Innovate Ag Pty Ltd
77a Rose Street, Wee Waa, NSW, 2388
(02) 6795 3787 info@innovate-ag.com.au
w: innovate-ag.com.au

SERO-X® PESTICIDE

400 g/L *Clitoria Ternatea* Extract

VERTICILLIUM WILT TECHNICAL GUIDE

APVMA registered

For Management of Verticillium Wilt in Cotton

Safe for bees, pollinators and predators

LABEL CLAIM:

For the control or suppression of a range of insect pests, including green mirids, silver leaf white fly (biotype b), heliothis, diamondback moth and two spotted spider mite, in cotton, lucerne, brassicas, cucurbits and tomatoes as specified in the Directions for Use table.

Also for use in cotton for the reduction in formation of the microsclerotia of *Verticillium dahliae* assisting in the management of Verticillium wilt.

April 2023 pg/VdDNA Trial Update

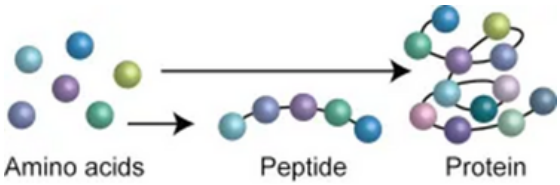
For short video summary and more information please click here or scan QR code

SCAN FOR MORE INFO

APVMA Approval no: 81070/129496

SERO-X: Why it works, the power of Peptides

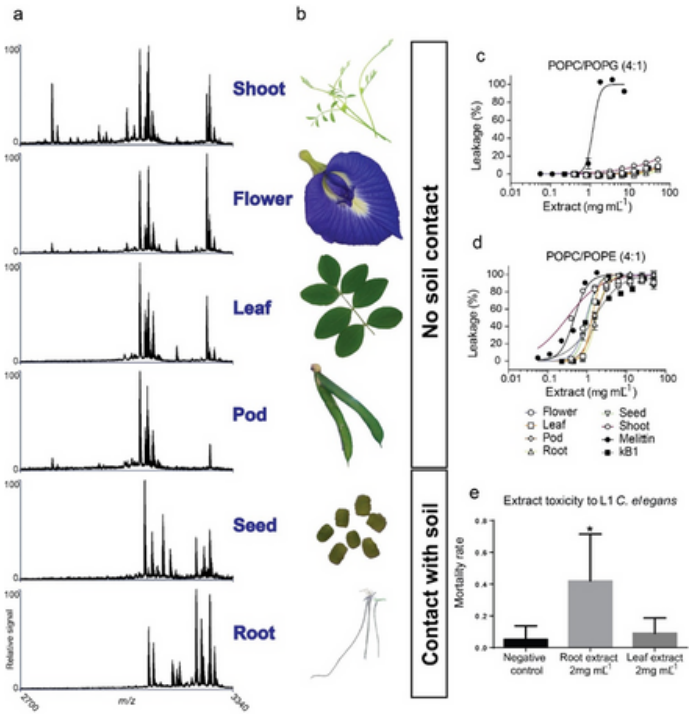
The active constituent in Sero-X is an extract of *Clitoria ternatea* (Butterfly Pea). *Clitoria ternatea* Extract is a complex mix of the compounds from the plant, some of which are the bioactive and ultra stable cyclic peptides called cyclotides.



Proteins and peptides are the working molecules of life, they make up the fundamental machinery that runs most biological processes.

More than 70 bioactive peptides have been discovered in Butterfly Pea, exhibiting chemical diversity based on the plant part they originate from, such as leaves, stems, roots, or seeds. These compounds possess unique biological activities. Butterfly pea generates them for defence against pests and diseases, with each playing specific roles.

As research on these compounds progresses, Innovate AG, in partnership with the University of Queensland's Institute for Molecular Bioscience, aims to further understand and characterise their modes of action and functions.



Gilding et. al. New Phytologist (2016) 210: doi: 10.1111/nph.13789

The ARC Centre of Excellence for Innovations in Peptide and Protein Science (CIPPS) is a leading national research centre. Their mission involves exploring new proteins and peptides from Australia's diverse plant and animal life, deciphering their biological functions, and developing novel proteins and peptides to tackle challenges in health, agriculture, and industry.

Innovate Ag is a proud industry partner, collaborating with international experts to harness the potential of peptides and proteins for the greater good. Learn more about them at https://cipps.org.au/

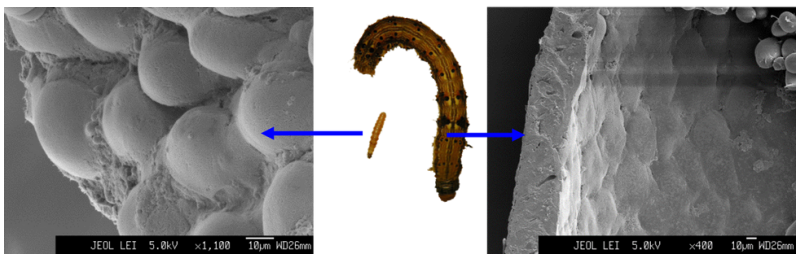
CIPPS
CENTRE FOR INNOVATIONS IN PEPTIDE AND PROTEIN SCIENCE
AUSTRALIAN RESEARCH COUNCIL CENTRE OF EXCELLENCE

SERO-X vs INSECTS:

Sero-X is approved for use in cotton against *Helicoverpa spp*., Silverleaf whitefly (biotype b) (*Bemisia tabaci*), and Green mirid (*Creontiades dilutus*). It offers control through three distinct modes of action:

1. Anti-feedant: Reduces plant damage by causing starvation and lowering pest viability.
2. Direct Mortality: The active compounds disrupts the pest's cell membrane walls.
3. Oviposition deterrent: Modifies pest behaviour, negatively impacting egg-laying.

Mode of action in phytophagous insects of cylcotides involves membrane interaction



Courtesy of Prof D Craik
The University Of Queensland AUSTRALIA

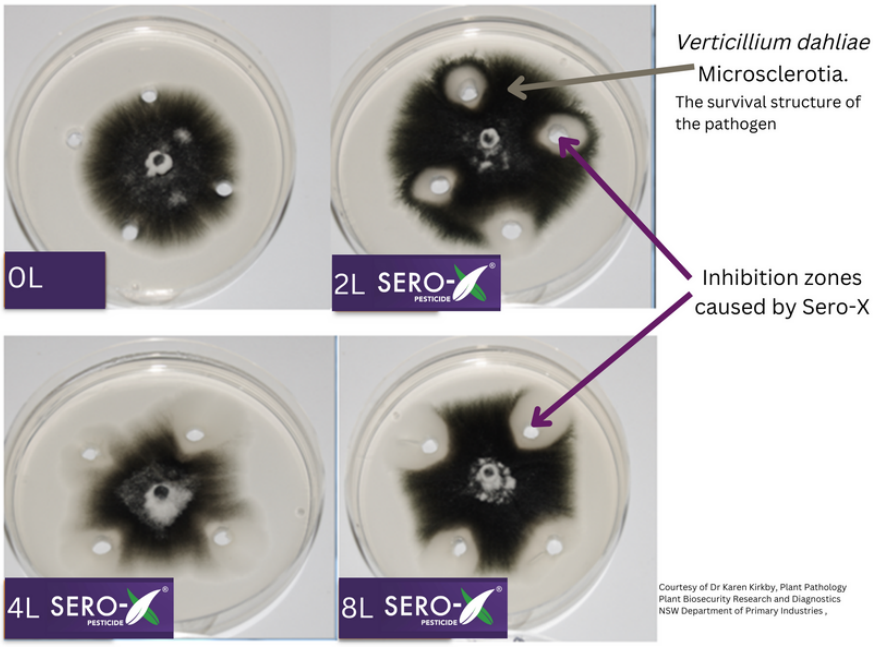
Barbeta *et. al.PNAS* 2008, 105, 1221

Electron micrographs show gut damage to insects after feeding trials.

SERO-X vs VERTICILLIUM WILT: The path to registration

Laboratory Trials - 2016-1018

Dr. Karen Kirkby and her team initiated replicated laboratory assays in 2016-17. Dr. Kirkby has been working for the NSW Department of Primary Industries as a plant pathologist at the Australian Cotton Research Institute in Narrabri since 2010, focusing on pathogens affecting important agricultural crops.



Terminology Explained

Pathogen - *Verticillium dahliae*

Inoculum - included all parts of the pathogen (conidia, hyphae or microsclerotia)

Microsclerotia – mass of melanised cells (**propagule).**

- This is the survival structure
- Contain food reserves for extended survival (>14 years)
- Resistant to harsh conditions

PPG - propagules per gram of dry soil

pgDNA/gm -picograms of DNA per gram of soil.

The assays showed suppression of microsclerotia at all application rates, whether applied as a spray or drench.

2017-18-19 Field Trials

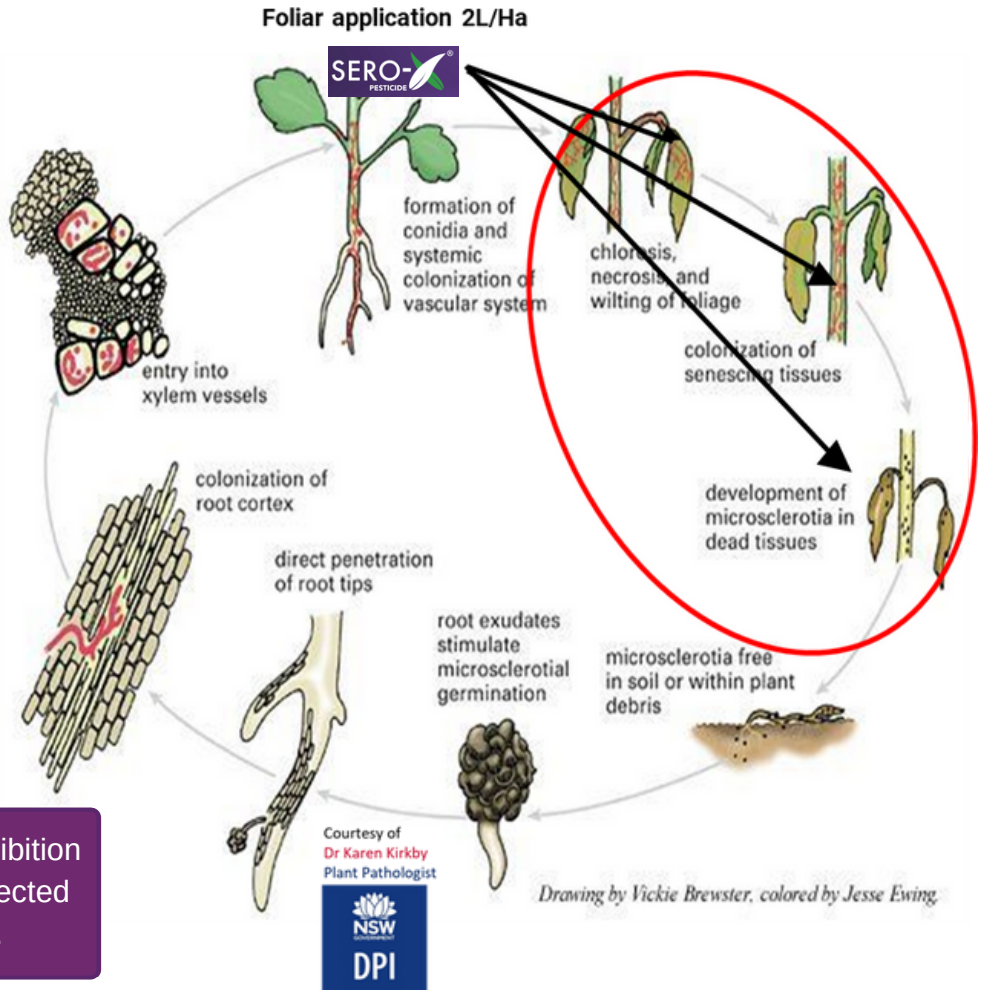
Replicated field trials commenced 2017-18

Field trials were conducted by Dr Karen Kirkby and the team at NSW DPI over 2 seasons and across 2 valleys.

Application & trial design was targeted to prevent microsclerotia developing in infected plant tissue.

Over 3,200 soil samples were taken from each trial area.

The data presented here is a testament and summary of their hard work.



Research strategy focused on the inhibition of microsclerotia development on infected plant tissue returning to the soil.

Applications and method:

Foliar sprays were applied at intervals by a ground rig or by aircraft to foliage

1. December or when majority of plants are between first square and first flower.
2. February or when majority of plants are between mid to late flowering.
3. With first defoliation.

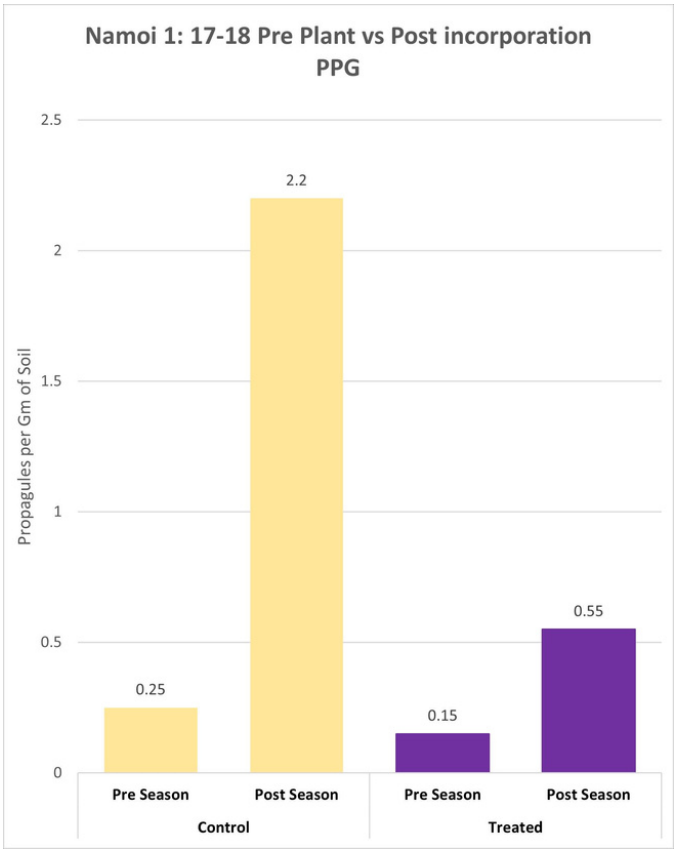
Inoculum levels in the soil (propagules per gram of soil) were measured in treated and untreated, and replicated across 3 Time points

1. Pre-planting of the cotton (Pre Season)
2. During the growing season
3. Post-harvest Post incorporation of the plant material into the soil (Post Season)

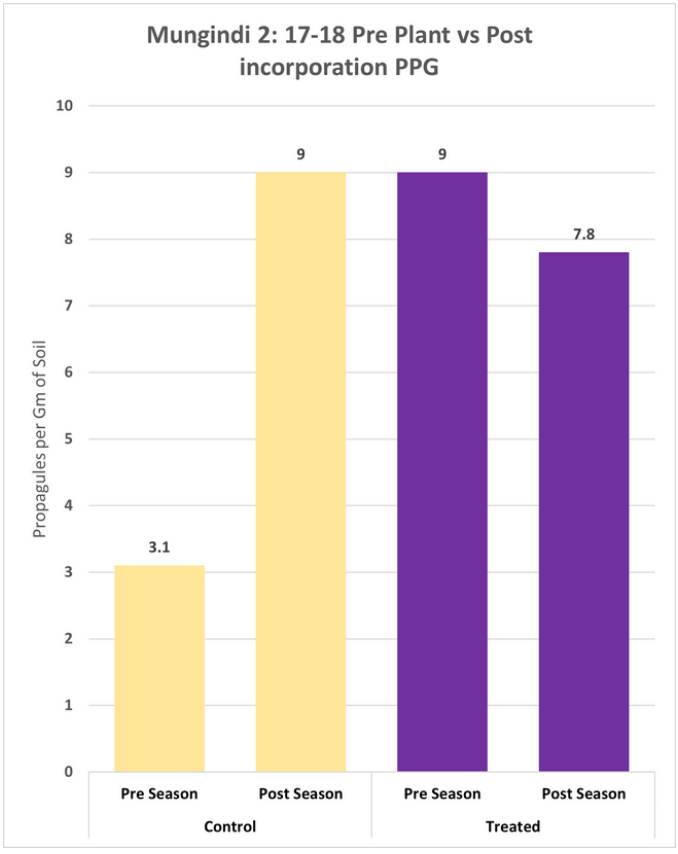
The effect of Sero-X was measured by the comparative differences between the inoculum in the soil pre plant and post incorporation of the soil.

2017-18-19 Results

Results 2017-18: Season where conditions saw an **increasing** population of Inoculum

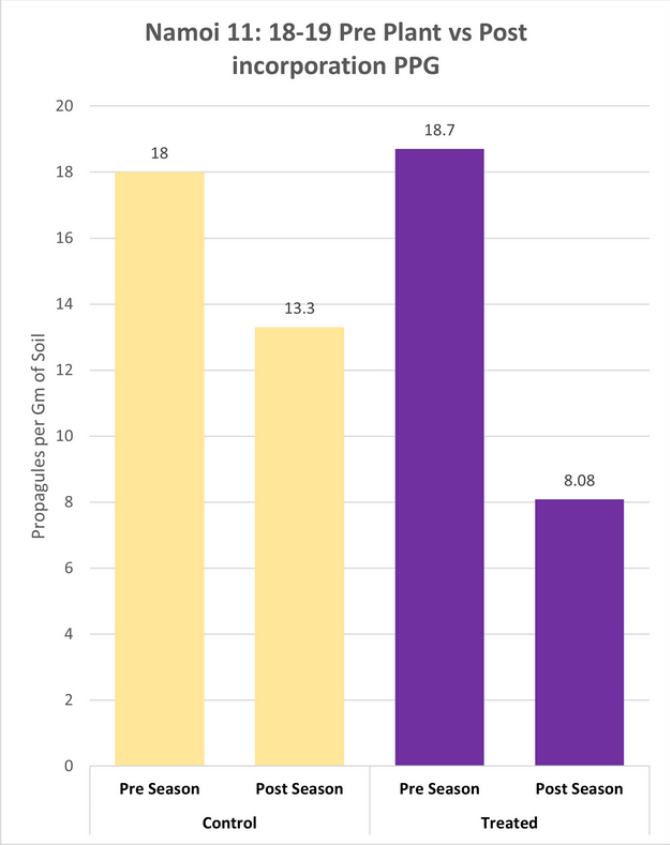


N1: Increase in PPG in both treated and untreated blocks however the rise in the treated area was significantly lower.

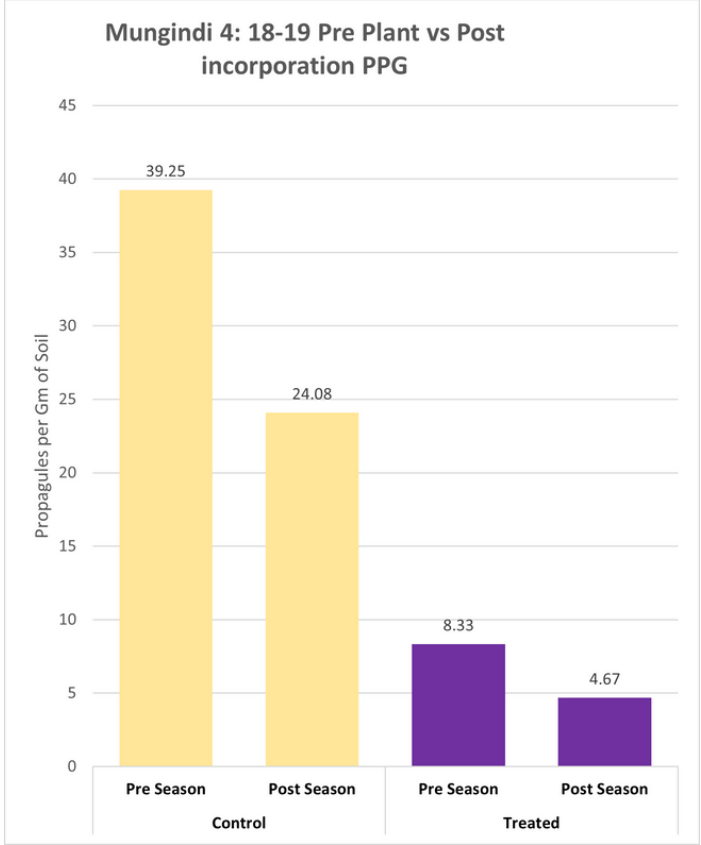


M2: Reduced PPG, though not significant, in the treated blocks whilst the untreated control significantly increased.

Results 2018-19: Season where conditions generally saw a **decreasing** population of Inoculum



N11: in a season where the PPG was decreasing naturally there was an increased reduction.



M4: In a season where the PPG was reducing naturally there was an increase in that reduction though in this case not significantly.

2017-18-19 Field Trials: Conclusion

1. **In seasons with increasing soil inoculum levels, Sero-X can limit the increase or reduce the levels of inoculum in the soil.**
2. **In seasons with a natural reduction in inoculum, Sero-X can accelerate the reduction process.**

APVMA Conclusions:

Field and laboratory trial data confirm that Sero-X Pesticide containing 400g/L Clitora ternatea extract, provides effective suppression of microsclerotia of Verticillium dahliae in cotton and would assist in management of verticillium wilt as an alternative to crop rotation.

Acknowledgements:

Innovate Ag thanks Dr Karen Kirkby, Sharlene Roser, the late Peter Lonergan and NSW DPI for going above and beyond in these trials.

Ongoing research: 2020-21

pgVdDNA Abundance Assessment and Crown Analytics Services

PPG Measuring Propagules per gram of soil (PPG) to assess the impact of Sero-X for APVMA registration was labour intensive and is not commercially available.

Crown Analytical Services (CAS) partnered with SARDI in 2011 to provide the qPCR PREDICTA® B soil and stubble borne disease DNA tests in the northern growing region. They have since continued to work with SARDI to develop a testing protocol for measuring the *Verticillium dahliae* pathogen.

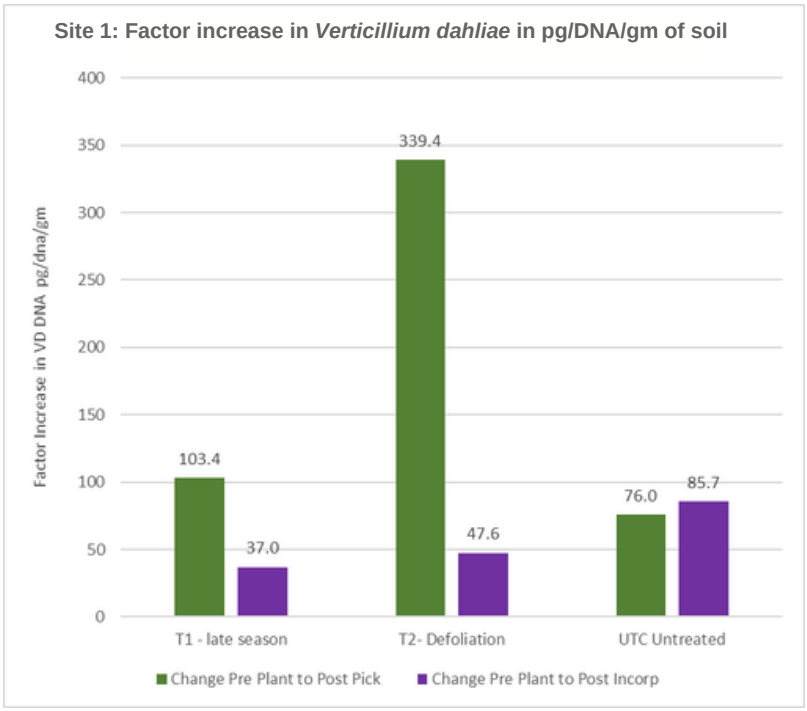
The measurement includes the DNA of all stages of the pathogen including conidia, hyphae, and microsclerotia.

The goal of these trials is to determine if CAS and DNA Abundance measurement can assess the impact of Sero-X in a commercially scalable way, providing the industry with a tool for management decisions on the use of Sero-X and exploring new application strategies.

PREDICTA® from SARDI gives a result from a sample of soil in Picograms of DNA per Gram of soil (pgDNA/gm/sample). It provides a simple and efficient testing process, where one soil sample can be analysed for multiple diseases.

Large plot Scoping Trials: 2 Sites – 2020-21

Aim: Monitor changes in DNA Abundance in the soil over time to determine the impact Sero-X has on DNA levels and thus assess the viability of DNA abundance measurement.



Results: Site 1

The change in DNA levels between picking and incorporating showed that the defoliation application may have been the most effective treatment due to the highest level of DNA reduction.

Conclusions of 2020-21

A single 2 L/ha application of Sero-X at both site 1 and site 2 demonstrated a reduction in the increase of *Verticillium dahliae* DNA levels in the soil at the end of the season. However, the analysis was limited by the number of data points.

Need for further research

A randomised, replicated field trial with more data points is needed to assess the appropriateness of this measurement technique and to evaluate the efficacy of a single application and/or lower rates. This will allow for a comparison of its effectiveness to the current label use pattern.

Large Scale Randomised Complete Block 2021-22-23 -pg VdDNA Abundance.

With the conclusions from 2020-21 in mind and in co-operation with Crown Analytical Services and the Grower, a site with a known history of *Verticillium dahliae* inoculum was selected for a large scale replicated trial

Aims:

1. Determine rate and timing response of Sero-X in inhibiting microsclerotia.
2. Assess the appropriateness of DNA abundance measurement for Sero-X's mode of action.
3. Provide enough data points from replicated & randomised treatments for statistical analysis.
4. Minimise the effect of spatial variability across the paddock

Trial Design Random Complete Block

5 Treatments

Name	Rate L/ha	Description
1 Lo T1	2 x 500ml + 1L/ha	Low label rate
2 Do T2	1 x 2L/ha	Defoliation
3 La T3	3 x 2L/ha	Current label
4 HrT4	3 x 1L/ha	Half label rate
5 Utc T5	Untreated Control	No Treat

3 Replications
0.96 ha for each treatment block

Sample Dates (SD)

SD 1 Pre plant - 18/10/21. - Our starting point for each Data point

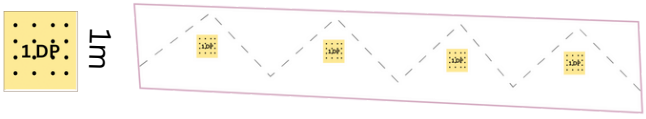
SD 2 Post Pick – 01/06/22. Pre treatment Effect. Prior to incorporation of the plant material into the soil we are not expecting any treatment effect to be evidenced.

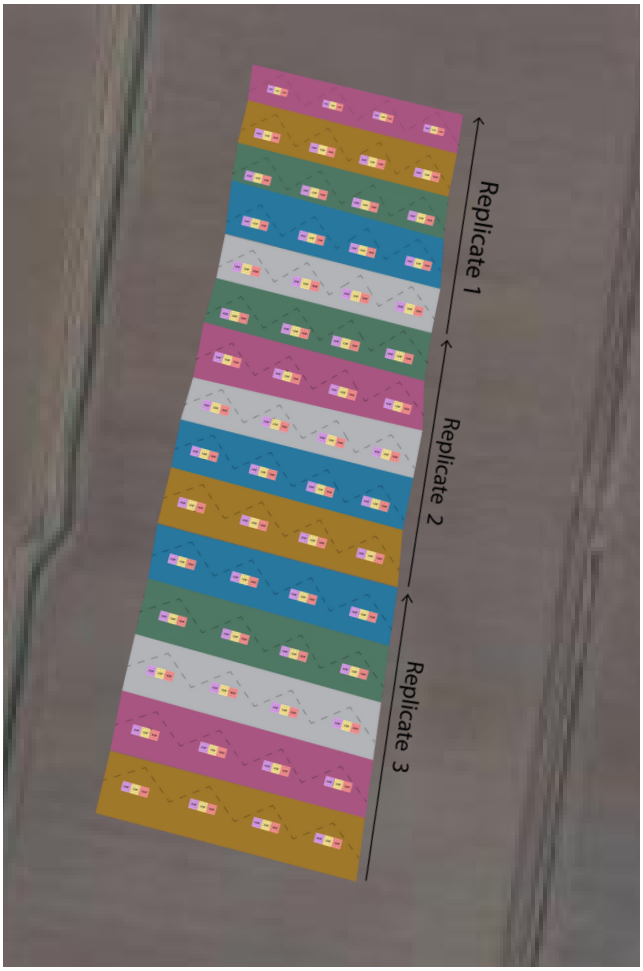
SD 3 Post incorporation – 11/11/22. Post incorporation of the plant material into the soil. First measurable treatment effect. Also becomes an SD1 for the next season

.

At SD 1 and SD 2: Per treatment block

- 4 GPSlocated Data Points (GPS DP)
  - Each DP is 1 composite made up of 16 sub samples from 1 sqM
- Broad sampled Data Point (Broad DP)
  - 1 composite made up of 50 sub samples taken in a zig zag pattern across the whole treatment block
- Total 5 DPS per Treatment block = 75 for the trial area

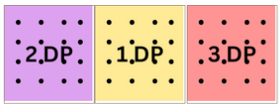




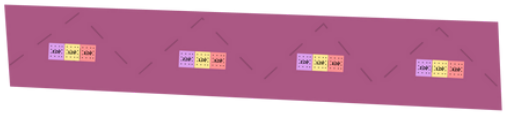
With thanks to the grower and to CAS, a field with prior history of Verticillium wilt incidence was identified and trial laid out. Applications by air @ 30lt/ha, thanks to Exact Aviation)

Results from SD1 and SD2 showed us that the variation in the data would not give us meaningful results so at Sample Date 3 a different sampling protocol was implements

- 4 GPSloc's x 3 Data Points (12 DP per treatment block)
  - Each DP is 1 composite made up of 16 sub samples from 1 sqM with another sample collected 1m up and 1m down the row

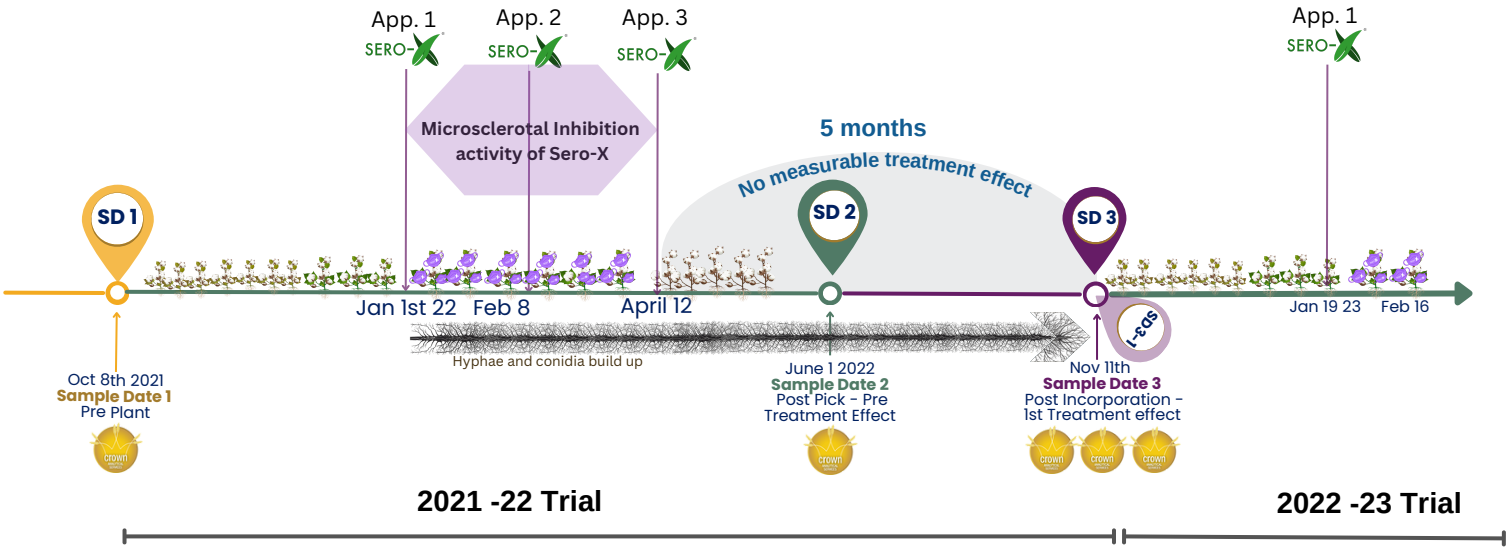


- Broad sampled Data Point (Broad DP)
  - 1 composite made up of 50 sub samples taken in a zig zag pattern across the whole treatment block
- Total 13 DPs per treatment block = 195 DP for the trial Area



Timeline for large scale RCB Trial

- Microsclerotial inhibition lasts ~14 days after each Sero-X treatment, leaving gaps in growing season where microsclerotia may develop.
- Parasitic life stages, (hyphae and conidia), not affected by Sero-X, can survive in soil for up to 4 months, depending on conditions.
- After this period, a 2-3 week timeframe exists where DNA from dead hyphae and conidia cells is still detectable by qPCR.
- This results in an approximate 5 month window where Sero-X treatment effects cannot be accurately measured through DNA comparison.



2021-2022 Results

SD 1 & SD 2 pgVdDNA/gm/Sample

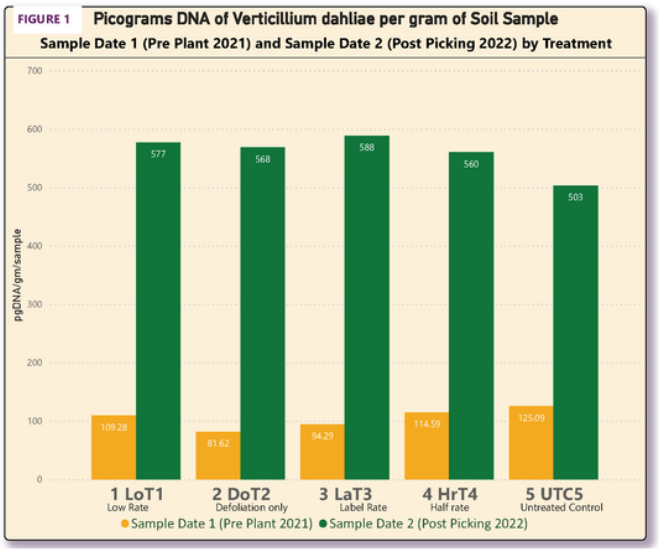
At SD1 Pre plant 2022 the mean across 15 Treatment Blocks was 104.97 pgVdDna/gm/sample.

StDev across the 75 Datapoints was 40.77

At SD2 Post Pick the mean was 559 and the StDev was 223.

All treatments experienced an increase in Verticillium dahliae DNA abundance from Sample Date 1 (pre-plant) to Sample Date 2 (post-pick). **(Fig 1)**

There was no statistical difference between the treatments, with the UTC 5 Untreated control having the lowest build-up and Treatment 2 having the highest.

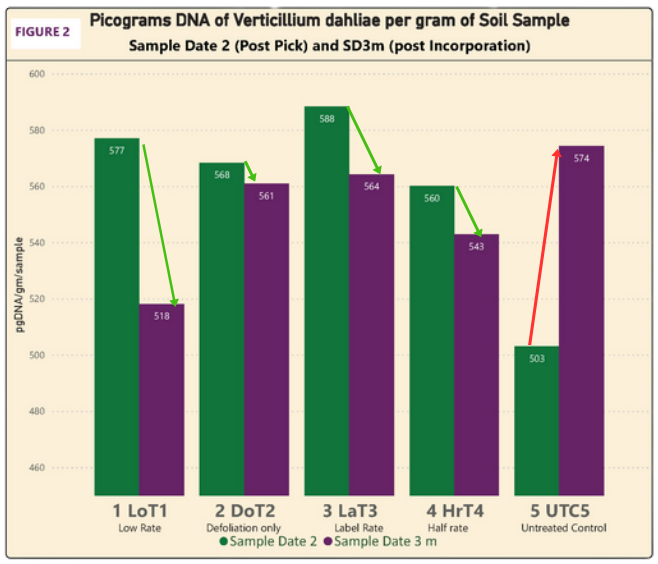


SD 2 & SD 3 pgVdDNA/gm/Sample

At SD3 Post incorporation and the first potentially measurable treatment the 4 x treated area's had a combined mean of 530 pg, vs 574 pg in UTC.

The mean of 3 DPS at each GPSloc was used to provide fair balance to SD3, and there was no statistically significant difference between the treatments with the StDev being 232pcDNA/gm/Soil.

All four Sero-X treatments had declining abundance of Verticillium Dahlia DNA from Sample Date 2 (post pick) to Sample Date 3 (post incorporation), whereas the untreated control increased. **(Fig 2)**



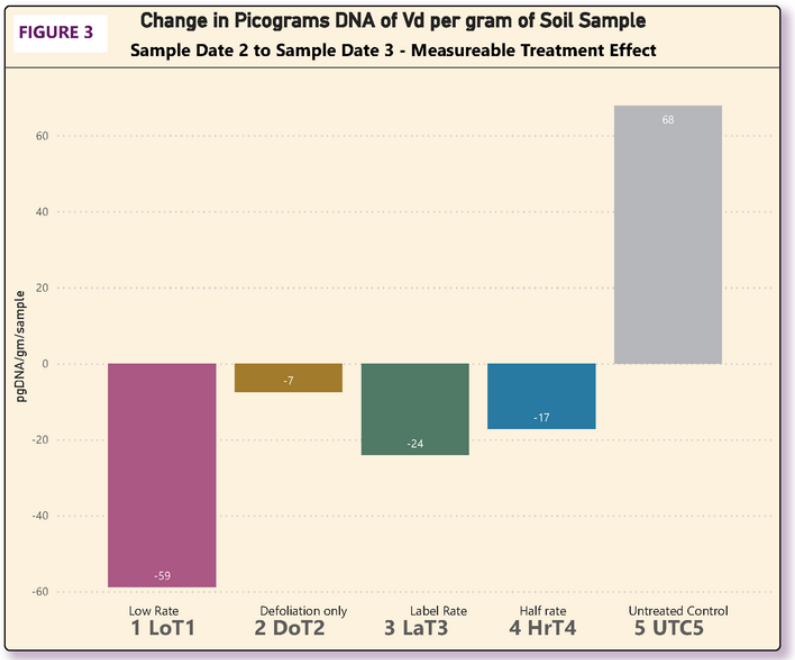
Results Aim 1: Rate and timing response

The Change in DNA abundance between SD 2 Post Picking and SD 3 Post incorporation is the first chance we have to asses the effect of Sero-X (fig 3)

Sero-X was shown to reduce the abundance of DNA of *Verticillium dahliae* across all 4 treatments.

- **10.21%** : largest decrease in 1 LoT1 - a low rate of 2 x 0.5L/ha + 1L/ha at defoliation = 2L/ha
- **5.35 %** : decrease in 3 La T3 - Label rate of 3 x2L/ha = 6L/ha
- **13.5% :** Increase in 5 UTC5 - Untreated control

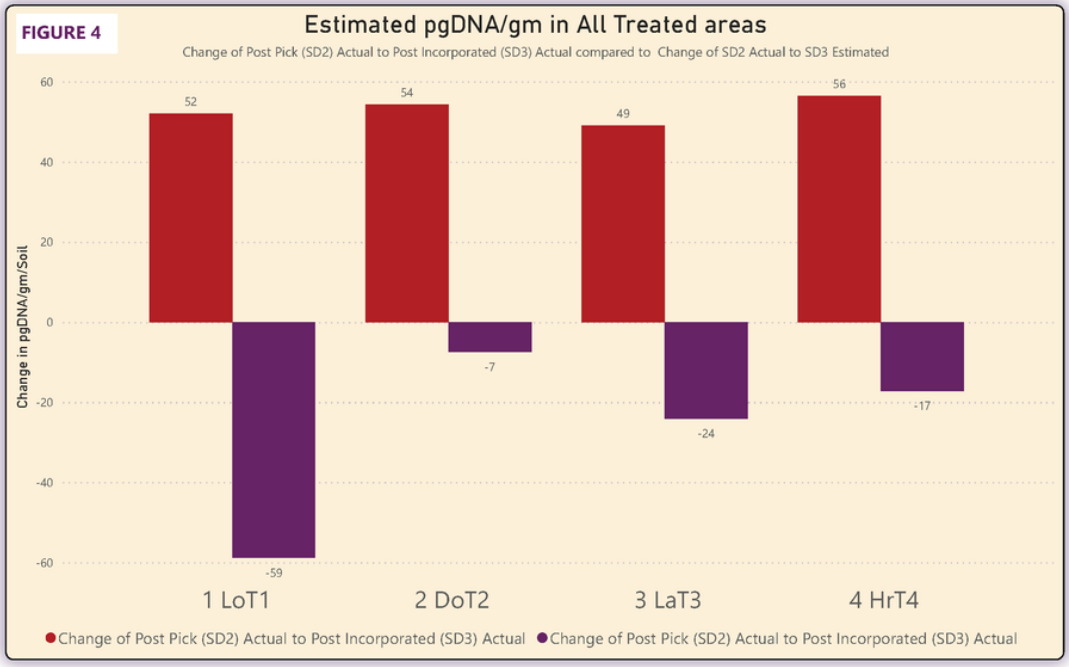
Though not statistically significant, Low rate treatments were shown to be as effective as label rate treatments.



Model of the effect Sero-X

To gain a better understanding of Sero-X's true effect, we need to estimate what would have happened if it had not been applied.

A regression analysis revealed a statistically significant positive correlation between Sample Date 2 (SD2) and Sample Date 3 (SD3) in the untreated control group. Using this information, a model that incorporated standard deviations was developed and run 200,000 times to simulate possible outcomes for the treatment blocks without Sero-X application (SD3yR). Figure 4 provides a comparison of the actual results with the simulated outcomes.



The model revealed for example the following:

- Treatment 1 (Lo T1) was likely to have experienced a 52 pgDNA increase in Verticillium dahliae ; instead, it reduced by 58 pgDNA, representing a 21% reduction.
- Treatment 3 (La T3) was likely to rise 49 pgDNA and instead dropped 24
- The defoliation-only treatment reduced Vd DNA by 10%.

These findings suggest that Sero-X application played a critical role in reducing the *Verticillium dahliae* DNA abundance in the treated plots, as opposed to the natural course of the disease progression in the untreated control.

Results Aim 2: Effectiveness of Measurement

This trial demonstrates the potential of DNA abundance testing in detecting the impact of Sero-X on the microsclerotia of *Verticillium dahliae*. However, certain limitations and factors can affect the results:

- Spatial variability & Sample collection: Accurate results depend on collecting samples from representative areas in the field and at the appropriate resolution.
- Analysis of Data: Repeated measure, paired, or "point to point" time change analysis is critical in assessing the influence of any intervention, including crop rotations or Sero-X, on DNA abundance.
- Viable vs nonviable DNA: measurement cannot differentiate between viable and nonviable pathogen cells which must be considered when interpreting results.

Results Aim 3 & 4: Statistical significance & spatial variability.

- Increasing the number of data points at each GPS location did not decrease variability but demonstrated the nature of the variability.
- Distribution of variability seemed statistically normal enough to predict a variable with reasonable accuracy.

Increase in Untreated Blocks at Sample Date 3:

During the fallow period from post-picking (SD2) to post-incorporation (SD3), soil inoculum levels might decrease, but untreated controls showed increased DNA abundance. This could be due to factors like wet weather-induced microsclerotia germination. While this germination doesn't increase propagules, it does increase DNA as hyphae develop seeking hosts. Without a host, hyphae die, and excess DNA breaks down

Key Takeaways:

- 3 applications of Sero-X appear to be effective at reducing the level of *Verticillium dahliae* DNA, with low rates of performing at least as well as higher rates.
- Defoliation-only treatment was less effective in this trial compared to previous years, likely due to the early onset of symptoms in the season.
- Climate conditions prior to testing can affect DNA levels in the soil.
- Spatial variability in *Verticillium dahliae* will make analyzing changes over time challenging. Adaptable sampling methods with appropriate resolution and taking into account local environmental considerations can help overcome these issues.

Next Steps

- As part of ongoing research efforts, Innovate Ag will continue to measure the treatment effect of Sero-X on the test blocks, with a cotton crop planted in November 2022 and post-pick (SD4-2) and post-incorporation assessments (SD5-3) planned.
- Cotton picker yield data from 22-23 and NDRE+NDVI time series analysis along with incidence and severity scoring to provide another layer of information to demonstrate the effect that Sero-X has on crop health and yield in following crops.

A question for the whole cotton industry is :
what are the risk thresholds for DNA abundance, and how can reducing DNA levels benefit cotton growers?

Determining this is an essential step in understanding the practical implications and advantages of using DNA abundance as measurement to guide management strategies of *Verticillium dahliae* in the field..

The Future

In partnership with The University of Queensland Institute for Molecular Bioscience, we at Innovate Ag and our sister company Growth Agriculture are investing heavily in understanding more about the nature of and role these cyclotides will play in agricultural production here and around the world.

It is only with the support of growers and the industry as a whole that we can invest in this type of unique, world first research and we thank you for your support.

Closing Remarks & acknowledgments

Innovate Ag believe that it is vital for the cotton industry as a whole to be involved in developing appropriate integrated management practice's for controlling Verticillium Wilt, Sero-X should always be used in conjunction with these.

We are encouraged by the results and feedback from growers and consultants who have incorporated Sero-X into their management approach. For more information, please don't hesitate to contact us.

We thank all co-operators in this often painstaking work including Dr Karen Kirkby, Sharlene Roser, Exact Aviations, Rob Long and Jenny Brooks from Crown Analytical Services, Prof David Craik and the research group at UQ IMB and the whole team at Growth Agriculture. Without the efforts of these people research like this can not happen.