The Verticillium wilt problem in Australian cotton

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10

11   **Abstract**

12   *Verticillium dahliae* is a soil-borne phytopathogen and the causal agent of

13   Verticillium wilt. It affects many agriculturally important crops around the world,

14   including cotton. In Australia, the billion-dollar cotton industry is increasingly

15   impacted by Verticillium wilt. Internationally it has been reported that the defoliating

16   *V. dahliae* Vegetative Compatibility Group (VCG) 1A causes severe damage to

17   cotton. In Australia however, the non-defoliating VCG2A is causing more severe

18   damage to crops in fields than the defoliating VCG1A. This review examines the

19   current research to understand the Australian *V. dahliae* situation, including current

20   classification systems, genetic analyses and management strategies. It appears that

21   virulence cannot be defined solely by VCG in Australian *Verticillium dahliae* isolates

22   causing disease in cotton, and that the industry must continually adapt their practices

23   in order to keep the disease under control.

24

25 **Key words**

26 *Verticillium*; cotton; *Gossypium hirsutum*; *V. dahliae*

27

28 **Introduction**

29 In Australia, cotton is a growing billion-dollar industry. Cotton yields have increased

30 from 500 kg per hectare in the 1960's to 2000 kg per hectare in 2013 (Hamilton

31 2016). Cotton crops are largely furrow irrigated, grown on alkaline clay soils and tend

32 to be located near flood plains. There is often reduced or minimum tillage, tail-water

33 recirculated and in some areas permanent bed systems (Kirkby et al. 2013).

34 Sustainability and growth of the cotton industry is reliant on improved cotton

35 varieties, management of soil and water resources, and control of weeds, insect and

36 diseases (Constable 2004). Although Verticillium wilt in Australian cotton is

37 generally well managed, other countries have seen economic losses of 50% or more

38 (Wu and Subbarao 2014). The average incidence levels of Verticillium wilt caused by

39 *V. dahliae* in Australian cotton are relatively low but yield losses can vary between 10

40 and 62% in some fields (Holman et al. 2016). However, the recent discovery of the

41 defoliating VCG1A and the disease severity of the non-defoliating VCG2A present an

42 additional problem for management of Verticillium wilt as incidences rise (Chapman

43 et al. 2016; Dadd-Daigle et al. 2020; Jensen and Redfern 2017; Kirkby et al. 2013).

44 Hence, Verticillium wilt is becoming a major concern for the Australian cotton

45 industry.

46

47 *Verticillium dahliae*

48 *Verticillium* encompasses a group of soil-borne ascomycetes. As of 2011, ten

49 *Verticillium* species have been described (Inderbitzin et al. 2011), including *V.*

50 *dahliae,* the main causal agent of Verticillium wilt. *Verticillium dahliae* is responsible

51 for disease in over 400 plant species across the world. These include many

52 economically important crops such as olives, tomatoes, potatoes, lettuce and cotton

53 (Bhat and Subbarao 1999; Inderbitzin et al. 2011).

54

55 The life cycle of *V. dahliae* allows it to persist on farms for many years. It survives in

56 soil in highly melanised resistant structures, known as microsclerotia, for over 10

57 years (Davis et al. 1994; Klosterman et al. 2009). These microsclerotia germinate in

58 the presence of host plants, producing hyphae that penetrate the root cortex and reach

59 the xylem. As hyphae and conidia grow within the xylem, the plant host can express

60 symptoms of wilting, necrosis and leaf discolouration (Klimes et al. 2015). As

61 symptoms progress, *V. dahliae* enters a saprophytic phase where the infection

62 expands to other tissues, such as leaves, and a mass production of microsclerotia

63 occurs. The extent of symptoms can depend on the susceptibility of the host and the

64 infecting strain of *V. dahliae*. While some plants suffer severe wilting and necrosis,

65 other infections are less severe, allowing the plant to recover (Daayf 2015).

66

67 Historically, the characterisation and classification of *V. dahliae* has been based on

68 the symptoms exhibited by the host plant, or by the interaction of pathogen virulence

69 and host resistance genes. Consequently, this has led to the use of host-specific

70 terminology and classification, resulting in a number of different classification

71 systems. *Verticillium dahliae* strains infecting tomato and cotton are divided into

72 "races", classified by the presence or absence of the *Ave1* gene (Hu et al. 2015;

73 Maruthachalam et al. 2010). Strains from cotton are also categorised into defoliating

74 (D) and non-defoliating (ND) pathotypes (Daayf et al. 1995). While the D and ND

75 pathotypes largely align to races 1 and 2, respectively, this is not true for all strains

76 and the systems are generally not used interchangeably (Hu et al. 2015). Host-specific

77 pathology groups also include "eggplant pathotype", "tomato pathotype", "mint

78 pathotype" and "sweet pepper pathotype" (Dung et al. 2012; Komatsu et al. 2001;

79 Papaioannou et al. 2013b). While these classifications are generally understood in

80 studies that focus on strains infecting a single host type, complexity arises when

81 investigating *Verticillium* strains independently of the plant host they infect.

82 Currently, there is only one system that classifies all *V. dahliae* strains into groups,

83 known as Vegetative Compatibility Groups (VCGs).

84

85 **Vegetative Compatibility Groups (VCGs) in *Verticillium dahliae***

86 VCGs are determined by strain interaction and describe the formation of prototrophic

87 heterokaryons, a fusion of two genetically distinct cells that occurs when two hyphal

88 cells meet (Puhalla and Mayfield 1974). While not molecularly characterised in *V.*

89 *dahliae*, related fungal models have shown that two sets of gene loci, known as *vic*

90 (vegetative incompatibility) and *het* (heterokaryon incompatibility) govern the

91 process. For isolates to form a heterokaryon, the alleles at the *het* or *vic* loci must be

92 identical (Jiménez-Gasco et al. 2013). In practice, the VCG determination process

93 requires that *V. dahliae* strains are mutated to become nitrogen non-utilizing "*nit*

94 mutants". Mutants strains, one or two with known and the other with an unknown

95 VCG, are placed on opposite sides of a minimal media agar plate and monitored for

96 signs of prototrophic growth. If the mutant isolates are able to form heterokaryons,

97 which allow growth on minimal media, the unknown isolate is assigned the same

98 VCG as the known isolate (Joaquim and Rowe 1990). This method has led to the

99 identification of five VCGs in *V. dahliae*, namely, VCG1 2, 3, 4 and 6, with VCG1

100   and VCG2 further characterised into A and B subgroups, and VCG4 into A, B and

101   AB (Papaioannou and Typas 2015; Strausbaugh 1993).

102

103   Vegetative Compatibility Groups have been used to track the evolution and

104   movement of *V. dahliae*. Several groups found that isolates within VCGs are

105   phylogenetically similar (Collado-Romero et al. 2006) or fit a clonal reproductive

106   model (Dung et al. 2013; Milgroom et al. 2014). Others argued that although isolates

107   of the same VCG may be genetically similar, they are often phylogenetically distant,

108   with members of different subgroups being more closely related (Jiménez-Gasco et al.

109   2013). In most instances VCGs are monophyletic, with some exceptions such as

110   VCG2B (Collado-Romero et al. 2008). Following these studies, the origin of the *V.*

111   *dahliae* species has been speculated to be in Europe (Short et al. 2015), while the

112   virulent VCG1A has been traced back to North America (Milgroom et al. 2016).

113

114   Different plant hosts are often associated with different *V. dahliae* VCGs. VCG2A is

115   known to be highly pathogenic to tomato (Tsror et al. 2001), VCG2B is highly

116   aggressive in mint (Dung et al. 2013), VCG4A is highly pathogenic to potato (El-

117   Bebany et al. 2013), and VCG1A is virulent in olives (Dervis et al. 2007). In cotton, it

118   has generally been reported that VCG1A causes significant damage while VCG2A

119   and VCG4B are less virulent, although there have been some reports of VCG2B

120   causing damage (Dervis and Bicici 2005; Dervis et al. 2008; Elena 1999; Jiménez-

121   Gasco et al. 2013; Korolev et al. 2001).

122

123   While VCGs are currently the most widespread method to describe *V. dahliae*

124   populations, the genetics behind VCGs in *V. dahliae* are not well understood. In their

attempt to create a high-throughput VCG screening method, Papaioannou and Typas

(2015) also sought to understand the genetic relationship between the two, "strong"

and "weak", heterokaryon reactions observed. These authors found that weak

interactions tend to be unstable, but there is still a transfer of genetic material,

suggesting that they may be vegetatively compatible. Although many other studies

acknowledge that weak reactions occur, most regard only strong interactions as

compatible (Strausbaugh 1993). This could impact the reliability of results examining

relatedness amongst VCGs and highlights a need for a narrower classification system

that does not suffer from these issues. Additionally, as the VCG determination process

is labour intensive and time-consuming, several groups have attempted to develop

alternative methods (Collado-Romero et al. 2009; El-Bebany et al. 2013; Papaioannou

et al. 2013a). However, currently, no molecular method is as reliable as the traditional

method.

Verticillium dahliae in Australian cotton

Since 1983, *Verticillium*-infected plant samples have been collected and *V. dahliae*

isolates maintained and stored in the culture collection of the NSW Department of

Primary Industries (Kirkby et al. 2013). The average incidence of Verticillium wilt

has generally been low throughout NSW. The incidence rose from 5.5% in 2013/2014

to 7.1% in 2014/2015 and 6.3% in the 2015/2016 season (Chapman et al. 2016).

Disease symptoms are becoming more severe in some patches of Verticillium wilt,

with yield reductions reported to be greater than 6 bales/ha. There are concerns that

this increase in severity is related to the ND VCG2A strain reported in 2014 (Dadd-

Daigle et al. 2020; Smith et al. 2014).

150   It was previously thought that only one VCG type, ND VCG4B, was present in

151   Australia, but in 2014, ND VCG2A was identified (Smith et al. 2014). Following the

152   discovery of ND VCG2A, analysis of *V. dahliae* historical samples taken from the

153   NSW Department of Primary Industries culture collection revealed the presence of the

154   D VCG1A (Chapman et al. 2016). The D VCG1A has been the cause of severe

155   disease and crop loss overseas (Jiménez-Díaz et al. 2006). However, despite the

156   presence of VCG1A in the historical samples, typical VCG1A disease presentation,

157   including the typical crop losses and complete defoliation of infected plants, has not

158   been a widespread observation in Australia. It is not clear what is causing the

159   disparity between the severity of D VCG1A and ND VCG2A disease in Australia and

160   overseas. It is possible, given that VCG2A has been shown to infect weeds commonly

161   found on cotton fields (Yildiz et al. 2009), that VCG2A *V. dahliae* has simply become

162   the most prevalent strain on Australian cotton fields, amplified by the polyetic nature

163   of the pathogen, and has acquired the ability to defoliate cotton plants. However,

164   further analysis of the relationship of genetics to pathogenicity and disease severity in

165   Australian *V. dahliae* VCGs is required.

166

167   **Insights from *Verticillium dahliae* genome sequencing**

168   In 2011 the *V. dahliae* VdLs.17 and *V. albo-atrum* genomes were sequenced using the

169   whole genome shotgun approach via Sanger sequencing (Klosterman et al. 2011).

170   Although the two ~ 33 Mb genomes were highly similar, there were four 300 kb

171   regions in *V. dahliae* which had no synteny with *V. albo-atrum*. These regions were

172   denoted "Lineage Specific" (LS) regions. The LS regions were found to be highly

173   repetitive and represented over 50% of all identifiable transposable elements

174   contained in *V. dahliae.* Faino et al. (2015) used PacBio long read sequences to create

175 a "gapless" genome and have since suggested that there are problems with the initial

176 *V. dahliae* VdLs.17 sequence. These authors argue that their method of genome

177 assembly helps to prevent problems associated with repetitive regions that cause

178 issues when assembling shorter contigs. Using PacBio sequencing, the VdLs.17

179 genome was re-assembled. The newly constructed genome indicates that 12% is

180 composed of repetitive regions, four times higher than was previously thought.

181

182 With the availability of a *V. dahliae* reference genome, there is an increasing

183 understanding of what makes *V. dahliae* such an adaptable pathogen with a broad host

184 range. There are suggestions that transposons could be a major reason for the genomic

185 diversity observed and that they contribute to the *V. dahliae* "plastic genome" driving

186 adaption to new plant hosts (Amyotte et al. 2012; Faino et al. 2016). This is supported

187 by de Jonge et al. (2013) who compared the VdLs.17 reference strain with 10 *V.*

188 *dahliae* genomes taken from geographically separate regions and hosts. The study

189 revealed that despite the genomes being highly similar, chromosome rearrangements

190 had occurred between all strains. Using RNA-seq data and deletion studies, they

191 showed that effector genes present in the LS regions were important to the

192 development of disease (de Jonge et al. 2013; de Jonge et al. 2012), suggesting that

193 chromosome rearrangements and these LS regions could contribute to *V. dahliae*'s

194 adaptation to new hosts. Jin et al. (2017) explored the organism's use of alternative

195 splicing and developed their own algorithms, alongside previously available software,

196 to analyse *V. dahliae* cDNA sequences for common splicing events. They found that

197 *V. dahliae* has one of the most sophisticated splicing systems in eukaryotes, outside of

198 animals, and believe that this alternative splicing could explain some of *V. dahliae's*

199 plasticity.

200

201 There are an increasing number of studies suggesting that horizontal gene transfer

202 plays an important role in *V. dahliae's* success as a pathogen. An analysis of *V.*

203 *dahliae* isolated from cotton in China, revealed the presence of a virulence gene

204 believed to have originated in *Fusarium oxysporum*, a related fungal pathogen often

205 found infecting cotton on the same farm (Chen et al. 2017). Their deletion

206 experiments found that removal of this gene affected the ability of the *V. dahliae*

207 strain to infect cotton, but not lettuce or tomato, highlighting it's ability to acquire

208 new virulence genes as it expands to different hosts. There has also been evidence of

209 *V. dahliae* acquiring genes from the host plant and from bacteria (de Jonge et al.

210 2012; van Kooten et al. 2019). These studies used phylogenetic analysis to look for

211 candidate genes that are found outside the *Verticillium* spp. They found numerous

212 candidate genes of bacterial and plant origin, many of which could potentially aid *V.*

213 *dahliae* in getting past the host plant's defences.

214

215 **Management strategies for the control of Verticillium wilt**

216 The nature of *V. dahliae* infection makes elimination of the pathogen difficult,

217 however, multiple management strategies have been applied over the years. As the *V.*

218 *dahliae* life cycle is dependent on microsclerotia present in crop soil, currently the

219 two main strategies target either the soil itself, for example by soil fumigation, or the

220 plants through development of resistant varieties (Short et al. 2015). Soil fumigation

221 aims to eliminate microsclerotia in crop soil. Traditionally, methyl bromide was used

222 to control pathogen populations, but was classified as a Class 1 stratospheric, ozone-

223 depleting substance and international regulations dictated by the Montreal Protocol

224 now restrict the use of this chemical (Martin 2003). Multiple studies have explored

225   alternatives, including green manures, anaerobic soil disinfection and anaerobic

226   digestion. Green manure is a method utilising volatile components from plant waste to

227   reduce the number of microsclerotia (Yohalem and Passey 2011). Anaerobic soil

228   disinfection uses microbial activity from agricultural or horticultural waste products,

229   combined with mulched plastics, to deplete available oxygen in soil, creating

230   anaerobic conditions to prevent fungal growth (Goud et al. 2004). Anaerobic

231   digestion uses liquid digestate, a by-product from biogas production, as a bio-fertiliser

232   to control microsclerotia levels (Wei et al. 2016). However, the suitability of these

233   methods in commercial processes is still questionable. While, green manures and

234   anaerobic digestion are still relatively new and understudied, the well-studied

235   variants, such as *Brassica sp.*, are deemed insufficient (Neubauer et al. 2014) and

236   anaerobic soil disinfection is not currently economically viable (Wei et al. 2016).

237

238   Production of resistant cotton varieties is a key strategy in the prevention of

239   Verticillium wilt. The development of resistant varieties in Australia has been

240   ongoing for more than 30 years, with the release of Sicala V-1 in 1990, and Sicala V-

241   2 in 1994 (Liu et al. 2013). Despite successes with Sicala V-2 and subsequent

242   varieties derived from it, the incidence of Verticillium wilt has continued to rise in

243   recent years (Kirkby et al. 2013). This could be linked to the temperature tolerance, as

244   currently the *V. dahliae* resistance in available cotton varieties breaks down when

245   temperatures drop below 22˚C (Quinn et al. 2018). Although there is ongoing research

246   into Verticillium resistance (Li et al. 2018; Li et al. 2019; Zhang et al. 2018), the

247   development of new cotton varieties that provide adequate yield is slow, and the

248   current varieties do not provide a substantial increase in resistance (Dadd-Daigle et al.

249   2020). Also, without a rapid diagnostic system that classifies *V. dahliae* into groups

250 meaningful for Australian cotton, it is difficult to develop targeted and effective

251 strategies.

252

253 Currently, crop rotation is one of the methods used to help manage Verticillium wilt

254 on cotton farms in Australia. Crop rotation is the practice of varying the successive

255 crops in a particular field to assist in the control of disease and weed management.

256 Each crop varies in its susceptibility to certain pathogens. The success of crop rotation

257 relies on initial inoculum levels in the soil, the number of rotations with non-host

258 crops and the wetting and drying cycles that assist in the breakdown of inoculum in

259 the soil (Wheeler et al. 2019). For example, most cotton farmers rotate with barley or

260 sorghum as they are not listed as host crops for *V. dahliae*. While commodity prices

261 are the short-term driving force, farms with high disease levels are looking at rotation

262 to ensure cotton remains sustainable in the long term (K. Kirby, personal

263 communication, September 2016). The current recommendations to growers are long

264 rotations with moderate irrigation to reduce overall pathogen levels and prevent

265 widespread movement of the microsclerotia (Holman et al. 2016; Scheikowski et al.

266 2019).

267

268 The development of real-time PCR protocols to determine microsclerotial load from

269 soil samples should assist with managing crop rotation practices (Banno et al. 2011;

270 Gharbi et al. 2016). Removal of the rotational crop plant debris has also been shown

271 to reduce the number of microsclerotia in the soil, but does sacrifice soil health

272 (Chawla et al. 2012). However, the known host range of *V. dahliae,* both symptomatic

273 and asymptomatic, is expanding as the pathogen comes into contact with new plant

274 species. There have been instances where a symptomless host has exhibited extensive

275   vascular colonization and so contributes to the microsclerotial load despite the lack of

276   symptoms (Wheeler and Johnson 2016). This makes selection of a suitable rotation

277   crop more complex and highlights the need for a better understanding of the genomics

278   of *V. dahliae*. In some instances, after multiple years of crop rotation followed by a

279   cotton crop, the incidence of Verticillium wilt rises to match those found on farms

280   that have had continuous cotton growth (Wheeler et al. 2019).

281

282   Given that the current attempts to mitigate Verticillium wilt on cotton farms is

283   becoming increasingly ineffective, new strategies need to be explored for use in

284   Australia. One area that hasn't been well examined in Australian cotton is the use of

285   endophytes as a biological control. The idea behind this strategy is to pre-infect the

286   plants with a microbe that will inhabit the same niche as *V. dahliae,* preventing

287   infection by the pathogen. This has been explored with both bacterial and fungal

288   endophytes (Li et al. 2012). Vagelas and Leontopoulos (2015) used the less virulent

289   *V. nigrescens* to take up the niche usually filled by *V. dahliae*, preventing the

290   infiltration of conidia by the more virulent species, while Yuan et al. (2017) looked at

291   using unrelated fungal species as seed treatments. Although both studies saw a

292   reduction in *V. dahliae* caused Verticillium wilt, the use of *Penicillium*

293   *simplicissimum* and *Leptosphaeria* sp. also saw an increase in cotton seed production

294   as the number of cotton bolls increased (Yuan et al. 2017). As endophytes have been

295   shown to be beneficial in other areas of crop sustainability, such as protection from

296   insect pests and abiotic stress (Lugtenberg et al. 2016), this area could be hugely

297   beneficial to the Australian cotton industry which is often heavily impacted by water

298   availability.

299

300

Improving future understanding of the Verticillium wilt problem in Australia

The nature of Verticillium wilt in Australian cotton is an interesting problem. Large patches of severe Verticillium wilt have been found to be caused by the ND VCG2A (Dadd-Daigle et al. 2020; Jensen and Redfern 2017), which is contrary to reporting on other cotton farms around the world. This could be dependent on factors other than the isolate, such as the Australian environment, or the farming conditions, and is an area that warrants further exploration. While studies to further examine the Australian *V. dahliae* population are currently being conducted, no study to date has indicated what causes the difference in disease potential between Australian and international cotton crops. In addition, the genetic analyses are revealing an increasing number of methods by which *V. dahliae* can adapt. It is no wonder that strategies that work some of the time, such as crop rotation or the use of resistant varieties, are becoming less effective (Kirkby et al. 2013; Wheeler et al. 2019).

There is an increasing need for new mitigation strategies or the development of new cotton varieties resistant to Verticillium wilt. However, in order to create and implement these strategies, the current classification system needs to be improved to better represent the *V. dahliae* present on Australian cotton farms. Characterisation of the genetics controlling virulence has improved the classification of VCGs within related *Fusarium* sp. by increasing molecular clarity between isolates and developing new classification systems (Carvalhais et al. 2019). Although there is still some debate surrounding the best tools to diagnostically identify virulent *Fusarium oxysporum* strains (Magdama et al. 2019), a similar molecular understanding could improve the VCG classification system within *V. dahliae* by establishing narrower

325   classifications or by implementing a new system based on virulence genes unrelated

326   to VCGs.

327

328   Future research to improve Verticillium wilt on Australian cotton farms needs to

329   largely build on current research efforts. An improved system for quantification of

330   inoculum in soils and a better understanding of the inoculum to disease thresholds for

331   different VCGs can clarify the effectiveness of crop rotation (Wheeler et al. 2019).

332   While an improved understanding of the environmental conditions and how current

333   farming methods impact Verticillium wilt on Australian farms can help inform best

334   farming practices (Kirkby et al. 2013). It is only through continued development of

335   new tools and a better understanding of *V. dahliae* genetics to rapidly analyse

336   Verticillium wilt samples that growers may be able to stay ahead of the pathogen,

337   preventing a situation where yield loss due to disease outweighs potential yield.

338

344

345   **References**

346   Amyotte SG, Tan X, Pennerman K, del Mar Jimenez-Gasco M, Klosterman SJ, Ma L-
347   J, Dobinson KF, Veronese P (2012) Transposable elements in
348   phytopathogenic *Verticillium spp.*: insights into genome evolution and
349   inter- and intra-specific diversification BMC Genomics 13:1-20
350   doi:10.1186/1471-2164-13-314
351   Banno S, Saito H, Sakai H, Urushibara T, Ikeda K, Kabe T, Kemmochi I, Fujimura M
352   (2011) Quantitative nested real-time PCR detection of *Verticillium*

353 *longisporum* and *V. dahliae* in the soil of cabbage fields Journal of General
354   Plant Pathology 77:282-291 doi:10.1007/s10327-011-0335-9
355 Bhat RG, Subbarao KV (1999) Host Range Specificity in *Verticillium dahliae*
356   Phytopathology 89:1218-1225 doi:10.1094/PHYTO.1999.89.12.1218
357 Carvalhais LC, Henderson J, Rincon-Florez VA, O'Dwyer C, Czislowski E, Aitken
358   EAB, Drenth A (2019) Molecular Diagnostics of Banana Fusarium Wilt
359   Targeting Secreted-in-Xylem Genes Frontiers in Plant Science 10
360   doi:10.3389/fpls.2019.00547
361 Chapman TA, Chambers GA, Kirkby K, Jiménez-Díaz RM (2016) First report of the
362   presence of *Verticillium dahliae* VCG1A in Australia Australasian Plant
363   Disease Notes 11:1-4 doi:10.1007/s13314-016-0197-2
364 Chawla S, Woodward JE, Wheeler TA (2012) Influence of *Verticillium dahliae*
365   Infested Peanut Residue on Wilt Development in Subsequent Cotton
366   International Journal of Agronomy 2012:1-5 doi:10.1155/2012/212075
367 Chen JY, Liu C, Gui YJ, Si KW, Zhang DD, Wang J, Short Dylan PG, Huang JQ, Li NY,
368   Liang Y, Zhang WQ, Yang L, Ma XF, Li TG, Zhou L, Wang BL, Bao YM,
369   Subbarao Krishna V, Zhang GY, Dai XF (2017) Comparative genomics
370   reveals cotton-specific virulence factors in flexible genomic regions in
371   *Verticillium dahliae* and evidence of horizontal gene transfer from
372   *Fusarium* New Phytologist 217:756-770 doi:10.1111/nph.14861
373 Collado-Romero M, Berbegal M, Jiménez-Díaz RM, Armengol J, Mercado-Blanco J
374   (2009) A PCR-based 'molecular tool box' for in planta differential
375   detection of *Verticillium dahliae* vegetative compatibility groups infecting
376   artichoke Plant Pathology 58:515-526 doi:10.1111/j.1365-
377   3059.2008.01981.x
378 Collado-Romero M, Mercado-Blanco J, Olivares-García C, Jiménez-Díaz RM (2008)
379   Phylogenetic Analysis of Verticillium dahliae Vegetative Compatibility
380   Groups Phytopathology® 98:1019-1028 doi:10.1094/PHYTO-98-9-1019
381 Collado-Romero M, Mercado-Blanco J, Olivares-García C, Valverde-Corredor A,
382   Jiménez-Díaz RM (2006) Molecular Variability Within and Among
383   *Verticillium dahliae* Vegetative Compatibility Groups Determined by
384   Fluorescent Amplified Fragment Length Polymorphism and Polymerase
385   Chain Reaction Markers Phytopathology 96:485-495
386   doi:10.1094/PHYTO-96-0485
387 Constable G (2004) Research's contribution to the evolution of the Australian
388   cotton industry Proceedings of the 4th International Crop Science
389   Congress Brisbane, Australia
390 Daayf F (2015) Verticillium wilts in crop plants: Pathogen invasion and host
391   defence responses Can J Plant Pathol 37:8-20
392   doi:10.1080/07060661.2014.989908
393 Daayf F, Nicole M, Geiger J-P (1995) Differentiation of *Verticillium dahliae*
394   populations on the basis of vegetative compatibility and pathogenicity on
395   cotton European Journal of Plant Pathology 101:69-79
396   doi:10.1007/BF01876095
397 Dadd-Daigle P, Kirkby K, Collins D, Cuddy W, Lonergan P, Roser S, Chowdhury
398   PR, Labbate M, Chapman TA (2020) Virulence not linked with vegetative
399   compatibility groups in Australian cotton *Verticillium dahliae* isolates
400   Australian Journal of Crop Science 14:633-640

401 Davis JR, Pavek JJ, Corsini DL, Sorensen LH, Schneider AT, Everson DO,
402   Westermann DT, Huisman OC (1994) Influence of continuous cropping of
403   several potato clones on the epidemiology of Verticillium wilt of potato
404   Phytopathology 84:207-214 doi:10.1094/Phyto-84-207
405 de Jonge R, Bolton MD, Kombrink A, van den Berg GCM, Yadeta KA, Thomma
406   BPHJ (2013) Extensive chromosomal reshuffling drives evolution of
407   virulence in an asexual pathogen Genome Research 23:1271-1282
408   doi:10.1101/gr.152660.112
409 de Jonge R, Peter van Esse H, Maruthachalam K, Bolton MD, Santhanam P, Saber
410   MK, Zhang Z, Usami T, Lievens B, Subbarao KV, Thomma BPHJ (2012)
411   Tomato immune receptor *Ve1* recognizes effector of multiple fungal
412   pathogens uncovered by genome and RNA sequencing Proceedings of the
413   National Academy of Sciences 109:5110-5115
414   doi:10.1073/pnas.1119623109
415 Dervis S, Bicici M (2005) Vegetative compatibility groups in *Verticillium dahliae*
416   isolates from cotton in Turkey Phytoparasitica 33:157-168
417   doi:10.1007/BF03029975
418 Dervis S, Erten L, Soylu S, Tok FM, Kurt S, Yıldız M, Soylu EM (2007) Vegetative
419   compatibility groups in *Verticillium dahliae* isolates from olive in western
420   Turkey European Journal of Plant Pathology 119:437-447
421   doi:10.1007/s10658-007-9183-z
422 Dervis S, Kurt S, Soylu S, Erten L, Mine Soylu E, Yıldız M, Tok FM (2008)
423   Vegetative compatibility groups of *Verticillium dahliae* from cotton in the
424   southeastern anatolia region of Turkey Phytoparasitica 36:74-83
425   doi:10.1007/BF02980750
426 Dung JKS, Peever TL, Johnson DA (2012) Verticillium dahliae Populations from
427   Mint and Potato Are Genetically Divergent with Predominant Haplotypes
428   Phytopathology® 103:445-459 doi:10.1094/PHYTO-06-12-0133-R
429 Dung JKS, Peever TL, Johnson DA (2013) *Verticillium dahliae* Populations from
430   Mint and Potato Are Genetically Divergent with Predominant Haplotypes
431   Phytopathology 103:445-459 doi:10.1094/PHYTO-06-12-0133-R
432 El-Bebany AF, Alkher H, Adam LR, Daayf F (2013) Vegetative compatibility of
433   *Verticillium dahliae* isolates from potato and sunflower using nitrate non-
434   utilizing (*nit*) mutants and PCR-based approaches Can J Plant Pathol 35:1-
435   9 doi:10.1080/07060661.2012.702128
436 Elena K (1999) Genetic Relationships Among *Verticillium dahliae* Isolates from
437   Cotton in Greece Based on Vegetative Compatibility European Journal of
438   Plant Pathology 105:609-616 doi:10.1023/A:1008771112068
439 Faino L, Seidl MF, Datema E, van den Berg GC, Janssen A, Wittenberg AH,
440   Thomma BP (2015) Single-Molecule Real-Time Sequencing Combined
441   with Optical Mapping Yields Completely Finished Fungal Genome MBio
442   6:e00936-00915 doi:10.1128/mBio.00936-15
443 Faino L, Seidl MF, Shi-Kunne X, Pauper M, van den Berg GCM, Wittenberg AHJ,
444   Thomma BPHJ (2016) Transposons passively and actively contribute to
445   evolution of the two-speed genome of a fungal pathogen Genome
446   Research 26:1091-1100 doi:10.1101/gr.204974.116
447 Gharbi Y, Barkallah M, Bouazizi E, Cheffi M, Krid S, Triki MA, Gdoura R (2016)
448   Development and validation of a new real-time assay for the
449   quantification of *Verticillium dahliae* in the soil: a comparison with

450   conventional soil plating Mycological Progress 15:1-13
451   doi:10.1007/s11557-016-1196-6
452 Goud J-KC, Termorshuizen AJ, Blok WJ, van Bruggen AHC (2004) Long-Term
453   Effect of Biological Soil Disinfestation on Verticillium Wilt Plant Disease
454   88:688-694 doi:10.1094/PDIS.2004.88.7.688
455 Hamilton D (2016) Reaping the benefits of innovation - the Australian cotton
456   story Agricultural Science 28:18-24
457 Holman S, Kirkby K, Smith L, Hartnett H (2016) Vert update: The latest in vert
458   research CottonInfo fact sheet September 2016
459 Hu X-P, Gurung S, Short DPG, Sandoya GV, Shang W-J, Hayes RJ, Davis RM,
460   Subbarao KV (2015) Nondefoliating and Defoliating Strains from Cotton
461   Correlate with Races 1 and 2 of *Verticillium dahliae* Plant Disease
462   99:1713-1720 doi:10.1094/PDIS-03-15-0261-RE
463 Inderbitzin P, Bostock RM, Davis RM, Usami T, Platt HW, Subbarao KV (2011)
464   Phylogenetics and Taxonomy of the Fungal Vascular Wilt Pathogen
465   *Verticillium*, with the Descriptions of Five New Species PLoS ONE
466   6:e28341 doi:10.1371/journal.pone.0028341
467 Jensen M, Redfern R (2017) Breaking the Verticillium cycle vol Winter 2017.
468   Cotton Research and Development Corporation,
469 Jiménez-Díaz RM, Mercado-Blanco J, Olivares-García C, Collado-Romero M,
470   Bejarano-Alcázar J, Rodríguez-Jurado D, Giménez-Jaime A, García-Jiménez
471   J, Armengol J (2006) Genetic and Virulence Diversity in *Verticillium*
472   *dahliae* Populations Infecting Artichoke in Eastern-Central Spain
473   Phytopathology 96:288-298 doi:10.1094/PHYTO-96-0288
474 Jiménez-Gasco MdM, Malcolm GM, Berbegal M, Armengol J, Jiménez-Díaz RM
475   (2013) Complex Molecular Relationship Between Vegetative
476   Compatibility Groups (VCGs) in *Verticillium dahliae*: VCGs Do Not Always
477   Align with Clonal Lineages Phytopathology 104:650-659
478   doi:10.1094/PHYTO-07-13-0180-R
479 Jin L, Li G, Yu D, Huang W, Cheng C, Liao S, Wu Q, Zhang Y (2017) Transcriptome
480   analysis reveals the complexity of alternative splicing regulation in the
481   fungus *Verticillium dahliae* BMC Genomics 18:130 doi:10.1186/s12864-
482   017-3507-y
483 Joaquim TR, Rowe RC (1990) Reassessment of Vegetative Compatibility
484   relationships among strains of *Verticillium dahliae* using nitrate-
485   nonutilizing mutants Phytopathology 80:1160-1166 doi: 10.1094/Phyto-
486   80-1160
487 Kirkby KA, Lonergan PA, Allen SJ (2013) Three decades of cotton disease surveys
488   in NSW, Australia Crop and Pasture Science 64:774-779
489   doi:10.1071/CP13143
490 Klimes A, Dobinson KF, Thomma BPHJ, Klosterman SJ (2015) Genomics Spurs
491   Rapid Advances in Our Understanding of the Biology of Vascular Wilt
492   Pathogens in the Genus *Verticillium* Annual Review of Phytopathology
493   53:181-198 doi:10.1146/annurev-phyto-080614-120224
494 Klosterman SJ, Atallah ZK, Vallad GE, Subbarao KV (2009) Diversity,
495   pathogenicity, and management of *Verticillium* species Annu Rev
496   Phytopathol 47:39-62 doi:10.1146/annurev-phyto-080508-081748
497 Klosterman SJ, Subbarao KV, Kang S, Veronese P, Gold SE, Thomma BPHJ, Chen Z,
498   Henrissat B, Lee Y-H, Park J, Garcia-Pedrajas MD, Barbara DJ, Anchieta A,

499   de Jonge R, Santhanam P, Maruthachalam K, Atallah Z, Amyotte SG, Paz Z,
500   Inderbitzin P, Hayes RJ, Heiman DI, Young S, Zeng Q, Engels R, Galagan J,
501   Cuomo CA, Dobinson KF, Ma L-J (2011) Comparative Genomics Yields
502   Insights into Niche Adaptation of Plant Vascular Wilt Pathogens PLoS
503   pathogens 7:e1002137 doi:10.1371/journal.ppat.1002137

504 Komatsu T, Sumino A, Kageyama K (2001) Characterization of *Verticillium*
505   *dahliae* Isolates from Potato on Hokkaido by Random Amplified
506   Polymorphic DNA (RAPD) and REP-PCR Analyses Journal of General Plant
507   Pathology 67:23-27 doi:10.1007/PL00012982

508 Korolev N, Pérez-Artés E, Bejarano-Alcázar J, Rodríguez-Jurado D, Katan J, Katan
509   T, Jiménez-Díaz RM (2001) Comparative Study of Genetic Diversity and
510   Pathogenicity Among Populations of *Verticillium Dahliae* from Cotton in
511   Spain and Israel European Journal of Plant Pathology 107:443-456
512   doi:10.1023/A:1011212426447

513 Li CH, Shi L, Han Q, Hu HL, Zhao MW, Tang CM, Li SP (2012) Biocontrol of
514   verticillium wilt and colonization of cotton plants by an endophytic
515   bacterial isolate J Appl Microbiol 113:641-651 doi:10.1111/j.1365-
516   2672.2012.05371.x

517 Li NY, Ma XF, Short DPG, Li TG, Zhou L, Gui YJ, Kong ZQ, Zhang DD, Zhang WQ, Li
518   JJ, Subbarao KV, Chen JY, Dai XF (2018) The island cotton NBS-LRR gene
519   GbaNA1 confers resistance to the non-race 1 *Verticillium dahliae* isolate
520   Vd991 Molecular Plant Pathology 19:1466-1479 doi:10.1111/mpp.12630

521 Li ZK, Chen B, Li XX, Wang JP, Zhang Y, Wang XF, Yan YY, Ke HF, Yang J, Wu JH,
522   Wang GN, Zhang GY, Wu LQ, Wang XY, Ma ZY (2019) A newly identified
523   cluster of glutathione S-transferase genes provides Verticillium wilt
524   resistance in cotton Plant J 98:213-227 doi:10.1111/tpj.14206

525 Liu S, Reid P, Stiller W, Constable G (2013) The contribution of new varieties to
526   cotton yield improvement. CSIRO Plant Industry, Narrabri

527 Lugtenberg BJ, Caradus JR, Johnson LJ (2016) Fungal endophytes for sustainable
528   crop production FEMS Microbiol Ecol 92 doi:10.1093/femsec/fiw194

529 Magdama F, Monserrate-Maggi L, Serrano L, Sosa D, Geiser DM, Jiménez-Gasco
530   MdM (2019) Comparative analysis uncovers the limitations of current
531   molecular detection methods for Fusarium oxysporum f. sp. cubense race
532   4 strains PLOS ONE 14:e0222727 doi:10.1371/journal.pone.0222727

533 Martin FN (2003) Development of alternative strategies for management of
534   soilborne pathogens currently controlled with methyl bromide Annu Rev
535   Phytopathol 41:325-350 doi:10.1146/annurev.phyto.41.052002.095514

536 Maruthachalam K, Atallah ZK, Vallad GE, Klosterman SJ, Hayes RJ, Davis RM,
537   Subbarao KV (2010) Molecular Variation Among Isolates of *Verticillium*
538   *dahliae* and Polymerase Chain Reaction-Based Differentiation of Races
539   Phytopathology 100:1222-1230 doi:10.1094/PHYTO-04-10-0122

540 Milgroom MG, del Mar Jiménez-Gasco M, Olivares-García C, Jiménez-Díaz RM
541   (2016) Clonal Expansion and Migration of a Highly Virulent, Defoliating
542   Lineage of *Verticillium dahliae* Phytopathology 106:1038-1046
543   doi:10.1094/PHYTO-11-15-0300-R

544 Milgroom MG, Jiménez-Gasco MdM, Olivares García C, Drott MT, Jiménez-Díaz RM
545   (2014) Recombination between Clonal Lineages of the Asexual Fungus
546   *Verticillium dahliae* Detected by Genotyping by Sequencing PLoS ONE
547   9:e106740 doi:10.1371/journal.pone.0106740

548 Neubauer C, Heitmann B, Müller C (2014) Biofumigation potential of
549   Brassicaceae cultivars to *Verticillium dahliae* European Journal of Plant
550   Pathology 140:341-352 doi:10.1007/s10658-014-0467-9
551 Papaioannou IA, Dimopoulou CD, Typas MA (2013a) Structural and phylogenetic
552   analysis of the rDNA intergenic spacer region of *Verticillium dahliae* FEMS
553   microbiology letters 347:23-32 doi:10.1111/1574-6968.12215
554 Papaioannou IA, Ligoxigakis EK, Vakalounakis DJ, Markakis EA, Typas MA
555   (2013b) Phytopathogenic, morphological, genetic and molecular
556   characterization of a *Verticillium dahliae* population from Crete, Greece
557   European Journal of Plant Pathology 136:577-596 doi:10.1007/s10658-
558   013-0189-4
559 Papaioannou IA, Typas MA (2015) High-Throughput Assessment and Genetic
560   Investigation of Vegetative Compatibility in *Verticillium dahliae* Journal of
561   Phytopathology 163:475-485 doi:10.1111/jph.12345
562 Puhalla JE, Mayfield JE (1974) The Mechanism of Heterokaryotic Growth in
563   *Verticillium dahliae* Genetics 76:411-422
564 Quinn J, Eveleigh R, Ford B, Millyard J, Teague C, Barry C, Lee S, Devlin A,
565   McDonald C (2018) Verticillium Wilt. Facts on Friday vol October. Cotton
566   Seed Distributors, Wee Waa, Australia
567 Scheikowski L, Smith L, Vadakattu G, Shuey T, Kafle D (2019) Longer rotations
568   are required to reduce Verticillium where disease levels are high vol
569   December18-January19.
570 Short DPG, Sandoya G, Vallad GE, Koike ST, Xiao C-L, Wu B-M, Gurung S, Hayes RJ,
571   Subbarao KV (2015) Dynamics of *Verticillium* Species Microsclerotia in
572   Field Soils in Response to Fumigation, Cropping Patterns, and Flooding
573   Phytopathology 105:638-645 doi:10.1094/PHYTO-09-14-0259-R
574 Smith L, Scheikowski L, Bauer B, Lehane J, Allen S (2014) Detection of New
575   Pathogens in Australian Cotton Cotton Research and Development
576   Corporationon behalf of the 17th Australian Cotton Conference
577 Strausbaugh CA (1993) Assessment of Vegetative Compatibility and Virulence of
578   Verticillium dahliae Isolates from Idaho Potatoes and Tester Strains
579   Phytopathology 83:1253-1258
580 Tsror L, Hazanovsky M, Mordechi-Lebiush S, Sivan S (2001) Aggressiveness of
581   *Verticillium dahliae* isolates from different vegetative compatibility
582   groups to potato and tomato Plant Pathology 50:477-482
583   doi:10.1046/j.1365-3059.2001.00587.x
584 Vagelas I, Leontopoulos S (2015) Cross-protection of cotton against Verticillium
585   wilt by *Verticillium nigrescens* Emirates Journal of Food and Agriculture
586   27:687-691 doi:10.9755/ejfa.2015-04-047
587 van Kooten M, Shi-Kunne X, Thomma BPHJ, Depotter JRL, Seidl MF (2019) The
588   Genome of the Fungal Pathogen *Verticillium dahliae* Reveals Extensive
589   Bacterial to Fungal Gene Transfer Genome Biology and Evolution 11:855-
590   868 doi:10.1093/gbe/evz040
591 Wei F, Passey T, Xu X (2016) Effects of individual and combined use of bio-
592   fumigation-derived products on the viability of *Verticillium dahliae*
593   microsclerotia in soil Crop Protection 79:170-176
594   doi:http://dx.doi.org/10.1016/j.cropro.2015.09.008

595 Wheeler DL, Johnson DA (2016) Verticillium dahliae Infects, Alters Plant
596   Biomass, and Produces Inoculum on Rotation Crops Phytopathology®
597   106:602-613 doi:10.1094/PHYTO-07-15-0174-R
598 Wheeler TA, Bordovsky JP, Keeling JW (2019) The effectiveness of crop rotation
599   on management of Verticillium wilt over time Crop Protection 121:157-
600   162 doi:https://doi.org/10.1016/j.cropro.2019.03.021
601 Wu BM, Subbarao KV (2014) A Model for Multiseasonal Spread of Verticillium
602   Wilt of Lettuce Phytopathology 104:908-917 doi:10.1094/PHYTO-12-13-
603   0333-R
604 Yildiz A, Dogan M, Boz Ö, Benlioglu S (2009) Weed hosts of Verticillium dahliae
605   in cotton fields in Turkey and characterization of V. dahliae isolates from
606   weeds Phytoparasitica 37:171-178 doi:10.1007/s12600-009-0027-6
607 Yohalem D, Passey T (2011) Amendment of soils with fresh and post-extraction
608   lavender (*Lavandula angustifolia*) and lavandin (*Lavandula × intermedia*)
609   reduce inoculum of *Verticillium dahliae* and inhibit wilt in strawberry
610   Applied Soil Ecology 49:187-196
611   doi:http://dx.doi.org/10.1016/j.apsoil.2011.05.006
612 Yuan Y, Feng H, Wang L, Li Z, Shi Y, Zhao L, Feng Z, Zhu H (2017) Potential of
613   Endophytic Fungi Isolated from Cotton Roots for Biological Control
614   against Verticillium Wilt Disease PLoS ONE 12:e0170557
615   doi:10.1371/journal.pone.0170557
616 Zhang L, Wang M, Li N, Wang H, Qiu P, Pei L, Xu Z, Wang T, Gao E, Liu J, Liu S, Hu
617   Q, Miao Y, Lindsey K, Tu L, Zhu L, Zhang X (2018) Long noncoding RNAs
618   involve in resistance to *Verticillium dahliae*, a fungal disease in cotton
619   Plant Biotechnology Journal 16:1172-1185 doi:10.1111/pbi.12861
620