

***Ralstonia solanacearum* species complex in Australia**

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Abstract

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The *Ralstonia solanacearum* species complex (RSSC) causes vascular wilt of many crops and is considered one of the most destructive plant pathogenic bacteria worldwide. The species complex was recently resolved into a stable taxonomy of three species aligning with the previously determined phylotypes, namely *R. solanacearum* (phylotype II), *R. pseudosolanacearum* (phylotype I and III), and *R. syzygii* (phylotype IV). Knowing which *Ralstonia* species and subspecies are established in Australia is important to Australia's biosecurity and market access. The goal of this study was to analyse Australia's *Ralstonia* culture collections and to assign the isolates to the modern taxonomic groups. The results shed light on the identity, distribution, and pathogenicity of the *Ralstonia* strains in Australia. *Ralstonia solanacearum*, *R. pseudosolanacearum* phylotype I, and *R. syzygii* phylotype IV-11 are present in Australia but have limited geographic ranges. We identified two aberrant RSSC strains that have genetic similarity to *R. syzygii* based on sequevar analysis, but do not yield a phylotype IV multiplex PCR band, similar to the known aberrant strain ACH732. The aberrant strains may represent a novel species. Three new sequevars were determined, 72, 73 and 74. Several *Ralstonia* lineages remain undetected in Australia, providing evidence that they are absent. These include *R. pseudosolanacearum* phylotype III and the phylotype I mulberry infecting strains; *R. solanacearum* strains IIC and the Moko causing strains; and *R. syzygii* subsp. *celebesensis*, and *R. syzygii* subsp. *syzygii*. This study fulfilled Koch's postulates for the Australian strains, *R. solanacearum* wilted potato plants, and *R. pseudosolanacearum* wilted

blueberry plants, the hosts from which they were initially isolated.. The data supports the hypothesis that Australia has native and introduced strains of *Ralstonia*.

Keywords: Prokaryotes, Tropical plants, Vegetables, Pathogen diversity

Introduction

Members of the *Ralstonia solanacearum* species complex (RSSC) cause bacterial wilt and are considered one of the most destructive plant pathogenic bacteria globally (Mansfield et al. 2012). The complex causes wilt in over 390 plant species in over 75 families (Lowe-Power et al. 2020). Strains in the RSSC express different phenotypes; some strains have wide host ranges, and others are more host-specific with narrow host ranges. *Ralstonia*'s wide host range and global distribution make quantifying its economic impact difficult. Several strains cause specific diseases that result in significant yield loss and economic impact. For example, worldwide losses on potatoes due to brown rot caused by the IIB-1 strain are estimated to be around US\$1 billion annually (Elphinstone 2005).

Taxonomists and plant pathologists have historically struggled to create a functional classification system for the heterogeneous RSSC. The early classification systems were based on phenotype, and isolates were classified into races based on host range and biovar based on the pattern of carbon acidification (Buddenhagen et al. 1962; Hayward 1964). Although they persist in regulatory statutes, the race and biovar sub-categorization systems (Table 1) are now defunct because they do not align with the robust phylogenetic divisions revealed by whole-genome sequence analysis (Fegan and Prior 2005; Lowe-Power et al. 2020; Remenant et al. 2011; Sharma et al. 2022). The phenotype systems were of little practical use and were first replaced by the early molecular classification systems, including the RFLP Divisions (Table 1), and were superseded when the group was classified into molecular groupings called phylotypes and sequevars (Fegan and Prior 2005).

Recent studies based on gene sequencing more accurately reflect the evolutionary history of the lineages. This approach separated the strains into four broad genetic groups called phylotypes (Fegan and Prior 2005). Safni's polyphasic revision of this diverse group resulted

in a stable taxonomic framework of three species, encompassing the four phlotypes: *R. solanacearum*, corresponding to phlotype II; *R. pseudosolanacearum*, corresponding to phlotypes I and III; and *R. syzygii*, corresponding to phlotype IV (Safni et al. 2014). *Ralstonia syzygii* was further separated into three subspecies, namely, *R. syzygii* subsp. *celebesensis* (Blood disease of banana), *R. syzygii* subsp. *syzygii* (Sumatra disease of clove), and *R. syzygii* subsp. *indonesiensis* (bacterial wilt of Solanaceae and clove) (Safni et al. 2014).

The phlotypes were divided into sequevars or sequence variants as a within-species level classification system, and these are groups of isolates clustering based on analysis of the partial endoglucanase gene sequences (Fegan and Prior 2005). This sequevar system was broadly adopted, and strains from around the world were assigned in over 195 papers to 68 sequevars (Lowe-Power et al. 2020). However, Sharma's (2022) study has demonstrated that the sequevar system breaks down in the highly recombinogenic phlotype I (Sharma et al. 2022). Thus far, the sequevar system remains congruent to whole-genome phylogenetics in phlotype II and for some sequevars in the other phlotypes. Cellier et al. recently published a standardized protocol that should be used to reproducibly assign sequevars to strains based on *egl* sequences (Cellier et al. 2023). The *Ralstonia* complex is naturally DNA competent or naturally transformable, and the observed polyphyletic sequevars in phlotype I may be the result of horizontal gene transfer and recombination confounding the single gene-based sequevar phylogenetic system (Coupat et al. 2008; Lowe-Power et al. 2020; Wicker et al. 2012).

Classifying bacteria into a stable species-level nomenclature system remains challenging for any genera, and the within-species classification systems continue to be a minefield. The *R. solanacearum* species complex is no exception. Sharma's recent study developed a whole genome-based within-species level system, which assigns Life Identification Numbers (LINs) to *Ralstonia* strains (Sharma et al. 2022). This new LINs system requires whole genome

sequencing and has not yet been universally adopted. Sharma's 2022 paper identifies within-species phylogenetic groupings that correlate to LINs, but the groupings do not appear to circumscribe specific strains with distinct phenotypes associated with a particular disease. Several diseases associated with specific strains of *Ralstonia* remain absent from Australia and are of biosecurity concern, including Blood disease of banana (*R. syzygii* subsp. *celebensensis*), Sumatra disease of clove trees (*R. syzygii* subsp. *syzygii*), mulberry wilt (*R. pseudosolanacearum*, phylotype I), and Moko disease of banana (several *R. solanacearum* phylotype IIA & IIB lineages). The identity of these diseases, are based upon genotype and phenotype, and their presence can be confirmed using specific molecular diagnostic tests (Cellier et al. 2015; Pan et al. 2013; Rincón-Flórez et al. 2022; Trianom et al. 2018).

Australia's *Ralstonia* collections date back to the 1960s and originated from various host genera. However, the collection's identifications require revision using the most recent and universally accepted taxonomic classification systems. Consequently, this work aims to provide an up-to-date specimen-based inventory for the *Ralstonia* strains associated with plant hosts in Australia. Therefore, we sought to answer the following questions: i) What is the identity and geographic distribution of the *Ralstonia* strains in Australia? ii) What plant species have been associated with *Ralstonia* in Australia? iii) Are the Australian *Ralstonia* species pathogenic, and do they fulfil Koch's postulates? Answering these questions provides baseline data to improve biosecurity decision-making and underpins future Australian studies into this group of bacteria.

Materials and Methods

Origin, growth conditions, biochemical testing and storage of bacterial isolates

Origin of isolates. Isolates derived initially from plant hosts in Australia were retrieved from the NSW Plant Pathology and Mycology Herbarium (DAR) and the Plant Pathology Herbarium, QLD (BRIP). Generally, the 170 isolates retrieved with meta-data were listed as *Ralstonia solanacearum*, or *Ralstonia solanacearum* species complex, and some were identified to biovar (Table 2 and Supplementary table 1). The cultures were stored on cryobeads (BRIP) or as freeze-dried ampoules (DAR).

Growth media, conditions, and storage of isolates. The bacteria were revived, maintained, and isolated from plant material using Kelman's tetrazolium chloride medium (TZC) in Petri dishes (Kelman 1954). The TZC medium is casamino acid peptone glucose medium (CPG; 10 g L⁻¹ Peptone, 1 g L⁻¹ Casein hydrolysate, 5 g L⁻¹ D-Glucose, 16 g L⁻¹ Bacto agar) with the addition of 50 mg L⁻¹ 2,3,5 triphenyl tetrazolium chloride. The isolates were grown on CPG medium in Petri dishes for approximately 24 hours before biochemical testing, DNA extraction, inoculum preparation, or storage. The inoculated Petri dishes were incubated in the dark at 28 °C ± 1 °C. The isolates were stored in 2 mL microtubes containing sterile water at room temperature, and in cryovials (Microbank™, Pro-lab Diagnostics™, Canada) at – 80 °C ± 2 °C.

Culture morphology and Biochemical testing. The cultures were visually assessed after two days of incubation on TZC medium as fluidal, mutated, dry, flat, small colonies or large colonies, or a combination to assist with initial isolate screening and determine the likelihood of pathogenicity. On TZC medium, pathogenic RSSC cultures are usually fluidal and pink. However, some variation exists, and non-pathogenic mutants produce smaller red colonies that are non-fluidal (Fig. 1). *Ralstonia* isolates are Gram-negative and oxidase-positive. Therefore, the isolates were presumptively identified as Gram-negative using the potassium hydroxide test (Schaad et al. 2001), and as oxidase-positive using oxidase detection strips (MB0266, Oxoid™, Thermo Fisher Scientific™, United States).

DNA extraction and molecular identification

DNA extraction. DNA was extracted using the Isolate II genomic DNA Kit (Meridian Bioscience®, Cincinnati, US). Extraction was from single colony cultures according to the manufacturer's instructions with an additional step to prevent spin column blockage; the sample was centrifuged for 1 min at 2,700 g prior to the supernatant transfer to the DNA spin column.

Phylotype-specific PCR. To identify the isolates retrieved from the culture collection and the re-isolated bacterial cultures, the multiplex phylotype PCR was conducted to confirm that isolates are within the RSSC and to assign phylotype (Fegan and Prior 2005; Opina et al. 1997). Specifically, the PCR reaction contained 5 µL of the primer mix (Supplementary table 2), 12.5 µL of MyFi™ Mix (Meridian Bioscience®, Cincinnati, US), and from 1 ng to 10 ng of template DNA in a 25 µl final reaction volume. The cycling conditions were an initial denaturation step of 95 °C for 2 min, and 30 cycles of 95 °C for 20 s, 59 °C for 30 s, and 72 °C for 20 s, followed by a final elongation at 72 °C for 3 min. The amplified PCR products were visualised using gel electrophoresis, and the amplicon length was estimated compared to a 1 Kb Plus DNA Ladder (Invitrogen™, Carlsbad, US). The isolates were assigned to phylotype based on the presence of two amplicons and their specific length. The 280-282 bp amplicon identified the isolate as a member of the RSSC, and the second amplicon identified the phylotype (Supplementary table 2).

Sequevar determination. The isolates retrieved from the culture collections were assigned to sequevar by partial endoglucanase (*egl*) gene sequencing and analysis using reference strains and standardised methodology (Cellier et al. 2023). A 750-bp amplicon from the partial *egl* gene was amplified and sequenced using the primers Endo-F (5'-ATGCATGCCGCTGGTCGCCGC-3') and Endo-R (5'-GCGTTGCCCGGCACGAACACC-3') according to previously published methodology (Fegan et al. 1998). Specifically, the PCR

reaction contained 0.4 μM of each primer, 12.5 μL of MyFi™ Mix (Meridian Bioscience®, Cincinnati, US), and from 1 ng to 10 ng of template DNA in a 25 μl final reaction volume. The cycling conditions were an initial denaturation step of 95 °C for 2 min, and 30 cycles of 95 °C for 20 s, 70 °C for 20 s, and 72 °C for 20 s, followed by a final elongation at 72 °C for 3 min. The PCR products were visualised by gel electrophoresis and sent for direct Sanger sequencing (AGRF, Sydney, Australia). The sequence data was assembled, trimmed and analysed using Geneious Prime® v. 2023. The isolates were assigned to sequevar, and the *egl* sequence data was deposited into the National Center for Biotechnology Information (NCBI) database and assigned accession numbers (Supplementary table 1).

16S rRNA gene region sequencing. The isolates that amplified a single amplicon in the phylotype PCR confirming RSSC were termed *Ralstonia* aberrant strains (BRIP 60866 and DAR 65892), and to confirm the phylogeny of these strains and the phylotype IV strain (DAR 77793), the 16S rRNA gene was amplified, sequenced and analysed. A 1450-bp amplicon from the 16S rRNA gene region was amplified and sequenced using the primers 27Fw (5'-AGAGTTTGATCMTGGCTCAG -3') and 1492Rv (5'-GGTTACCTTGTTACGACTT -3') (Lane 1991), and the same PCR reaction as for the *egl* region described previously. The cycling conditions were an initial denaturation step of 95 °C for 2 min, and 30 cycles of 95 °C for 20 s, 55 °C for 20 s, and 72 °C for 25 s, followed by a final elongation at 72 °C for 4 min. The PCR products were visualised, sequenced, assembled and trimmed as previously described. The 16S rRNA sequences were deposited and assigned accession numbers in the NCBI database (Supplementary table 3). A phylogenetic analysis of the 16S rRNA region was conducted for the *Ralstonia* aberrant strains (BRIP 60866 and DAR 65892) and the phylotype IV strain (DAR 77793) using sequence data downloaded from the NCBI database (Supplementary table 3). The sequences were trimmed and aligned using MUSCLE® and analysed with the Geneious Tree Builder® using the Tamura-Nei® genetic distance model, 1000 bootstraps, and *Ralstonia*

pickettii – type strain (LMG 6866) as the outgroup to build a neighbour-joining consensus tree in Geneious prime[®] v. 2023.

Isolate pathogenicity and Koch's postulates

Koch's postulates were conducted using a representative strain from each *Ralstonia* species identified from Australia and the two aberrant RSSC isolates to test disease causality. The pathogenicity of the isolates was tested using the same host from which they were initially isolated, and tomato, as the tomato plant, is considered a near-universal host and is susceptible to a wide range of strains (EPPO 2018). The isolates used for pathogenicity testing were *Ralstonia solanacearum* IIB-1 (DAR 49320) from potato, *Ralstonia pseudosolanacearum* I-18 (DAR 77786) from blueberry, *Ralstonia syzygii* IV-11 (DAR 77793) from blueberry, *Ralstonia solanacearum* species complex IV-74 (DAR 65892) from tomato, and *Ralstonia solanacearum* species complex IV-74 (BRIP 60866) from potato.

Plants. Tomato plants (*Solanum lycopersicum*) 'Gross lisse' obtained from The Plantsmith, Darwin, Australia, were grown in potting mix round in 11-cm diameter by 12-cm deep pots. Blueberry plants (*Vaccinium corymbosum x ashei x darrowi*) 'Sunshine blue' obtained from Plants Direct NT, Darwin, Australia, were grown in potting mix in round 20-cm diameter by 18-cm deep pots. Potato plants (*Solanum tuberosum*) 'Nadine' were grown from certified seed potatoes (Bloomin' Bulbs, Monbulk, Victoria, Australia). The potato tubers were sprouted in potting mix in round 100-cm diameter by 100-cm deep pots and thinned to one plant per pot. The plants were grown in a glasshouse (Berrimah Farm Science Precinct, Darwin, Australia) with a diurnal temperature range of 26 °C to 29 °C and exposed to natural light. The plants were fertilised (Osmocote[®] Landscape formula all-purpose, Scotts[™], Marysville, US), and watered regularly. The tomato plants were 25 to-40-cm tall and at the 6-9 leaf stage, the

blueberry plants were approximately 15-cm tall, and the potato plants were 12 to-15-cm tall and at the 7-9 leaf stage when inoculated to assess isolate pathogenicity.

Inoculum. The bacterial isolates were revived from the cultures stored in microtubes containing sterile water. The isolates were revived by streaking onto TZC medium in Petri plates. After 2-3 days of incubation, fluidal colonies were preferentially selected and grown on CPG medium in Petri plates for 1-day. The bacterial colonies grown on GPG medium were suspended in sterile deionised water and adjusted to an absorbance value of approximately 0.02 at OD600 using a spectrometer (BioPhotometer®, Eppendorf™, Hamburg, Germany), equating to a concentration of approximately 10^7 CFU.mL⁻¹. Following the inoculation, the inoculum was streaked onto TZC to confirm viability.

Inoculation. The plants were not watered from the day before to two days after inoculation. The roots of the plants were wounded by pushing a knife with a 2.5-cm wide by 16-cm long blade to the base of the pot 1-to 2-cm away from the stem, at two locations on the same side of the plant. Immediately following wounding, 200 mL of the inoculum at approximately 10^7 CFU.mL⁻¹ was poured over the wounded roots.

Each of the tested isolates (DAR 49320, DAR 77786, DAR 77793, DAR 65892, BRIP 60866) was inoculated onto a tomato plant and their original host, if not a tomato plant, and sterile deionised water was used as a negative control. Plants were placed into individual drip trays to prevent cross-contamination. Each treatment included 3 plants, and the whole experiment was repeated once, resulting in a total of 6 plants per treatment.

Disease assessment. Following inoculation, the plants were assessed daily for the first 2-weeks, then weekly for the development of external symptoms. Plants were considered diseased when visible external symptoms of wilt, leaf discolouration, or leaf necrosis developed. Following

symptom development, at least two plants from each treatment were processed for bacterial isolation. Asymptomatic plants were harvested after 4 weeks for tomato and potato and after 14 weeks for blueberry to assess for the development of internal symptoms, and isolations were completed to determine if the inoculated bacteria were present, thus confirming host colonisation. The pathogenicity of the *Ralstonia* strain is described as virulent or latent (Cellier and Prior 2010). A strain is considered virulent to a particular host plant if it causes wilt symptoms on at least one inoculated plant, and colonised plants that remain asymptomatic are latent infections (Cellier and Prior 2010).

Bacterial re-isolation from plant tissues and confirmatory PCR. For isolation, a 10-cm stem section beginning at the soil line was removed from the base of the plant; it was briefly dipped in 70% ethanol, rinsed in sterile distilled water, and dried with a paper towel. A 1-cm section of stem material from approximately 3-cm above the soil level was selected using aseptic techniques. The section was cut into small pieces, placed into a 7-mL vial containing 3-mL of sterile water, and incubated for 5 to 10-minutes. Approximately 40 μ L of the inoculum was streaked using a loop onto a Petri dish containing TZC medium and incubated at $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 2 to 3-days to obtain single colonies. A single colony with *Ralstonia*'s distinctive growth morphology (Fig. 1) was selected and streaked onto the CPG medium in a Petri dish and incubated for 24 hours before biochemical testing and DNA extraction, as described above. To complete Koch's postulates, the isolated bacteria were identified using the phylotype-specific PCR as described above.

Results

The 170 accessions retrieved from the Australian collections resulted in the identification of 153 strains within the *Ralstonia solanacearum* species complex (Table 2); the remainder were not viable or were not *Ralstonia*. Three phlotypes were detected, aligning with the RSSC species, namely, *R. pseudosolanacearum* (phlotype I), *R. solanacearum* (phlotype II), and *R. syzygii* (phlotype IV) using the phlotype-specific PCR (Table 2). Phlotype III was not detected in the accessions.

Ralstonia pseudosolanacearum was the most common species detected, with 124 isolates identified from the Northern Territory (NT), Queensland (QLD), and New South Wales (NSW) (Table 2 and Fig. 2). *Ralstonia solanacearum* was identified from 26 isolates originating from QLD, NSW, South Australia (SA), and Victoria (VIC) (Table 2 and Fig. 2). Only one isolate of *R. syzygii* was identified from the accessions and originated from NSW (Table 2 and Fig. 2).

Two isolates (DAR 65892, BRIP 60866a) produced only one band in the phlotype-specific PCR of 280-282 bp, indicating they were members of the RSSC. These strains failed to amplify a band that identifies their phlotype grouping. The phylogenetic analysis of the 16S rRNA for the aberrant strains described in this study, DAR 65892 and BRIP 60866a, and the previously described strain ACH732 suggested that they do not align with the clades representing the four phlotypes (Fig. 3). The *Ralstonia* isolates DAR 65892, and BRIP 60866a form a clade with 92% consensus support, indicating that they were phylogenetically similar. In contrast, the *egl* sequence analysis suggested that these three strains belong in phlotype IV, ACH 732 is sequevar IV-11, and DAR 65892 and BRIP 60866a form a novel sequevar, IV-74. These three strains all originate from Northern Australia and are hereafter referred to as the aberrant RSSC isolates.

The RSSC isolates were assigned sequevars using *egl* sequence analysis (Table 2). The *egl* phylogenetic analysis concurred with the phylotype-specific PCR results, except for the aberrant strains (DAR 65892 and BRIP 60866a). The *R. pseudosolanacearum* isolates were assigned to eight different sequevars: I-13, I-16, I-17, I-18, I-33, I-44, I-46, and included two new sequevars I-72 and I-73. Most *R. solanacearum* isolates were assigned to sequevar IIB-1, except for a single isolate assigned to IIA-50. The *R. syzygii* isolate (DAR 77793) was assigned to sequevar IV-11 (Table 2).

The Australian *Ralstonia* collections date back to 1965. *Ralstonia pseudosolanacearum* sequevars I-18 and I-16, and *R. solanacearum* IIB-1 and IIA-50 were identified from Queensland (QLD) in the 1960s (Table 2). In Nambour, QLD, the strain from ginger was identified as biovar 4 (Table 2 and Supplementary table 1). . It was in the late 1970s that the collection data confirmed the presence of *Ralstonia* from the Northern Territory (NT) and New South Wales (NSW). *Ralstonia pseudosolanacearum* was identified from Western Australia (WA) in the 1960s, although it is important to note that *Ralstonia* is considered eradicated from WA (Table 2) (EPPO 2019).

Ralstonia pseudosolanacearum was associated with 31 plant host species from 17 plant families (Table 2). In contrast, the *R. solanacearum* and the RSSC aberrant strains were only associated with potato (*Solanum tuberosum*) and tomato (*Solanum lycopersicum*) (Table 2). The single isolate of *Ralstonia syzygii* IV-11 (DAR 77793) was isolated from blueberry (*Vaccinium* sp.) (Table 2). Within *R. pseudosolanacearum* there is no apparent pattern between sequevar and host, although there does appear to be an association between sequevar and geographic location (Table 2). Queensland has the largest number of strains, including three sequevars not recorded elsewhere in Australia. The NT has the second largest number of strains, including two sequevars not recorded elsewhere in Australia, and NSW and WA have the lowest number of strains and no unique sequevars (Table 2).

The inoculated isolates of *R. solanacearum* (DAR 49320) and *R. pseudosolanacearum* (DAR 77786) caused symptoms in their original hosts, potato and blueberry, respectively, and in the near-universal host indicator tomato (Table 3 and Fig. 4 and Fig. 5). Following inoculation *Ralstonia syzygii* IV-11 (DAR 77793) and the aberrant *Ralstonia* strain IV-74 (BRIP 60866) caused wilt symptoms in tomato plants but not in their original hosts (Table 3). The identity of the bacterium re-isolated from the inoculated symptomatic plants was confirmed as the inoculated strain using the phylotype-specific PCR.

Inoculation with *R. solanacearum* (DAR 49320) caused colonisation and wilt in 100% of the potato and tomato plants. In blueberry plants inoculated with *R. pseudosolanacearum* (DAR 77786) only 33% exhibited wilt symptoms, yet the bacteria was recovered from all plants. This is in contrast to the inoculated tomato indicator plants where all plants were colonised and showed wilting (Table 3). *Ralstonia syzygii* IV-11 (DAR 77793) colonised 83% of the inoculated blueberry plants, but they remained healthy, and colonised 100% of the inoculated tomato plants, causing 83% to wilt (Table 3). Inoculation of tomato plants with the aberrant *Ralstonia* isolate IV-74 (BRIP 60866) resulted in 100% colonisation and caused wilt in 83% of the plants, but the strain did not wilt the original host, potato (Table 3). The bacterium re-isolated from the colonised healthy plants was identified as the inoculated strain using the phylotype-specific PCR.

The time before the first wilt symptoms occur varied between the *Ralstonia* strains and the hosts (Table 4). Inoculation of *R. pseudosolanacearum* (DAR 77786) first caused wilt in tomato plants after 5 days, but it took 84 days for the blueberry plants to develop symptoms. *Ralstonia solanacearum* (DAR 49320), *R. syzygii* IV-11 (DAR 77793) and the aberrant *Ralstonia* isolate IV-74 (BRIP 60866) first caused wilt in tomato plants from 6-7 days following inoculation. The *Ralstonia* isolates that caused disease in potato and tomato plants produced the same set of symptoms, including leaf and plant wilt, leaf necrosis, brown staining

of vascular tissue, and plant death (Fig. 4). The symptoms in blueberry include plant stunting, leaf fall, leaf discolouration and leaf tip necrosis (Fig 5).

Discussion

The three RSSC species, *R. solanacearum* (IIB-1 and IIA-50), *R. pseudosolanacearum* (I), and *R. syzygii* (IV-11) are present in Australia but have restricted geographic ranges. The *Ralstonia* species' host range in Australia varied. *Ralstonia pseudosolanacearum* has a wide host range in contrast to *R. solanacearum* and *R. syzygii*, with narrow host ranges. The three species within the RSSC and the aberrant RSSC strain IV-74 (BRIP 60866) all caused wilt symptoms in tomato plants. *Ralstonia solanacearum* caused wilt in potato plants, and *R. pseudosolanacearum* caused wilt in blueberry plants, the hosts they were initially isolated from, thus satisfying Koch's postulates.

The collection data reveals that the first specimen-based records were from Queensland (QLD) in the 1960s of *R. pseudosolanacearum* and *R. solanacearum* and from Western Australia (WA) of *R. pseudosolanacearum*. However, anecdotal or non-specimen-based records indicate that bacterial wilt was present before the 1960's in Australia. Reports of potato brown rot presumably caused by *R. solanacearum* IIB-1 strain (syn: R3 Bv2, *Bacillus solanacearum*, *Pseudomonas solanacearum*, *Bacillus vascularum solani*) date back to 1891 in QLD, 1911 in the Koo-Wee-Rup swamp area of Victoria, and to 1919 in WA (Faggian R 2004; Hayward 1975; Herbert 1919; Simmonds 1966). *Ralstonia solanacearum* IIB-1 is the cold tolerant, narrow host range, clonal pandemic strain that has spread globally with seed potatoes from its origin in the Andes and continues to cause significant potato crop losses worldwide (Janse 1996; Wicker et al. 2012).

According to the EPPO database, *R. solanacearum* on potatoes is present in New South Wales, Northern Territory (NT), QLD, and Victoria (EPPO 2023). However, there are no anecdotal or specimen-based records of *R. solanacearum sensu stricto* in the NT (Hayward 1975; Pitkethley 1981); therefore, its presence is reported in error. South Australia was free of potato brown rot (IIB-1) until the incursion in 1982 (Akiew 1982), and since then, the disease has remained under active control (EPPO 2023). Bacterial wilt has not been detected in WA since 1987, and *R. solanacearum* and *R. pseudosolanacearum* are considered eradicated (EPPO 2019). Bacterial wilt is also not reported from Tasmania.

Hayward and Pegg (2013) assert that a consignment of ginger imported from China in 1955 was responsible for the outbreak of ginger wilt in Nambour, QLD, the same year and attribute the disease outbreak to RSSC Bv4. This conclusion concurs with the specimen-based records reported here from the 1960s that show *R. pseudosolanacearum* Bv4 was isolated from ginger plants in Nambour, QLD. The investigation in the 1960s yielded Bv4 associated with rapid wilt and Bv3 associated with slow wilt of ginger (Hayward et al. 1967; Hayward and Pegg 2013), suggesting that more than one strain was associated with ginger bacterial wilt. Pegg and Moffett (1971), also demonstrated that the strains of *R. pseudosolanacearum* that caused ginger wilt have a broad host range (Pegg and Moffett 1971).

Strains of *R. solanacearum* and *R. pseudosolanacearum* have been present in Australia prior to the first specimen based records of the 1960s. In QLD, bacterial wilt was recorded on *Zinnia elegans* and *Dahlia pinnata* in 1896 (Simmonds 1966), and the specimen based record confirms *R. pseudosolanacearum* on *Zinnia elegans* in QLD in 1977. In the NT, bacterial wilt (Bv4) was reported from capsicum, tomato, eggplant and potato from 1961 (Heaton and Benson 1968), concurring with the specimen-based records confirming the presence of *R. pseudosolanacearum* from 1977. Movement of plant materials such as potato tubers and ginger plants have been associated with long-distance dissemination of *R. solanacearum* IIB-1 and *R.*

pseudosolanacearum (Buddenhagen 1986; Kelman et al. 1994), and the importation of these crops may be responsible for subsequent disease outbreaks in Australia.

Additional bacterial wilt host-plant associations anecdotally reported in the NT and QLD are not in the specimen-based records reported here. Anecdotal records for the NT include *Bignonia magnifica*, *Canavalia ensiformis* (jack bean), *Canavalia gladiata* (sword bean), *Stylosanthes* spp. and *Acacia difficilis* (Pitkethley 1981), and for QLD include *Xanthium pungens* (Noogoora burr), *Physalis minima* (wild gooseberry), *Solanum mauritianum* (wild tobacco tree), and *Physalis peruviana* (cape gooseberry) (Pegg and Moffett 1971). The presence of anecdotal records not listed in the specimen-based list suggests that the host range in Australia could be significantly wider than reported here for *R. pseudosolanacearum*.

Ralstonia pseudosolanacearum is associated with a wide range of hosts in Australia, which concurs with previous descriptions of this species (Lowe-Power et al. 2020). This species is highly recombinogenic, contributing to its ability to infect new hosts (Wicker et al. 2012), and worldwide, it has reportedly infected approximately 95 different plant host species (Lowe-Power et al. 2020), significantly more hosts than the 31 specimen-based records here reported. This incongruence may be due to several factors, including 1) unreported hosts and anecdotal host records that lack corresponding specimens-based records, 2) host species absent from Australia, and 3) the strains present in Australia lack pathogenicity for those additional hosts.

Safni's (2014) taxonomic review of the *Ralstonia solanacearum* species complex determined that phylotype I and phylotype III strains fall within one species, namely, *Ralstonia pseudosolanacearum*. This taxonomy and nomenclature was universally accepted and adopted until Liu (2023) proposed to classify *Ralstonia solanacearum* phylotype I strains as a new species *Ralstonia nicotianae* sp. nov. (Liu et al. 2023). This proposed change in nomenclature was swiftly rejected by the *Ralstonia* research community that maintain phylotype I and

phylotype III are subgroups of *R. pseudosolanacearum* based on genomic similarity and evolutionary relationships (Lowe-Power et al. 2023). Consequently, the bacterial isolates present in Australia are named *R. pseudosolanacearum* phylotype I, not *R. nicotianae* sp. nov.

The two aberrant RSSC strains (DAR 65892 and BRIP 60866) originating from northern Australia and investigated during this study failed to amplify the phylotype band in the phylotype-specific PCR (Fegan and Prior 2005). This phenomenon has been previously described for only one strain isolated from northern Australia, ACH732 (Fegan and Prior 2005; Prior and Fegan 2005). The 16S rDNA phylogenetic alignment suggested that all three aberrant strains fall outside the previously described phylotype clades and that DAR 65892 and BRIP 60866 may represent a new clade and possibly a new phylotype and species. Philippe Prior previously suggested the name “*Ralstonia haywardii*” in honour of Alan Christopher Hayward, the Australian plant pathologist who led pioneering work on *Ralstonia* diversity. Should further phylogenetic analysis reveal that these aberrant strains clearly form a species or subspecies then we propose to name this unique group ‘*Ralstonia haywardii*’ or subsp. ‘*haywardii*’ with the type strain ACH732.

The three aberrant *Ralstonia* isolates from northern Australia are unique, and no similar isolates have been detected elsewhere in the world. These aberrant isolates may be native to Australia or introduced and whole genome sequence analysis should shed light on this. Wickers' (2012) study using the coalescent approach considered the aberrant strain ACH732 from Australia to be a single ancestral genotype. In addition, several lines of molecular evidence support the theory that the RSSC evolved from phylotype IV strains originating in Australia / Indonesia (Fegan and Prior 2005; Wicker et al. 2012). Further analysis is required to determine if the aberrant strains are ancestral to the previously described phylotypes within the RSSC, and to determine if the data supports the hypothesis that the RSSC originated from Australian strains (Wicker et al. 2012).

The taxonomy of *Ralstonia syzygii* is in need of review, as the group is more diverse than the current subspecies nomenclature of *celebesensis*, *syzygii*, and *indonesiensis* suggests. Particularly, *R. syzygii* subsp. *indonesiensis* appears to be polyphyletic with distinct but unresolved lineages as is apparent in Safni's 16S-23S rRNA ITS phylogenetic alignment (Safni et al. 2014). The *R. syzygii* isolate from blueberry DAR 77793 detected in the current study and the aberrant strain ACH732 have been described within the phylotype sequevar grouping of IV-11 (Wicker et al. 2012), suggesting that both isolates are related to each other and may be native to Australia. The *Ralstonia* grouping of IV-11 most likely forms a distinct lineage that differs phylogenetically from the *R. syzygii* subsp. *indonesiensis* type strain of UQRS 464T (= LMG 27703T = DSM 27478T 5PSI 07T).

Only one isolate of *R. syzygii* IV-11 has been recorded in an Australian specimen collection suggesting that this strain is not commonly recovered from diseased plants in Australia. The isolate originated from blueberry but was only able to cause disease on tomato plants in our assays, thus surveys for the strain should focus on tomato. Extensive surveys targeting *Ralstonia syzygii* IV-11 have not been conducted in the geographic location where the isolate was detected. However, it is possible that the strain has been replaced by more competitive or virulent lineages of *Ralstonia* such as *R. solanacearum* or *R. pseudosolanacearum* and may have died out.

Symptom development was significantly delayed for the woody blueberry plants compared to the tomato and potato plants in the current study. Herbaceous plants, including potatoes, tomatoes, and bananas, typically develop wilt symptoms within three weeks of inoculation (Ray et al. 2022). In other studies, the symptoms of blueberry wilt developed within 6 weeks of incubation (Norman et al. 2018; Oliver et al. 2023), yet the symptoms took 12 weeks to develop in the current study. Both *R. pseudosolanacearum* and *R. syzygii* (IV-11) were virulent, causing wilt symptoms on tomato within 6 days. Both isolates were pathogenic on blueberry,

with *R. pseudosolanacearum* causing wilt and thus being virulent and *R. syzygii* (IV-11) causing a latent or endophytic infection. Variation in susceptibility to bacterial wilt is reported for *Vaccinium* spp. (Conner et al. 2022). Therefore, the significant delay in symptom development reported in the current study may be due to some resistance in the blueberry variety 'Sunshine blue'. The delayed symptoms demonstrate the importance of incubating woody plants for an extended time and determining the presence of a latent infection through isolation.

Even though strains of all three species of *Ralstonia* are present in Australia, it is clear that they have restricted geographic distributions that, in some instances, are maintained through state/territory quarantine restrictions. Australia has a robust biosecurity system and has previously successfully eradicated specific strains from particular areas. Moko disease caused by *R. solanacearum* (Race 2, Bv1), introduced on a shipment of Heliconia from Hawaii in 1989, was successfully eradicated from a property in Cairns, Australia (Hyde et al. 1992). In WA, bacterial wilt was eradicated after all infected properties were quarantined for five years (EPPO 2019). The data suggest that native and introduced strains are present in Australia. Further research using molecular tools to analyse the population is warranted to determine the origin and diversity of the strains present within Australia. Assigning LINs to isolates within phylotype I using whole genome sequencing (WGS) would enable within-species identification for this group and facilitate the identification of strains absent from Australia (Sharma et al. 2022).

This study provides a baseline of data based on molecular phylogenetic identification of specimen-based records, forming a reliable RSSC pest list for Australia. Importantly, this baseline data reveals that several significant strains or groups of strains remain undetected in Australia, providing evidence of absence, including *R. pseudosolanacearum* phylotype III, the phylotype I mulberry infecting strains; *R. solanacearum* strains IIC and the Moko causing strains; and *R. syzygii* subsp. *celebesensis* and *R. syzygii* subsp. *syzygii*. The *Ralstonia* pest list,

here reported, supports Australia's robust biosecurity system by improving the ability to detect new and emerging plant diseases and by providing information to support disease eradication efforts. It also underpins future *Ralstonia* research in Australia.

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Figures and Tables

Table 1. The early *Ralstonia solanacearum* species complex classification systems were phenotypic, races are based on supposed host range, and biovars are based on carbonacidification; the complex was also divided based on restriction fragment length polymorphism (RFLP) into two divisions, table adapted from (Schaad et al. 2001).

Race	Host range	Geographic distribution	Biovar	RFLP Division
1	Wide	Asia, Australia	3,4	I
1	Wide	Americas	1	II
2	<i>Musa</i> spp.	Caribbean, Brazil, Philippines	1	II
3	Primarily potato	Worldwide	2	II
4	Ginger	Asia	3,4	I
5	Mulberry	China	5	I

Table 2. Specimen-based list of the Australian *Ralstonia solanacearum* species complex isolates identified to species, phylotype, sub-phylotype and sequevar. Data includes herbarium collection number, culture isolation date, plant host common name and species, and geographic location. The isolate in bold represents a historic record only as *R. pseudosolanacearum* is considered eradicated from Western Australia.

Collection # ^a	Host common	Host species	State ^d	City / Town	Date	<i>Ralstonia</i> species	Seq. ^b
BRIP 38727a	Ornamental ginger	<i>Alpinia sp.</i>	QLD	Gordonvale	1990	<i>R. pseudosolanacearum</i>	I-13
BRIP 60393	Custard apple	<i>Annona reticulata</i>	QLD	Walkamin	2014	<i>R. pseudosolanacearum</i>	I-13
BRIP 71183	Chia	<i>Salvia hispanica</i>	QLD	Silkwood	2020	<i>R. pseudosolanacearum</i>	I-13
DAR 34821	Capsicum cv. grossum	<i>Capsicum annuum</i>	NT	Darwin	1979	<i>R. pseudosolanacearum</i>	I-16
BRIP 38813	Fireweed	<i>Crassocephalum crepidioides</i>	QLD	Tanawha	1970	<i>R. pseudosolanacearum</i> ^c	I-16
BRIP 38798a	Tobacco	<i>Nicotiana tabacum</i>	QLD	Innisfail	1976	<i>R. pseudosolanacearum</i>	I-16
DAR 61727	Tomato	<i>Solanum lycopersicum</i>	NSW	Crawford	1988	<i>R. pseudosolanacearum</i>	I-16
BRIP 67085	Tomato cherry	<i>Solanum lycopersicum</i>	NT	Darwin	2018	<i>R. pseudosolanacearum</i>	I-16
BRIP 67082	Eggplant	<i>Solanum melogena</i>	NT	Jingili	2018	<i>R. pseudosolanacearum</i>	I-16
BRIP 60872a	Black nightshade	<i>Solanum nigrum</i>	QLD	Perwillowen	1968	<i>R. pseudosolanacearum</i>	I-16
BRIP 60775a	Ginger	<i>Zingiber officinale</i>	QLD	Coes Creek	1966	<i>R. pseudosolanacearum</i>	I-16
BRIP 38790a	Ginger	<i>Zingiber officinale</i>	QLD	Nambour	1968	<i>R. pseudosolanacearum</i> ^c	I-16
BRIP 34834	Ginger	<i>Zingiber officinale</i>	QLD	Nambour	1969	<i>R. pseudosolanacearum</i> ^c	I-16
BRIP 34848	Ginger	<i>Zingiber officinale</i>	QLD	Nambour	1969	<i>R. pseudosolanacearum</i> ^c	I-16

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DAR 34807	Capsicum cv. Grossum	<i>Capsicum annuum</i>	NT	Nightcliff	1978	<i>R. pseudosolanacearum</i>	I-17
DAR 34820	Chili	<i>Capsicum sp.</i>	NT	Nightcliff	1979	<i>R. pseudosolanacearum</i>	I-17
DAR 34814	Flowering shrub	<i>Galphimia glauca</i>	NT	Darwin	1979	<i>R. pseudosolanacearum</i>	I-17
BRIP 75374	Tomato	<i>Solanum lycopersicum</i>	QLD	Moreton	1992	<i>R. pseudosolanacearum</i>	I-17
DAR 34822	Tomato	<i>Solanum lycopersicum</i>	NT	Darwin	1979	<i>R. pseudosolanacearum</i>	I-17
BRIP 67084	Eggplant	<i>Solanum melongena</i>	NT	Driver	2018	<i>R. pseudosolanacearum</i>	I-17
BRIP 75384	Custard apple	<i>Annona reticulata</i>	QLD	Muchilba	2015	<i>R. pseudosolanacearum</i>	I-18
DAR 64837	Annona	<i>Annona sp.</i>	QLD	Palmwoods	1983	<i>R. pseudosolanacearum</i>	I-18
BRIP 34874	Sugar apple	<i>Annona squamosa</i>	QLD	Palmwoods	1983	<i>R. pseudosolanacearum</i>	I-18
BRIP 34877	Sugar apple	<i>Annona squamosa</i>	QLD	Palmwoods	1983	<i>R. pseudosolanacearum</i>	I-18
BRIP 34873	Sugar apple	<i>Annona squamosa</i>	QLD	Unknown	1983	<i>R. pseudosolanacearum</i>	I-18
BRIP 34905	Sugar apple	<i>Annona squamosa</i>	QLD	Mareeba	1984	<i>R. pseudosolanacearum</i>	I-18
BRIP 38700	Alexandra palm	<i>Archontophoenix alexandrae</i>	QLD	Clifton Beach	1989	<i>R. pseudosolanacearum</i>	I-18
BRIP 38805	Capsicum	<i>Capsicum annuum</i>	QLD	Redlands	1977	<i>R. pseudosolanacearum</i>	I-18
BRIP 60870a	Capsicum	<i>Capsicum annuum</i>	QLD	Redlands	1985	<i>R. pseudosolanacearum</i>	I-18
BRIP 67077a	Capsicum	<i>Capsicum annuum</i>	QLD	Ayr	2018	<i>R. pseudosolanacearum</i>	I-18
BRIP 67077b	Capsicum	<i>Capsicum annuum</i>	QLD	Ayr	2018	<i>R. pseudosolanacearum</i>	I-18
BRIP 67077c	Capsicum	<i>Capsicum annuum</i>	QLD	Ayr	2018	<i>R. pseudosolanacearum</i>	I-18
BRIP 75376	Chili	<i>Capsicum sp.</i>	QLD	Mareeba	1993	<i>R. pseudosolanacearum</i>	I-18
DAR 65958	Eucalypt	<i>Eucalyptus pellita</i>	QLD	Innisfail	1992	<i>R. pseudosolanacearum</i>	I-18
BRIP 75333	Eucalypt	<i>Eucalyptus urophylla</i>	QLD	Innisfail	1992	<i>R. pseudosolanacearum</i>	I-18
BRIP 75375	Eucalypt	<i>Eucalyptus urophylla</i>	QLD	Innisfail	1992	<i>R. pseudosolanacearum</i>	I-18
BRIP 34998a	Heliconia	<i>Heliconia sp.</i>	QLD	Mossman	1988	<i>R. pseudosolanacearum</i>	I-18
BRIP 38701a	Heliconia	<i>Heliconia sp.</i>	QLD	Cairns	1989	<i>R. pseudosolanacearum</i>	I-18
BRIP 38701b	Heliconia	<i>Heliconia sp.</i>	QLD	Cairns	1989	<i>R. pseudosolanacearum</i>	I-18
BRIP 38703	Heliconia	<i>Heliconia sp.</i>	QLD	Cairns	1989	<i>R. pseudosolanacearum</i>	I-18
DAR 64839	Heliconia	<i>Heliconia sp.</i>	QLD	Cairns	1989	<i>R. pseudosolanacearum</i>	I-18
BRIP 75364	Heliconia	<i>Heliconia sp.</i>	QLD	Crystal Cascades	1989	<i>R. pseudosolanacearum</i>	I-18
BRIP 38703a	Heliconia	<i>Heliconia sp.</i>	QLD	Redlynch	1989	<i>R. pseudosolanacearum</i>	I-18
BRIP 75363	Heliconia	<i>Heliconia sp.</i>	QLD	Redlynch	1989	<i>R. pseudosolanacearum</i>	I-18
BRIP 38736a	Heliconia	<i>Heliconia sp.</i>	QLD	Cairns	1990	<i>R. pseudosolanacearum</i>	I-18
BRIP 60873a	Heliconia	<i>Heliconia sp.</i>	QLD	Mossman	1990	<i>R. pseudosolanacearum</i>	I-18
BRIP 75366	Heliconia	<i>Heliconia sp.</i>	QLD	Mossman	1990	<i>R. pseudosolanacearum</i>	I-18
BRIP 75326	Heliconia	<i>Heliconia sp.</i>	QLD	Redlynch	1990	<i>R. pseudosolanacearum</i>	I-18
BRIP 75329	Heliconia	<i>Heliconia sp.</i>	QLD	Redlynch	1990	<i>R. pseudosolanacearum</i>	I-18
BRIP 75369	Heliconia	<i>Heliconia sp.</i>	QLD	Redlynch	1990	<i>R. pseudosolanacearum</i>	I-18
BRIP 65194a	Succulent plant	<i>Kalanchoe sp.</i>	QLD	Park Ridge	2016	<i>R. pseudosolanacearum</i>	I-18
DAR 77241	Lettuce	<i>Lactuca sativa</i>	QLD	Morayfield	~ 2006	<i>R. pseudosolanacearum</i>	I-18
BRIP 75370a	Banana	<i>Musa sp. x</i>	QLD	South Johnstone	1991	<i>R. pseudosolanacearum</i>	I-18
DAR 65956	Banana	<i>Musa sp. x</i>	QLD	Innisfail	1992	<i>R. pseudosolanacearum</i>	I-18
DAR 64900	Tobacco	<i>Nicotiana tabacum</i>	QLD	Mareeba	1966	<i>R. pseudosolanacearum</i>	I-18
BRIP 34966	Tobacco	<i>Nicotiana tabacum</i>	QLD	Mareeba	1986	<i>R. pseudosolanacearum</i>	I-18
DAR 76146	Olive	<i>Olea europaea</i>	NSW	Denman	2004	<i>R. pseudosolanacearum</i>	I-18
DAR 61441	Tomato	<i>Solanum lycopersicum</i>	NSW	Maroota	1988	<i>R. pseudosolanacearum</i>	I-18
BRIP 60867a	Tomato	<i>Solanum lycopersicum</i>	QLD	Redlands	1970	<i>R. pseudosolanacearum</i>	I-18
BRIP 38803	Tomato	<i>Solanum lycopersicum</i>	QLD	Redlands	1977	<i>R. pseudosolanacearum</i>	I-18
BRIP 47222a	Tomato	<i>Solanum lycopersicum</i>	QLD	Nambour	1979	<i>R. pseudosolanacearum</i>	I-18

BRIP 60869a	Tomato	<i>Solanum lycopersicum</i>	QLD	Brisbane	1984	<i>R. pseudosolanacearum</i>	I-18
BRIP 75323	Tomato	<i>Solanum lycopersicum</i>	QLD	Clohesy River	1990	<i>R. pseudosolanacearum</i>	I-18
BRIP 75330	Tomato	<i>Solanum lycopersicum</i>	QLD	Herberton	1991	<i>R. pseudosolanacearum</i>	I-18
BRIP 75334	Tomato	<i>Solanum lycopersicum</i>	QLD	Herberton	1992	<i>R. pseudosolanacearum</i>	I-18
BRIP 75335	Tomato	<i>Solanum lycopersicum</i>	QLD	Mareeba	1992	<i>R. pseudosolanacearum</i>	I-18
BRIP 75381	Tomato	<i>Solanum lycopersicum</i>	QLD	Emerald Ck	2010	<i>R. pseudosolanacearum</i>	I-18
BRIP 76152a	Tomato	<i>Solanum lycopersicum</i>	QLD	Evelyn	2021	<i>R. pseudosolanacearum</i>	I-18
DAR 64896	Tomato	<i>Solanum lycopersicum</i>	QLD	Buderim	1965	<i>R. pseudosolanacearum</i>	I-18
BRIP 75365	Tomato	<i>Solanum lycopersicum</i>	QLD	Dimbulah	1989	<i>R. pseudosolanacearum</i>	I-18
BRIP 76150a	Tomato	<i>Solanum lycopersicum</i>	QLD	Tolga	1990	<i>R. pseudosolanacearum</i>	I-18
DAR 65896	Tomato	<i>Solanum lycopersicum</i>	QLD	Currumbin	1992	<i>R. pseudosolanacearum</i>	I-18
DAR 65853	Tomato cherry	<i>Solanum lycopersicum</i>	NSW	Glenorie	1991	<i>R. pseudosolanacearum</i>	I-18
DAR 65854	Tomato cv. Pirate	<i>Solanum lycopersicum</i>	NSW	Glenorie	1991	<i>R. pseudosolanacearum</i>	I-18
BRIP 75331	Tomato cv. Pirate	<i>Solanum lycopersicum</i>	QLD	Bundaberg	1991	<i>R. pseudosolanacearum</i>	I-18
BRIP 75371	Tomato cv. Pirate	<i>Solanum lycopersicum</i>	QLD	Bundaberg	1991	<i>R. pseudosolanacearum</i>	I-18
BRIP 75373	Tomato cv. Pirate	<i>Solanum lycopersicum</i>	QLD	Ravenshoe	1992	<i>R. pseudosolanacearum</i>	I-18
DAR 61442	Tomato cv. Summit	<i>Solanum lycopersicum</i>	NSW	Maroota	1988	<i>R. pseudosolanacearum</i>	I-18
BRIP 60868a	Eggplant	<i>Solanum melongena</i>	QLD	Nambour	1977	<i>R. pseudosolanacearum</i>	I-18
BRIP 75368	Eggplant	<i>Solanum melongena</i>	QLD	Lockhart River	1990	<i>R. pseudosolanacearum</i>	I-18
BRIP 75327	Eggplant	<i>Solanum melongena</i>	QLD	Paddy's Green	1990	<i>R. pseudosolanacearum</i>	I-18
BRIP 75379	Eggplant	<i>Solanum melongena</i>	QLD	Innisfail	1995	<i>R. pseudosolanacearum</i>	I-18
BRIP 75337	Eggplant	<i>Solanum melongena</i>	QLD	Mackay	2007	<i>R. pseudosolanacearum</i>	I-18
BRIP 75407	Eggplant	<i>Solanum melongena</i>	QLD	Ingham	1988	<i>R. pseudosolanacearum</i>	I-18
DAR 61444	Black nightshade	<i>Solanum nigrum</i>	NSW	South Maroota	1988	<i>R. pseudosolanacearum</i>	I-18
BRIP 60862a	Potato	<i>Solanum tuberosum</i>	QLD	Bundaberg	1965	<i>R. pseudosolanacearum</i>	I-18
DAR 64898	Potato	<i>Solanum tuberosum</i>	QLD	Atherton	1966	<i>R. pseudosolanacearum</i>	I-18
DAR 65872	Potato	<i>Solanum tuberosum</i>	WA	Manjimup	1967	<i>R. pseudosolanacearum</i>	I-18
BRIP 60871a	Bird of paradise	<i>Strelitzia reginae</i>	NSW	Somersby	1987	<i>R. pseudosolanacearum</i>	I-18
DAR 58719	Bird of paradise	<i>Strelitzia reginae</i>	NSW	Somersby	1987	<i>R. pseudosolanacearum</i>	I-18
BRIP 75380	Marigold	<i>Tagetes sp.</i>	QLD	Cairns	1998	<i>R. pseudosolanacearum</i>	I-18
BRIP 76151a	Blueberry	<i>Vaccinium sp.</i>	QLD	Tolga	2016	<i>R. pseudosolanacearum</i>	I-18
DAR 77786	Blueberry cv. Sharpeblue	<i>Vaccinium sp.</i>	NSW	Woolgoolga	2007	<i>R. pseudosolanacearum</i>	I-18
DAR 64897	Ginger	<i>Zingiber officinale</i>	QLD	Coes Creek	1966	<i>R. pseudosolanacearum</i>	I-18
BRIP 38799	Ginger	<i>Zingiber officinale</i>	QLD	Tanawha	1970	<i>R. pseudosolanacearum</i> ^c	I-18
BRIP 38801	Ginger	<i>Zingiber officinale</i>	QLD	Tanawha	1970	<i>R. pseudosolanacearum</i> ^c	I-18
BRIP 38808a	Zinnia	<i>Zinnia elegans</i>	QLD	Brisbane	1977	<i>R. pseudosolanacearum</i>	I-18
DAR 34817	Tomato	<i>Solanum lycopersicum</i>	NT	Melville Island	1978	<i>R. pseudosolanacearum</i>	I-33
DAR 34808	Zinnia	<i>Zinnia elegans</i>	NT	Nakara	1978	<i>R. pseudosolanacearum</i>	I-33
DAR 34809	Tomato	<i>Solanum lycopersicum</i>	NT	Berrimah	1979	<i>R. pseudosolanacearum</i>	I-44
BRIP 75372	Anthurium	<i>Anthurium sp.</i>	QLD	Aloomba	1992	<i>R. pseudosolanacearum</i>	I-46
BRIP 75332	Tomato	<i>Solanum lycopersicum</i>	QLD	Walkamin	1992	<i>R. pseudosolanacearum</i>	I-46
BRIP 75338	Potato	<i>Solanum tuberosum</i>	QLD	Tolga	2009	<i>R. pseudosolanacearum</i>	I-46

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BRIP 75336	Peanut	<i>Arachis hypogaea</i>	QLD	Southedge	1993	<i>R. pseudosolanacearum</i>	I-72
BRIP 75325	Cobblers peg	<i>Bidens pilosa</i>	QLD	Southedge	1990	<i>R. pseudosolanacearum</i>	I-72
BRIP 34819	Capsicum	<i>Capsicum annuum</i>	QLD	Redlands	1981	<i>R. pseudosolanacearum</i>	I-72
BRIP 34889	Tobacco	<i>Nicotiana tabacum</i>	QLD	Mount Abbott	1983	<i>R. pseudosolanacearum</i>	I-72
BRIP 34948	Tobacco	<i>Nicotiana tabacum</i>	QLD	Southedge	1985	<i>R. pseudosolanacearum</i>	I-72
BRIP 38655a	Tobacco	<i>Nicotiana tabacum</i>	QLD	Mareeba	1988	<i>R. pseudosolanacearum</i>	I-72
BRIP 75367	Tobacco	<i>Nicotiana tabacum</i>	QLD	Southedge	1990	<i>R. pseudosolanacearum</i>	I-72
BRIP 75328	Tomato	<i>Solanum lycopersicum</i>	QLD	Mareeba	1990	<i>R. pseudosolanacearum</i>	I-72
BRIP 75324	Black nightshade	<i>Solanum nigrum</i>	QLD	Southedge	1990	<i>R. pseudosolanacearum</i>	I-72
DAR 34818	Mountford's wattle	<i>Acacia mountfordiae</i>	NT	Berrimah	1978	<i>R. pseudosolanacearum</i>	I-73
DAR 34824	Pumpkin butternut	<i>Cucurbita moschata</i>	NT	Middle Point	1978	<i>R. pseudosolanacearum</i>	I-73
DAR 34813	Zucchini	<i>Cucurbita pepo</i>	NT	Berrimah	1977	<i>R. pseudosolanacearum</i>	I-73
DAR 34816	Zucchini	<i>Cucurbita pepo</i>	NT	Middle Point	1978	<i>R. pseudosolanacearum</i>	I-73
DAR 34819	Zucchini	<i>Cucurbita pepo</i>	NT	Middle Point	1978	<i>R. pseudosolanacearum</i>	I-73
DAR 34812	Tomato	<i>Solanum lycopersicum</i>	NT	Elcho Island	1978	<i>R. pseudosolanacearum</i>	I-73
DAR 64828	Tomato	<i>Solanum lycopersicum</i>	NSW	Glenorie	1990	<i>R. pseudosolanacearum</i>	I-73
DAR 34811	Tomato	<i>Solanum lycopersicum</i>	NT	Darwin	1979	<i>R. pseudosolanacearum</i>	I-73
DAR 34815	Tomato	<i>Solanum lycopersicum</i>	NT	Darwin	1979	<i>R. pseudosolanacearum</i>	I-73
DAR 34810	Eggplant	<i>Solanum melongena</i>	NT	Tiwi	1978	<i>R. pseudosolanacearum</i>	I-73
BRIP 67079	Eggplant	<i>Solanum melongena</i>	NT	Alawa	2018	<i>R. pseudosolanacearum</i>	I-73
DAR 86208	Eggplant	<i>Solanum melongena</i>	NT	Middle point	2022	<i>R. pseudosolanacearum</i>	I-73
BRIP 60861a	Potato	<i>Solanum tuberosum</i>	QLD	Mulgowie	1965	<i>R. solanacearum</i>	IIA-50
DAR 35670	Tomato	<i>Solanum lycopersicum</i>	NSW	Macquarie Fields	1981	<i>R. solanacearum</i>	IIB-1
DAR 34823	Tomato	<i>Solanum lycopersicum</i>	NSW	Concord	1978	<i>R. solanacearum</i>	IIB-1
DAR 35671	Tomato	<i>Solanum lycopersicum</i>	NSW	Macquarie Fields	1981	<i>R. solanacearum</i>	IIB-1
DAR 65856	Tomato	<i>Solanum lycopersicum</i>	NSW	Corrimal East	1990	<i>R. solanacearum</i>	IIB-1
DAR 49320	Potato	<i>Solanum tuberosum</i>	NSW	Narrandera	1984	<i>R. solanacearum</i>	IIB-1
DAR 49855	Potato	<i>Solanum tuberosum</i>	NSW	Coleambally	1985	<i>R. solanacearum</i>	IIB-1
DAR 49854	Potato	<i>Solanum tuberosum</i>	NSW	Yanco	1985	<i>R. solanacearum</i>	IIB-1
DAR 69850a	Potato	<i>Solanum tuberosum</i>	NSW	Morpeth	1994	<i>R. solanacearum</i>	IIB-1
BRIP 60864a	Potato	<i>Solanum tuberosum</i>	QLD	Kingaroy	1966	<i>R. solanacearum</i>	IIB-1
DAR 64899	Potato	<i>Solanum tuberosum</i>	QLD	Kingaroy	1966	<i>R. solanacearum</i>	IIB-1
BRIP 60865a	Potato	<i>Solanum tuberosum</i>	QLD	Atherton	1974	<i>R. solanacearum</i>	IIB-1
BRIP 34942a	Potato	<i>Solanum tuberosum</i>	QLD	Glenore Grove	1985	<i>R. solanacearum</i>	IIB-1
DAR 65929	Potato	<i>Solanum tuberosum</i>	SA	Angle Vale	1992	<i>R. solanacearum</i>	IIB-1
DAR 65930	Potato	<i>Solanum tuberosum</i>	SA	Hindmarsh Island	1992	<i>R. solanacearum</i>	IIB-1
DAR 65919	Potato	<i>Solanum tuberosum</i>	SA	unknown	1992	<i>R. solanacearum</i>	IIB-1
BRIP 75378	Potato cv. Pontiac	<i>Solanum tuberosum</i>	QLD	Tolga	1993	<i>R. solanacearum</i>	IIB-1
BRIP 75377	Potato cv. Red port	<i>Solanum tuberosum</i>	QLD	Tolga	1993	<i>R. solanacearum</i>	IIB-1
BRIP 75382	Potato (Seed)	<i>Solanum tuberosum</i>	Vic.	Victoria	2010	<i>R. solanacearum</i>	IIB-1
BRIP 75383	Potato (Seed)	<i>Solanum tuberosum</i>	Vic.	Victoria	2010	<i>R. solanacearum</i>	IIB-1
DAR 41371	Potato cv. Pontiac	<i>Solanum tuberosum</i>	NSW	Euroley Bridge	1983	<i>R. solanacearum</i>	IIB-1
DAR 49859	Potato cv. Pontiac	<i>Solanum tuberosum</i>	NSW	Murray Downs	1985	<i>R. solanacearum</i>	IIB-1
DAR 65918	Potato cv. Saturna	<i>Solanum tuberosum</i>	SA	Angle Vale	1992	<i>R. solanacearum</i>	IIB-1

DAR 65920	Potato cv. Saturna	<i>Solanum tuberosum</i>	SA	Loxton	1992	<i>R. solanacearum</i>	IIB-1
DAR 65922	Potato cv. Saturna	<i>Solanum tuberosum</i>	SA	Parilla	1992	<i>R. solanacearum</i>	IIB-1
DAR 33374	Potato cv. Sebago	<i>Solanum tuberosum</i>	NSW	Freemans Reach	1979	<i>R. solanacearum</i>	IIB-1
DAR 77793	Blueberry	<i>Vaccinium sp.</i>	NSW	Woolgoolga	2008	<i>R. syzygii</i>	IV-11
DAR 65892	Tomato	<i>Solanum lycopersicum</i>	NT	Darwin	1978	RSSC aberrant	IV-74
BRIP 60866a	Potato	<i>Solanum tuberosum</i>	QLD	Atherton	1974	RSSC aberrant	IV-74

^aHerbaria, NSW Plant Pathology and Mycology Herbarium (DAR), Plant Pathology Herbarium, QLD (BRIP). ^bSeq. includes phylotype, sub-phylotype, and sequevar categorisation. ^cBiovar-4 as reported in herbarium accession data. ^dAustralian states; Queensland (QLD), Northern Territory (NT), New South Wales (NSW), Western Australia (WA), South Australia (SA), Victoria (VIC).

Table 3. The pathogenicity of five representative *Ralstonia* strains was assessed by wounded root inoculation of potted host plants. The strain was inoculated onto its original host and the near-universal host tomato. Plants were monitored for wilt symptoms, potato and tomato plants were harvested after 4 weeks, and blueberry plants were harvested after 14 weeks. Asymptomatic plants were assessed for latent infections to determine the colonisation index (CI).

Treatment; strain (phylotype-sequevar)	Tomato		Potato		Blueberry	
	W ^a	CI ^b	W ^a	CI ^b	W ^a	CI ^b
<i>R. solanacearum</i> DAR 49320 (IIB-1)	6/6	100	6/6*	100	-	-
<i>R. pseudosolanacearum</i> DAR 77786 (I-18)	6/6	100	-	-	2/6*	100
<i>R. syzygii</i> DAR 77793 (IV-11)	5/6	100	-	-	0/6*	83
RSSC aberrant BRIP 60866 (IV-74)	5/6	100	0/6*	33	-	-
RSSC aberrant DAR 65892 (IV-74)	0/6*	33	-	-	-	-
Control – mock inoculated	0/6	-	0/6	-	0/6	-

^aWilted plants (W), the number of wilted plants/number of inoculated plants. ^bColonisation Index (CI) = % of wilted plants + % of plants confirmed with latent infection. *The plant species that the bacterial strain was initially isolated.

Table 4. The time (days) taken for wilt symptoms to first appear following wounded root inoculation of potted plants with the representative *Ralstonia* species complex strains. Plants were inspected visually for wilt symptoms; potatoes and tomato plants were monitored for 4 weeks, and blueberry plants were monitored for 14 weeks.

Bacterium	Host ^a	Day of first wilt symptoms post inoculation ^b									
		3	4	5	6	7	8	9	10	28	84
<i>R. solanacearum</i>	T	0	0	0	1	6	6	6	6	6	-
DAR 49320 (IIB-1)	P	0	0	0	3	4	6	6	6	6	-
<i>R. pseudosolanacearum</i>	T	0	5	5	5	6	6	6	6	6	-
DAR 77786 (I-18)	B	0	0	0	0	0	0	0	0	0	2
<i>R. syzygii</i>	T	0	0	0	1	5	5	5	5	5	-
DAR 77793 (IV-11)	B	0	0	0	0	0	0	0	0	0	0
RSSC aberrant	T	0	0	0	0	1	2	3	5	5	-
BRIP 60866 (IV-74)	P	0	0	0	0	0	0	0	0	0	-
RSSC aberrant	T	0	0	0	0	0	0	0	0	0	-
DAR 65892 (IV-74)											

^aHost abbreviations; T = tomato, P = potato, B = blueberry. ^bNumber of wilted plants out of the 6 inoculated.

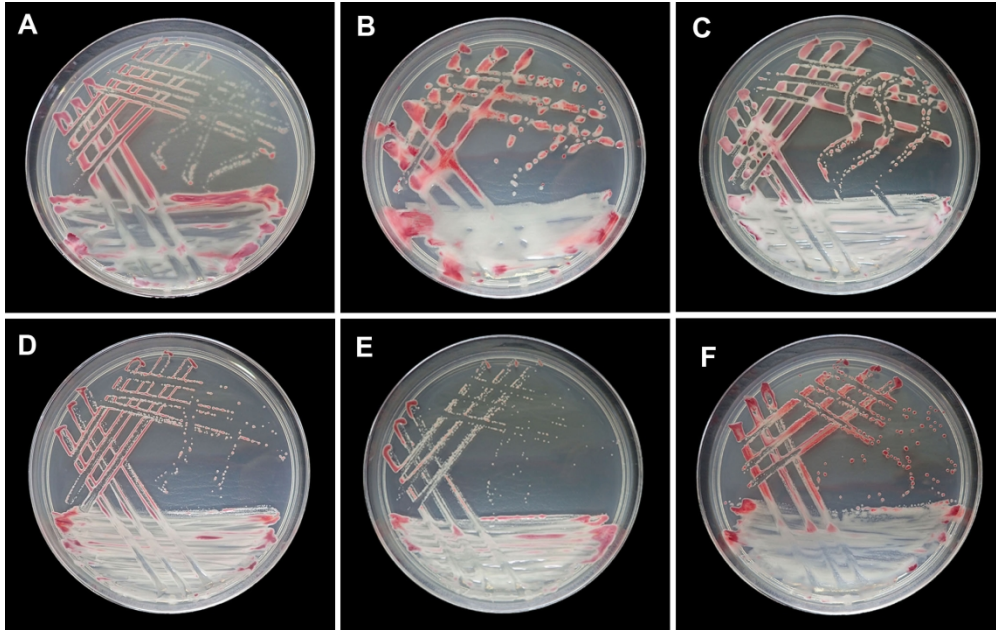
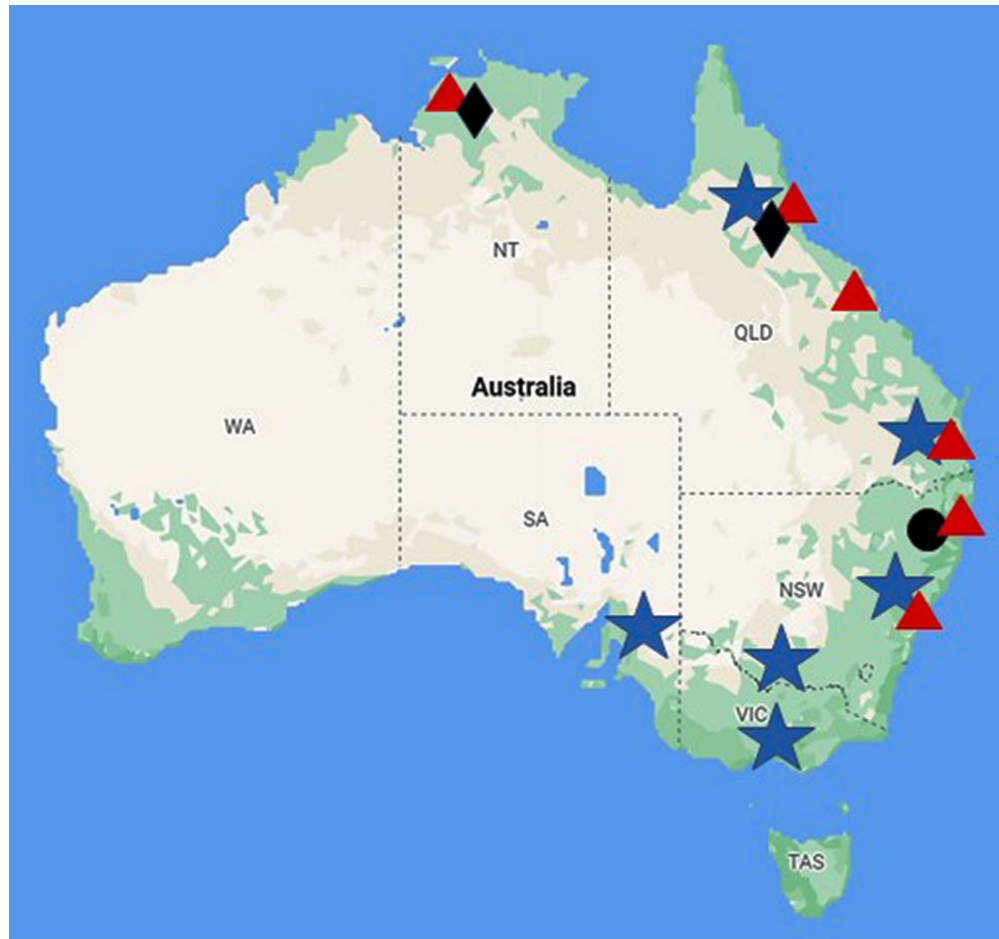


Fig. 1. Colony morphology of *Ralstonia* isolates grown on Kelman's tetrazolium chloride medium after two days at approximately 28°C. A, *Ralstonia solanacearum* DAR 49320 (IIB-1). B, *Ralstonia pseudosolanacearum* DAR 77786 (I-18). C, *Ralstonia syzygii* DAR 77793 (IV-11). D, *Ralstonia aberrant* (IV-74) BRIP 60866. E, *Ralstonia aberrant* (IV-74) DAR 65892. F, *R. solanacearum* BRIP 60861a (IIA-50). Scale bar = 10mm.

177x111mm (300 x 300 DPI)



Geographic distribution of *Ralstonia solanacearum* (blue star), *Ralstonia pseudosolanacearum* (red triangle), *Ralstonia syzygii* IV-11 (black circle), and *Ralstonia aberrant* IV-74 (black diamond) within Australia. The map indicates presence in the Australian states of Queensland (QLD), South Australia (SA), New South Wales (NSW), Victoria (VIC), and the Northern Territory (NT). The *Ralstonia solanacearum* species complex is not present in Western Australia (WA) or Tasmania (TAS). Background, Google Maps [online, 2023].

85x79mm (300 x 300 DPI)

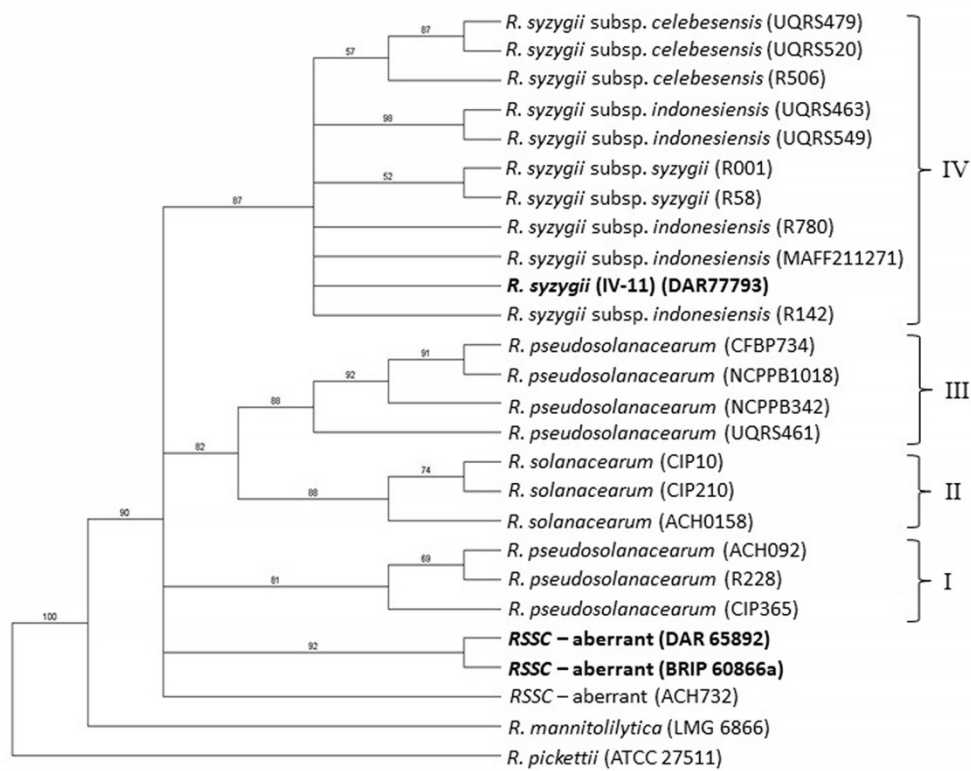


Fig. 3. Phylogenetic analysis of 16S rRNA sequence data, showing the position of the *Ralstonia solanacearum* species complex (RSSC) aberrant strains DAR65892 and BRIP60866. The Australian isolates sequenced as part of the current study are highlighted in bold, and the alignment includes the previously described Australian aberrant strain ACH732. Neighbour-joining consensus tree, with *Ralstonia pickettii* as the outgroup and 1000 bootstraps, nodes show consensus support (%), and the strain collection codes are in parenthesis. The brackets indicate the phylotype groupings.

177x141mm (300 x 300 DPI)

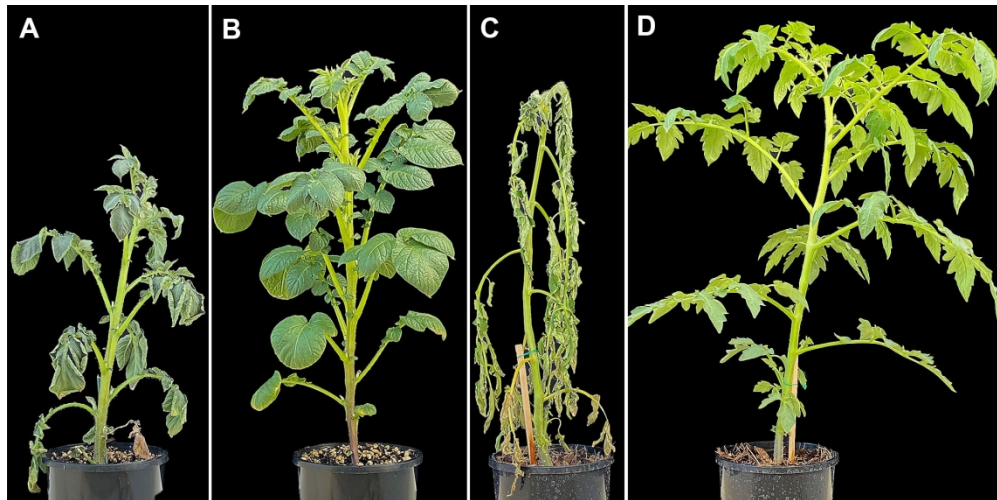


Fig. 4. Characteristic symptoms of bacterial wilt in potato and tomato plants, the leaves collapse, and the lower leaves become necrotic; symptom progression results in plant death. A, Potato plant 13 days after inoculation with *R. solanacearum* DAR 49320 (IIB-1). B, Potato plant mock-inoculated. C, Tomato plant 8 days after inoculation with *R. pseudosolanacearum* DAR 77786 (I-18). D, Tomato plant mock-inoculated.

177x88mm (300 x 300 DPI)

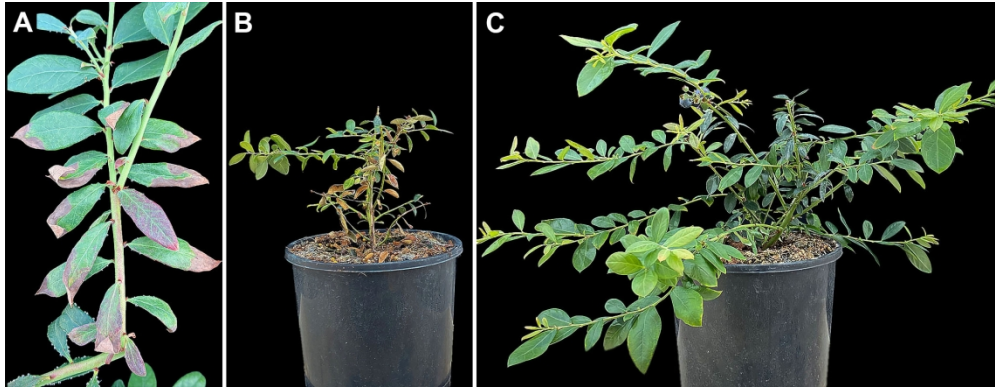


Fig. 5. Symptoms of bacterial wilt in blueberry plants inoculated with *R. pseudosolanacearum* DAR 77786 (I-18) after 14 weeks. A, Symptoms of bacterial wilt in blueberries include leaf discolouration and leaf tip necrosis. B, Blueberry plant with advanced symptoms of leaf browning and necrosis, leaf drop, and plant stunting. C, Blueberry plant mock-inoculated.

177x68mm (300 x 300 DPI)

Collection #	Species identity	Phylotype - sequevar	Host common	*Biovar	NCBI accession
BRIP 38727a	<i>R. pseudosolanacearum</i>	I-13	Ornamental ginger		PP378335
BRIP 60393	<i>R. pseudosolanacearum</i>	I-13	Custard apple	Bv3	PP378284
BRIP 71183	<i>R. pseudosolanacearum</i>	I-13	Chia		PP378290
DAR 34821	<i>R. pseudosolanacearum</i>	I-16	Capsicum cv. grossum		PP378241
BRIP 38813	<i>R. pseudosolanacearum</i>	I-16	Fireweed	Bv4	PP378305
BRIP 38798a	<i>R. pseudosolanacearum</i>	I-16	Tobacco		PP378337
DAR 61727	<i>R. pseudosolanacearum</i>	I-16	Tomato		PP378309
BRIP 67085	<i>R. pseudosolanacearum</i>	I-16	Tomato cherry		PP378289
BRIP 67082	<i>R. pseudosolanacearum</i>	I-16	Eggplant		PP378287
BRIP 60872a	<i>R. pseudosolanacearum</i>	I-16	Black nightshade		PP378346
BRIP 60775a	<i>R. pseudosolanacearum</i>	I-16	Ginger		PP378339
BRIP 38790a	<i>R. pseudosolanacearum</i>	I-16	Ginger	Bv4	PP378336
BRIP 34834	<i>R. pseudosolanacearum</i>	I-16	Ginger	Bv4	PP378279
BRIP 34848	<i>R. pseudosolanacearum</i>	I-16	Ginger	Bv4	PP378280
DAR 34807	<i>R. pseudosolanacearum</i>	I-17	Capsicum cv. grossum		PP333990
DAR 34820	<i>R. pseudosolanacearum</i>	I-17	Chili		PP378240
DAR 34814	<i>R. pseudosolanacearum</i>	I-17	Flowering shrub		PP378234
BRIP 75374	<i>R. pseudosolanacearum</i>	I-17	Tomato		PP378272
DAR 34822	<i>R. pseudosolanacearum</i>	I-17	Tomato		PP378242
BRIP 67084	<i>R. pseudosolanacearum</i>	I-17	Eggplant		PP378288
BRIP 75384	<i>R. pseudosolanacearum</i>	I-18	Custard apple		PP378331
DAR 64837	<i>R. pseudosolanacearum</i>	I-18	Annona		PP378311
BRIP 34874	<i>R. pseudosolanacearum</i>	I-18	Sugar apple		PP378295
BRIP 34877	<i>R. pseudosolanacearum</i>	I-18	Sugar apple	Bv3	PP378296
BRIP 34873	<i>R. pseudosolanacearum</i>	I-18	Sugar apple		PP378294
BRIP 34905	<i>R. pseudosolanacearum</i>	I-18	Sugar apple		PP378297
BRIP 38700	<i>R. pseudosolanacearum</i>	I-18	Alexandra palm	Bv3	PP378332
BRIP 75380	<i>R. pseudosolanacearum</i>	I-18	Marigold		PP378327
BRIP 38805	<i>R. pseudosolanacearum</i>	I-18	Capsicum		PP378283
BRIP 60870a	<i>R. pseudosolanacearum</i>	I-18	Capsicum		PP378344
BRIP 67077a	<i>R. pseudosolanacearum</i>	I-18	Capsicum		PP378291
BRIP 67077b	<i>R. pseudosolanacearum</i>	I-18	Capsicum		PP378292
BRIP 67077c	<i>R. pseudosolanacearum</i>	I-18	Capsicum		PP378285
BRIP 75376	<i>R. pseudosolanacearum</i>	I-18	Chili		PP378278
DAR 65958	<i>R. pseudosolanacearum</i>	I-18	Eucalypt		PP378321
BRIP 75333	<i>R. pseudosolanacearum</i>	I-18	Eucalypt		PP378273
BRIP 75375	<i>R. pseudosolanacearum</i>	I-18	Eucalypt		PP378274
BRIP 34998a	<i>R. pseudosolanacearum</i>	I-18	Heliconia	Bv3	PP378333
BRIP 38701a	<i>R. pseudosolanacearum</i>	I-18	Heliconia	Bv3	PP378299
BRIP 38701b	<i>R. pseudosolanacearum</i>	I-18	Heliconia	Bv3	PP378300
BRIP 38703	<i>R. pseudosolanacearum</i>	I-18	Heliconia		PP378301
DAR 64839	<i>R. pseudosolanacearum</i>	I-18	Heliconia		PP378312
BRIP 75364	<i>R. pseudosolanacearum</i>	I-18	Heliconia		PP378251
BRIP 38703a	<i>R. pseudosolanacearum</i>	I-18	Heliconia		PP378250
BRIP 75363	<i>R. pseudosolanacearum</i>	I-18	Heliconia		PP378325
BRIP 38736a	<i>R. pseudosolanacearum</i>	I-18	Heliconia	Bv3	PP378348
BRIP 60873a	<i>R. pseudosolanacearum</i>	I-18	Heliconia		PP378347
BRIP 75366	<i>R. pseudosolanacearum</i>	I-18	Heliconia		PP378254
BRIP 75326	<i>R. pseudosolanacearum</i>	I-18	Heliconia		PP378259

BRIP 75329	<i>R. pseudosolanacearum</i>	I-18	Heliconia		PP378263
BRIP 75369	<i>R. pseudosolanacearum</i>	I-18	Heliconia		PP378261
BRIP 65194a	<i>R. pseudosolanacearum</i>	I-18	Succulent plant		PP378350
DAR 77241	<i>R. pseudosolanacearum</i>	I-18	Lettuce		PP378323
BRIP 75370a	<i>R. pseudosolanacearum</i>	I-18	Banana		PP378266
DAR 65956	<i>R. pseudosolanacearum</i>	I-18	Banana		PP378320
DAR 64900	<i>R. pseudosolanacearum</i>	I-18	Tobacco		PP378316
BRIP 34966	<i>R. pseudosolanacearum</i>	I-18	Tobacco		PP378298
DAR 76146	<i>R. pseudosolanacearum</i>	I-18	Olive		PP378322
DAR 61441	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378306
BRIP 60867a	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378341
BRIP 38803	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378304
BRIP 47222a	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378349
BRIP 60869a	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378343
BRIP 75323	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378253
BRIP 75330	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378265
BRIP 75334	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378275
BRIP 75335	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378276
BRIP 75381	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378330
BRIP 76152a	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378307
DAR 64896	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378313
BRIP 75365	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378252
BRIP 76150a	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378264
DAR 65896	<i>R. pseudosolanacearum</i>	I-18	Tomato		PP378319
DAR 65853	<i>R. pseudosolanacearum</i>	I-18	Tomato cherry		PP378247
DAR 65854	<i>R. pseudosolanacearum</i>	I-18	Tomato cv. Pirate		PP378317
BRIP 75331	<i>R. pseudosolanacearum</i>	I-18	Tomato cv. Pirate		PP378267
BRIP 75371	<i>R. pseudosolanacearum</i>	I-18	Tomato cv. Pirate		PP378268
BRIP 75373	<i>R. pseudosolanacearum</i>	I-18	Tomato cv. Pirate		PP378270
DAR 61442	<i>R. pseudosolanacearum</i>	I-18	Tomato cv. Summit		PP378245
BRIP 60868a	<i>R. pseudosolanacearum</i>	I-18	Eggplant		PP378342
BRIP 75368	<i>R. pseudosolanacearum</i>	I-18	Eggplant		PP378258
BRIP 75327	<i>R. pseudosolanacearum</i>	I-18	Eggplant		PP378260
BRIP 75379	<i>R. pseudosolanacearum</i>	I-18	Eggplant		PP378326
BRIP 75337	<i>R. pseudosolanacearum</i>	I-18	Eggplant		PP378328
BRIP 75407	<i>R. pseudosolanacearum</i>	I-18	Eggplant		PP378249
DAR 61444	<i>R. pseudosolanacearum</i>	I-18	Black nightshade		PP378246
BRIP 60862a	<i>R. pseudosolanacearum</i>	I-18	Potato		PP378340
DAR 64898	<i>R. pseudosolanacearum</i>	I-18	Potato		PP378315
DAR 65872 ^c	<i>R. pseudosolanacearum</i>	I-18	Potato		PP378318
BRIP 60871a	<i>R. pseudosolanacearum</i>	I-18	Bird of paradise		PP378345
DAR 58719	<i>R. pseudosolanacearum</i>	I-18	Bird of paradise		PP378244
BRIP 76151a	<i>R. pseudosolanacearum</i>	I-18	Blueberry		PP378248
DAR 77786	<i>R. pseudosolanacearum</i>	I-18	Blueberry cv. Sharpeblue		PP378324
DAR 64897	<i>R. pseudosolanacearum</i>	I-18	Ginger		PP378314
BRIP 38799	<i>R. pseudosolanacearum</i>	I-18	Ginger	Bv4	PP378302
BRIP 38801	<i>R. pseudosolanacearum</i>	I-18	Ginger	Bv4	PP378303
BRIP 38808a	<i>R. pseudosolanacearum</i>	I-18	Zinnia		PP378338
DAR 34817	<i>R. pseudosolanacearum</i>	I-33	Tomato		PP378237
DAR 34808	<i>R. pseudosolanacearum</i>	I-33	Zinnia		PP333991

DAR 34809	<i>R. pseudosolanacearum</i>	I-44	Tomato		PP333992
BRIP 75372	<i>R. pseudosolanacearum</i>	I-46	Anthurium		PP378269
BRIP 75332	<i>R. pseudosolanacearum</i>	I-46	Tomato		PP378271
BRIP 75338	<i>R. pseudosolanacearum</i>	I-46	Potato		PP378329
BRIP 75336	<i>R. pseudosolanacearum</i>	I-72	Peanut		PP378277
BRIP 75325	<i>R. pseudosolanacearum</i>	I-72	Cobblers peg		PP378257
BRIP 34819	<i>R. pseudosolanacearum</i>	I-72	Capsicum		PP378293
BRIP 34889	<i>R. pseudosolanacearum</i>	I-72	Tobacco	Bv3	PP378281
BRIP 34948	<i>R. pseudosolanacearum</i>	I-72	Tobacco		PP378282
BRIP 38655a	<i>R. pseudosolanacearum</i>	I-72	Tobacco		PP378334
BRIP 75367	<i>R. pseudosolanacearum</i>	I-72	Tobacco		PP378256
BRIP 75328	<i>R. pseudosolanacearum</i>	I-72	Tomato		PP378262
BRIP 75324	<i>R. pseudosolanacearum</i>	I-72	Black nightshade		PP378255
DAR 34818	<i>R. pseudosolanacearum</i>	I-73	Mountford's wattle		PP378238
DAR 34824	<i>R. pseudosolanacearum</i>	I-73	Pumpkin butternut		PP378243
DAR 34813	<i>R. pseudosolanacearum</i>	I-73	Zucchini		PP378233
DAR 34816	<i>R. pseudosolanacearum</i>	I-73	Zucchini		PP378236
DAR 34819	<i>R. pseudosolanacearum</i>	I-73	Zucchini		PP378239
DAR 34812	<i>R. pseudosolanacearum</i>	I-73	Tomato		PP378232
DAR 64828	<i>R. pseudosolanacearum</i>	I-73	Tomato		PP378310
DAR 34811	<i>R. pseudosolanacearum</i>	I-73	Tomato		PP378231
DAR 34815	<i>R. pseudosolanacearum</i>	I-73	Tomato		PP378235
DAR 34810	<i>R. pseudosolanacearum</i>	I-73	Eggplant		PP378230
BRIP 67079	<i>R. pseudosolanacearum</i>	I-73	Eggplant		PP378286
DAR 86208	<i>R. pseudosolanacearum</i>	I-73	Eggplant		PP378308
BRIP 60861a	<i>R. solanacearum</i>	IIA-50	Potato		PP447361
DAR 35670	<i>R. solanacearum</i>	IIB-1	Tomato		PP447364
DAR 34823	<i>R. solanacearum</i>	IIB-1	Tomato		PP447343
DAR 35671	<i>R. solanacearum</i>	IIB-1	Tomato		PP447344
DAR 65856	<i>R. solanacearum</i>	IIB-1	Tomato		PP447349
DAR 49320	<i>R. solanacearum</i>	IIB-1	Potato		PP447346
DAR 49855	<i>R. solanacearum</i>	IIB-1	Potato		PP447366
DAR 49854	<i>R. solanacearum</i>	IIB-1	Potato		PP447365
DAR 69850a	<i>R. solanacearum</i>	IIB-1	Potato		PP447355
BRIP 60864a	<i>R. solanacearum</i>	IIB-1	Potato		PP447362
DAR 64899	<i>R. solanacearum</i>	IIB-1	Potato		PP447348
BRIP 60865a	<i>R. solanacearum</i>	IIB-1	Potato		PP447363
BRIP 34942a	<i>R. solanacearum</i>	IIB-1	Potato		PP447360
DAR 65929	<i>R. solanacearum</i>	IIB-1	Potato		PP447353
DAR 65930	<i>R. solanacearum</i>	IIB-1	Potato		PP447354
DAR 65919	<i>R. solanacearum</i>	IIB-1	Potato		PP447367
BRIP 75378	<i>R. solanacearum</i>	IIB-1	Potato cv. Pontiac		PP447357
BRIP 75377	<i>R. solanacearum</i>	IIB-1	Potato cv. Red Port		PP447356
BRIP 75382	<i>R. solanacearum</i>	IIB-1	Potato (Seed)		PP447358
BRIP 75383	<i>R. solanacearum</i>	IIB-1	Potato (Seed)		PP447359
DAR 41371	<i>R. solanacearum</i>	IIB-1	Potato cv. Pontiac		PP447345
DAR 49859	<i>R. solanacearum</i>	IIB-1	Potato cv. Pontiac		PP447347
DAR 65918	<i>R. solanacearum</i>	IIB-1	Potato cv. Saturna		PP447350
DAR 65920	<i>R. solanacearum</i>	IIB-1	Potato cv. Saturna		PP447351
DAR 65922	<i>R. solanacearum</i>	IIB-1	Potato cv. Saturna		PP447352
DAR 33374	<i>R. solanacearum</i>	IIB-1	Potato cv. Sebago		PP447342

DAR 77793	<i>R. syzygii</i>	IV-11	Blueberry	PP347759
DAR 65892	RSSC aberrant	IV-74	Tomato	PP347758
BRIP 60866a	RSSC aberrant	IV-74	Potato	PP347760

*Biovar (Bv) data obtained from the culture collection accession details.

Supplementary table 2. The phylotype-specific PCR multiplex reaction includes a set of primers at specific reaction concentrations (Fegan and Prior 2005; Opina et al. 1997). The presence of a 280-282 base-pair amplicon confirms the presence of a *Ralstonia solanacearum* species complex strain; the second amplicon identifies the phylotype group.

Target	Primer	Sequence	Reaction concentration	Size bp
Phylotype I	Nmult21:1F	CGTTGATGAGGCGCGCAATTT	0.24 μ M	144
Phylotype II	Nmult21:2F	AAGTTATGGACGGTGGAAGTC	0.24 μ M	372
Phylotype III	Nmult23:AF	ATTACSAGAGCAATCGAAAGATT	0.72 μ M	91
Phylotype IV	Nmult22:InF	ATTGCCAAGACGAGAGAAGTA	0.24 μ M	213
All phylotypes	Nmult22:RR	TCGCTTGACCCTATAACGAGTA	0.24 μ M	NA
<i>R. solanacearum</i> species complex	759	GTCGCCGTCAACTCACTTTCC	0.16 μ M	280 or
	760	GTCGCCGTCAGCAATGCGGAATCG	0.16 μ M	282

Species identity	Phylotype - sequevar	Collection #	Host common	Origin	NCBI accession
<i>R. syzygii</i> ^a	IV-11	DAR 77793	Blueberry	Australia	PP326220
RSSC aberrant ^a	IV-74	DAR 65892	Tomato	Australia	PP326219
RSSC aberrant ^a	IV-74	BRIP 60866a	Potato	Australia	PP326221
RSSC aberrant	IV-11	ACH732	Tomato	Australia	U27983
<i>R. pseudosolanacearum</i>	I-18	ACH 092	Ginger	Australia	U27985
<i>R. pseudosolanacearum</i>	I-45	CIP 365	Potato	Philippines	U28220
<i>R. pseudosolanacearum</i>	I/-	R288	Mulberry	China	U27984
<i>R. pseudosolanacearum</i>	III	UQRS461	Pelargonium	Reunion	KC757037
<i>R. pseudosolanacearum</i>	III/ -	NCPPB 1018	Potato	Angola	AF207893
<i>R. pseudosolanacearum</i>	III/20	NCPPB 342	Tobacco	Zimbabwe	AF207896
<i>R. pseudosolanacearum</i>	III/-	CFBP 734	Potato	Madagascar	AF207895
<i>R. solanacearum</i>	II/ -	ACH 0158	Potato	Australia	U28224
<i>R. solanacearum</i>	II/ -	CIP10	Potato	Peru	U28227
<i>R. solanacearum</i>	II/25	CIP210	Potato	Brazil	U28222
<i>R. syzygii</i> subsp. <i>indonesiensis</i>	IV-10	R142	Clove	Indonesia	U28233
<i>R. syzygii</i> subsp. <i>indonesiensis</i>	IV/-	R780	Potato	Indonesia	U28232
<i>R. syzygii</i> subsp. <i>indonesiensis</i>	IV/-	UQRS463	Tomato	Indonesia	KC757056
<i>R. syzygii</i> subsp. <i>indonesiensis</i>	IV/-	UQRS549	Potato	Indonesia	KC757060
<i>R. syzygii</i> subsp. <i>indonesiensis</i>	IV/8	MAFF211271	Potato	Japan	AB024609
<i>R. syzygii</i> subsp. <i>syzygii</i>	IV-9	R 001	Clove	Indonesia	U28237
<i>R. syzygii</i> subsp. <i>syzygii</i>	IV-9	R 58	Clove	Indonesia	U28238
<i>R. syzygii</i> subsp. <i>celebesensis</i>	BDB	R 506	Banana	Indonesia	U28234
<i>R. syzygii</i> subsp. <i>celebesensis</i>	BDB	UQRS520	Banana	Indonesia	KC757067
<i>R. syzygii</i> subsp. <i>celebesensis</i>	BDB	UQRS479	Banana	Indonesia	KC757063
<i>Ralstonia pickettii</i> - Type		ATCC 27511			AY741342
<i>Ralstonia mannitolilytica</i> - Type		LMG 6866			AJ270258

^aIsolates sequenced as part of the current study.