

Making sense of the *Verticillium* classification tangle

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AT A GLANCE...

Verticillium wilt (VW) caused by *Verticillium dahliae* is an important cotton disease. In NSW, the disease is detected across the state, but it is more prevalent in northern valleys of the Namoi and Gwydir. The national disease survey funded by CRDC and led by Dr Linda Smith from Department of Agriculture and Fisheries, Queensland during the 2016 to 2019 seasons revealed that the highest average VW incidence in NSW and QLD was 30 per cent and 4 per cent, respectively. Yield losses of up to 50 per cent have been reported in some fields. The disease has been reported worldwide across many cotton growing countries such as China, Spain, Turkey and the US. In China, the losses of cotton lint yield may be as high as 80 per cent. In Turkey, VW significantly reduced cotton yield by 15.93 per cent. Cotton yield loss due to the VW disease was estimated up to 480 million bales over a period of 1990–2014 in the US.

VERTICILLIUM wilt in cotton is associated with both the non-defoliating (ND) pathotype (strain) of *V. dahliae* which has been detected throughout NSW, and the defoliating (D) pathotype which was more prevalent in the northern valleys of NSW. We also hear of classifications such as VCG 1A or VGA 2A – standing for vegetative compatible group. All these different classifications may have caused some confusion. This article attempts to provide further information about these classifications and what they really mean for disease management strategies.

Pathotype classifications

Verticillium dahliae is a soilborne fungus and is the most ubiquitous and notorious species within the *Verticillium* genus. The fungus is commonly associated with vascular wilt diseases of up to 400 host plant species, including cotton. The disease incidence and severity are highly dependent on multiple factors such as cultivars grown and virulence of *V. dahliae*.

The virulence of *V. dahliae* was commonly thought to be



Photo 1: Complete defoliation of a cotton plant was associated with the D pathotype.



Photo 2: Leaves were desiccated and remained attached to a dead cotton plant, caused by the ND pathotype.

associated with its pathotypes (D or ND). The D pathotype was deemed to be highly aggressive, inciting defoliation, and was lethal to cotton (Photo 1). Though D pathotype can infect an array of plant crops, defoliation is commonly recorded on cotton, olive and okra. On the other hand, the ND pathotype was considered less aggressive and did not lead to defoliation.

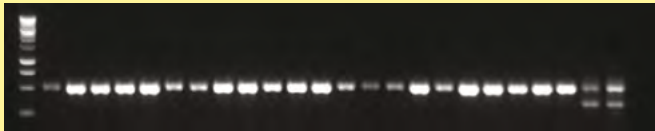
But independent research funded by CRDC from our group and Dr. Karen Kirkby's team at NSW DPI has revealed that *V. dahliae* isolates recovered from Australian cotton and designated as D and ND pathotypes were found to be equally lethal to cotton (Photo 2). In Australia, partial defoliation was also incited in cotton plants infected with the ND pathotype (Photo 3).

In term of genetics, the two pathotypes carry two different DNA genomes and PCR (polymerase chain reaction) assays can give a rapid and accurate differentiation of D and ND pathotypes (Figure 1). In 2019, the unique gene fragment was found which

was associated with a compound that induces defoliation by altering abscisic acid sensitivity or hormone disruption. Deletion of this unique gene fragment in D pathotype isolates resulted in the ND phenotype.

The two pathotypes differ in temperature responses in the lab.

FIGURE 1: Molecular pathotyping of *V. dahliae* recovered from cotton in 2019–20 season in NSW. Gel electrophoresis indicated the predominance of ND pathotype (single ~500 bp band) in comparison to D pathotype (double bands).



Gel ladder for size reference was on the first left.



Photo 3: Under severe infection, field cotton plants infected with the ND pathotype also showed some leaf defoliation. Only a specific molecular marker could provide an accurate and reliable identification of D and ND pathotypes associated with Verticillium wilt of field cotton.

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In most cases, both D and ND isolates showed optimal growth at 25°C. In a growth assessment conducted by Dr. Linda Smith from DAF QLD, growth rates varied, but as a whole ND isolates grew faster than D isolates between 20–0°C. On the other hand, our unpublished data indicated that the D isolates tended to grow faster than the ND isolates, especially at a higher temperature (30°C). Other researchers have also found that D isolates grew significantly faster than the ND isolates at 30°C. They also found that the D isolates were more aggressive on cotton than ND isolates in a 30/30°C (day/night) testing regime. So they concluded that the D pathotype may be better adapted to a higher temperature (30°C).

During our late season disease survey in the past two years, we recovered a total of 120 *V. dahliae* isolates in a hot dry year in the 2018–19 season compared to 311 isolates in a wetter year in 2019–20. Of the collection, D isolates accounted for approximately 34 per cent and 14.5 per cent in 2018–19 and 2019–20 seasons, respectively. This suggests that the Australian D pathotype may also have an adaptation to hot dry conditions. But more data must be collected for any further conclusive recommendations.

As mentioned previously, both D and ND pathotypes are detected in Australian cotton. In many cases, we also detected both pathotypes in the same fields in NSW. During the survey in the 2017–18 and 2018–19 seasons, we also detected a low number of cotton stems infected with both the D and ND pathotypes in NSW. Co-occurrence of both pathotypes is not an uncommon event in other crops and it was previously reported in cotton and olive fields in Spain.

According to Dr. Carlos Traperero, a former postdoctoral researcher at CSIRO Narrabri, commercial Australian cotton varieties respond similarly to D and ND pathotypes; but some of the new germplasm being evaluated responds differently to these pathotypes and increased resistance to one pathotype may result in increased susceptibility to the other pathotype. To date, there has not been a highly resistant cultivar against both pathotypes, especially to the D pathotype. Due to limited resistant resources to D pathotype, it will be challenging to breed Verticillium resistant varieties in cases where both D and ND pathotypes are present.

But before this raises an alarm, a thorough assessment should be made of the damage that co-infection may cause. The co-infection incidence under natural field conditions was detected at a low level in the past two seasons. We continue to carry out CRDC-funded disease surveillance, which has been ongoing for over 30 years in NSW, to enable the monitoring of the occurrence and distribution of the co-infection of both D and ND pathotypes on cotton.

VCG classifications

VCG stands for Vegetative Compatible Group and is a common marker for studying the genetic diversity in ascomycota fungi such as *Verticillium* and *Fusarium*. But the relationship between VCGs and genetic diversity within *V. dahliae* is highly complex.

There has not been a reliable test developed for rapid and accurate detection and identification of VCGs in *Verticillium* and VCG tests in Australia are still outsourced to international collaborations and services. VCG characterisation of *V. dahliae* is considered an expensive, time-consuming and laborious process.

Now for the detail

Worldwide, six different VCGs (VCG1 to VCG6) have been found for *V. dahliae* from various hosts and geographic locations. VCGs 1, 2 and 4 are also subdivided into subgroups A and B based on the vigor of compatible formations, and they are globally distributed and associated with a wide host range.

Verticillium wilt of cotton worldwide has been attributed to VCGs 1, 2 and 4 (and in VCG 3 in overseas reports).

In Australia, VCG 1A, VCG 2A and VCG 4B have been recorded in cotton – mainly VCGs 1A and 2A. Similar VCGs were also reported on cotton grown in China, Greece, Spain and US. But VCG 1B isolates were found in cotton grown in Greece in 2006 and VCG 3 isolates, which were originally obtained from potato in the US, were additionally reported for the first time on cotton in China in 2015. We don't yet know how virulent these strains may be in cotton, so field surveillance of Verticillium wilt will continue to play an essential role in monitoring and early detection of new strains.

In relation to pathotype designations, the D pathotype has been found in VCG 1A isolates only, while all other VCGs were found to have the ND pathotype.

To summarise, we have recorded both of the pathotypes and three different VCGs in Australian cotton crops and these have exhibited a range of virulence on cotton. We hope that further studies will shed more light on the nature of variations among and within pathotypes/VCGs in *V. dahliae* and will eventually contribute to more effective disease management.

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