

Unearthing potential of biocontrol to reduce Verticillium wilt

BUTTERFLY pea plant (*Clitoria ternatea*) plant extracts have been shown to inhibit the development of *Verticillium* inoculum in both laboratory and field trials. The pathogen causing Verticillium wilt in cotton produces microsclerotia which are the main survival structures that serve as the primary inoculum source for infecting crops, volunteer plants and weeds.

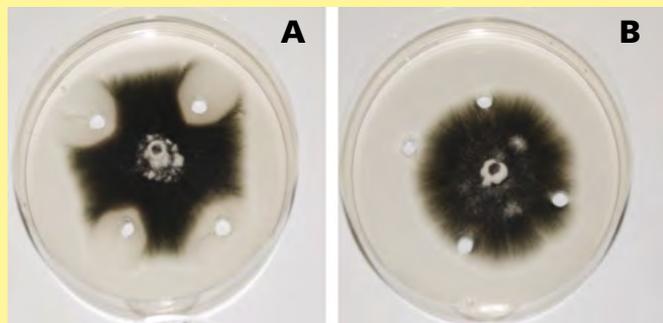
The microsclerotia form in the infected and dying plant tissue and return to the ground in season and following defoliation. Microsclerotia are capable of persisting in the soil for more than 14 years without a host plant. These multicelled melanised survival structures remain dormant until conditions are right to germinate. Because of this, microsclerotia are considered important targets for control.

“The search for products that kill the microsclerotia or inhibit their production has been going on for decades in a bid to control Verticillium wilt,” says Dr Karen Kirkby, Cotton Plant Pathologist with NSW DPI.

Karen and her team conducted laboratory assays using an extract from the plant *Clitoria ternatea* (CT Extract) which contains a suite of bioactive compounds called cyclotides, which are known to have fungicidal activity. “What I saw when examining the Petri plates containing both the pathogen and the extract was exciting.”

Each Petri dish containing a selective media had four plugs removed. In the treated dishes the CT Extract was placed in the wells and in the untreated dishes distilled water was added as a control. A four mm plug of the *Verticillium* pathogen was placed in the centre. After incubating at temperatures ideal for the pathogen, clear inhibition zones, where no microsclerotia were formed, surrounded each well in the CT Extract treated dishes, while microsclerotia formed uninhibited in the control plates. The results of the laboratory experiments that were repeated many times indicated that the CT Extract was preventing the development of microsclerotia (Figure 1).

FIGURE 1: Laboratory assay showing microsclerotia development inhibited in a 2%v/v CT Extract treated Petri plate (A) and no inhibition in the untreated control plate (B)



The next step was to test the theory that targeted applications of CT Extract formulated at 400 g/L within the growing season suppressed the number of microsclerotia returning to soil following incorporation. Three fields that had a history of

Verticillium wilt were selected in 2017–18 along with a further two fields in 2018–19 season. Three fields were in the Namoi and two in the Mungindi region. Foliar applications were applied at 2 litres per hectare in December 2017, in February and at defoliation in each season.

Inoculum levels naturally fluctuate throughout the cotton growing season. It was important to consider this when setting up the field trials. Soil samples were collected pre-planting in October each year to establish the starting levels of inoculum in the soil. Further soil samples were collected in the control and foliar plots in December, March and May in the 2017–18 season and in February and May in the 2018–19 season. Soil samples were air dried for up to four weeks from collection, rolled to a uniform fine texture and mixed thoroughly before being isolated. The number of viable microsclerotia were quantified and reported as propagules per gram (ppg) of dry soil.



Karen Kirkby.

Widetract Picking Unit Lift Frame

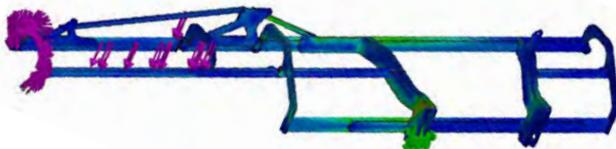


JOHN Deere Cotton Pickers have become a mainstay for cotton farmers in Australia. Many customers require different configurations from standard and so Widetract have applied their engineering expertise to developing a range of products to suit these machines. The latest addition is a complete new unit lift frame that includes full ground-following automation while eliminating the shortfalls of original and modified frames.

RCG Contracting using their new unit lift frame near Gunnedah

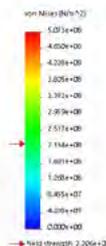
Challenges facing Operators

- Inability to pick 6 rows in one pass if the row spacings are greater than 40"
- Rearward flex of the outer units during normal operation, amplified when extended for skip rows or wider rows than 1m.
- Inability to accurately follow the ground contour at 'greater than standard' widths.
- Excess loads are applied to the frame carrying arms, pins and adjustment bolts, and ultimately to the frame of the machine due to the extra weight combined with the design of the attachment configuration.
- Sagging frames, broken or worn attachment pins and bent rails due to the bouncing and occasional ground contact over time.
- Excessive wheel contact when the picker does not correctly match due to pass overlap.
- Downtime removing sections of the frame and the inconvenience of having to move those parts separately when transporting the machine.



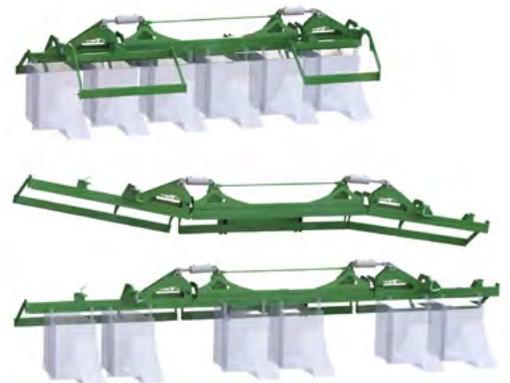
Key features

- 3-piece frame eliminates the original 'broken centre' design which causes most of the fatigue damage.
- Forward folding wings for ease of switching from a narrow configuration out to a wide configuration.
- Legal road travel width of 5.5m can be achieved by folding the outer sections forward and around in front of the picking units, without having to haul heavy components on a separate vehicle.
- Increased strength and vastly decreased rearward flex under heavy picking conditions.
- Superior ground following capability due to the natural contour following ability of a hinged design.
- Reduced strain on the machine chassis and frame compared to original frames.



Design notes

- Uses original John Deere control parts for ground following functionality. OEM replaceable.
- The kit is completely removable (compatible with 7760 models through to the current cp690).
- Kits can be tailored to suit individual needs.
- Metric and imperial row indexing holes on our frame for easy setup.
- Maximum visibility while picking.
- Frame moves the picking units forward 75mm which allows for fitment of a broader range of tyre options.



Adjustable wide and narrow configurations

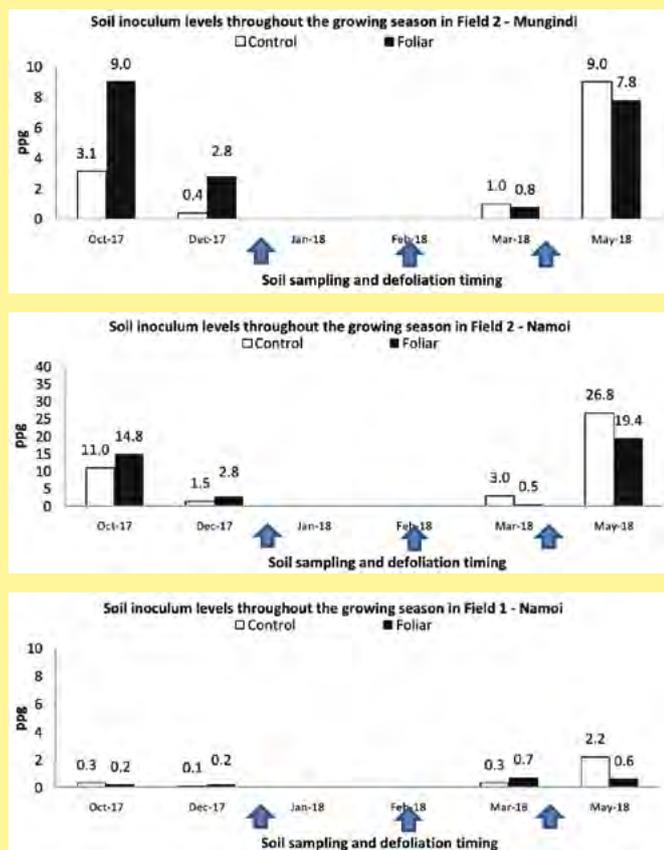


Applying CT extract in mid December.

The results of the laboratory Petri plate assays helped to determine when the foliar applications should be applied in the field. "I wanted the product on the plants at times when symptoms are most observed."

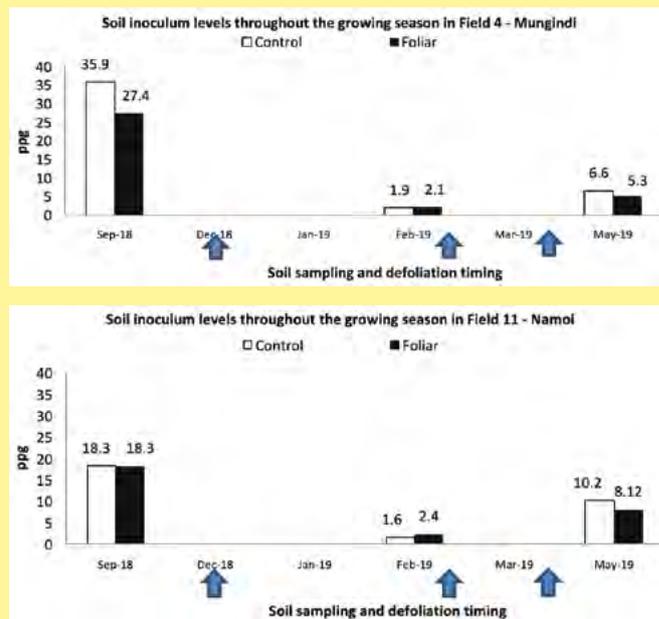
Three applications were chosen with the first being applied mid-December. It is not uncommon for Verticillium wilt symptoms low on the plant to be seen before Christmas. The second application was in February when symptoms become obvious throughout the

FIGURE 2: Fluctuating inoculum levels – high pre-plant and reducing before increasing post defoliation in three field trial



The increase in the ppg in May was less in plots treated with foliar applications of CT extract compared with control plots in all three fields.

FIGURE 3: Fluctuating inoculum levels – high pre-plant and reducing before increasing post defoliation in the two field trials



The increase in the ppg in May was less in plots treated with foliar applications of CT extract compared with control plots in both fields.

crop. The third application was with defoliation. "I was targeting applications to inhibit the development of microsclerotia in dying plant tissue before it was returned to the soil."

We were particularly interested in the change in inoculum levels between pre-incorporation in February 2017–18 and March 2018–19 and post-incorporation in May. This is when infected plant tissue falls to the ground following defoliation and incorporation.

In each of the fields in 2017–18, the number of microsclerotia returned to the soil post incorporation was less in the plots treated with foliar applications compared to the untreated control plots (Figure 2). The incidence of Verticillium wilt in Mungindi Field 2 was 46 per cent, Namoi Field 2 was 60 per cent and lowest in Namoi Field 1 was 2 per cent.

Similar results were obtained in 2018–19 in Mungindi Field 4 where the average disease incidence was 35 per cent and in Namoi Field 11 where disease incidence was high at 84 per cent. The number of microsclerotia returned to the soil post incorporation was less in the plots treated with foliar applications compared to the untreated control plots (Figure 3).

This exciting trial work has only been possible with the continued support of Innovate Ag.

CT EXTRACT

- Demonstrated inhibitory effects in all laboratory assays.
- Reduced the number of microsclerotia isolated from the soil following incorporation in all five field trials.
- Innovate Ag of Wee Waa, NSW are the owners and developers of products made from *Clitoria ternatea* Extracts and are developing a registration package for the APVMA.
- Innovate Ag is supporting further research including an application for an ARC Linkage Grant with the Institute of Molecular Bio-science and University of Queensland.