Australasian Plant Pathology (2019) 48:637–650
https://doi.org/10.1007/s13313-019-00667-7

ORIGINAL PAPER

Seed tuber incidence, identification and pathogenicity of *Verticillium* species infecting potatoes in South East Australia

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Received: 12 May 2019 / Accepted: 15 October 2019 / Published online: 6November2019

Abstract

Verticillium wilt, caused by the soilborne fungi *Verticillium dahliae* and *Verticillium albo-atrum*, is a serious disease of potato as well as many other crops. Potato seed tuber surveys (2010 to 2012) from Victoria and Tasmania, Australia identified *V. dahliae*, *V. albo-atrum* and *V. tricorpus* infecting the stem end vascular tissue of seed tubers. The species were identified by traditional morphology and phylogenetic analysis of the ITS region. Isolation of *V. dahliae* within a seed lot varied greatly and ranged from 0 to 55%. *Verticillium* spp. were isolated from the stem-end vascular tissue of tubers from seed lots from Victoria and Tasmania with an overall percent infection of 27.7 (*V. dahliae*), 8.4 (*V. albo-atrum*) and 4.8 (*V. tricorpus*). *Verticillium dahliae* was isolated from 11% of tubers with discoloured stem-end vascular tissue and 3.3% of tubers without stem-end vascular decolourisation suggesting that tuber stem end vascular discolouration symptoms were not a reliable indication of Verticillium wilt infection. In glasshouse trials, *V. dahliae* isolates from different geographical locations varied in pathogenicity during infection of susceptible potato cv Shepody, moderately resistant cv Ranger Russet and eggplant cv Black Beauty. The majority of *V. dahliae* isolates were highly aggressive in potato and eggplant, especially the Tasmanian *V. dahliae* isolates. Infected plants of cv Shepody inoculated with *V. albo-atrum* and *V. tricorpus* showed typical wilt symptoms however, the severity of infection caused by *V. tricorpus* was substantially lower compared to highly aggressive isolates of *V. dahliae*. In eggplant, *V. dahliae* isolates also varied in pathogenicity in terms of disease severity but all isolates significantly ($p \leq 0.05$) reduced eggplant growth.

Keywords Verticillium wilt · *Verticillium* spp. · Seed tuber incidence · Multigene phylogeny · Pathogenicity

Introduction

Verticillium wilt is an economically important disease and occurs everywhere that potatoes are commercially grown. *Verticillium dahliae* Klebahn and *V. albo-atrum* Reinke & Berthold are the main causal agents of the disease (Rowe and Powelson 2002). Symptoms of Verticillium wilt of potato include wilting, chlorosis and necrosis of lower leaves and premature senescence of the plant (Johnson and Dung 2010). An Australian study by Powney et al. (2005) showed that around one third of all commercial processing potato crops were infected with *V. dahliae*. Harding and Wicks (2007) subsequently reported that *V. dahliae* was the only pathogen infecting potatoes in Australia. These two investigations confirmed that *V. dahliae* was the major species causing wilt of potatoes in South East Australia and reported that *V. albo-atrum* was absent, which is in contrast to the situation in USA and Canada where *V. albo-atrum* and *V. dahliae* were detected together (Uppal et al. 2007; Pegg and Brady 2002).

Seed tuber infection by *V. dahliae* is the main source of pathogen transmission into clean potato production regions (Dung et al. 2013) and may lead to the introduction of a highly aggressive strain of *V. dahliae* into a region where previously no *Verticillium* had been detected or where fumigation had been applied to reduce the inoculum in the soil (Johnson and Dung 2010). In Australia, the seed certification standard does not check for seed tuber infection by Verticillium and therefore, the incidence of infected seed tubers within potato production regions is unknown. Nair et al. (2016) demonstrated tubers naturally infected by *V. dahliae* were causing

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Verticillium wilt and contributing to increased levels of soil inoculum resulting in subsequent progeny tuber infection in seven potato cultivars.

Easton et al. (1972) reported the incidence of *Verticillium* spp. within seed lots ranging between 1 to 2% and overall infection by *V. albo-atrum* and *V. dahliae* at 39% of 244 seed lots tested in Washington, USA. In UK, MacGarvie and Hide (1966) found that 79% of 225 certified seed potato lots were infected with *Verticillium* spp. other than *V. albo-atrum* or *V. dahliae* with most isolations made from young tuber sprouts. Surveys conducted in 1995 and 1996 in North American potato production regions detected *V. dahliae* in 29% of the seed lots (Omer et al. 2000). In Israel, imported seed tubers from Europe were found to be free of *V. dahliae* but seed lots within the potato production regions had an incidence of 22% (Nachmias and Krikun 1984). A further seed tuber survey in Israel reported that one third of the seed lots were infected with *V. dahliae* and 10% of seed lots had more than 5% of tubers infected (Tsror et al. 1999). In Japan, *V. tricorpus* was isolated from tubers (Ebihara et al. 2003) and was found to be mildly pathogenic to potato.

Correct species identification is important for determining the ecological roles of *Verticillium* spp. and for the development of diagnostic methods (Hyde et al. 2014). Earlier studies showed resting structures (dark resting mycelium, microsclerotia and chlamydospores) as the main characteristic to differentiate *Verticillium* spp. (Isaac 1949). Other studies differed in the usefulness of identifying species based on morphology and abundance of microsclerotia because these structures varied depending on the growth medium and culture conditions (Goud et al. 2003). Molecular techniques have been used to identify *Verticillium* spp. and for phylogenetic comparisons (Collins et al. 2003; Fahleson et al. 2004; Qin et al. 2006; Collado-Romero et al. 2008). Inderbitzin et al. (2011a), described 10 species of *Verticillium*, five of which were new, using four-gene phylogenetic analyses, morphological description, and herbarium material as type specimens. Recent reports based on phylogenetic analyses also proposed that morphology of resting structures had limited use in identifying *Verticillium* at the species level (Zare et al. 2007; Inderbitzin et al. 2011a). For example, *V. tricorpus* was divided into three species, *V. tricorpus*, *V. isaacii* and *V. klebahnii*, that were morphologically indistinguishable (Inderbitzin et al. 2011a). Hyde et al. (2014) recommended the sequence of the internal transcribed spacer region nrDNA be used to identify *Verticillium* species. In Australia, comprehensive studies have not been carried out using phylogenetic analysis to characterize *Verticillium* spp.

Verticillium dahliae has a wide host range including woody plants, vegetables, field crops, herbaceous plants, ornamentals, shrubs and weeds (Fradin and Thomma 2006). Based on susceptibility, 410 plant species that include nearly 80 plant genera have been recorded as being infected by *Verticillium* spp., particularly in temperate and cool temperate regions (Pegg and Brady 2002). *Verticillium* is not host specific and isolates from a particular host may cause various degrees of symptoms in other hosts particularly within cultivars, but usually disease symptoms are more severe on the host from which the pathogen was isolated (Bhat and Subbarao 1999). Difference in the pathogenic variability was observed among *V. dahliae* isolates when inoculated onto potato cv Russet Burbank (Uppal et al. 2007). The pathogen was also found in roots of other plant species, including plants that did not become systemically infected (Resende et al. 1994). Eggplant seedlings are often used in pathogenicity trials for *Verticillium* spp. because the plant is very susceptible and disease development is rapid on this host (Korolev et al. 2000).

The incidence of *Verticillium* spp. found in the vascular tissue of potato tubers in certified seed lots in Australia is not known. The lack of knowledge about pathogenicity of different species of *Verticillium* that infect potatoes is significant for the effective management of Verticillium wilt in Australia. The objectives of this research were therefore to (i) determine the incidence of *Verticillium* spp. within the seed lots of potatoes obtained from seed potato production areas across Victoria and Tasmania, (ii) characterise *Verticillium* spp. using morphology and phylogenetic analysis; and (iii) study the pathogenicity of isolates of *Verticillium* spp.

Materials and methods

Incidence of seed lot infection

Seed source and isolation

Certified seed tubers that had been produced by commercial seed growers in Victorian and Tasmanian potato production areas were obtained from 2010 to 2012. In 2010, 33 seed lots from Victoria; in 2011, 16 lots from Victoria and 10 lots from Tasmania; and in 2012, 11 lots from Victoria and 13 from Tasmania were selected. A total of 83 seed lots (20 tubers/lot) were selected in an unbiased manner as growers volunteered samples for assessment.

Tubers were washed with high pressure water to remove surface soil, surface sterilized in 0.5% ai NaOCl for 5 min. and then rinsed in sterile distilled water. The tubers were numbered individually, transverse cut at the stolen end 3–5 mm beneath the stem end of each tuber and vascular discolouration recorded. Four pieces of vascular tissue (approx 5 mm diameter) per tuber were excised aseptically from the vascular tissue (Omer et al. 2000) then placed on either Soil Pectate Tergitol (SPT) (Hawke and Lazarovits 1994) or Ethanol Potassium Amoxicillin Agar medium (EPAA) (Mansoori 2011) and incubated at 23 °C in the dark for 15 days.

Morphological and molecular identification of seed tuber isolates

Verticillium spp. were identified by sub-culturing colonies onto potato dextrose agar (PDA; DIFCO Laboratories). Conidia were collected from each isolate with a sterile loop and streaked onto water agar. After one day colonies grown from single spores were transferred onto PDA in 90 mm Petri dishes to ensure genetic uniformity, and were incubated in darkness at 23 °C for 4 weeks. Cultures were examined under the stereo and compound microscope for the presence of conidiophores, microsclerotia, dark resting mycelia and chlamydospores characteristics of *Verticillium* spp. and identified based on published descriptions (Hawksworth 1970; Hawksworth and Talboys 1970a; Hawksworth and Talboys 1970b; Zare et al. 2007; Inderbitzin et al. 2011a). All the single spored cultures were stored on PDA slants in 10 mL plastic tubes at 4 °C and were used for all subsequent studies. Eighteen isolates that were difficult to identify based on morphological characteristics were identified based on nrDNA of the Internal Transcribed Spacer (ITS) gene sequences following Hyde et al. (2014).

For DNA extraction, isolates were grown in 10 mL V8 broth or Czapek Dox broth in McCartney bottles and incubated at room temperature for 2 weeks or until a mycelial mat was formed on top of the broth. DNA extractions were performed with 100 mg of mycelia of each mono-conidial isolate using the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. The nrDNA ITS regions were amplified using primers ITS 1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). PCR was performed either on a MJ Research PTC-100 Thermal Cycler or Eppendorf Thermalcycler. The PCR reactions were performed in a total volume of 25 μL including 50 ng genomic DNA, 5 μL of 10× PCR buffer containing 25 mM $MgCl_2$, 0.2 mM dNTP, 0.2 μM of each primer and 1 unit of Taq DNA polymerase (Bioline Australia). The cycling conditions consisted of 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 30 s, 72 °C for 30 s and elongation at 72 °C for 6 min. PCR products were also purified using QIAquick® PCR Purification Kit (QIAGEN) according to the manufacturer's instructions. The forward and reverse PCR products were sequenced at the Australian Genome Research Facility (AGRF, Melbourne).

All sequences were checked manually and edited when necessary using MEGA version 5 (Tamura et al. 2011). To identify sequences, they were compared against those sequences already found in the databases using the BLAST search in GenBank. ITS sequences revealed highest similarity with type and reference strains of *Verticillium* spp., as classified by Inderbitzin et al. (2011a).

Characterisation of *Verticillium* spp.

Fungal isolates and morphological characterisation

The taxonomy of three *Verticillium* spp. isolated from potato was described using both morphological characters and molecular analysis. The isolates included four of *V. albo-atrum* and two of *V. tricorpus* that had been isolated from the vascular tissue of seed tubers from the 2012 seed potato survey from Victoria and Tasmania (Table 1). Nineteen isolates of *V. dahliae*; and one of *V. tricorpus* isolated from either potato tubers or petiole tissue in 2005 were obtained from the culture collection of Department of Primary Industries, Knoxfield, Victoria.

For morphological description, the fungal isolates were grown on PDA for four weeks before being examined under stereo and compound microscopes. For compound microscope examination, fungal structures were mounted on glass slides in water and photographs were taken with a Leica DFC295 camera, using Leica application software.

Molecular characterisation and phylogenetic analysis

All the sequences were aligned in MEGA 7 (Kumar et al. 2016) using ClustalW v.2.0 (Larkin et al. 2007) and phylogenetic trees constructed. Phylogenetic relationships of *Verticillium* spp. was inferred by using Maximum Likelihood method based on the Kimura 2-parameter model (Kimura 1980). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) were shown next to the branches. Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selected the topology with superior log likelihood value. All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). All consensus sequence data were deposited in GenBank® under accession numbers KC592062 to KC592090. Reference extype and epitype sequences for *V. dahliae* (HQ206718), *V. albo- atrum* (JN188016), *V. tricorpus* (JN187993), *V. alfalfae* (JN187971), *V. nonalfalfae* (JN187973), *V. nibilum* (JN188011), *V. zaregamsianum* (JN188005), *V. isaacii* (HQ206873) and *V. klebahnii* (JN187967) were used for ITS phylogenetic analysis (Inderbitzin et al. 2011a; Inderbitzin et al. 2011b). The trees were rooted with *G. nigrescens*.

Pathogenicity of *Verticillium* spp. isolates in potato and eggplant

Plant materials and fungal isolates

Tissue culture plantlets of potato cv Shepody, susceptible to *V. dahliae* (Arbogast et al. 1999), and moderately resistant cv

Table 1 Hosts, geographic locations, *Verticillium* spp. and the GenBank accession numbers of sequences used for taxonomy

Isolate code	Host	Location	Species	ITS
VICVd36*	Potato petiole	Cora Lynn, Victoria	*V. dahliae*	KC592062
VICVd54*	Potato petiole	Ballarat, Victoria	*V. dahliae*	KC592063
VICVd69	Potato petiole	Ballarat, Victoria	*V. dahliae*	KC592064
VICVd72	Potato petiole	Ballarat, Victoria	*V. dahliae*	KC592065
VICVd74	Potato petiole	Ballarat, Victoria	*V. dahliae*	KC592066
VICVd85*	Potato petiole	Colac-Otway, Victoria	*V. dahliae*	KC592067
SAVd7*	Potato petiole	Mt Gambier, South Australia	*V. dahliae*	KC592068
SAVd8	Potato petiole	Mt Gambier, South Australia	*V. dahliae*	KC592069
SAVd10	Potato petiole	Mt Gambier, South Australia	*V. dahliae*	KC592070
SAVd15	Potato petiole	Mt Gambier, South Australia	*V. dahliae*	KC592071
SAVd16*	Potato petiole	Mt Gambier, South Australia	*V. dahliae*	KC592072
SAVd20	Potato petiole	Mt Gambier, South Australia	*V. dahliae*	KC592073
SAVd21	Potato petiole	Mt Gambier, South Australia	*V. dahliae*	KC592074
SAVd12*	Potato petiole	Robe, South Australia	*V. dahliae*	KC592075
SAVd13	Potato petiole	Robe, South Australia	*V. dahliae*	KC592076
SAVd14	Potato petiole	Robe, South Australia	*V. dahliae*	KC592077
TASVd24*	Potato petiole	Devonport, Tasmania	*V. dahliae*	KC592078
TASVd25*	Potato petiole	Devonport, Tasmania	*V. dahliae*	KC592079
TASVd27*	Potato petiole	Devonport, Tasmania	*V. dahliae*	KC592080
VICVaa1*	Potato tuber	Ballarat, Victoria	*V. albo-atrum*	KC592083
VICVaa2	Potato tuber	Ballarat, Victoria	*V. albo-atrum*	KC592084
TASVaa1*	Potato tuber	Lileah, Tasmania	*V. albo-atrum*	KC592085
TASVaa2	Potato tuber	Winkleigh, Tasmania	*V. albo-atrum*	KC592086
TASVt1*	Potato tuber	Yolla, Tasmania	*V. tricorpus*	KC592087
TASVt2*	Potato tuber	Mt Seymour, Tasmania	*V. tricorpus*	KC592088
VICVt143	Potato tuber	Cora Lynn, Victoria	*V. tricorpus*	KC592089
PD322**	Lettuce	USA	*V. dahliae*	HQ206718
PD489**	Alfalfa	USA	*V. alfalfae*	JN187971
PD742**	Soil	UK	*V. nubilum*	JN188011
PD660**	Lettuce	USA	*V. isaacii*	HQ206873
PD592**	Irish Potato	Japan	*V. nonalfalfae*	JN187973
PD747**	Potato soil	Canada	*V. albo-atrum*	JN188016
PD736**	Lettuce	Japan	*V. zaregamsianum*	JN188005
PD690**	Garden Tomato	UK	*V. tricorpus*	JN187993
PD401**	Lettuce	USA	*V. klebahnii*	JN187967

* isolates used for pathogenicity study

**ex-type (ex-epitype) strains type culture isolates shown in the phylogenetic trees

Ranger Russet (Whitworth and Davidson 2008) were planted in pasteurized sand for 15 days to establish the root system. Eggplant seedlings (cv Black Beauty) were grown in seedling mix (Yates, NSW, Australia) from seed and maintained on benches in a glasshouse until plants reached the 4-leaf stage (4-week-old). Two sets of experiments were conducted as follows.

i) Pathogenicity of *V. dahliae* isolates

Nine *V. dahliae* isolates were tested for pathogenicity in potato and eggplant using three isolates each from Victoria (VICVd36, VICVd54, VICVd85), South Australia (SAVd7, SAVd16, SAVd12) and Tasmania (TASVd24, TASVd25, TASVd27) in August to November 2011 and experiments were repeated in April to July 2012.

ii) Pathogenicity of *V. dahliae, V. albo-atrum* and *V. tricorpus* isolates

The pathogenicity of the high (VICVd25) and low (VICVd54) aggressive isolates of *V. dahliae* isolates were compared to the *V. albo-atrum* (VICVaa1 and TASVaa1) and *V. tricorpus* (TASVt1 and TASVt2) isolates from potato. The isolates of *V. albo-atrum* and *V. tricorpus* were isolated from the vascular tissue of seed tubers from Victoria and Tasmania in 2012.

Inoculum production and inoculation

Inoculum was prepared by growing isolates on PDA in 90 mm Petri plates at 23 °C for four weeks. Spore suspensions were prepared by adding 10 mL of sterile distilled water (SDW) to each plate and scraping the cultures with a spatula. The resulting spore suspensions were filtered through double layers of cheesecloth, and then adjusted to 10^5 conidia per mL using a haemocytometer.

Tissue culture seedlings of cvs Shepody and Ranger Russet, and eggplant seedlings, were gently removed from sand or soil, roots washed under running tap water, then dipped for at least five min into the spore suspension of each isolate. For uniform distribution of conidia, the spore suspension was thoroughly shaken every minute. Roots were dipped in sterile distilled water (SDW) as a control. The inoculated seedlings were transplanted immediately to 2.8 L pots for potatoes and 0.5 L pots for eggplants containing pasteurised potting mix and then placed on glasshouse benches. The plants were grown under glasshouse conditions at 22–25 °C. Natural light was supplemented with incandescent lamps for a photoperiod of 16 h. Plants were watered once a day and fertilised weekly with AquasolTM (N:P:K = 2.8:1:4.5). All the treatments were arranged in the glasshouse as a randomized complete block design with four replicates per isolate.

Disease assessment and petiole isolation

Symptom severity on potatoes and eggplants were recorded at weekly intervals starting from 2 weeks after inoculation on a scale of 0–5, where 0 = no symptoms, 1 = chlorosis of lower leaves, 2 = moderate (30–50% of leaves) wilt with severe chlorosis, 3 = moderate wilt and necrosis, 4 = severe (>50% of leaves) wilt and necrosis, and 5 = death of plant (Tsror and Hazanovsky 2001).

Potato and eggplant leaflets were collected 2-weeks after inoculation and the petioles were surface sterilized in 0.5% ai NaOCl for 1 min and rinsed twice with sterile distilled water. Four, 4 mm wide cross sections were cut at equal intervals from each petiole and plated onto EPAA medium (Mansoori 2011) and incubated at 23 °C in dark. After 15 days, the resulting fungal colonies were observed using stereo and compound microscopes and identified based on morphological characters such as verticilliate conidiophores and resting structures.

Plant height, aerial biomass in eggplant

Plant height (cm) measured from soil level to apical meristem of eggplants was measured 7-weeks after inoculation. Then the eggplants were destructively sampled. For dry weight of roots, plants were lifted from the potting mix and dipped in a bucket of water to separate the potting mix and further cleaned in gently running tap water. The roots and aerial parts were oven-dried at 65 °C for 3 days and dry weight for each plant was recorded.

Statistical analysis

One-way ANOVA was performed on disease severity ratings, plant height, aerial biomass and root weight. Means separations were made using Fisher's Least Significant Difference (LSD) at $P \leq 0.05$. Tests for significant differences among isolates were analysed using the statistical package Minitab 16 (Minitab 2010). The frequency of infected seed lots was compared across 20 cultivars using Pearson χ^2 test in Genstat for each cultivar and overall to test whether stem end vascular discolouration was correlated with *V. dahliae* recovery. A Chi-square goodness of fit test was conducted to assess the observed distribution and expected probability of *V. dahliae* recovery within the seed lots.

Results

Incidence of seed lot infection

The majority of *Verticillium* isolates were identified based on morphological characteristics on SPT and EPAA medium. The eight isolates of *V. albo-atrum*, and 4 *V. tricorpus* were confirmed by the ITS gene sequence analysis. Overall, based on the molecular identification and formation of resting structures and colour of colonies, three *Verticillium* spp. (*V. dahliae*, *V. albo-atrum* and *V. tricorpus*) were identified. On both media, *V. dahliae* microsclerotia were dark brown to black and regularly or irregularly distributed throughout the colonies which were sharply differentiated from the hyaline mycelium. The hyphae of *V. albo-atrum* differentiated into thick-walled melanised cells that became dark resting mycelia. *Verticillium tricorpus* formed large and irregular shaped microsclerotia and resting mycelium after 15 days of incubation. Most of the *V. tricorpus* isolates produced a yellow to orange pigment that diffused into the culture medium. *Colletotrichum coccodes*, *Fusarium* spp. and *Plectospaerella cucumerina* were also frequently isolated from the vascular tissue of seed tuber.

In 2010, 33.3% of seed lots collected from Ballarat and Cora Lynn regions of Victoria were infected with *V. dahliae*, which was the only species isolated from the vascular tissue of

seed tubers. In 2011, 31% of seed lots were infected with *V. dahliae* while 6% of seed lots were infected with *V. albo-atrum*. *V. tricorpus* was not identified in 2010 or 2011. In 2012, *V. dahliae* (18%), *V. albo-atrum* (9%) and *V. tricorpus* (9%) were isolated from 11 seed lots in Victoria (Table 2).

From the Tasmanian seed tuber survey in 2011, *V. dahliae* (10%), *V. albo-atrum* (20%) and *V. tricorpus* (20%) were isolated from the vascular tissue of seed tubers from 10 seed lots. In 2012, infection by *V. dahliae* was comparatively higher with 30.7% seed lots infected from 13 seed lots. A greater percentage of seed lots in Tasmania were infected by *V. albo-atrum* (23%) compared to Victoria (Table 2).

In Victoria, overall percent infections of seed lots from 2010 to 2012 were 30% for *V. dahliae*, 3.3% for *V. albo-atrum*, 1.6% for *V. tricorpus* out of 60 seed lots tested. Overall infection of *V. albo-atrum* (21.7%) and *V. tricorpus* (13%) were higher in Tasmanian seed potatoes. In general, Tasmania had a higher level of seed lots infected than Victoria2.

Tubers from 20 cultivars were assessed for the presence of *Verticillium* spp. Overall percent infection of seed lots from Victoria and Tasmania were 27.7 (*V. dahliae*), 8.4 (*V. albo*-atrum) and 4.8 (*V. tricorpus*) (Table 3). Based on Pearson χ^2 analysis, *Verticillium* recovery from seed lots were not dependent on cultivar (*V. dahliae* $\chi^2 = 125.96$, $df = 120$, $P = 0.337$; *V. albo-atrum* $\chi^2 = 83.98$, $df = 80$, $P = 0.359$; *V. tricorpus* $\chi^2 = 62.98$, $df = 60$, $P = 0.371$). The likely hood of recovering the different species of *Verticillium* was significantly different according to Pearson χ^2 analysis (*V. dahliae* and *V. albo-atrum* recovery, $\chi^2 = 64.31$, $df = 24$, $P < 0.001$; *V. dahliae* and *V. tricorpus* recovery, $\chi^2 = 52.24$, $df = 18$, $P < 0.001$; *V. albo-atrum* and *V. tricorpus* recovery, $\chi^2 = 46.13$, $df = 12$, $P < 0.001$).

Vascular infection by *V. dahliae* within a seed lot varied greatly and ranged from 0 to 55%. A significant proportion (66%) of seed lots were pathogen free according to Pearson χ^2 test ($\chi^2 = 186.8$, $df = 6$, $P = 0.000$). Over 12% of seed lots tested ranged from 0 to 5% infection within the seed lots. Only one seed lot had more than 50% of seed tubers infected with *V. dahliae* (Table 4).

Stem-end vascular discoloration was correlated with presence of the *V. dahliae* ($\chi^2 = 146.97$, $df = 119$, $P = 0.042$). Of 416 vascular discoloured tubers tested, 11% were infected with *V. dahliae* (Table 5). Of 1244 tubers without vascular discolouration 3.3% were infected with *V. dahliae* ($\chi^2 = 356.90$, $df = 306$, $P = 0.134$). There was no apparent correlation with incidence of infection and cultivar ($\chi^2 = 357$, $df = 340$, $P = 0.252$). The greatest percentage of cultivar infected was more likely a result of a greater sampling for that particular genotype. In general, the percent recovery rate of *V. dahliae* from vascular discoloured tubers was 0–35.7% and non vascular discoloured tubers was 0–11.7% (Table 5).

Characterisation of *Verticillium* spp.

Morphology

All *Verticillium* isolates produced flocculose colonies with conidiophores which were more or less erect with verticilliate branches. Conidia were borne in small clusters at the tip of each phialide. Based on the size of conidia, formation of resting structures, and colour of colonies on PDA, three *Verticillium* spp. (*V. dahliae*, *V. albo-atrum*, *V. tricorpus*) were identified from potatoes and representative isolates were described in Table 6. The morphological description was similar to the ex-type strains or voucher strains of *Verticillium* spp. (Inderbitzin et al. 2011a).

ITS sequencing and phylogenetic analysis

Sequences for the ITS region for all 27 isolates were deposited in GenBank® (Table 1). The dendogram based on ITS gene sequences showed the separation of the *Verticillium* spp. into clades with high boot strap values. *V. tricorpus* and *V. albo-atrum* being more closely related than to *V. dahliae*. The phylogenetic tree based on ITS differentiated *Verticillium* species and the isolates of *V. dahliae*, *V. albo-atrum* and *V. tricorpus* were similar to the ex-type strains with high boot strap values (Fig. 1).

Pathogenicity

Pathogenicity of *V. dahliae* isolates on potatoes

The two potato cultivars inoculated with nine isolates of *V. dahliae* exhibited typical Verticillium wilt symptoms ranging

Table 2 Proportion of seed lots infected by *Verticillium* spp. in the stem end vascular tissue of tubers collected from Victoria and Tasmania (2010–2012)

	2010		2011				2012			
	No of seed lots tested	*V. dahliae*	No of seed lots tested	*V. dahliae*	*V. albo-atrum*	*V. tricorpus*	No of seed lots tested	*V. dahliae*	*V. albo-atrum*	*V. tricorpus*
Victoria	33	11 (33.3%)	16	5 (31%)	1 (6%)	0	11	2 (18%)	1 (9%)	1 (9%)
Tasmania	0	0	10	1 (10%)	2 (20%)	2 (20%)	13	4 (30.7%)	3 (23%)	1 (7%)

Table 3 Percent overall infection of *Verticillium* spp. isolated from stem end vascular tissue of 83 seed lots containing tubers from 20 cultivars in Victoria and Tasmania (2010–2012)

Cultivar	No of seed lot tested	No of seed lots from which *Verticillium* spp. isolated and percent infection		
		V. dahliae	*V. albo-atrum*	*V. tricorpus*
Russet Burbank	23	8 (34.7%)	3 (13%)	0
Innovator	14	5 (35.7%)	2 (14%)	2 (14%)
Atlantic	11	4 (36.3%)	0	0
Ranger Russet	7	2 (28.5%)	0	1 (14.2%)
Trent	4	1 (25%)	0	0
Pike	4	0	0	0
Nadine	3	0	0	0
Sebago	2	1 (50%)	0	0
Kennebec	2	0	0	0
Catani	2	0	0	0
Simcoe	2	0	0	0
Harmony	1	0	0	0
Nicola	1	0	1 (100%)	1 (100%)
Desiree	1	0	0	0
Topcat	1	0	0	0
Moon light	1	0	0	0
Bondi	1	1 (100%)	1 (100%)	0
Nooksack	1	1 (100%)	0	0
Shepody	1	0	0	0
Wont Scab	1	0	0	0
Total	83	23	7	4
Percent of seed lot infection		27.7	8.4	4.8

Association between: Cultivars and *V. dahliae* recovery: $\chi^2 = 125.96$, $df = 120$, $P = 0.337$; Cultivars and *V. albo-atrum* recovery: $\chi^2 = 83.98$, $df = 80$, $P = 0.359$; Cultivars and *V. tricorpus* recovery: $\chi^2 = 62.98$, $df = 60$, $P = 0.371$; *V. dahliae* and *V. albo-atrum* recovery: $\chi^2 = 64.31$, $df = 24$, $P < 0.001$; *V. dahliae* and *V. tricorpus* recovery: $\chi^2 = 52.24$, $df = 18$, $P < 0.001$; *V. albo-atrum* and *V. tricorpus* recovery: $\chi^2 = 46.13$, $df = 12$, $P < 0.001$

from chlorosis to necrosis which started from the margin of the leaf then along the main vein followed by wilting. The disease symptoms were first observed on the lower leaves and appeared earlier on the susceptible cv Shepody, 2 weeks after inoculation (wai), compared to 3 wai on the moderately resistant cv Ranger Russet. The disease symptoms progressed faster at 4 wai on cv Shepody than on cv Ranger Russet. At 6 wai, in the 2011 trial, disease severity was high for the cv Shepody inoculated with TASVd24, 25 and 27 isolates with death of the plants (rating of 5 and 4.75 ± 0.25). In the 2012 trial, the disease severity was not as high with no plant death (Table 7) with infection by isolate TASVd25 and 27 being the most virulent producing a disease severity rating of 3.75 ± 0.25 and 4.25 ± 0.25 in Shepody plants. On Ranger Russet, similar trends were observed as in the 2011 trial with TASVd27 being the most virulent causing the plants to die (rating of 5) and TASVd24 producing a rating of 4.

Table 4 Isolation of *V. dahliae* from stem end vascular tissue of tubers within the seed lots

Percent tubers infected/seed lot	Number of seed lots infected*	χ^2***
0	55 (66.2%**)	156.97
0–5	10 (12%)	0.29
6–10	5 (6%)	3.96
11–20	4 (4.8%)	5.20
21–30	4 (4.8%)	5.20
31–40	4 (4.8%)	5.20
41–60	1 (1.2%)	9.94

*a total of 83 seed lots (20 tubers/lot) tested

**Percentage of total tested

***$\chi^2 = 186.8$, $df = 6$, $P = 0.000$

In the 2012 trial, TASVd25 was the most virulent on Ranger Russet with a rating of 4 (3.75 ± 0.25), while all other isolates, except VICVd54, only producing a disease severity rating of 2 (1.75 ± 0.25). VICVd54 was less virulent in Ranger Russset only showing moderate infection (rating of 1 (0.5 ± 0.28)). The isolate VICVd54 caused mild symptoms on infected plants in both trials. No Verticillium wilt symptoms were observed in non-inoculated control plants, nor was the pathogen reisolated (Table 7).

Table 5 *V. dahliae* isolation from tuber stem ends showing brown vascular discolouration and without vascular discolouration

Cultivars (A*)	With stem end vascular discolouration			Without stem end vascular discolouration		
	Number of vascular discoloured tubers (B*)	*V. dahliae* recovered (C*)	Per cent of recovery	Number of tubers without vascular discolouration (D*)	*V. dahliae* recovered (E*)	Per cent of recovery
Russet Burbank	126	16	12.6	334	14	4.1
Innovator	56	7	12.5	224	9	4
Ranger Russet	46	6	13	94	0	0
Atlantic	39	8	20.5	181	9	4.9
Catani	25	0	0	15	0	0
Trent	24	4	16.6	56	6	10.7
Pike	18	0	0	62	0	0
Nooksack	14	5	35.7	6	0	0
Desiree	13	0	0	7	0	0
Nadine	11	0	0	49	0	0
Simcoe	11	0	0	29	0	0
Kennebec	9	0	0	31	0	0
Shepody	7	0	0	13	0	0
Sebago	6	0	0	34	4	11.7
Bondi	4	0	0	16	0	0
Nicola	2	0	0	18	0	0
Topcat	2	0	0	18	0	0
Harmony	2	0	0	18	0	0
Wont Scab	1	0	0	19	0	0
Moonlight	0	0	0	20	0	0
Total	416	46	11	1244	42	3.3

*Pearson χ^2 association between: A and B: $\chi^2 = 357$, $df = 340$, $P = 0.252$; A and C: $\chi^2 = 146.94$, $df = 140$, $P = 0.327$; A and D: $\chi 2 = 378$, $df = 360$, $P = 0.247$; A and E: $\chi^2 = 104$, $df = 100$, $P = 0.347$; B and D: $\chi^2 = 356.90$, $df = 306$, $P = 0.024$; D and E: $\chi^2 = 356.90$, $df = 306$, $P = 0.134$; B and C: $\chi^2 = 146.97$, $df = 119$, $P = 0.042$

Pathogenicity of *V. dahliae* isolates on eggplants

In 2011 all isolates, with the exception of VICVd54, induced Verticillium wilt symptoms 2 wai in eggplant seedlings. The disease symptoms appeared on the lower leaves first and then symptoms progressed in the subsequent weeks. At 7 wai, the disease severity of plants inoculated with TASVd25 isolate was high with an incidence severity scale of 4 (severe wilt and necrosis). At 7 wai, in both trials, TASVd25 inoculated plants induced higher disease severity compared to other treatments. In 2012, disease severity was less (rating of 2) in plants inoculated with VICVd36, SAVd12 and TASVd24 isolates; and a rating of 1 for plants inoculated with VICVd54. However, TASVd25 was the most virulent producing a disease severity rating of 4, similar to the 2011 trial. *V. dahliae* was recovered and morphologically identified from petioles of

Table 6 Morphological and cultural characteristics of *Verticillium* spp. isolates from potato

Verticillium isolates	Colour of colony	Resting structure	Conidia Size
TASVd25 (*V. dahliae*)*	White to black	Microsclerotia spherical to elongate (15–68 μm)	2.5–6 × 1.25–3 μm
TASVaa1 (*V. albo-atrum*)*	Dark grey to black	Dark Resting mycelium 3.5–7 μm	2.5–5.5 × 1.25–3 μm
TASVt1 (*V. tricorpus*)*	Yellow to black	Microsclerotia elongated to Spherical (55–90 μm), dark resting mycelium (3.5–7 μm). Chlamydospores 7.5–11 μm	3.5–8 × 1.5–3.5 μm

* Representing isolates

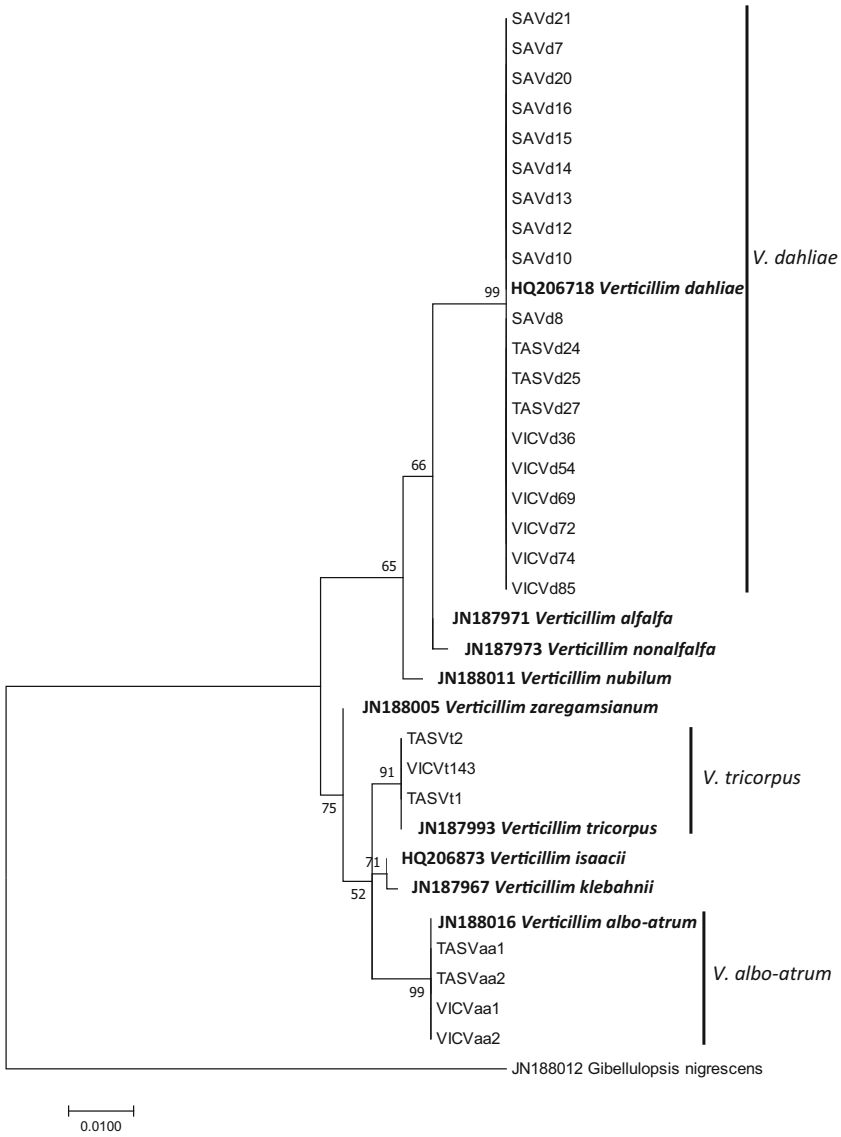

Fig. 1 Dendogram based on ITS sequences of Australian *Verticillium* isolates and comparison of type and ex-type strains (in bold) of 9 *Verticillium* spp. (Inderbitzin et al. 2011a; Inderbitzin et al. 2011b) using the Maximum likelihood method with 1000 boot strap replicates. The tree is rooted with *G. nigrescens*

infected plants. The pathogen was not isolated from un-inoculated control plants (Table 8).

All isolates of *V. dahliae* (including the least virulent isolate VICVd54) in both trials significantly reduced eggplant height, aerial biomass and below ground root mass compared to non-inoculated control. In 2011, TASVd25 was the most virulent isolate resulting in significant ($p \leq 0.05$) reduction of plant height and aerial biomass at 7 wai (Table 8). Although, the root weight was significantly reduced compared to most other isolates, there was no significant difference between TASVd25, TASVd24 and SAVd12. In contrast in 2012, TASVd25 and TASVd27 significantly reduced the height but no significant difference was detected between most isolates for aerial biomass and root weight (Table 8). Nevertheless, aerial biomass of plants inoculated with TASVd25 was significantly lower compared to isolates VICVd54 and SAVd7.

Pathogenicity of *V. dahliae*, *V. albo-atrum* and *V. tricorpus* on potato

Plants inoculated with the high and low aggressive isolates of *V. dahliae* showed typical Verticillium wilt symptoms 2 wai however, at 6 wai plants inoculated with the highly aggressive isolate (TASVd25) of *V. dahliae* showed disease severity of 4 compared to the plants inoculated with the less aggressive isolate (VICVd54) which had a rating of 2. Both isolates of *V. albo-atrum* also induced wilt symptoms at 2 wai, and at 6 wai symptoms of yellowing of lower leaves, necrosis and wilting of plants, were similar to the symptoms produced by *V. dahliae* inoculated plants but with a disease severity scale of 3 (moderate wilt and necrosis). Plants inoculated with *V. tricorpus* isolates showed mild symptoms on potato 3 wai, then at 6 weeks, plants showed moderate wilt with severe

Table 7 Verticillium wilt severity recorded on potatoes cvs Shepody and Ranger Russet six weeks after inoculation

Treatment	Disease severity in potato*			
	2011		2012	
	Shepody	Ranger Russet	Shepody	Ranger Russet
VICVd36	3.75 ± 0.25b	1.75 ± 0.25d	3 ± 0b	1.75 ± 0.25bc
VICVd54	1.75 ± 0.25d	0.5 ± 0.28e	1.75 ± 0.25c	0.5 ± 0.28d
VICVd85	3.75 ± 0.25b	3 ± 0c	1.75 ± 0.25c	2 ± 0bc
SAVd7	3.75 ± 0.25b	2.75 ± 0.25c	1.75 ± 0.25c	1.5 ± 0.28c
SAVd16	3 ± 0c	2.75 ± 0.25c	1.75 ± 0.25c	2 ± 0bc
SAVd12	3.75 ± 0.25b	1.75 ± 0.25d	3 ± 0b	2.25 ± 0.25b
TASVd24	4.75 ± 0.25a	3.75 ± 0.25b	3 ± 0b	2 ± 0bc
TASVd25	4.75 ± 0.25a	4.75 ± 0.25a	3.75 ± 0.25a	4 ± 0a
TASVd27	5 ± 0a	3 ± 0c	4.25 ± 0.25a	2.25 ± 0.25b
Control	0 ± 0e	0 ± 0e	0 ± 0d	0 ± 0d

*Four plants were used in each treatment. Values are means ± SEM, means with the same letter within a column are not significantly different ($P < 0.05$) as analysed by one-way ANOVA. Disease severity index was rated on each plant with the following scale: 0 = no symptoms, 1 = chlorosis of lower leaves, 2 = moderate (30–50% of leaves) wilt with severe chlorosis, 3 = moderate wilt and necrosis, 4 = severe (more than 50% of leaves) wilt and necrosis, and 5 = death of plant (Tsror and Hazanovsky 2001)

chlorosis (rating 2) (Table 9). All the *Verticillium* spp. were pathogenic to potato and were re-isolated from the petioles of plants. The pathogen was not reisolated from non-inoculated control plants.

Discussion

Seed lot incidence

The incidence of infection by *Verticillium* spp. in seed lots was high in the seed tubers originating from various cultivars and geographic locations in Victoria and Tasmania. Based on morphological characters and molecular analysis, three *Verticillium* spp. were identified viz., *V. dahliae*, *V. albo-atrum* and *V. tricorpus*.

Overall infection by *V. dahliae* in the seed lots was around 28%, which is similar to the reports in Turkey and North America where nearly 30% of seed lots were infected with *V. dahliae* (Göre et al. 2015; Omer et al. 2000). Infected certified seed tubers can readily transmit significant levels of *Verticillium* spp. into potato growing regions and establish new infection (Easton et al. 1972; Krikun and Orion 1979). The high percentage rate in the seed tuber lots in Victoria and Tasmania may have indicated that *V. dahliae* was widely distributed across all Australian potato growing areas. Pegg and Brady (2002) reported that *Verticillium* spp. can be isolated from infected potatoes growing in temperate and subtropical zones of the USA. There was a higher frequency of *V. albo-atrum* in Tasmanian seed potatoes (21.7%) than in Victoria. However, overall percent infection of seed lots in Victoria and Tasmania were similar at 7%. This was the first report of *V. albo-atrum* detected in potato in Australia for over 47 years. In 1967, *V. albo-atrum* was identified as a causal organism of Verticillium wilt of potatoes in Australia (Harrison 1967). However, surveys by Harding and Wicks (2007) reported that only *V. dahliae* was isolated from the

Table 8 Verticillium wilt severity and the effect of *V. dahliae* isolates on eggplant (cv Black Beauty) height (cm), aerial biomass (g) and root weight (g) seven weeks after inoculation

Treatment	2011				2012			
	Disease severity*	Plant height	Arial biomass (dry weight)	Root biomass (dry weight)	Disease severity	Plant height	Arial biomass (dry weight)	Root biomass (dry weight)
VICVd36	2.5 ± 0.28 ^{B}	9.0 ^{B}	4.23 ^{BC}	1.25 ^{B}	1.75 ± 0.25 ^{B}	13.7 ^{D}	1.40 ^{BC}	0.65 ^{BC}
VICVd54	1.75 ± 0.25 ^{B}	9.2 ^{B}	3.86 ^{BCD}	1.26 ^{B}	0.5 ± 0.289 ^{C}	15.7 ^{BC}	1.90 ^{B}	0.85 ^{B}
VICVd85	1.75 ± 0.25 ^{B}	10.1 ^{B}	2.90 ^{BCD}	0.74 ^{BCD}	1.75 ± 0.25 ^{B}	13.7 ^{D}	1.50 ^{BC}	0.65 ^{BC}
SAVd7	1.75 ± 0.25 ^{B}	9.5 ^{B}	3.12 ^{BCD}	0.78 ^{BCD}	1.75 ± 0.25 ^{B}	14.2 ^{CD}	1.80 ^{B}	0.80 ^{B}
SAVd16	1.75 ± 0.25 ^{B}	10.0 ^{B}	4.70 ^{B}	1.21 ^{BC}	1.75 ± 0.25 ^{B}	16.2 ^{B}	1.40 ^{BC}	0.67 ^{BC}
SAVd12	2.5 ± 0.28 ^{B}	6.6 ^{B}	2.20 ^{D}	0.55 ^{DE}	1.75 ± 0.25 ^{B}	14.7 ^{BCD}	1.70 ^{BC}	0.75 ^{B}
TASVd24	2.5 ± 0.28 ^{B}	9.8 ^{B}	2.50 ^{CD}	0.61 ^{CDE}	2 ± 0 ^{B}	13.5 ^{DE}	1.40 ^{BC}	0.75 ^{B}
TASVd25	4 ± 0.40 ^{B}	4.1 ^{C}	0.28 ^{E}	0.08 ^{E}	4 ± 0 ^{A}	10.0 ^{F}	0.80 ^{C}	0.35 ^{C}
TASVd27	1.75 ± 0.25 ^{B}	10.2 ^{B}	3.88 ^{BCD}	1.06 ^{BCD}	2 ± 0 ^{B}	11.7 ^{EF}	1.40 ^{BC}	0.57 ^{BC}
Control	0 ± 0 ^{C}	14.2 ^{A}	8.17 ^{A}	2.65 ^{A}	0 ± 0 ^{C}	18.2 ^{A}	4.10 ^{A}	1.50 ^{A}

*Four plants were used in each treatment. Disease severity values are means ± SEM. Means with the same letter within a column are not significantly different ($P < 0.05$) as analysed by one-way ANOVA. Disease severity index was rated on each plant with the following scale: 0 = no symptoms, 1 = chlorosis of lower leaves, 2 = moderate (30–50% of leaves) wilt with severe chlorosis, 3 = moderate wilt and necrosis, 4 = severe (more than 50% of leaves) wilt and necrosis, and 5 = death of plant (Tsror and Hazanovsky 2001)

Table 9 Verticillium wilt severity (0–5 scale) (Tsror and Hazanovsky 2001) recorded on potatoes cv Shepody with different *V. dahliae* (TASVd25 and VICVd54), *V. albo-atrum* (VICVaa1 and TASVaa1) and *V. tricorpus* (TASVt1 and TASVt2) isolates compared to a non- inoculated control six weeks after inoculation

Isolates	Species	Disease severity*
TASVd25	*V. dahliae*	4.25 ± 0.25 ^{A}
VICVd54	*V. dahliae*	1.75 ± 0.25 ^{C}
TASVaa1	*V. albo-atrum*	2.75 ± 0.25 ^{B}
VICVaa1	*V. albo-atrum*	2.75 ± 0.25 ^{B}
TASVtr1	*V. tricorpus*	1.75 ± 0.25 ^{C}
TASVtr2	*V. tricorpus*	1.5 ± 0.28 ^{C}
Control	–	0 ± 0 ^{D}

*Four plants were used in each treatment. Values are means ± SEM, means with the same letter within a column are not significantly different ($P < 0.05$) as analysed by one-way ANOVA

petiole of infected plants in South East Australia. Powney et al. (2005) identified *V. dahliae, V. tricorpus* and *G. nigrescens* from infected potatoes in Australia implicating *V. dahliae* as the major pathogen of potato in South East Australia. According to the Australian Department of Agriculture, Fisheries and Forestry (DAFF), *V. albo-atrum* was present in South Australia, Tasmania, Victoria and Queensland (DAFF 2012). However, work by Walker (1990) authenticated the records only for potato from South Australia, Tasmania and Victoria. *V. albo-atrum* is a major pathogen of lucerne, hops and some vegetables including tomato, eggplant and cucurbits (Jabnoun-Khiareddine et al. 2006; Kim et al. 2001).

Verticillium tricorpus was also identified from the vascular tissue of seed tubers from Victoria (1.6%) and Tasmania (13%) with the overall infection of 4.8%. In UK, MacGarvie and Hide (1966) found 72% of infected seed stock contained *V. tricorpus*, but not *V. dahliae* or *V. albo-atrum*. Similarly *V. tricorpus* was identified from potato tubers in Japan and was found to be mildly pathogenic to potato (Ebihara et al. 2003).

The fact that pathogenic *Verticillium* spp. were readily found in the certified seed lots in Victoria and Tasmania indicated the likely movement of infected seed tubers to commercial production areas across Australia. However, in Australia the seed certification scheme does not require testing for *Verticillium* spp. The reason for not testing for *Verticillium* in the seed tuber might have been due to the difficulties of assessing the pathogen in the internal vascular region of seed tuber on a large scale (Harding and Wicks 2007). Once the pathogen has been introduced into a field, it can be moved from field to field during distribution, transport and planting of seed tubers (Dung et al. 2013).

Verticillium dahliae was recovered from tubers with discoloured stem-end vascular tissue as well as from tubers without discoloured vascular tissue. However a higher chance of *V. dahliae* recovery was observed from tubers with discoloured stem-end vascular tissue, suggesting that tuber stem end vascular discolouration symptoms were not a reliable indication of Verticillium wilt infection. This was similar to the observation of McKay (1926) who reported that tuber stem end discolouration was not reliable for the separation of infected from healthy tubers. Vascular discoloration can result from stress, physiological or other pathogens unrelated to Verticillium wilt disease (Rowe and Powelson 2002; Baribeau 1952; Isaac and Harrison 1968; Thanassoulopoulos and Hooker 1968). A study by Wang and Bethke (2013) reported that *V. dahliae* infected plants had a higher chance of severe stem-end chip defect, which is a serious quality concern for the USA potato chip industry.

Morphology and phylogenetic analysis

Based on cultural and morphological characteristics, ITS phylogeny, and pathogenicity assays, *V. dahliae*, *V. albo-atrum* and *V. tricorpus* were confirmed as the major Verticillium pathogens of potatoes in SE Australia. The taxonomic description of these species was similar to the published descriptions of *Verticillium* spp. (Hawksworth 1970; Hawksworth and Talboys 1970a, 1970b; Zare et al. 2007; Inderbitzin et al. 2011a).

Morphological groupings (based on the size of conidia, formation of resting structures, and colour of colonies on PDA) were in concordance with phylogenies derived from the molecular data. However, there was no significant difference between the conidial size among the four morphological groups making conidial size morphology a poor descriptor for separating species. Previous studies have also shown the lack of variation in morphology of conidia as inadequate to separate *Verticillium* spp. (Rowe 1995; Inderbitzin et al. 2011a; Jabnoun-Khiareddine et al. 2010).

Variability in culture characteristics of *V. dahliae* was high within the SE Australian isolates grown on PDA medium with microsclerotia being the most variable ranging from scarce to abundant; and mycelium varying from cottony and dense, to thin and floccose. Similar findings have been previously observed for *V. dahliae* (Katan 2000; Jabnoun-Khiareddine et al. 2010; Blanco-Lopez et al. 2005). *Verticillium albo-atrum* produced dark resting mycelia, no microsclerotia while *V. tricorpus* formed brown colonies on PDA. In some *V. tricorpus* isolates, occasionally yellow colonies were observed on PDA. *Verticillium tricorpus* produced three types of resting structures: chlamydospores, dark resting mycelia and microsclerotia. The microsclerotia of *V. tricorpus* were irregular in shape compared to *V. dahliae* microsclerotia. Differences in morphology of *V. tricorpus* have also been noted by Korolev and Katan (1999), Qin et al. (2008) and Jabnoun-Khiareddine et al. (2010). Walker (1990) reported that earlier identifications may have failed to distinguish between *V. dahliae* and *V. albo-atrum*, hence assumed that

diseases reported as Verticillium wilt were caused by *V. albo-atrum*.

Molecular characterisation using ITS sequences validated the taxonomy of *V. dahliae, V. albo-atrum* and *V. tricorpus* based on traditional morphological characters. The ITS phylogenetic tree revealed three monophyletic groups corresponding to these three species which was similar to the phylogenetic tree produced by Inderbitzin et al. (2011a) and Hyde et al. (2014). The ITS gene sequences of Australian *Verticillium* spp. were similar to the type or ex-type strains designated for *Verticillium* spp. by Inderbitzin et al. (2011a). According to Inderbitzin et al. (2011a) ITS sequence could distinguish 9 *Verticillium* spp. except *V. longisporum*. *Verticillium longisporum* isolates only had one ITS allele consistent with all other *Verticillium* spp. and hence this gene sequence could not retrace the evolution of this species (Inderbitzin et al. 2011b). Neither the four gene phylogenetic analysis nor the single ITS phylogenetic tree were able to differentiate *V. longisporum* alleles D2 and D3 from *V. dahliae* (Inderbitzin et al. 2011b). Nevertheless, the phylogenetic tree based only on ITS provided better discrimination to differentiate *Verticillium* spp. (Hyde et al. 2014). The phylogenetic tree also confirmed the relationship of *G. nigrescens* to the *Verticillium* spp. There has been some debate as to the identity and phylogeny of *G. nigrescens* which was regarded as *Verticillium nigrescens* until recent taxonomic studies considered this as a separate species (Inderbitzin et al. 2011a; Zare et al. 2007).

Pathogenicity

Verticillium dahliae isolates from different geographical locations varied in pathogenicity on both potato cultivars and eggplant. Isolates caused higher disease severity in cv Shepody (susceptible) than in the cv Ranger Russet (moderately resistant) which was consistent to the results obtained by Arbogast et al. (1999) and Jansky (2009) who showed variation in cultivar resistance to Verticillium wilt. The disease severity was higher in potato than on eggplant. Less disease severity in the second experiment may have been due to the time of the year when length of daylight was shorter and cooler weather conditions which may have slowed down the rate of infection and colonisation. Nevertheless, the ranking of isolates based on pathogenicity was maintained in both experiments with TASVd25 the most aggressive in both potato and eggplant. These results were in agreement with other studies reporting pathogenic variability within *V. dahliae* isolates on a range of hosts (Strausbaugh 1993; Resende et al. 1994; Daayf et al. 1995; Dobinson et al. 2000; Uppal et al. 2007). Mansoori et al. (1995), Meyer et al. (1994) and Palmer et al. (2005) reported that pathogenic variability of *V. dahliae* was related to pathogenic factors such as toxins which induced the symptoms in host plants.

The majority of *V. dahliae* isolates from potato were highly pathogenic in eggplant especially the Tasmanian *V. dahliae* isolate (TASVd25). Compared to the symptom severity in potatoes, eggplants showed less disease severity in both trials. In eggplant in 2011, the highly pathogenic isolate caused severe disease hence plant height, aerial biomass and root weight were reduced. Bhat and Subbarao (1999) reported that disease scoring in combination with measuring growth parameters could provide an accurate evaluation of the pathogenicity of *V. dahliae* in various host plants. Eggplant has been shown to be a preferential host for *V. dahliae* (Resende et al. 1994; Mansoori et al. 1995; Jabnoun-Khiareddine et al. 2006) and eggplant seedlings have been used in pathogenicity trials for *Verticillium* spp. (Korolev et al. 2000) because disease development is rapid in this host. The eggplant seedling bioassay was less time consuming and more cost effective compared to using tissue culture generated potato plants in a pathogenicity screening trial. *Verticillium* has a wide host range and isolates vary in their level of pathogenicity on a particular host and even cultivars within a species (Alkher et al. 2009; Bhat and Subbarao 1999). Cross-pathogenicity studies of the isolates from different host plants in Australia needs to be conducted with a broad range of isolates and hosts.

In potato cv Shepody, disease severity was generally higher for *V. dahliae* and *V. albo-atrum* compared to *V. tricorpus* isolates which were less aggressive. However, although the disease severity caused by *V. tricorpus* was substantially lower, wilting did occur on infected plants similar to the degree of wilting of the less aggressive isolates of *V. dahliae*. This supported previous studies that showed that *V. tricorpus* caused low Verticillium wilt symptoms (Heinz and Platt 2000; Robinson et al. 2006; Nair et al. 2015). *Verticillium tricorpus* was isolated from the petioles of the infected plants, which was in contrast to the finding of Mahuku et al. (1999) who reported that *V. tricorpus* was unsuccessful in colonizing the potato stems. In other reports, *V. tricorpus* was considered as a soil saprotroph and a weak pathogen of tomato, potato, eggplant and other plants (Isaac 1967; Pegg and Brady 2002; Barbara and Clewes 2003; Ebihara et al. 2003).

In conclusion, seed tuber surveys from South East Australia identified three *Verticillium* spp. (*V. dahliae, V. albo-atrum* and *V. tricorpus*) infecting the stem end vascular tissue of seed tubers with an overall incidence of around 30%. The species were identified by morphological characteristics and phylogenetic analysis of the ITS region. *V. dahliae* isolates varied in pathogenicity on both potato cultivars (Shepody and Ranger Russet). All *Verticillium* spp. were pathogenic on potato cv. Shepody. In eggplants, majority of *V. dahliae* isolates from potato were highly pathogenic and significantly reduced plant height, aerial biomass and below ground root mass. The eggplant seedling bioassay was less time consuming and more cost effective compared to using tissue culture generated potato plants in a pathogenicity screening trial hence can be used as a bait plant for Verticillium wilt pathogenicity studies.

Acknowledgments We would like to thank Fran Richardson, Mark Wardzynski for helping glasshouse evaluation of isolates and Dr. Jacqueline Edwards and Dr. Dolf de Boer, DJPR, Victoria for constructive discussion. The research was a part of a multi-pronged research drive through the Australian Potato Research Program (Phase 2), funded by Horticulture Innovation Australia Limited using the processing potato industry levy and matched funds from the Federal Government.

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