

**Verticillium wilt in Australian cotton:
Examining the relationship between Australian *Verticillium dahliae* isolates
and virulence**

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Certificate of Authorship

I, Pearl Dadd-Daigle, declare that this thesis, submitted in fulfillment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science, School of Life Sciences at the University of Technology Sydney, is wholly my own work unless otherwise reference or acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

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Signature:

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Statement

This thesis is by compilation. The first research chapter and the literature review are published papers listed in the section below. The second research chapter is a completed manuscript and has been submitted to the Australasian Plant Pathology Journal. The third research chapter is a publication style manuscript that is being prepared for journal submission. Figure and Table numbers have been edited from the original publications to match the chapter numbering of the thesis.

List of publications

Paper 1; Chapter 2

The Verticillium wilt problem in Australian cotton

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Australasian Plant Pathology. 2021 50:129–135

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Paper 2; Chapter 3

Virulence not linked with vegetative compatibility groups in Australian cotton

Verticillium dahliae isolates

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Abbreviations

VCG	Vegetative Compatibility Groups
D	Defoliating
ND	Non-Defoliating
ISSR	InterSequence Simple Repeats

Abstract

Verticillium wilt, caused by the soil-borne phytopathogen *Verticillium dahliae*, affects many agriculturally important crops around the world. In Australia, the cotton industry, worth, on average, between \$2-3 billion a year, is increasingly impacted by Verticillium wilt. The fungal pathogen is characterised into Vegetative Compatibility Groups (VCG) and further into defoliating and non-defoliating pathotypes. *Verticillium dahliae* defoliating VCG1A is reported to cause severe damage to cotton internationally, while non-defoliating VCG2A is responsible for only mild to moderate disease symptoms. In Australian cotton however, the non-defoliating VCG2A is causing more severe damage to crops in the field than the defoliating VCG1A.

A selection of isolates taken from the New South Wales (NSW) Department of Primary Industries Verticillium collection were used to infect cotton plants in controlled greenhouse conditions. The plants, encompassing four varieties, were monitored over a period of seven weeks and the disease progress scored bi-weekly. Analysis of the disease scores revealed that although disease progression is slower in plants infected with non-defoliating VCG2A isolates, both Australian defoliating VCG1A and non-defoliating VCG2A are able to kill cotton plants in glasshouse trials. Cotton variety had minimal impact on disease outcomes. This was the first confirmed report of an Australian non-defoliating VCG2A causing plant mortality in cotton plants outside of the field.

Eighty-four isolates from the NSW Department of Primary Industries Verticillium historical collection were further analysed using InterSequence Simple Repeats (ISSR). The PCR-based method resulted in a molecular fingerprint for each isolate, which was then used to produce a phylogenetic tree. Within the tree the isolates clustered into three main groups, one composed of non-defoliating VCG2A isolates, another made of both non-defoliating VCG2A and non-defoliating VCG4B, and the third contained only defoliating VCG1A isolates. These groups were labelled “Defoliating-like”, “Non-defoliating”, and “Defoliating”, respectively. Further glasshouse trials to examine the virulence of isolates in each group confirmed that the virulent VCG2A isolates all fell within the “Defoliating-like” group, the virulent

VCG1A isolates within the “Defoliating” group, and all non-virulent isolates in the “Non-defoliating” group. The inclusion of American *V. dahliae* defoliating VCG1A isolates and eight Israeli isolates of varying VCG into the ISSR study revealed that the Australian isolates appear to cluster separately and suggests that Australian *V. dahliae* isolates could be unique to Australia.

To further examine the differences between Australian and international defoliating VCG1A *V. dahliae*, four isolates were DNA sequenced using both Nanopore Minion and Illumina sequencing platforms to produce whole genomes. Isolates were analysed with 13 publicly available *V. dahliae* isolates using phylosift to build a phylogenetic tree, and gene content examined using ProgressiveMauve alignments to determine where the genes differed. The comparisons found minimal differences between the four Australian isolates, but when compared with the other 13, they appear more genetically distant. Additionally, Australian VCG1A isolates lack a set of genes identified as being involved in defoliation of cotton plants. This work highlights, for the first time, genetic differences between Australian and international defoliating VCG1A *V. dahliae*.