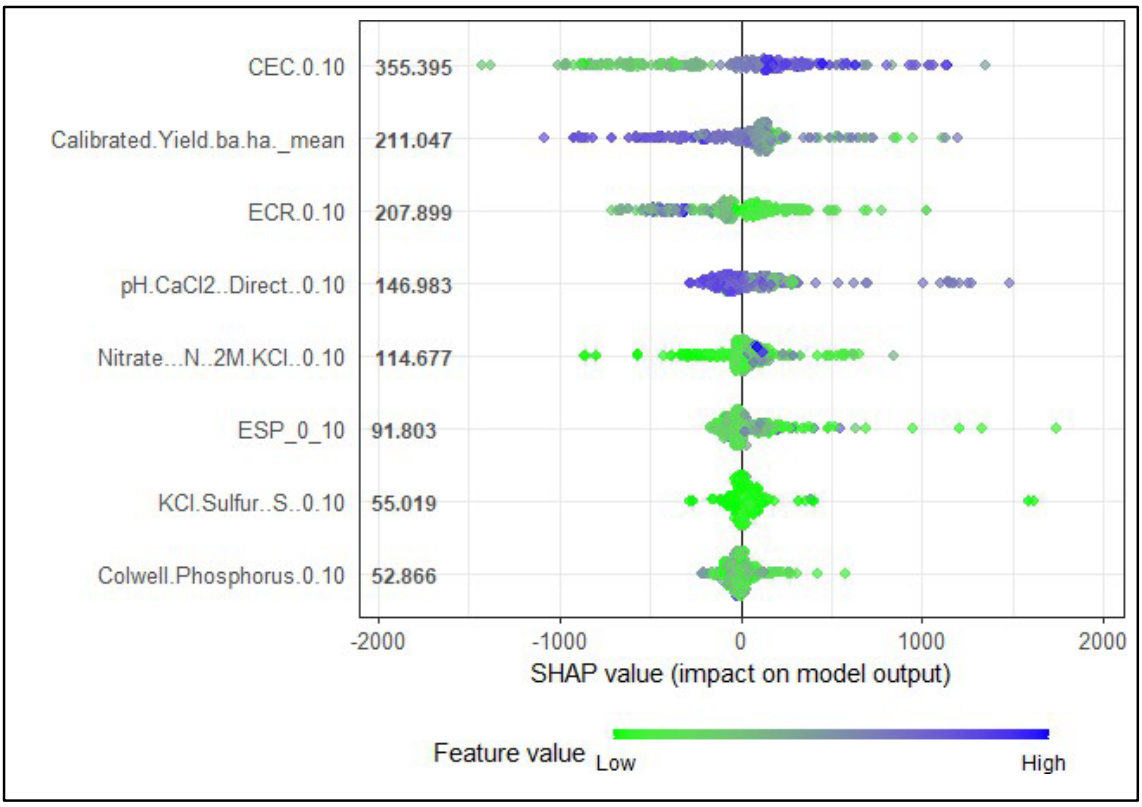


Figure 22: SHAP plot analysis of Verticillium inoculum post pick – All Years, All sites

SHAP summary plots from the XGBoost model to understand drivers of Verticillium post-pick (pgDNA/g) across all sites and seasons. The position on the x-axis is determined by the SHAP value, which represents the feature effect on yield for each yield observation. The colour indicates the feature value from low to high. Position on the y-axis is ordered by decreasing mean absolute SHAP value for each feature. (P. Filippi, USYD)

Nutrition Interactions – Summary of correlation plots

Total N							
2020/21	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
AV1	N/A	N/A	N/A	N/A	N/A	0.83	N/A
CP4	-0.18	-0.11	-0.17	-0.04	0.15	-0.18	-0.45
Glen 3N	-0.06	0.02	0.21	0.21	0.23	0.01	0.33
Glen 3S	-0.1	0.28	-0.13	0.35	0.03	0.45	0.04
Riv P12	N/A	N/A	N/A	N/A	N/A	-0.11	N/A
T8	-0.36	-0.14	-0.37	-0.48	0.6	0.19	0.46
2021/22	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
B4	-0.16	0.24	-0.19	-0.08	-0.22	N/A	N/A
CP2	0.2	-0.1	N/A	0.32	-0.04	0.19	0.12
W12	0.19	0.09	N/A	N/A	0	0.09	-0.05
H2N	-0.22	0.07	-0.14	-0.25	-0.18	-0.02	N/A
M5	N/A	0.22	N/A	N/A	-0.09	0.06	0.19
R07	-0.06	-0.39	-0.59	-0.5	N/A	N/A	N/A
2022/23	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
CP4	0.04	0.27	N/A	N/A	0.02	0.15	-0.15
P12	0	0.07	N/A	0.01	N/A	-0.06	N/A
W7	-0.09	0.19	N/A	N/A	-0.01	-0.09	-0.03
AV1	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Phosphorus (0-10)							
2020/21	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
AV1	0.18	0.03	N/A	0.03	N/A	0.09	N/A
CP4	-0.13	-0.13	-0.28	-0.2	-0.24	-0.14	-0.25
Glen 3N	0	0.24	0.21	0.4	0.48	0.16	0.18
Glen 3S	0.48	0.06	0.45	-0.55	0.12	0.26	0.16
Riv P12	-0.32	0.07	N/A	-0.27	N/A	-0.21	N/A
T8	0.34	0.6	0.54	0.67	-0.13	-0.28	-0.4
2021/22	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
B4	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CP2	N/A	N/A	N/A	N/A	N/A	N/A	N/A
W12	N/A	N/A	N/A	N/A	N/A	N/A	N/A
H2N	N/A	N/A	N/A	N/A	N/A	N/A	N/A
M5	N/A	N/A	N/A	N/A	N/A	N/A	N/A
R07	N/A	N/A	N/A	N/A	N/A	N/A	N/A

2022/23	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
CP4	0.08	0.03	N/A	N/A	-0.11	0.07	0.04
P12	-0.06	-0.05	N/A	0.15	N/A	-0.03	N/A
W7	-0.19	-0.31	N/A	N/A	-0.16	0.22	-0.37
AV1	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Potassium (0-10)

2020/21	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
AV1	0.39	0.43	N/A	0.54	N/A	0	N/A
CP4	0.23	0.05	-0.1	-0.03	0.17	0.15	0.54
Glen 3N	-0.02	0.09	-0.04	0.28	0.46	-0.05	0.11
Glen 3S	0.38	0.13	-0.25	-0.11	0.01	0.24	0.11
Riv P12	0.14	0.29	N/A	-0.06	N/A	-0.53	N/A
T8	0.34	0.62	0.61	0.83	-0.13	-0.16	-0.35
2021/22	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
B4	0.26	0.01	0.07	0.18	0.17	N/A	N/A
CP2	-0.21	0.34	N/A	0.25	0.09	0.33	0.37
W12	0.16	0	N/A	N/A	0.09	-0.23	-0.25
H2N	0.31	0.75	0.49	0.51	-0.09	-0.04	N/A
M5	N/A	0.34	N/A	N/A	0	-0.39	-0.45
R07	0.21	-0.24	-0.07	0.05	N/A	N/A	N/A
2022/23	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
CP4	0.45	0.2	N/A	N/A	0.21	0.19	0.22
P12	0.42	0.21	N/A	-0.13	N/A	0.06	N/A
W7	-0.39	-0.36	N/A	N/A	-0.29	0.08	-0.44
AV1	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Sodium (0-10)

2020/21	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
AV1	0.23	0.4	N/A	0.4	N/A	0.04	N/A
CP4	0.43	0.18	-0.05	-0.03	0.29	0.31	0.83
Glen 3N	-0.19	-0.21	-0.4	0.06	-0.23	-0.32	-0.19
Glen 3S	-0.28	-0.25	-0.37	0.45	-0.57	-0.47	-0.55
Riv P12	0.16	-0.05	N/A	0.25	N/A	0.28	N/A
T8	0.23	0.72	0.61	0.86	-0.31	-0.17	-0.48
2021/22	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
B4	0.28	0.26	0.65	0.69	0.08	N/A	N/A
CP2	0.38	0.2	N/A	0.15	0.06	0.16	0.15
W12	0.14	0.14	N/A	N/A	0.2	0.36	0.38
H2N	-0.26	0.06	-0.36	-0.36	0.15	0.01	N/A
M5	N/A	-0.01	N/A	N/A	0.7	0.23	0.58

R07	-0.07	0.2	-0.03	-0.23	N/A	N/A	N/A
2022/23	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
CP4	0	0.36	N/A	N/A	0.39	0.28	0.49
P12	-0.28	-0.04	N/A	-0.07	N/A	-0.1	N/A
W7	0.66	0.6	N/A	N/A	0.26	-0.1	0.44
AV1	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Magnesium (0-10)							
2020/21	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
AV1	0.41	0.35	N/A	0.65	N/A	-0.07	N/A
CP4	0.36	0.05	-0.42	0.1	0.18	0.32	0.75
Glen 3N	-0.26	-0.23	-0.26	0.27	-0.12	-0.34	-0.19
Glen 3S	-0.06	-0.6	-0.97	-0.36	-0.32	-0.37	-0.42
Riv P12	0.34	0.05	N/A	0.02	N/A	-0.01	N/A
T8	0.27	0.71	0.58	0.82	-0.24	-0.24	-0.47
2021/22	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
B4	0.32	0.1	0.17	0.2	0.06	N/A	N/A
CP2	0.07	0.43	N/A	0.13	0.05	0.24	0.21
W12	0.07	0.17	N/A	N/A	0.23	0.43	0.3
H2N	0.19	0.07	-0.02	0.13	-0.07	0.18	N/A
M5	N/A	-0.04	N/A	N/A	0.71	0.32	0.65
R07	0.18	-0.04	0.32	0.18	N/A	N/A	N/A
2022/23	BRR pre	BRR Post	BRR Inc	BRR Sev	Vd Pre	Vd Post	Vd % inc
CP4	0.18	0.4	N/A	N/A	0.39	0.29	0.65
P12	-0.07	0.05	N/A	-0.1	N/A	0.13	N/A
W7	0.47	0.55	N/A	N/A	0.18	-0.12	0.22
AV1	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Tables 2-8: Summary tables of correlation between nutrient and disease factor for each field.

These tables represent summaries of the correlations between nutrient and the measured disease factor for Black Root Rot and Verticillium. Unfortunately, the ground was too hard and dry in southern NSW at the time of sampling to get the hand held pneumatic corer to penetrate down to 60cm, therefore all readings for AV1 and P12 in 2020/21 are only to 10cm. Given there are so many fields and specific interactions we will concentrate on two fields as case studies for nutrient x disease interactions, namely AV1 for Black Root Rot and T8 for Verticillium in 2020/21.

Total N x BRR: All fields tested in 2020/21 had a slight negative correlation between Total Nitrogen to 60cm and BRR.

Total N x Verticillium: T8 showed a strong positive correlation between higher Total N levels and Verticillium pre plant, post pick and % incidence. The average starting N levels was 204 kg/ha N. In practical terms high N is associated with elevated Verticillium expression and inoculum.

Phosphorus X BRR: At AV1 there was a positive correlation for BRR pre plant but inconclusive for the BRR post pick and Severity.

Phosphorus X Verticillium: At T8 the was a consistent negative correlation between Phosphorus and Verticillium pre plant, post pick and % incidence.

Potassium x BRR: At AV1 there was a positive correlation for BRR pre plant, post pick and Severity.

Potassium x Verticillium: At T8 the was a consistent negative correlation between Phosphorus and Verticillium pre plant, post pick and % incidence.

Sodium x BRR: At AV1 there was a consistent positive correlation between Sodium BRR pre plant, post pick and Severity.

Sodium x Verticillium: At T8 the was a consistent negative correlation between Sodium and Verticillium pre plant, post pick and % incidence.

Magnesium x BRR: At AV1 there was a consistent positive correlation between Magnesium and BRR pre plant, post pick and Severity.

Magnesium x Verticillium: At T8 the was a consistent negative correlation between Magnesium and Verticillium pre plant, post pick and % incidence.

Development of the defoliating and non-defoliating diagnostic test

In the first year of this project (2020/21), CAS collaborated with CRDC, CSD and CSIRO, using the services of SARDI to successfully develop a diagnostic test to distinguish between the defoliating and non-defoliating strains of Verticillium dahliae. Two pathotype specific qPCR tests were designed and evaluated in the lab, then compared to the current non distinguishing Vd test. The correlation between the pathotype specific “single gene” (kDNA copies/g) test and species specific “multi-gene” (pgDNA/g) test was strong (R^2>0.92), albeit approximately 32 times less sensitive, which poses some limitations to its use as a predictive soil test. On the other hand, it is extremely useful as a diagnostic tool later in the season for both soil and plant tissue.

For more information regarding the test development, see the Summary report in the attached notes.

Change of inoculum DNA - pre planting and post picking

Growing cotton increases the inoculum load of diseases in the soil. It is more incremental for Black Root Rot but can be explosive for Verticillium. In these fields, BRR inoculum levels doubled as a result of growing cotton, whereas Verticillium numbers increased by an alarming 48 times.

2020/21	Black Root Rot				Verticillium			
Field	**Pre**	**Post**	**Increase**	**% increase**	**Pre**	**Post**	**Increase**	**% increase**
AV1	24	76	52	217				
CP4	0.4	23	22.6	5650	3.2	65	61.8	1931
G3 N&S	0.11	1	0.89	809	13.4	616	602.6	4497
P12	20	77	57	285				
T8	0.4	3.4	3	750	55	3626	3571	6493
Average	9	36	27	302%	24	1436	1412	5915%

2021/22	Black Root Rot				Verticillium			
Field	**Pre**	**Post**	**Increase**	**% increase**	**Pre**	**Post**	**Increase**	**% increase**
CP2	0.4	31.4	31	7750	3.5	387.5	384	10971
M5					14.7	375	360.3	2451
W12	0.6	7.7	7.1	1183	3.2	113.4	110.2	3444
H2N	12	55.5	43.5	363				
B4	65	114	49	75				
R07								
Average	20	52	33	167%	7	292	285	3993%

2022/23	Black Root Rot				Verticillium			
Field	**Pre**	**Post**	**Increase**	**% increase**	**Pre**	**Post**	**Increase**	**% increase**
CP4	9	6.1	-2.9	-32	16	906	890	5563
AV1	32.8	49.2	16.4	50				
W7	1	71	70	7000	44	711	667	1516
P12	33.8	91.9	58.1	172				
Average	19	55	35	185%	30	809	779	2595%

Cumulative Totals

Black Root Rot				Verticillium			
Pre	**Post**	**Increase**	**% increase**	**Pre**	**Post**	**Increase**	**% increase**
200	607	408	**204%**	137	6800	6663	**4849%**

Table 9,10: % Change of soil inoculum pre-plant and post picking

The increase in inoculum population, especially Verticillium, depends on the seasonal conditions of which the crop is subject to during its development. It must be noted, the past three seasons have been relatively mild and wet, and conducive to the increased production of microsclerotia in the canopy.

Discussion

The formation of the disease steering committee was a sound initiative by CRDC, whereby those personnel who agreed to join the committee were issued a Terms of Reference, signed a confidentiality agreement, and attended the first 2 meetings. Unfortunately, this initiative did not proceed past July 2021, thereby losing the momentum for a mechanism of feedback to CRDC.

These layers of data sampled and collated from over 400 GPS referenced points, enable a number of metrics to be analysed. This project has concentrated on:

- Soil inoculum x disease symptoms (regionalised correlation analysis)
- Soil inoculum x Yield (correlation plots, boxplots and SHAP plots – All sites)
- Soil inoculum x nutrient (correlation analysis, boxplots and SHAP plots – All sites)
- % change in soil inoculum levels after a cotton crop

Please refer to the interpretive machine learning report appendix prepared by Patrick Filippi, University of Sydney, for the creation of a predictive model and to understand how these variable impacted yield in terms of the nature, degree and magnitude of change.

The soil inoculum x disease symptom data was regionalised, whereby Black Root Rot as assessed in the southern fields and Verticillium for the northern fields. This was to account for the regions where each disease inoculum is at higher pre plant populations and the regional climate has a more profound influence on the symptoms displayed. Another factor was a concern regarding the calibration units assessed for BRR on the northern fields in Year 1. Regionalising the data overcame that concern.

Black Root Rot and Verticillium soil inoculum levels increased during all three growing seasons. In particular Verticillium post-pick soil populations were higher than at planting by an average of 48-fold. The explosive nature of soil inoculum loading is a real challenge for growers to manage. How long and what measures need to be taken to bring those levels down to an acceptable level? What agronomic practices are there to fast track the breakdown of inoculum, in conjunction with crop rotation, so that cotton growers can grow cotton in the most sustainable way?

The simplicity of comparing digital maps, looking for visual similarities and differences is a simple, quick and effective method of looking for agronomic relationships. Delta Ag assisted with the initial preparation of these digital images. Copies of these comparison maps are included as an appendix. PCT AgCloud has been a useful tool for data storage and in time will enable growers and consultants to compare data layers and to run basic analytics to identify the key drivers of disease and yield on their own fields and farms.

A redeeming feature of this project is that these GPS reference points can be used to direct future project and to add to what data has already been complied. Essentially this dataset becomes a baseline.

The project did run into some minor difficulty, mainly due to accessing the availability of the pathologists for our Black Root Rot assessments. The COVID pandemic and state border lockdowns restricted movements over the course of 2020 and 2021. Floods made site access difficult in 2022/23. Additionally, the pathologists were busy with their own survey work and were unable to complete all of our GPS referenced grid sampling, especially as the sampling methodology differs from their random field testing methodology. Despite early assurance and including NSW DPI in the

project initially, they found it difficult to include our assessment work as part of their schedule, accordingly there are gaps in our dataset regarding Black Root Rot incidence and severity. It was extremely disappointing that NSW DPI chose to request a release from this project during the first year.

Verticillium assessments were more successful, with consistent protocols and timing for stem cuts giving reliable % incidence readings. Some growers opted to mulch and root-cut immediately within days of picking, so those fields were often assessed prior to picking, after the crop had been defoliated. All stem browning characterised by the speckled vascular spotting was classified as Verticillium. If there were low levels of Fusarium or other wilt diseases, these were not obvious and were not counted separately.

The pathogenic virulence of a strain of Verticillium has a bearing on the damage it causes to the vascular system and therefore its yield impact. Stem cut samples were forwarded to QDAF for assessment.

Geospatial variability: Using a 2 hectare grid, a $1m^2$ area is tested to represent the remaining 20,000 m^2 in that sector of the field. Verticillium can vary from plant to plant within the $1m^2$ and even more within the 2 hectares. Additionally, the GPS units, whilst using a Bluetooth Garmin GLO 2 receiver sensor, with an estimated accuracy of 2 metres, there will be some minor variation in the relocation of sites. As with all testing methodologies – more sample sites reduce the variability and error.

Ground truthing with satellite imagery and previous yield maps will help give clues whether the inoculum maps will be a predictive indicator of future crop performance. This is a service that consultants should be offering for their clients as an endeavour to manage the agronomic variables that a crop will face and to take measures to optimise crop performance.

One disease can overshadow the impact of another. In the warmer northern NSW valleys, NSW DPI surveys indicate Verticillium and Fusarium are the dominant pathogens, whereas the southern NSW regions are dominated by Black Root Rot. Potentially there should be different thresholds set for northern and southern valleys for BRR.

The defoliating and non-defoliating qPCR test (D x ND) that differentiates between the isolates is of cursory interest for growers and consultants at this stage. The CSIRO cotton breeding team is actively utilising this technology as a diagnostic tool. CAS have assisted CSIRO by scrutinising heatmaps of the D x ND tests to identify suitable field trial sites dominated by specific isolates, then designed higher resolution grids for their research.

More research should be undertaken on managing the degradation of inoculum during the fallow period. This data showed Verticillium soil inoculum levels to be 48 times higher after picking than they were prior to planting. Obviously, this is a function of the epidemiology of the disease, microsclerotia would be present in the vast amounts of leaf and petiole (trash). Are there mechanisms to hasten the breakdown process of that trash to reduce inoculum carryover.

Nutrient relationships depend on their specific critical levels. Nitrogen for example – adequate N is required for plant function and growth, yet luxuriant N will cause excessive vegetative growth and may in fact promote expression of the disease. The dataset from this project showed Nitrogen to have the greatest impact on crop yield and a marginal relationship between Total Nitrate (0-60cm) and Verticillium inoculum pre plant was observed but had a negligible relationship with Verticillium post, indicating that the Nitrogen was at adequate but not luxuriant levels.

There were some strong correlations between the inherent soil properties such as pH and Cation Exchange Capacity (CEC) and disease. As demonstrated in *Figures 20-23*, lower pH soils (ie more acidic) generally had higher BRR levels and lower Verticillium levels, which coincides with the regional distribution of this disease.

As demonstrated in *Figures 22,23,* soils with higher CEC had the highest level of impact on Verticillium inoculum pre plant and post pick. This relationship may well be contingent with the fact that higher CEC soils also have a higher clay percentage and water holding capacity.

Magnesium and Sodium are two cations that become subsoil constraints at elevated levels, by hindering soil hydraulic conductivity. *Tables 5,6* demonstrate an inconsistent correlation between Sodium and Magnesium with disease inoculum levels and symptoms, however these did not lead to any significant impact on crop yield.

Despite a general understanding that N/K imbalances can exacerbate vascular diseases such as Verticillium, there was no trend observed in this dataset between Potassium levels and disease expression or crop yield. Similarly, there were no trends observed for Phosphorus or Sulphur.

For research purposes, this technology has considerable merit and provides plenty of opportunities. To achieve the best research outcomes, it makes sense to eliminate as many background variables as possible. Site selection can be contingent on baseline disease inoculum pressure, then the series of treatments are applied, with a result for the efficacy of the treatment but there is also the result of the impact on inoculum levels.

Examples of projects to explore could include:

- Disease x Rotation crops
    - Crop type (RWI Disease initiative)
    - Cash crop v Ground covers.
    - Termination timing
- Disease x Irrigation management.
    - Timing of first irrigation for BRR
    - Furrow v Bankless channel v Overhead
- Disease x Nutrient management (especially N, P, K)
    - Disease x N rates and timing
- Disease x Cultivation (and land levelling)
- Disease x Products
    - Seed dressings,
    - Foliar fungicides and biological products

Having a reliable tool to measure soil inoculum will assist in trialling and validating new and novel products, crops and varieties. In addition to assessing the efficacy of a chemical or agronomic treatment, its impact on the resultant carryover inoculum is another valuable layer of data for the cotton farming system. The Richard Williams disease initiative is a perfect example, where this tool measures the impact of alternative rotation crops on inoculum levels, aiming to devise a sustainable cotton cropping sequence.

So long as the specific disease diagnostic is in place, this technology brings its own form of biosecurity. For instance, any incursion of Verticillium into the southern valleys will be detected on fields. Growers and consultants can be alerted of the incursion and steps can be made to mitigate its

spread. Testing more pathogens such as Fusarium and potentially Reniform Nematodes for Qld, will be enormously beneficial for the industry.

Over time with more testing, naturally suppressive soils will become evident. From this project, chemical and physical soil property indicators have been identified to have differing impacts on the expression of disease symptoms and subsequent yield. Suppressive soils will be those which either:

- Defy the buildup of pathogenic soil-borne inoculum despite growing disease susceptible crops such as cotton, or
- Demonstrate consistently high yield performance in the presence of known inoculum levels and virulence.

Agronomic and farm management decision making is a major contributing factor to crop performance – choosing the most resistant varieties, appropriate N rates, time of sowing, time of first irrigation relative to soil type and time periods that soils remain saturated.

The biological balance of the soil may be a factor, either as a solitary factor or as co-contributor with the physical and chemical properties. We welcome any future work with Dr Gupta Vadakattu to further explore this quest to define what soil properties determine a disease suppressive soil. Are these soils Healthy? ….or just balanced and resilient?

Verticillium symptom assessments (% incidence as stem cuts) correlated with inoculum in most cases, most significantly in 2020/21. Black Root Rot incidence showed a weaker correlation than severity. In 2020/21 southern NSW fields displayed 100% incidence despite a range of inoculum levels, whereas the severity ratings could be ranked against the predictive soil inoculum populations.

Conclusion

DNA is a reliable indicator of background disease inoculum levels. The value of that background disease inoculum is then a reliable indicator of spatial disease risk and subsequent crop yield so long as other crop production variables are eliminated or accounted for such as:

- The pathogenic virulence of the strain of Verticillium
- Fusarium
- Plant population and uniformity.
- Irrigation management
- Nutrient availability and distribution – spatially and at depth
- Compaction
- Weather conditions

The cotton industry needs access to a diagnostic test for *Fusarium oxysporum (F.ov)*. Fusarium is the dominant pathogen in southern Queensland and parts of the Macintyre and Gwydir Valleys. Not knowing the levels of *F.ov* thwarts the accuracy and scientific benefit of the Vert/BRR test in those valleys. This should be an imperative for future funding and research.

Inoculum maps are already being used to assist:

- Target trial locations onto sites with known levels of background disease:
- CSD to improve the site location of their V Rank and disease management trials.
- CSD to assess the impact of certain Xtend Flex varieties have on resultant inoculum.

- Chemical companies targeting low, medium or high levels of disease inoculum for efficacy trials.
- Chemical companies testing products for efficacy can now also add the treatments impact on resultant inoculum.
- Source sites with either Defoliating or Non Defoliating dominance.
- Monitor the impact of alternative crops on disease inoculum levels. CAS are the primary service provider for monitoring disease levels as part of the Richard Williams Disease Initiative to identify crop management practices that can assist with on-farm disease management. It is a grower participatory project to encourage growers and consultants to assess the value and cost of alternative practices across different farming systems and environments.

It is premature to make any recommendations on the predictive Yield Loss Risk as determined by DNA inoculum levels for BRR and Verticillium. Further research, taking into account the strain of Verticillium, is required for any yield loss thresholds to be set. Having said that, it does enable prioritisation of fields, to be planted to cotton, based on inoculum population densities and spatial distribution.

Cotton consultants now have these DNA diagnostics as a reliable tool for the detection, measurement and management of cotton disease on behalf of their clients. Consultants have the responsibility of protecting their clients crops from biotic threats, of which disease is often the most damaging. Disease management takes a long term and integrated approach. No longer should the grower be solely responsible for crop sequencing and field preparation, consultants need to be actively involved to in crop management earlier during the preceding years and during the fallow period. Consultants can use these disease inoculum measurements, to run their own trials and case studies.

- to establish their own field thresholds,
- assess the efficacy of specific agronomic practices such as crop rotations, N management, irrigation management and
- assess any yield impact of crop products and/or the impact of resultant inoculum levels.

Over time, by taking many more data points and with the advent of Interpretive Machine Learning, these and other data sets will provide the basis for establishing not only the direct interactions of Inoculum x Disease x Yield, but also their responses to specific weather conditions. For example, for a particular field with known inoculum levels, physical and chemical soil properties and elevation…what will the yield outcome be in a Decile 2 rainfall (hot and dry) year v a Decile 9 rainfall year (wet and cool). This could be further refined into specific weather Deciles for rainfall and temperature during specific crop stages (Day Degrees) x Month. Intuitively then and ultimately, yield loss estimates could be used relative to known objective measurements and according to a long range weather forecast. Similar retrospective findings could be associated with the confirmation of the virulence of the strain of Verticillium for each field.

Resource Use Efficiency

Disease has been the missing spatial digital data layer. Now it is available and readily accessible, it is up to the collective cotton industry as to how to best use its potential.

As growers and consultants start to use and trust the validity of the spatial DNA disease data (heatmaps), it can be used to identify soils or management practices that suppress inoculum levels. The heatmaps can also play an integral role in improving resource-use efficiency to grow cotton. In known disease hotspots, factors such as

- Seed
- Nutrients, especially N, P, K,
- Water, (although difficult in flood irrigated fields)
- Growth regulants,
- Defoliants

can all be scaled to a level that matches yield potential. Greenhouse gas emissions will be more closely scrutinised, so demonstrating that resources are being spatially strategically placed on zones that match production will reduce wastage thereby improving resource use efficiency.

Publications, Presentations and Extension activities

Presentations:

CCA Seminar, Narrabri, June 2021

Soil DNA Testing in Cotton:

CSD Lower Namoi Field Day, February 2021

Managing Cotton Disease using DNA

AgTech Pitch, Moree, July 2022

Soil DNA testing on Cotton

Australian Cotton Conference, Gold Coast, August 2022

Measure, Map and Manage cotton disease inoculum using soil DNA.

Southern Field Day, Cottoninfo, CSD (At Farm 5 Field B4), November 2022

Soil DNA Testing for BRR and Vert

At the CSIRO Black Root Rot early stage breeding trial,…at a site CAS identified.

SMAG grower meeting Wee Waa 2022

Using Soil DNA to Measure, Map and Manage Verticillium Wilt and Black Root Rot

NAB Agribusiness team (Narrabri, Moree) November 2022

The challenge of Cotton Disease

Publications:

Australian CottonGrower, August 2021

An Industry First: Soil DNA Testing for Cotton Disease

1 pager in CSD Namoi Field day handbook, February 2021, February 2023

Managing Cotton Disease using DNA

References:

Holman, S., Kirkby, K., Smith, L., & Hartnett, H. (2016). Vert Update: The latest in vert research. https://www.cottoninfo.com.au/sites/default/files/documents/Vert%20update%20%28long%29%20-%20August%202016%20v3.pdf