

Longer rotations are required to reduce *Verticillium* where disease levels are high

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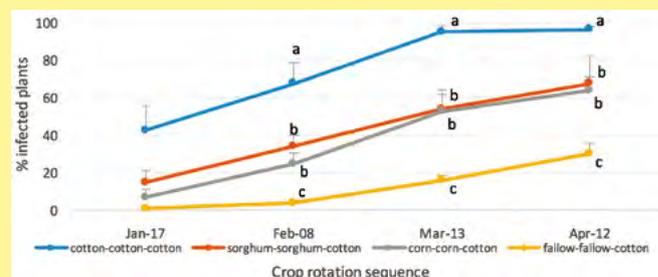
VERTICILLIUM wilt continues to be a major disease issue for many Australian cotton growing regions, resulting in significant yield losses. The soilborne fungus causing the disease, *Verticillium dahliae*, has a wide host range and can persist in the soil for up to 14 years. Management of the disease requires an integrated approach that aims to reduce the soil inoculum load. Crop rotation with non-hosts is one strategy that could be used.

Department of Agriculture and Fisheries (DAF) Queensland trials have examined the impact of one and two year rotations out of cotton on disease incidence and development in the following cotton crop. The trials were conducted near North Star in NSW and included rotations of sorghum, corn or bare fallow compared to continuous cotton. The effect of cropping on soil microbial diversity was also investigated, as high microbial taxonomic and functional diversity is associated with suppression of disease.

The two trials commenced in the 2015–16 season in adjoining areas of the same field where a high level of *Verticillium* wilt was present. In this first year, one trial (Trial 1) was flood irrigated and the other (Trial 2) was grown dryland. Both trials were fully irrigated thereafter. Disease incidence, as determined by the presence of vascular stem browning, was assessed in the cotton treatments in both trials at the end of the first season. While not significantly different, there was a trend for lower disease in the dryland cotton (63 per cent diseased plants) compared to where cotton had been grown under flood irrigation (71 per cent diseased plants).

This concurs with findings from overseas studies that have shown that as you irrigate more frequently the incidence of disease increases. Drought stressed cotton typically suffers less infection by *Verticillium dahliae* than irrigated cotton and this is likely to be related to irrigation cooling the soil and enhancing

FIGURE 1: Trial 2 – disease progress in cotton following two years of rotation with non-hosts, cotton or bare fallow during the latter part of the 2017–18 season (half the trial only from the head ditch end was assessed for all sampling dates for this study)



NB: Due to a planting error, replications of the sorghum-sorghum rotation in the 2015–16 to 2016–17 season were actually corn-sorghum, fallow-sorghum or sorghum-sorghum: one replication of the corn-corn rotation was actually sorghum-corn. Treatments within the same sampling time followed by different letters are significantly different, $p < 0.05$.

pathogen survival and infection. Irrigation scheduling should therefore be closely monitored and overwatering avoided.

In Trial 1, cotton was planted across all treatments in the second and final year of the trial. In Trial 2, the same crops were sown back into the same plot in the second year, before cotton was planted across all treatments in the following season. Disease development and progress was monitored in cotton throughout this final season in Trial 2 by assessing the amount of stems with vascular stem browning at several time periods (Figure 1). *Verticillium* infection was confirmed by isolation of the pathogen from stem sections back in the laboratory.



Typical vascular stem discolouration.



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Longer term rotations with alternative summer crops may be necessary to reduce high levels of *Verticillium*.

This study showed that lower inoculum levels delayed disease development. At the first intensive assessment made on January 17, continuous cotton had at least twice as many diseased plants as the other treatments, and there were minimal (0.8 per cent) infected plants in the cotton following two years of fallow (Figure 1). As the season progressed this trend continued. There were significantly more diseased plants in assessments made from February until the end of the season (Figure 1), where three years of continuous cotton had been grown compared to cotton following either two years of fallow or non-host crops.

Figure 2 shows the end of season disease incidence assessed in cotton grown after one year of rotation crops (Trial 1). While a one-year rotation with corn, sorghum or a bare fallow lowered disease compared to continuous cotton, the difference was not significant. Disease incidence was still 74 per cent where cotton had been planted after sorghum and corn compared to 81 per cent where cotton followed cotton.

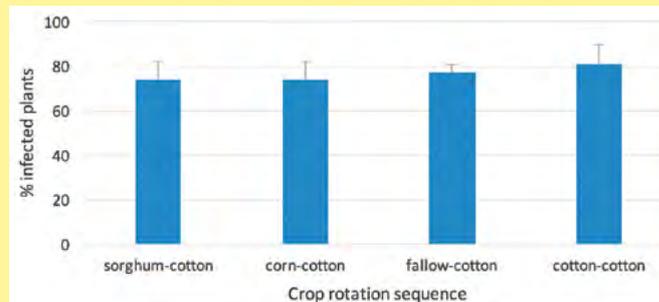
Soil was collected pre-plant and post-harvest during both trials and sent to SARDI (South Australian Research and Development Institute) for analysis to quantify, using molecular methods (PreDicta Pt), the amount of *Verticillium dahliae* DNA present per gram of soil (http://www.pir.sa.gov.au/research/services/molecular_diagnostics/predicta_pt). Supporting what was observed in the field, when measured post-harvest, the quantity of *V. dahliae* DNA peaked after the first year of irrigated cotton (Figure 3) compared to where cotton was grown dryland (Figure 4). In Trial 2 (Figure 4), the higher quantity of *V. dahliae* DNA under continuous cotton correlated with the disease incidence observed in the field during the final season. For research purposes this is a useful tool to monitor general trends following different management strategies.

Different crop types can influence the abundance and composition of soil microbial populations due to the quantity and quality of carbon substrates they add for microbial use. There is also a plant type based variation in the members of microbial communities they promote. Soil was collected after the two years of rotation sequences but before cotton was planted across all treatments (Trial 2). Results after two years of rotation sequences showed that surface soils from the fallow-fallow rotation had the lowest abundance of fungal populations and overall catabolic diversity of soil microbial communities, whereas the crop sequence that included sorghum showed the highest values (Figure 5).

Although the fallow-fallow rotation reduced the pathogen level and disease incidence it seemed to cause a significant reduction in general microbial catabolic diversity (Figure 5b) and activity levels (data not shown), potentially causing a reduction in soil biological functions. Such a decline in overall microbial populations and activities in the long-term could potentially make soils more conducive to soilborne diseases as seen from

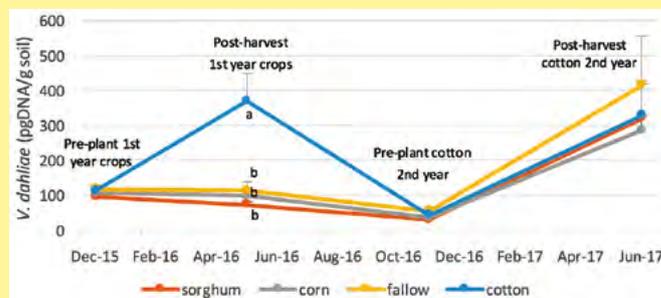
the results from the crop rotation trials at ACRI (data not shown). These results suggest that management of *Verticillium* wilt through crop sequences that include other crops, may be a better option as they not only reduce disease incidence but also maintain overall soil biological health.

FIGURE 2: Trial 1 – disease incidence in cotton following one year of rotation with corn, sorghum, cotton or bare fallow



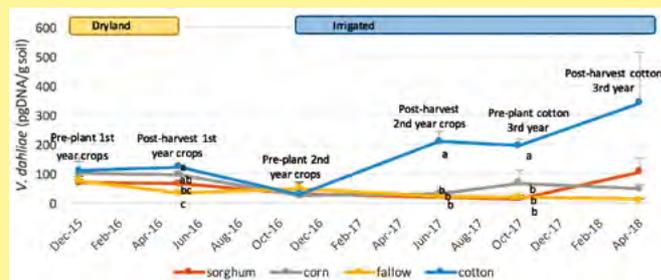
NB: Not significantly different at $p < 0.05$.

FIGURE 3: Trial 1 – quantity of *Verticillium dahliae* (pgDNA/g soil) measured during one year of crop sequences (2015–16) followed by cotton (2016–17) in a fully irrigated trial



Treatments within the same sampling time followed by different letters are significantly different, $p < 0.05$.

FIGURE 4: Trial 2 – quantity of *Verticillium dahliae* (pgDNA/g soil) measured during two seasons of rotation sequences (2015–17) followed by cotton (2017–18). December 2015 to May 2016 was dryland cropping and November 2016 to April 2018 was irrigated cropping.



NB: Due to a planting error, replications of the sorghum-sorghum rotation in the 2015–16 to 2016–17 season were actually corn-sorghum, fallow-sorghum or sorghum-sorghum: one replication of the corn-corn rotation was actually sorghum-corn. Treatments within the same sampling time followed by different letters are significantly different, $p < 0.05$.



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Verticillium wilt infected plant.

In summary

The management of Verticillium wilt requires an integrated approach that ultimately reduces the soil inoculum levels in the soil. These trials have shown that crop rotation can reduce disease but more than one year out of cotton is required

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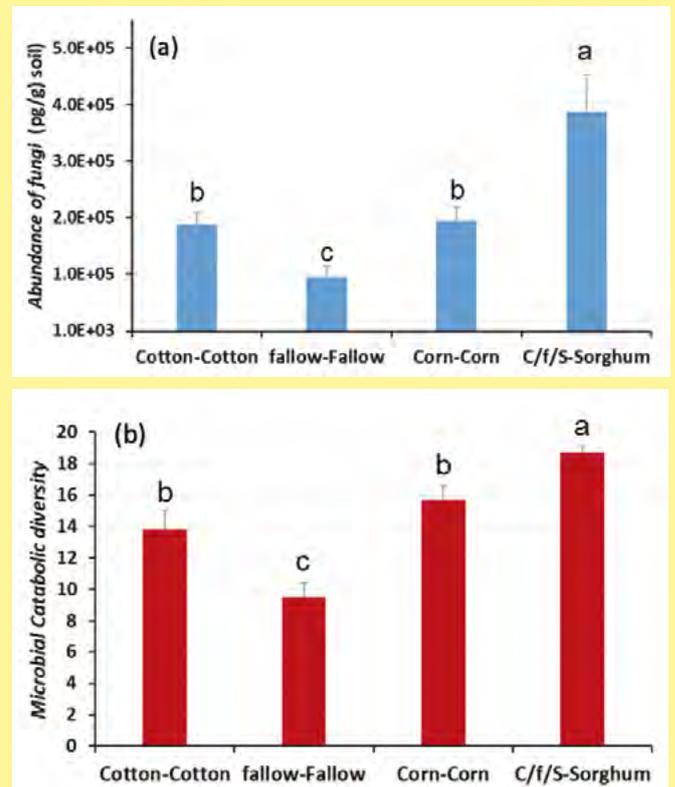
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FIGURE 5: Effect of two years of crop rotation sequences on total soil fungal populations (a) and microbial catabolic diversity (b) in the surface 10 cm soil



Bars with different letters are significantly different from each other at $P < 0.05$.
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where disease levels are high. While two years of crop rotation significantly reduced disease compared to continuous cotton, more than half the plants were still infected with *Verticillium dahliae* where sorghum and corn had been grown in rotation. While this is significantly better than 95 per cent infected plants under a continuous cotton rotation, even longer rotations may be required to sufficiently lower the soil population of Verticillium.

Even though cotton will continue to yield well in warmer seasons, it should be remembered that inoculum is continuing to build under back to back cotton. This could have severe yield implications especially if a cool wet mid-season occurs that is conducive to Verticillium wilt. Where possible, an appropriately chosen crop rotation program for each individual field that reduces disease incidence and maintains overall soil biological health should be considered and used as part of an overall management plan for this disease. Ideally it would be wise to instigate rotation options earlier rather than later because it is much harder to lower soil populations of this pathogen once they become high.

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