Preliminary pathogenicity screening of *Verticillium* spp. on kiwifruit in New Zealand

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Abstract Plant-pathogenic *Verticillium* species have been present in New Zealand for many years, and have been considered minor wilt pathogens of kiwifruit. However, an outbreak of *Verticillium nonalfalfae* (previously identified as *Verticillium alboatrum*) causing wilt and death of the kiwifruit cultivar *Actinidia chinensis* var. *chinensis* 'Hort16A' in Chile has raised questions around the pathogenicity and significance of New Zealand *Verticillium* species. This study investigated the pathogenicity of New Zealand isolates of *Verticillium* spp. to 'Hort16A'. Three isolates of *Verticillium dahliae* and one of *V. alboatrum sensu stricto*, previously recovered from kiwifruit in New Zealand, were tested for pathogenicity against 'Hort16A' by artificial inoculation of young vines. Disease assessments were carried out monthly. Symptoms observed ranged from minor wilt to vine death. The *V. alboatrum* isolate appeared the most aggressive. Although there is evidence of some pathogenicity on kiwifruit within this group of isolates from *Verticillium* species in New Zealand, they appear less aggressive than those recorded in Chile. However, this cannot be confirmed without testing isolates from both countries concurrently under the same conditions.

Keywords *Verticillium dahliae*, *Verticillium alboatrum*, *Verticillium nonalfalfae*, *Actinidia chinensis*

INTRODUCTION

The genus *Verticillium* comprises a group of plant-pathogenic fungi that are of economic importance to a variety of agricultural crops in many parts of the world. The soil-borne nature of *Verticillium* spp. makes control of diseases caused by these pathogens problematic. Some *Verticillium* species, such as *V. dahliae*, produce long-lived microsclerotia and are able to survive in the soil for more than a decade, adding to the difficulty of control of verticillium wilts (Daayf 2015).

In 2005, a new disease was seen causing sudden wilt, dieback and death of gold-fleshed kiwifruit (*Actinidia chinensis* var. *chinensis* 'Hort16A') in Chile. The Chilean outbreak of verticillium wilt was severe, with losses of up to 80% being reported in some orchards (Auger et al. 2009).

The symptoms were consistent with descriptions of vascular infection by *Verticillium* spp. in other plant species (Manning unpublished data). The causal pathogen was isolated and identified as *Verticillium alboatrum* (Fullerton & Young unpublished^a).

In 2014, *V. alboatrum* was taxonomically revised and split into three species (*V. alboatrum sensu stricto*, *V. alfalfae* and *V. nonalfalfae*) (Inderbitzin et al. 2011). The isolates of *V. alboatrum* from *Actinidia* in Chile have subsequently been re-classified as *V. nonalfalfae* (Kasson et al. 2014). Molecular characterisation of *V. nonalfalfae* isolates associated with lethal wilt of *Actinidia chinensis* in Chile by Kasson et al. (2014), placed them in a distinct intra-specific group designated multilocus sequence type 2 (MLST 2). Although *V. nonalfalfae* has been

found in New Zealand, MLST 2 has not been recorded (P. Johnston, Landcare Research, pers. comm.).

Verticillium wilt of kiwifruit caused by *Verticillium dahliae* was first recorded in New Zealand on *Actinidia chinensis* var. *deliciosa* ‘Hayward’ in 1982. The primary symptom was cane dieback and associated wood discoloration (Hill 1982). At this time, it was described as a minor disease (Brook 1990). Manning et al. (unpublished^b) reported *Verticillium dahliae* causing spongy bark disease of ‘Hort16A’; it was also associated with vine death of kiwifruit in Paengaroa and Whakatane, New Zealand. However, the significance of *Verticillium* spp. to the New Zealand kiwifruit industry is largely unknown.

This study aimed to test New Zealand isolates of *Verticillium* spp. for pathogenicity to kiwifruit, using young vines of ‘Hort16A’ as this cultivar was identified as the most susceptible in Chile (Fullerton et al. unpublished^c). This study was conducted after the New Zealand industry stopped growing ‘Hort16A’ (due to its susceptibility towards *Pseudomonas syringae* pv. *actinidiae* (Psa)) but the results are still relevant to the current kiwifruit industry in New Zealand.

MATERIALS AND METHODS

Plant material

One hundred ‘Hort16A’ plants were used in this study. All plants were grown from tissue-cultured plantlets obtained from Multiflora Laboratories, Auckland, New Zealand. The plantlets were potted into 8-cm diameter polythene planting bags and grown in glasshouse conditions at

The New Zealand Institute for Plant and Food Research Limited (PFR), Mount Albert Research Centre (average temperature of 25 ± 5°C).

The plants were moved to the PFR Pukekohe Research Station 6–9 months post-tissue culture and transferred to 12 cm diameter potting bags containing sterile potting mix with slow-release fertiliser and were irrigated via drippers. The plants remained there for the duration of the trial, tied to an overhead wire and maintained at 2 m in height by pruning.

Verticillium isolates

New Zealand isolates of *Verticillium* spp. were obtained from collections held by PFR (Table 1). All the isolates of *Verticillium* spp. used in this study were from kiwifruit (*Actinidia* species) and identified using standard morphological techniques.

Prior to inoculation of the ‘Hort16A’ vines, the isolates from each *Verticillium* species were grown on Oxoid malt extract agar (MEA) at room temperature for 7 days.

Representative isolates have been deposited into the Manaaki Whenua - Landcare Research International Collection of Microorganisms from Plants (ICMP) as ICMP 22720 (*V. alboatrum*, cc164) and ICMP 22666 (*V. dahliae*, MM765).

Inoculation

All vines were inoculated on 11 July 2017. Half of the vines were in an active growth phase, while half had entered dormancy. Ten vines of ‘Hort16A’ of each growth stage were inoculated with each treatment (four isolates of *Verticillium* spp. and a non-inoculated control).

Table 1 New Zealand isolates of *Verticillium* spp. used for pathogenicity testing. All isolates originate from kiwifruit (*Actinidia* spp.).

<i>Verticillium</i> sp.	Isolate ID	Collection date	Location	Isolated from
<i>V. alboatrum</i> *	cc164	May 2003	No. 1 Road, Te Puke	Leaf
<i>V. dahliae</i>	MM765	Feb 2009	Old Coach Rd, Te Puke	Leader
<i>V. dahliae</i>	MM766	Feb 2009	Old Coach Rd, Te Puke	Trunk, 0.2 m
<i>V. dahliae</i>	MM768	Feb 2009	Old Coach Rd, Te Puke	Trunk, 0.8 m

*The *V. alboatrum* isolate used in this study has since been identified using molecular techniques as *V. alboatrum sensu stricto* (P. Johnston, Landcare Research, pers. comm.)

An approximately 3-mm deep, downward-slanting cut was made on the main stem from the outer bark to the inner wood with a sterile scalpel, 5 cm above the soil. A 5-mm mycelial plug of inoculum was placed into the wound using a sterile scalpel and the inoculation site was wrapped with Parafilm® to allow local mycelial infection of vines by the various isolates of *Verticillium* species. The Parafilm was removed 48 h after inoculation. Plugs of sterile malt agar were used for all control vines.

Assessment and re-isolation

Assessments of the vines were made monthly. At each assessment date, any symptoms of disease such as dieback and wilt were recorded. At any stage of the trial, when a vine was found to exhibit severe symptoms (severe wilt, near total dieback, or death), isolations were performed to identify the cause of the symptoms. All remaining vines were harvested 9 months after inoculation. After harvest, the lower stem of each vine encompassing the inoculation point was split vertically and the extent of stem discoloration above and below the inoculation point measured.

To establish whether the inoculated *Verticillium*

spp. remained alive in the vine tissues, the stems were surface sterilised and small pieces of tissue from the inoculation point were placed onto Difco potato dextrose agar (PDA) amended with antibiotics (ampicillin and streptomycin). In addition, a short piece of stem (approximately 20 cm), including the inoculation point, was incubated at high humidity for 7 days, then examined for the formation of *Verticillium* spp. conidiophores on the cut surfaces. For vines that died, re-isolation was attempted at the inoculation site and at 1, 2 and 5 cm above the inoculation site and at 1 and 2 cm below the inoculation site, and from the roots.

RESULTS

A wide range of symptoms was observed during the experiment, from a minor wilt to death of the vine. The overall results are summarised in Figure 1. Results of stem inoculations from specific treatments are summarised in Figure 2. At no time did any of the non-inoculated control vines develop symptoms of verticillium wilt. Isolate cc164 (*V. alboatrum sensu stricto*) appeared to be more aggressive than the three *V. dahliae* isolates used in this study. All ten vines inoculated with

Figure 1 Number of active and dormant *Actinidia chinensis* var. *chinensis* 'Hort16A' plants that developed verticillium wilt symptoms in each treatment within 9 months of inoculation with *V. alboatrum* or *V. dahliae* (n=10).

Figure 2 Number of *Actinidia chinensis* var. *chinensis* ‘Hort16A’ plants that developed verticillium wilt symptoms of different severities in each treatment within 9 months of inoculation with *V. alboatrum* or *V. dahliae* (n=10).

isolate cc164 during active growth developed symptoms, with five dying. Conversely, of the vines inoculated with the three *V. dahliae* isolates, only one vine died (Fig. 2).

Many of the vines that developed symptoms of verticillium wilt recovered, particularly those inoculated with *V. dahliae*. Some of these recovering vines also developed vigorous regrowth at the crown below the inoculation site.

Verticillium species were recovered from 23 of the 27 vines (82%) that developed symptoms. Of the vines that died ($n=7$), isolates of *Verticillium* species were recovered up to 5 cm above the inoculation site of 5 of the 7 vines. Isolates of *Verticillium* species were recovered from the roots of only one vine during the experimental time frame (9 months). Of the 53 inoculated vines that remained asymptomatic throughout the duration of the experiment, *Verticillium* species were re-isolated from 25 (47%) of these.

DISCUSSION

The results from this study give an indication of the pathogenicity of *Verticillium alboatrum sensu stricto* and *V. dahliae* from 'Hort16A' kiwifruit in New Zealand. Also, they are consistent with observations of verticillium wilt by Manning et al. (unpublished^b) in orchards at Opotiki and Paengaroa, where relatively few vine deaths were observed despite there being large numbers of symptomatic vines from which *V. dahliae* was isolated. Although all of the isolates of *Verticillium* spp. tested in this study were pathogenic to some extent on 'Hort16A' kiwifruit, the isolates appeared to be less aggressive than those recorded in Chile.

A similar study in Chile on 'Hort16A' and 'Hayward' using Chilean isolates of *V. nonalfalfae* reported that 100% of inoculated vines developed typical wilt and dieback symptoms 8 weeks after inoculation. Eleven months after inoculation, budding of 'Hort16A' was completely inhibited while 'Hayward' budding was unaffected (Auger et al. 2011). However, 'Hayward' is tolerant in the field in Chile. The findings of this study, in conjunction with the observed resistance of Chilean 'Hayward' orchards, suggests that

invasive inoculation tests are not necessarily representative of symptoms observed in an orchard environment.

Of the four *Verticillium* isolates tested in this study, the isolate identified as *V. alboatrum* showed the greatest pathogenicity. Similar studies in Chile testing for pathogenicity of *Verticillium* spp. using local strains of *V. nonalfalfae* found high pathogenicity to the 'Hort16A' cultivar (Auger et al. 2011). The 'Hayward' cultivar is relatively tolerant to the disease, and 'Hayward' orchards do not appear affected by *V. nonalfalfae* in Chile (Auger et al. 2011). Investigating the pathogenicity of New Zealand *Verticillium* spp. with other cultivars of kiwifruit would give more insight into the potential threat these pathogens have on the kiwifruit industry in New Zealand.

Despite the presence of *V. nonalfalfae* in New Zealand, the highly pathogenic MLST 2 group of *V. nonalfalfae* identified in Chile has not been recorded in New Zealand. Furthermore, there have been no records of *V. nonalfalfae* on kiwifruit in New Zealand. Research investigating the potential threat of New Zealand species of *Verticillium* to a variety of host crops is ongoing (P. Johnston, Landcare Research, pers. comm.). Following the taxonomic changes within the *Verticillium* genus, there is limited knowledge of the host range of *V. alboatrum*. The only two isolates reported as *V. alboatrum sensu stricto* were from potato (Inderbitzin et al. 2011).

The results from pathogenicity screening suggest that the tested isolate of *V. alboatrum* has greater pathogenicity towards 'Hort16A' than *V. dahliae*. Interestingly, the isolate of *V. alboatrum* used in this study was originally collected from the surface of a leaf from a healthy kiwifruit vine, and there are no records of kiwifruit wilt in orchards caused by this species. Despite the pathogenicity of the *V. dahliae* isolates from this study appearing low, it has been associated with multiple, although sporadic, occurrences of problematic wilt of kiwifruit in New Zealand (Hill 1982; Manning et al. unpublished^b). However, the biggest threat of verticillium wilts in New Zealand remains the risk of introduction of *Verticillium nonalfalfae* MLST 2.

Future work for better understanding the potential threat of New Zealand isolates of *Verticillium* spp. could include investigating any genetic similarities and differences between the New Zealand and Chilean isolates using the protocols of Kasson (2014). Testing of the Chilean and New Zealand *Verticillium* species concurrently would give greater confidence in the differences in pathogenicity between the species. Ensuring the prevention of the introduction of highly pathogenic strains of *Verticillium* species (such as the *V. nonalfalfae* MLST 2 group from *Actinidia* sp. in Chile) into New Zealand is essential to protecting the kiwifruit industry from verticillium wilts.

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ENDNOTES

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