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Evaluation of spider lilies (*Hymenocallis* spp.) as sentinel plants for orthotospoviruses

Hsu-Yao Chao[1*], Ralf G. Dietzgen[1], John E. Thomas[1] and Andrew D. W. Geering[1*]

Abstract

Spider lilies (genus *Hymenocallis*) are perennial herbaceous plants native to tropical and subtropical America and are popular landscaping plants in similar climes throughout the world. They are susceptible to infection by at least five different orthotospoviruses, and display prominent symptoms of infection, suggesting that they may be useful as sentinel plants for monitoring the presence of orthotospoviruses in the environment. In this study, surveys of spider lilies for orthotospoviral infections were done in three distinct Queensland regions. In southeast Queensland, spider lilies infected by tomato spotted wilt virus (TSWV) were found at 65 locations, whereas spider lilies infected by capsicum chlorosis virus (CaCV) were only found at two locations. Conversely, in central and far north Queensland, CaCV-infected spider lilies were found at 41 locations, while TSWV-infected spider lilies were only found at two locations. Phylogenetic analyses based on the complete nucleocapsid protein sequences indicated a population structure of the virus isolates that correlated with the geographical origins of the samples. Both non-systemic and systemic TSWV infections were identified in spider lilies, and some initially non-systemically infected spider lily plants ended up with systemic infection. We demonstrated that *Taeniothrips eucharii* (Whetzel), a common thrips species on spider lilies in southeast Queensland, can transmit TSWV and has likely amplified TSWV infections in spider lilies in this region. Overall, the results of this study suggest that spider lilies could be useful sentinel plants for indicating the presence of orthotospoviruses in the environment.

Keywords Tomato spotted wilt virus, Capsicum chlorosis virus, *Hymenocallis*, Spider lily, Orthotospovirus vector, *Taeniothrips eucharii*

Background

The genus *Orthotospovirus* (*Elliovirales*: *Tospoviridae*) contains many viruses that are serious threats to food and fibre production throughout the world, particularly in the tropics and subtropics. All orthotospoviruses are transmitted by thrips (Thysanoptera: Thripidae) in a persistent and propagative manner (Rotenberg and Whitfield 2018). In Australia, five orthotospoviruses are known, which are capsicum chlorosis virus (CaCV), impatiens necrotic spot virus (INSV), iris yellow spot virus (IYSV), Pterostylis blotch virus (PtBV), and tomato spotted wilt virus (TSWV) (Persley et al. 2006; Chao et al. 2022; Geering and Thomas 2022). Only PtBV is considered indigenous, the remainder originating from Eurasia or the Americas. Seven out of 18 known thrips vectors of orthotospoviruses have also been introduced into Australia, and these are *Frankliniella occidentalis* (Pergande), *F. schultzei* (Trybom), *Microcephalothrips abdominalis* (Crawford DL), *Taeniothrips eucharii* (Whetzel), *Scirtothrips dorsalis* Hood, *Thrips palmi* Karny, and *T. tabaci* Lindeman (Australian Government Department of Agriculture and Water Resources 2017; Mound and Tree 2020; Sharman et al. 2020).

Although TSWV has been present in Australia for over 100 years (Brittlebank 1919; Geering 2024), INSV

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is a recent introduction, first detected in 2010 on several ornamental plant species in a nursery on the north coast of New South Wales and a second time in lettuce crops in an outer southwest suburb of Sydney in 2018 (Geering and Thomas 2022). The virus was eradicated from the nursery in 2010, so the second record is considered an independent introduction of the pathogen, indicating that active movement pathways for orthotospoviruses into Australia still exist. The movement pathways for these viruses have not been clearly defined, but the most likely mode of transoceanic dispersal is through the legal or illegal importation of ornamental plants or plant propagules, which are infected and/or carry viruliferous thrips. Seed transmission of orthotospoviruses represents a second but far less likely movement pathway, as this mode of transmission has only been reported twice. The first and most convincing report involves soybean vein necrosis virus (SVNV) in soybean (*Glycine max* (L.) Merr.) (Groves et al. 2016). The second report involves seed transmission of TWSV in pepper (*Capsicum annuum* L.), but only the cotyledons, and not the true leaves, were infected, and the virus was only detected in the endosperm and not the embryo of the seed (Wang et al. 2022).

Many orthotospoviruses have very wide host ranges, and their thrips vectors are also highly polyphagous. These two biological features make surveillance and disease control difficult. Significant benefits could be obtained for disease control by using a suitable sentinel plant to signal when infection pressures are high and for delimitation surveys in surveillance for new orthotospovirus incursions. The three characteristics that would make a good sentinel plant for orthotospoviruses are: (i) they are susceptible to infection by a broad range of orthotospoviruses; (ii) they display distinctive and easily recognisable disease symptoms; and (iii) they are popular in cultivation, easy to propagate and are perennial in growth habit.

Hymenocallis spp. (Asparagales: Amaryllidaceae), commonly called spider lilies, are native to neotropical America and have a centre of diversity in Mexico (Tapia-Campos et al. 2012). They are bulbous, perennial, herbaceous plants that can be propagated sexually by seeds or asexually by bulb offsets, and micropropagation is also used for mass production (Tapia-Campos et al. 2012). Most cultivated spider lilies were taken from the Antilles and the coastline of the Gulf of Mexico by sailors in the sixteenth century and are sold in nurseries under various species names due to difficulties in identification (Smith and Flory 2002; Tapia-Campos et al. 2012). At least two forms of *Hymenocallis* are commonly grown in Queensland: one with narrow strappy leaves, widely known as *H. littoralis* (Jacq.) Salisb. but more likely to be *H. acutifolia* (Herb.) Sweet, and a second form with broader leaves, often referred to as *H. speciosa* Salisb. but also resembling a cultivar called *Hymenocallis* 'Tropical Giant' (King 2012).

Spider lilies are susceptible to infection by at least five species of orthotospovirus, including calla lily chlorotic spot virus, Hippeastrum chlorotic ringspot virus (HCRV), CaCV, INSV, and TSWV (Liu et al. 2010; Liu et al. 2012; Xu et al. 2013; Huang et al. 2017; Dietzgen et al. 2018). In Australia, there has been only a single record of an orthotospovirus infecting spider lilies, this being TSWV in plants growing in the Mt Coot-tha Botanic Gardens in Brisbane (Dietzgen et al. 2018). However, foliar ringspot symptoms are ubiquitous on spider lily plants throughout Queensland.

Spider lilies appear to have all the features that would make them a good sentinel plant for orthotospoviruses. The main objectives of this study were firstly, to investigate the diversity of orthotospoviruses infecting spider lilies in Queensland, and secondly, to determine whether there were any differences in the genetic structure of the orthotospovirus populations in three widely dispersed regions. *Taeniothrips euchari*, a species of thrips commonly associated with spider lilies, is shown for the first time to be a vector of TSWV. Finally, evidence is provided that TSWV can systemically infect spider lilies, although this does not always occur, with discrete ringspots most likely representing independent inoculation events.

Results

Disease surveys of spider lilies in Queensland

Disease surveys of spider lilies were conducted in three distinct regions of Queensland: southeast Queensland (Coolangatta to Noosa Heads), central Queensland (Sarina to Gumlu), and far north Queensland (Innisfail to Chillagoe) (Fig. 1). These regions are geographically distinct, being about 500–1500 km apart, and are separated by drier livestock grazing country. The suburbs/towns where orthotospovirus-infected leaf samples were collected are listed in Additional file 1: Table S1. A variety of disease symptoms were observed (Additional file 2: Figure S1), with ringspots being the most common, followed by streaks or irregular blotches. The symptomatic areas were either chlorotic, strongly pigmented red or necrotic, sometimes a mixture of all three.

Initially, symptomatic specimens from each region were tested using a broad-spectrum reverse transcription-polymerase chain reaction (RT-PCR) assay for orthotospoviruses, and species identification was done by amplicon sequencing. However, the sequencing results gradually revealed trends, with positive samples from southeast Queensland mostly infected with TSWV and conversely, CaCV dominating north

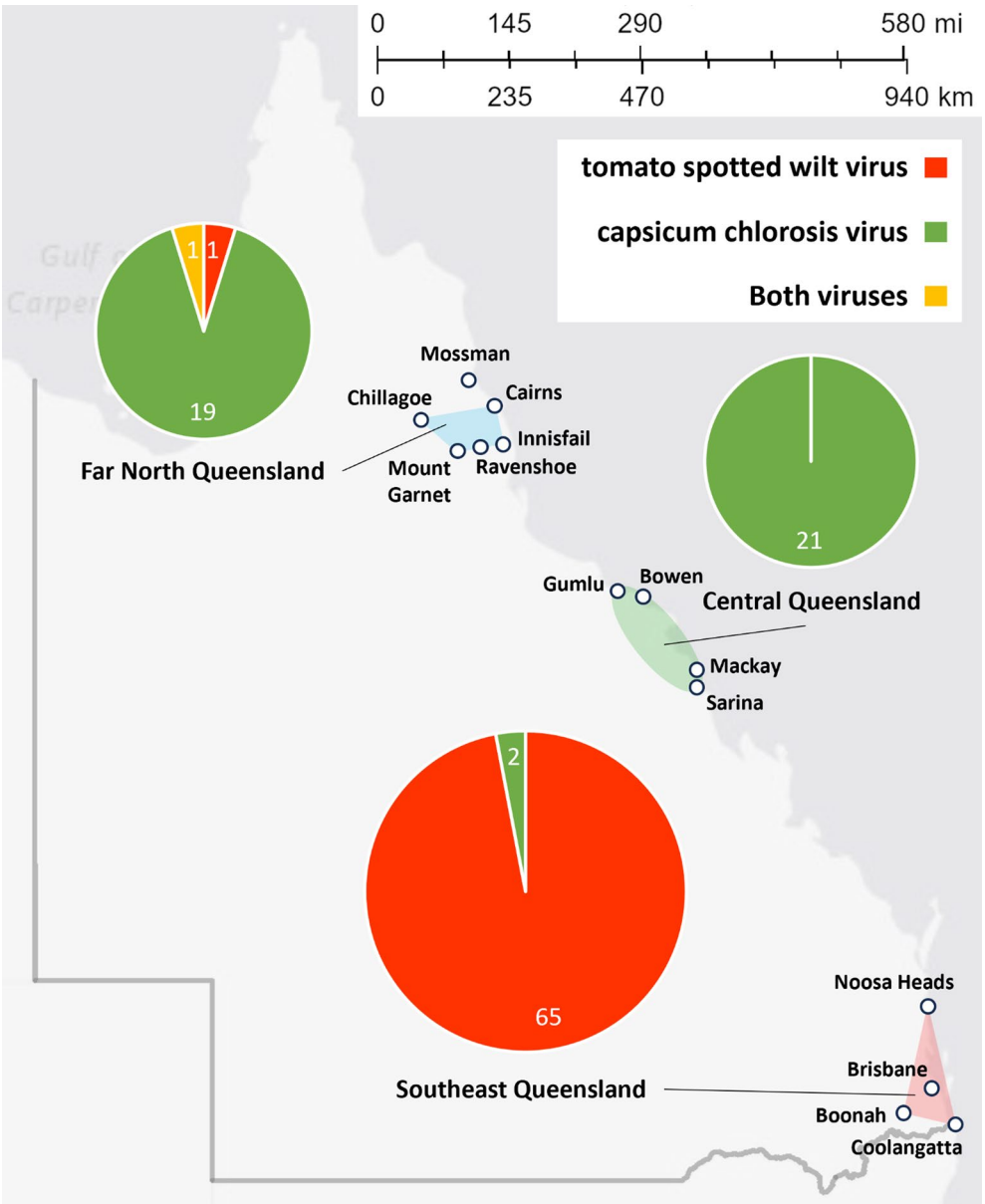

Fig. 1 Regions surveyed and numbers of locations where orthotospovirus-infected spider lilies were found in this study

of the Tropic of Capricorn, without other orthotospoviruses being detected. Single ringspots or blotches were tested, and no examples of mixed infections were observed. Small numbers of asymptomatic samples from each region were also tested, and negative test results were obtained. Given these preliminary results, the diagnostic approach shifted to using virus-specific primers targeting the nucleocapsid gene (*N*) of the two viruses. Each sample was tested with both the TSWV-specific and CaCV-specific primers, and no mixed infections were detected.

In southeast Queensland, orthotospovirus-infected spider lily plants were widespread across both metropolitan and rural areas, including the inner suburbs of Brisbane and the City of the Gold Coast. TSWV-infected spider lilies were identified at 65 different locations within 41 different suburbs and towns, while CaCV was only found at two locations: one in Brisbane (Darra) and the other in the City of the Gold Coast (Burleigh Heads) (Fig. 1 and Additional file 1: Table S1). In central Queensland, only CaCV was detected, and the distribution of CaCV-infected spider lilies was more localised.

No orthotospovirus-infected spider lilies were found in Mackay, the major city in the region. However, in Bowen, a town about 200 km northwest of Mackay that services a major vegetable growing area (including irrigated field tomatoes, sweet pepper, cucurbits and sweet corn), CaCV-infected spider lilies were very common, even in the centre of the town.

In far north Queensland, the distribution of orthotospovirus-infected spider lilies was again very localised. Along the high-rainfall, hot and humid tropical coastal strip from Innisfail to Mossman, where the main crops are sugarcane and Cavendish banana, orthotospovirus-infected spider lily plants were rare, found only at two sites (Babinda and Coorumba), with CaCV being the virus detected (Additional file 1: Table S1). In Cairns and Innisfail, the two major cities along this coast, spider lilies are common landscaping plants, but none displayed symptoms of orthotospoviral infection. Orthotospovirus-infected spider lily plants were more common on the Atherton Tableland to the west of Cairns and in even more westerly areas like Chillagoe and Mt Garnett. The Atherton Tableland is a major horticultural production zone for tropical fruit, sugarcane, coffee, mixed vegetables and flowers. CaCV-infected spider lilies were found at 18 different locations within seven different towns in this region. In Ravenshoe, TSWV-infected spider lilies were found at two locations, although at one of these locations, there was a mixture of CaCV and TSWV-infected plants in the same garden (Fig. 1). Ravenshoe is at a tropical latitude (17.56 S) but experiences cooler temperatures than surrounding regions since it is the highest point of the Atherton Tableland (930 m altitude).

Other ornamental host plants of orthotospoviruses in Queensland

Apart from spider lilies, other plant species showing symptoms typically associated with orthotospoviral infections were also included in the survey. Some of these samples were found near diseased spider lilies and were infected by the same virus species, suggesting vector transmission between the plants or from a third, identical source. For instance, in southeast Queensland, individual TSWV-infected agapanthus (*Agapanthus praecox* Willd.) in Toowong and swamp lily (*Crinum pedunculatum* R.Br.) in Surfers Paradise were found next to clumps of TSWV-infected spider lilies. Similarly, a CaCV-infected hippeastrum was found next to a CaCV-infected spider lily in Babinda in far north Queensland.

Other TSWV-infected ornamental plants found in southeast Queensland included bridal bouquet (*Plumeria pudica* Jacq.) from Cleveland, *Dahlia* 'Café au Lait' from Anstead, cast iron plants (*Aspidistra elatior* Blume) from Dutton Park, petunia (*Petunia*×*atkinsiana* (Sweet) W.H.Baxter) from Toowong and another agapanthus from Moorooka (Additional file 1: Table S1). Additionally, two CaCV-infected hoya plants, *Hoya australis* R.Br. and *H. macgillivrayi* F.M.Bailey, were also found in the Brisbane suburb of Yeerongpilly (Additional file 1: Table S1).

Genetic relationships of orthotospoviruses infecting spider lilies in Queensland

Three hypotheses were considered to explain the distribution of orthotospoviruses in spider lilies in Queensland. The first hypothesis suggests that infected plants have been divided through vegetative propagation practices and the progeny plants distributed over large distances by the nursery trade of infected plants. The second hypothesis states that each infected spider lily plant is the result of an independent transmission event. The final hypothesis combines the first two, proposing both horizontal and vertical transmission of the viruses between plants. To examine these hypotheses, clustering analysis was performed on the complete nucleocapsid protein sequences of TSWV and CaCV to identify population structures (i.e., clustering patterns) among the virus samples in this study.

The sequence clustering analysis based on complete N protein sequences showed that most of the TSWV samples collected in southeast Queensland formed a single clade that was sister to other TSWV isolates reported from capsicum plants in Bowen and Gatton, Queensland (Fig. 2). However, one of the southeast Queensland TSWV isolates collected from a spider lily plant in Boonah (TSWV sample number 1–1 from Boonah, Additional file 1: Table S1) and the bridal bouquet sample from Redland City (TSWV sample number 3–1 from Cleveland) formed part of the same clade as TSWV from capsicums in central (Bowen) and southeast (Gatton) Queensland.

The TSWV isolates from Ravenshoe in far north Queensland were more closely related to TSWV isolates recorded in Western Australia and South Australia than those found in spider lily plants in southeast Queensland (Fig. 2). Additionally, the TSWV isolate detected in *Dahlia* sp. 'Café au Lait' (TSWV sample number 1–1 from Anstead) also clustered with TSWV from other states (Fig. 2). According to the submitter of the sample, this dahlia plant was propagated from a tuber purchased in Victoria, and disease symptoms were apparent from the very first leaves, suggesting the origin of this virus isolate was likely also Victoria.

In the case of CaCV, all three previously reported CaCV sequences in Australia were from capsicum in Queensland. Together with the samples collected in this study, the CaCV sequences clustered into two clades

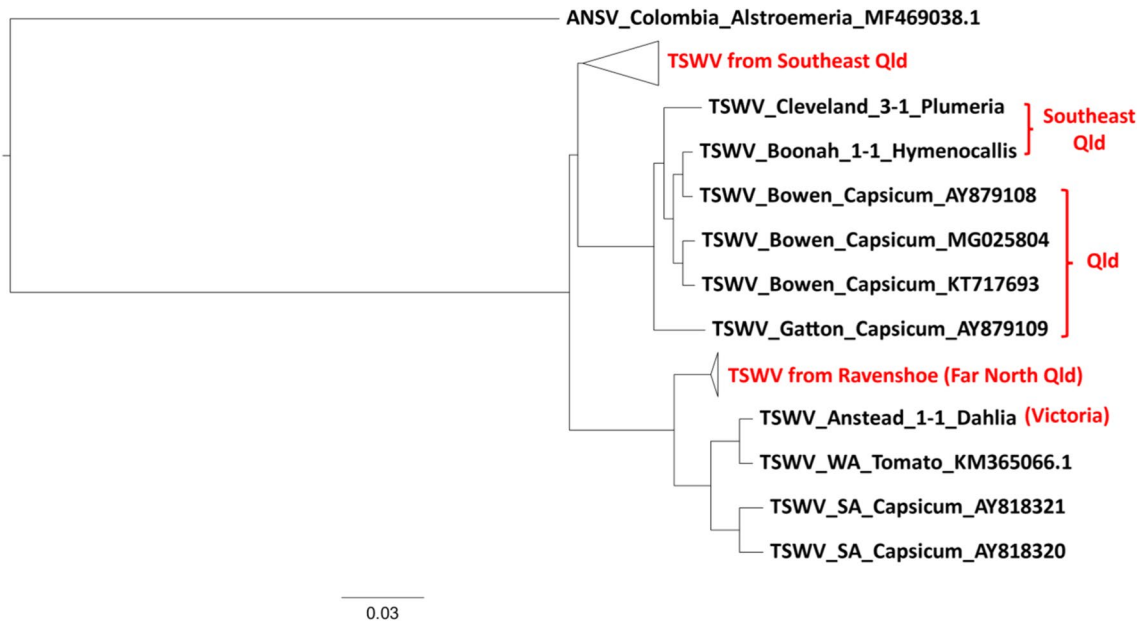

Fig. 2 Dendrogram representing the sequence clustering results for tomato spotted wilt virus (TSWV) nucleocapsid protein sequences. Sequences from this study are labelled in the format of TSWV_suburb/town_sample number_host genus, while sequences with a GenBank accession number are from other studies. The dahlia sample was collected in Anstead, Queensland, but the plant was known to be from Victoria. All sequences from this study (including those in collapsed clades) are described in Additional file 1: Table S1. ANSV, Alstroemeria necrotic streak virus; Qld, Queensland; WA, Western Australia; SA, South Australia

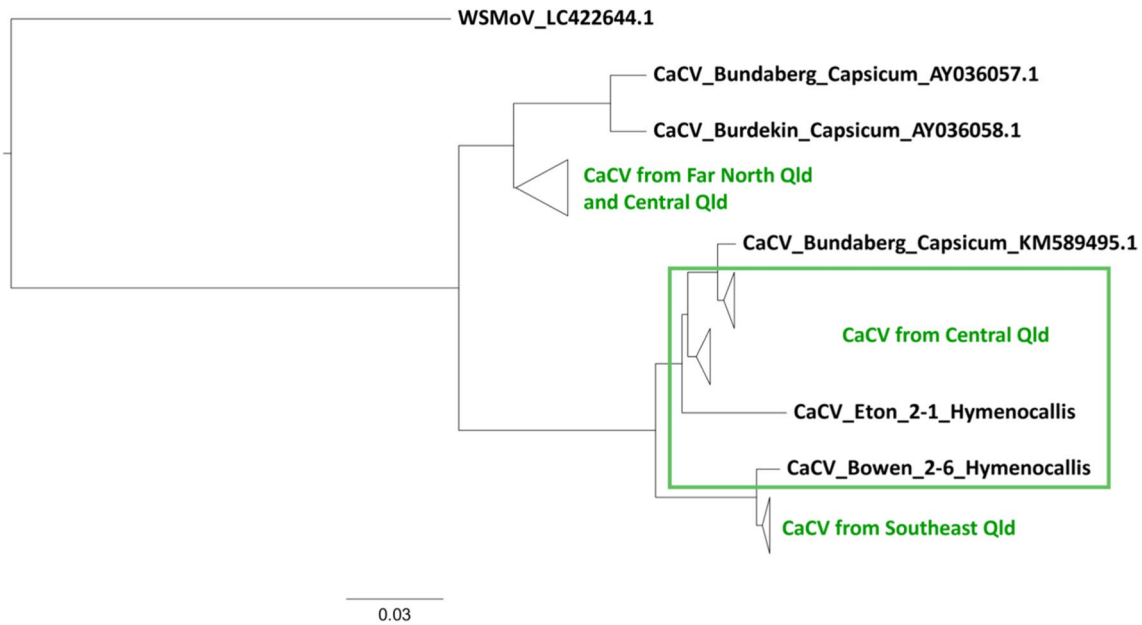

Fig. 3 Dendrogram representing the sequence clustering results for capsicum chlorosis virus (CaCV) nucleocapsid protein sequences. Sequences from this study are labelled in the format of CaCV_suburb/town_sample number_host genus, while sequences with a GenBank accession number are from other studies. All sequences from this study (including those in collapsed clades) are described in Additional file 1: Table S1. WSMoV, watermelon silver mottle virus; Qld, Queensland

(Fig. 3). One clade contained all sequences from south-east Queensland, including two spider lily and the two *Hoya* sp. samples, and one of the previously reported CaCV sequences (GenBank accession KM589495.1), while the other clade included all sequences from samples collected in far north Queensland and the other two previously reported CaCV sequences from capsicums (GenBank accessions AY036057.1 and AY036058.1). Interestingly, the sequences of samples from central Queensland were present in both clades, indicating the co-occurrence of both populations in the region. Like the case of TSWV, the clustering result also suggests that CaCV circulates between crops and ornamental plants given the presence of CaCV sequences from spider lilies highly similar to the CaCV sequence from capsicum (GenBank accession KM589495.1).

The clustering results of the TSWV and the CaCV sequences both showed a diversity of subpopulations among these viruses, and the clustering of sequences from different plant hosts provided strong evidence for the presence of vector transmission.

Detection of TSWV in spider lily plants

On many spider lily plants, discrete ringspots were observed on an otherwise green leaf lamina. It was hypothesised that each ringspot represented a local lesion that developed at the point of inoculation by thrips, and that the number of ringspots would reflect the infection pressure. To test this hypothesis, TSWV-infected spider lily leaves showing ringspot symptoms were collected, with the leaf lamina sampled at multiple spots from the leaf and tested for the presence of virus as illustrated in Fig. 4. The test results suggested that TSWV was restricted to the symptomatic areas. Such experiments were repeated with ten more leaves collected from naturally infected spider lily plants, including both *H. littoralis* and *H. speciosa*, and the same pattern of infection was observed.

To examine whether TSWV can systemically infect spider lilies, five plants (two *H. littoralis* and three *H. speciosa*) with discrete ringspot symptoms were dug up from gardens and maintained in the glasshouse. RT-PCR results consistent with Fig. 4 were obtained from the symptomatic leaves at the time of transplantation, suggesting a localised nature of TSWV in the plant. The first few newly emerged leaves were asymptomatic and tested negative for TSWV, but after approximately four months and the emergence of about nine uninfected and asymptomatic leaves, one *H. speciosa* plant became obviously symptomatic, and TSWV could be detected uniformly across the leaf lamina, no matter whether the area was showing symptoms or not (Fig. 5). Later, one *H. littoralis* plant also developed systemic infection. TSWV was detectable in the adventitious shoots of the two systemically infected plants, further confirming the systemic nature of the infection. The other three spider lily plants remained asymptomatic and uninfected over a total of 6 months of observation and testing.

Transmission of TSWV by *Taeniothrips eucharii*

Blue sticky traps were set up close to TSWV-infected spider lilies at five different locations in Brisbane. Only 33 thrips from these locations tested positive for TSWV, and each was identified as *Taeniothrips eucharii* by DNA barcoding (>99% pairwise nt sequence identity to *T. eucharii*, GenBank Accession KT946651.1).

As *T. eucharii* was not previously reported as a vector of TSWV, experiments were carried out to examine the

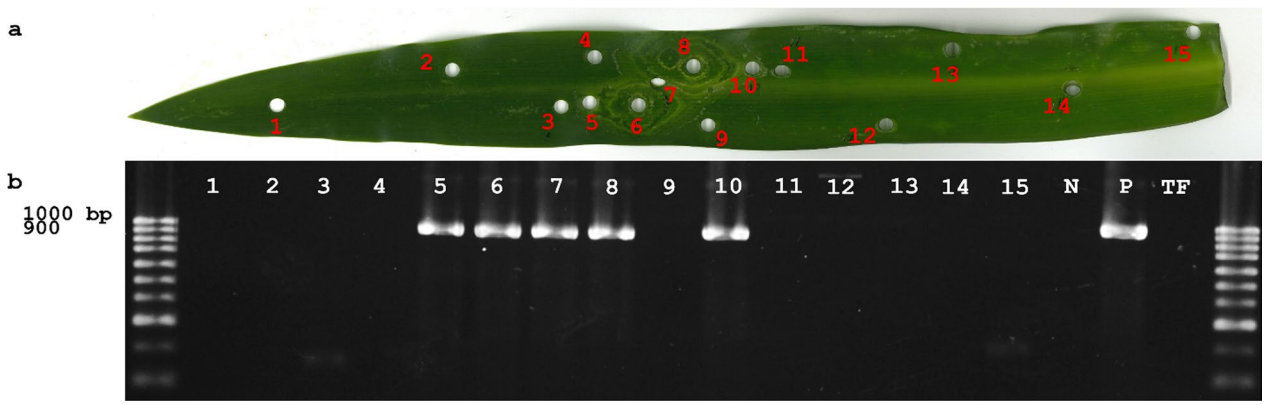

Fig. 4 Detection of tomato spotted wilt virus (TSWV) in a *Hymenocallis speciosa* leaf with discrete ringspots. **a** Image of the leaf showing the location of the tissue sampling sites (numbered 1 to 15) relative to the ringspot symptoms. **b** Detection of TSWV by reverse transcriptase-polymerase chain reaction. The lane number corresponds to the sampling site in **a**. The target amplicon is about 900 bp, and N, P and TF are the negative, positive and template-free controls, respectively. N was extracted from a TSWV-negative spider lily sample, and P from a TSWV-positive spider lily sample

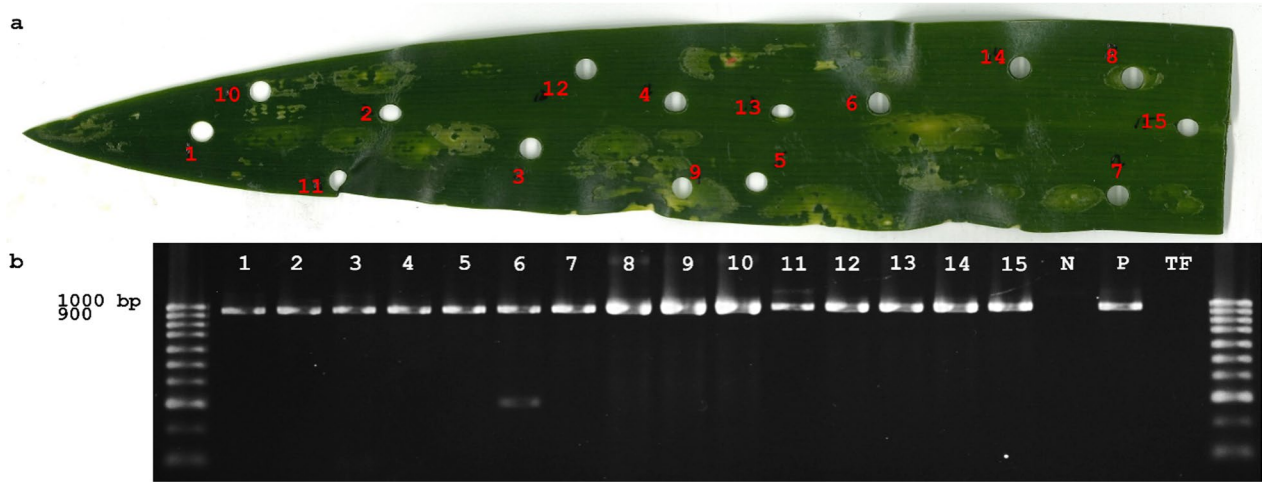

Fig. 5 Detection of tomato spotted wilt virus (TSWV) in a *Hymenocallis speciosa* leaf with systemic infection. **a** Image of the leaf showing the location of the tissue sampling sites (numbered 1 to 15) relative to the symptoms. **b** Detection of TSWV by reverse transcription polymerase chain reaction. The lane number corresponds to the sampling site in **a**. The target amplicon is about 900 bp, and N, P, and TF are the negative, positive, and template-free controls, respectively. N was extracted from a TSWV-negative spider lily sample, and P from a TSWV-positive spider lily sample

competency of this thrips species to transmit the virus. Eight out of 15 detached leaves of *Emilia sonchifolia* (L.) DC. showed disease symptoms (chlorotic and necrotic lesions) 7 days after inoculation with TSWV-viruliferous thrips, and these leaves tested positive for the virus by RT-PCR (Fig. 6). Additionally, three out of four *E. sonchifolia* plants became systemically infected after viruliferous thrips fed on the plants. Some of the newly emerged leaves that tested positive for TSWV showed chlorotic ringspot symptoms as well as various other chlorotic or necrotic lesions, while others were asymptomatic. In total, 11/19 (58%) of the individual thrips transmitted TSWV (Fig. 6b). The thrips were removed from the plants after the inoculation access period, and their identity as *T. eucharii* and the presence of TSWV were confirmed by DNA barcoding and RT-PCR (Fig. 6a), respectively.

Discussion

This study demonstrates that spider lilies are promising sentinel plants for surveillance of orthotospoviruses in the environment. Strong geographic structuring of the orthotospoviruses infecting spider lilies in Queensland was observed, and this likely reflects differences in the abundance of these viruses in the different regions, and even in different suburbs or towns in the same region. The most striking pattern in virus distribution was the complete reversal in the relative abundance of TSWV and CaCV as latitude decreased and the climate transitioned from subtropical (e.g., Brisbane) to tropical (e.g., Bowen). TSWV has been recorded as a pathogen of tomato and capsicum in Bowen between 1998 and 2015 (Persley et al. 2006; Moyle et al. 2016) but was not represented in the spider lily samples that were collected during our most recent surveys. However, CaCV has become the dominant orthotospovirus in this region since about 2018 (Gambley 2022), which agrees with our sampling results from spider lilies.

The reasons for the distinctive differences in orthotospovirus distribution in Queensland are unclear from this study, but several reasons can be speculated. The presence of TSWV correlated with cooler climates, as experienced in southeast Queensland and the very highest parts of the Atherton Tableland in far north Queensland. Climate strongly influences the distribution of thrips vectors species. For example, *Thrips palmi* is considered one of the main vectors of CaCV, and this thrips species is restricted to tropical and subtropical parts of the world including northern Australia, but it is absent in more temperate regions such as southern Australia (EPPO Global Database 2024). *Frankliniella schultzei* is now recognised to be a complex of closely related species that correspond to the colour morphs, and these colour morphs occupy geographically distinct areas (Hereward et al. 2017). In Australia, the brown and black morphs of *F. schultzei* occur to the south of the Tropic of Capricorn, while the yellow morph is the dominant form north of this line. The vector competencies of each of these colour morphs of *F. schultzei* to transmit the different orthotospoviruses have yet to be fully explored, but it has been demonstrated that the yellow morph does transmit CaCV at a very high efficiency (Sharman et al. 2020).

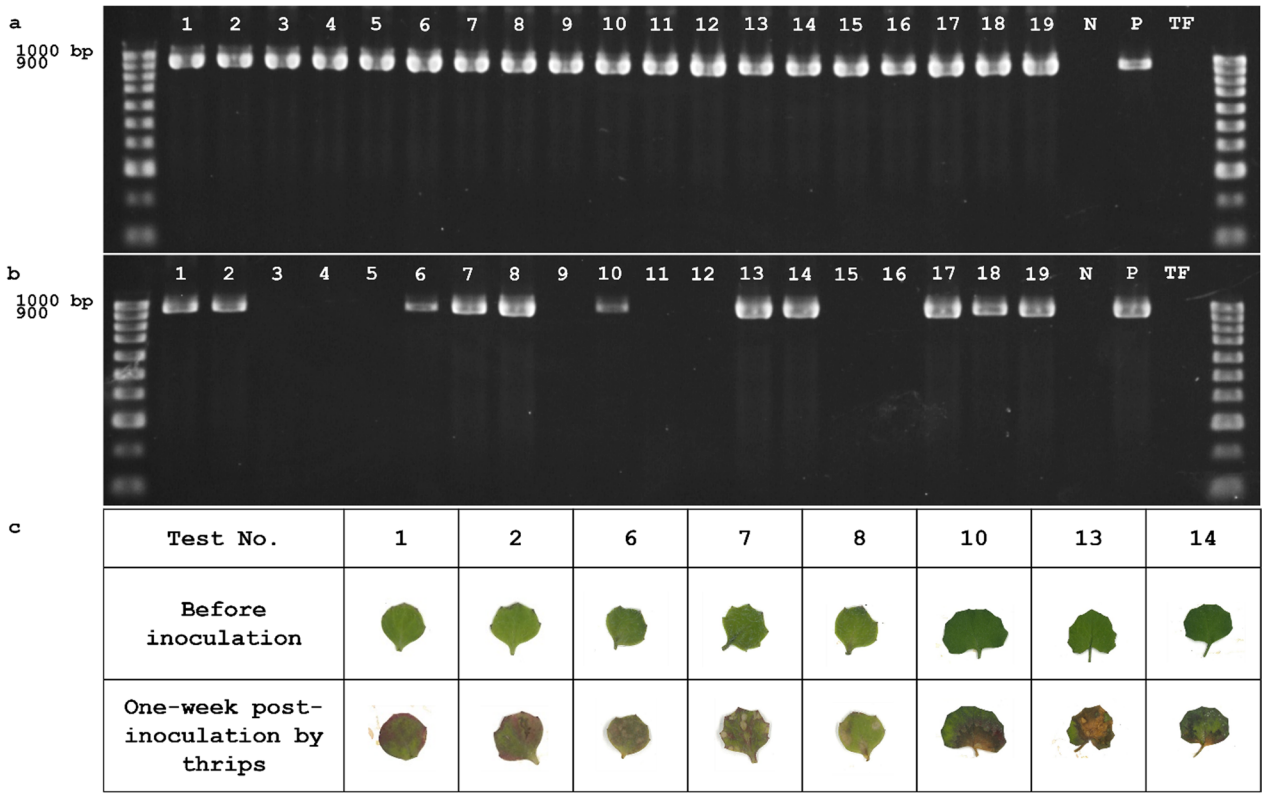

Fig. 6 Tomato spotted wilt virus (TSWV) transmission test results. **a** TSWV reverse transcription-polymerase chain reaction (RT-PCR) test results of *Taeniothrips eucharii* (Whetzel) used for inoculating *Emilia sonchifolia* (L.) DC. **b** TSWV RT-PCR test results of *E. sonchifolia* fed on by *T. eucharii*. **c** Symptoms before and after virus inoculation by thrips in *E. sonchifolia* leaves testing positive for TSWV. TSWV transmission tests 1 to 15 were from thrips to detached leaves, while tests 16 to 19 were from thrips to whole plants. The target amplicon is about 900 bp, and N, P, and TF are the negative, positive, and template-free controls, respectively. N was extracted from a TSWV-negative spider lily sample, and P from a TSWV-positive spider lily sample. The test numbers in panel **c** correspond to the lane numbers in panels **a** and **b**

Temperature may also affect the susceptibility of spider lily plants to infection by either CaCV or TSWV, and it certainly would influence what alternative host plant species for these viruses may be growing in the vicinity of the spider lilies.

The phylogenetic analyses also indicated that distinct genetic lineages of TSWV and CaCV infect spider lilies in the different regions of Queensland. For instance, the population of TSWV isolates from Ravenshoe in far north Queensland was distinct from that circulating in southeast Queensland. This result suggests that the prevalence of orthotospoviruses in spider lilies cannot simply be explained by vegetative plant propagation practices and vertical transmission of the virus from infected nuclear stocks to progeny plants. However, the possibility of a combination of vertical and horizontal transmission of orthotospoviruses cannot be dismissed, as it was demonstrated that spider lilies can sometimes become systemically infected by TSWV, and the virus spreads into the adventitious shoots (slips) that are used for propagation.

In this study, we demonstrated for the first time that *Taeniothrips eucharii* is a vector of TSWV. Combining this observation with the previous report of *T. eucharii* being a vector of HCRV (Xu et al. 2017) shows that this thrips species is capable of transmitting both Eurasian and American orthotospoviruses, and therefore it might act as a vector for even more orthotospoviruses such as CaCV. *T. eucharii* is thought to have originated in Asia but has now spread to the Americas, Australia, and Europe, possibly mediated by the horticultural trade (Mound and Tree 2008, 2020; Cavalleri and Lima 2024). Its known breeding hosts are restricted to the Amaryllidaceae (*Crinum, Dietes*, *Eucharis*, *Hymenocallis*, *Lycoris*, *Narcissus*, and *Zephyranthes*), the Asparagaceae (*Beaucarnea*, *Ophiopogon*, *Rohdea*, and *Sansevieria*), and the Liliaceae (*Lilium* and *Liriope*), on which it is associated with the flowers, leaf bases, and bulbs (Mound and Tree 2008; Cavalleri and Lima 2024). One would expect that *T. eucharii* would facilitate transmission of orthotospoviruses between plant species in these botanical families, but whether it would have a broader vectoring role in

vegetable crops is questionable. In this study, *T. eucharii* was shown to be capable of transmitting TSWV between spider lilies and *E. sonchifolia*, a member of the Asteraceae, but this was a no-choice feeding experiment and therefore probably not representative of what may happen in nature. *T. eucharii* would amplify orthotospoviral infections within stands of spider lilies, but other polyphagous thrips species would likely be responsible for the spread of the orthotospoviruses between spider lilies and other dicotyledonous plant species.

T. eucharii was the only TSWV-carrying thrips species that was trapped near the spider lilies in Brisbane, and the trapping was done in winter when the plants were not flowering. This suggests that *T. eucharii* is an important vector of TSWV infection in spider lilies because it indicates virus transmission outside the flowering season. The blossom of spider lilies only lasts for a short period, usually mid-late summer to early autumn. Although orthotospoviral infections were identified in other potential host plants of *T. eucharrii* including agapanthus, crinum, and hippeastrum, the incidence of infection was much lower than in spider lilies, suggesting that either the spider lilies are much more susceptible to orthotospoviruses than other related bulbous host plants, or alternatively they are much more attractive to *T. eucharrii*.

Conclusions

This study demonstrates the potential of using spider lilies as sentinel plants to monitor the presence of orthotospoviruses in a region. Spider lilies are grown throughout tropical and subtropical regions of the world, so the method would have broad applicability. However, spider lilies are frost-sensitive and therefore the method cannot be extended to temperate regions such as southern Australia. We also confirmed the ability of *T. eucharii* to transmit TSWV, highlighting the need for more research to increase our knowledge of thrips species capable of transmitting orthotospoviruses and to develop more comprehensive pest management strategies.

Methods

Plant and thrips surveys

From 2020 to 2023, leaves (mainly *Hymenocallis* spp.) with foliar chlorotic ringspots, spots, and blotches (examples shown in Additional file 2: Figure S1) were sampled from various locations in Queensland (Additional file 1: Table S1), mostly public spaces like council parks and roadside verges. In the laboratory, symptomatic portions of the leaf were dissected out using a razor blade or a biopsy punch, lyophilised, and stored at −20°C until virus testing by RT-PCR.

For ease of reference in this study, narrow-leaf-type and broad-leaf-type spider lilies are referred to as *H. littoralis* and as *H. speciosa*, respectively, despite the underlying taxonomic uncertainty. Five spider lily plants (two *H. littoralis* and three *H. speciosa*) showing symptoms were dug up from gardens in Brisbane and the Gold Coast and maintained in the glasshouse at the Ecosciences Precinct, Dutton Park, Queensland. These plants were transplanted into pots with commercial potting mix (Searles Premium) and sprayed with Confidor insecticide (active ingredient: imidacloprid) before placement in the glasshouse. A tablet formulation of Confidor was also mixed into the potting mix.

To identify the thrips species associated with TSWV transmission in spider lilies, blue sticky traps with Stikem Special (Seabright Laboratories, CA, USA) adhesive were set up next to clumps of infected plants at five locations in inner Brisbane, two in Dutton Park, one in St Lucia, one in Toowong, and one in Woolloongabba, during June to August 2022. Each trap was set up for a week, and thrips specimens were removed from the traps by immersing them in De-Solv-It Solution (Vardon Industries, Australia), cleaned with dishwashing liquid and then rinsed with water. The thrips were stored in 85% isopropanol before further PCR testing (see below).

Thrips transmission

Live thrips from the flowers of spider lilies that were tentatively identified by size and colour as *Taeniothrips euchari* were introduced onto a spider lily plant (*H. speciosa*) that was systemically infected with TSWV. The thrips and the plant were maintained in an insect rearing tent (BugDorm Cat. EM2E400) at ambient, outside temperature, to establish a viruliferous thrips colony. The thrips colony was maintained from February 2023 for 3 months before the TSWV transmission experiments to ensure that the thrips were exposed to TSWV for several generations. For the transmission experiments, *E. sonchifolia* was propagated using seeds collected from healthy plants and maintained in an insect-free growth chamber with a diurnal cycle of 16 h light and 8 h dark at 25°C and 50% humidity.

All thrips used in the TSWV transmission experiments were adult individuals. Before use, each thrips was starved overnight in a closed container. The individual thrips were then placed with one leaf or one plant (the whole plant with roots wrapped in wet tissue paper) of *E. sonchifolia* in the same container for 1–3 days depending on the extent of feeding damage that was observed. After the feeding step, the thrips were collected for TSWV testing and cytochrome c oxidase subunit I (COX1) DNA barcoding as described below. Each *E. sonchifolia* leaf was then floated on water in another closed container in the insect-free growth chamber for 7 days and then tested for TSWV, while each *E. sonchifolia* plant was first

incubated in a closed container for 2 weeks to ensure no emergence of nymphal thrips and then transplanted into potting mix, all done in the insect-free growth chamber. One month after transplanting, newly emerged leaves of the *Emilia* plants were tested for TSWV. The experiments were conducted using 15 detached leaves and four entire plants of *E. sonchifolia*.

Nucleic acid extraction

Total nucleic acid extractions were done using a BioSprint® 15 DNA Plant Kit (QIAGEN, Germany) as per the manufacturer's instructions, except that RNase A was not used during extraction. Lyophilised leaf samples were homogenised with a 5 mm-diameter stainless steel bead in 300 µL Buffer RLT in a 2-mL safe-lock tube, while thrips samples were instead homogenised with 0.5 mm-diameter glass beads. Sample homogenisation was carried out using a TissueLyser II (QIAGEN) at 30 Hz for 3 min.

RT-PCR detection of orthotospoviruses

Orthotospoviruses were initially identified by RT-PCR using a MyTaq™ One-Step RT-PCR Kit (Meridian Bioscience, Cincinnati OH, USA) and TospoRp-F/TospoRp-R primers (Table 1, A.D.W. Geering, unpublished), each at 0.4 µM final concentration, with other reagents added as per the manufacturer's instructions. These primers target conserved motifs in the RNA-dependent RNA polymerase gene (*RdRp*) and are broad-spectrum for orthotospoviruses. The thermocycling parameters were 45°C for 20 min, 95°C for 2 min, 40 cycles of 95°C for 15 s, 46°C for 15 s, and 72°C for 1 min, and one final extension cycle of 72°C for 5 min. Amplicon sequencing was done using the same PCR primers.

Table 1 Primers used in this study

Primer name[a]	Primer sequence	Amplicon size
PCR Primers (5′–3′)		
TospoRp-F[b]	GGCATCATRTAIATIGCIARRTAIACATT	~320 bp
TospoRp-R[b]	ATGGGIATITTYGAYTTYATGIGRYATGC	
TSWV-N-F1	AGCAAGCACAAGCAATAAAGATAAAGAAA	~930 bp
TSWV-N-R1	CGACTGCGGVATACAGAGTTG	
CaCV-N-F2	GGGAARATRAYYGGTGCAAG	532 bp
CaCV-N-R1	ACGTTTCCAKAGTAAWCACC	
CaCV-N-F1	ACATGAYAATAGATTAATATGAATTGACTTATAATTAAATATAAC	~950 bp
CaCV-N-R1	as above	
NAD5-F	GATGCTTCTTGGGGCTTCTT	325 bp from plant RNA
NAD5-R	GAGCTRATCCRAAATCMCCTACTCG	
CaCV-S-F	AGAGCAATCGRGGCWTCTAATAATTCAAG	~3.5 to 4 kb
CaCV-S-R	AGAGCAATCGAGGCRCTAATAAAATC	
C1-J-1751[c]	GGATCACCTGATATAGCATTYCC	~480 bp
C1-N-2191[d]	CCCGGTAAAATTAAAATATAAACTTC	
Sequencing Primers (5′–3′)		
TospoRp-F[b]	as above	
TospoRp-R[b]	as above	
TSWV-N-F2	TCRACIGAAGCAATRAGAGGTAA	
TSWV-N-R2	ATGACCTYCAGAAGGCTTGA	
CaCV-N-F2	as above	
CaCV-N-R2	GAYTGGACTTTYAAGAGRACAGA	
C1-J-1751[c]	as above	
C1-N-2191[d]	as above	
Reverse Transcription Primer (5′–3′)		
CaCV-S-RT	AGAGCAATCGAGGCRCTAAT	

[a] All primers were designed by the first author unless specifically stated otherwise

[b] Designed by A.D.W. Geering

[c] Lin et al. 2004

[d] Simon et al. 1994

For specific detection of TSWV, the TSWV-N-F1/TSWV-N-R1 primer pair (Table 1) was used, which allowed amplification of the entire *N* gene sequence. RT-PCR was done using a OneTaq® One-Step RT-PCR Kit (New England Biolabs, Ipswich MA, USA). Each primer was used at a final concentration of 0.8 µM, and the thermocycling conditions were 48°C for 30 min, 94°C for 1 min, 40 cycles of 94°C for 15 s, 50°C for 30 s, and 68°C for 1 min, and ending with 68°C for 5 min. Amplicon sequencing was done using the nested primers TSWV-N-F2 and TSWV-N-R2 (Table 1).

For specific detection of CaCV, the CaCV-N-F2/CaCV-N-R1 primer pair (Table 1) was used to amplify a portion of the *N* gene, using the same RT-PCR protocol as for TSWV detection. Amplifying the complete *N* gene of all CaCV isolates was more challenging than TSWV as the intergenic region of the S segment RNA is more variable in CaCV, and therefore either a one-step or two-step RT-PCR was used. For one-step reactions, the CaCV-N-F1 primer was paired with the CaCV-N-R1 primer (Table 1) using the same one-step RT-PCR protocol employed for specific detection of CaCV.

In the event of failure of the one-step RT-PCR, the cDNA of the entire S segment RNA of CaCV was synthesised with the primer CaCV-S-RT (Table 1) using Maxima H Minus Reverse Transcriptase (Thermo Fisher Scientific, Waltham MA, USA) as per the manufacturer's instructions. PCR was then done with the CaCV-S-F/CaCV-S-R (Table 1) primer pair to amplify the cDNA using either a LongAmp® Taq 2X Master Mix (New England Biolabs) or a PrimeSTAR® GXL DNA Polymerase (Takara Bio, Japan) as per the manufacturers' instructions. The thermocycling conditions used in LongAmp® reactions were 94°C for 30 s, 40 cycles of 94°C for 10 s, 55°C for 15 s, and 65°C for 4 min, and ending with 65°C for 10 min, while those used in PrimeSTAR® GXL reactions were 40 cycles of 98°C for 10 s, 55°C for 15 s and 68°C for 4 min. The primers used for sequencing the *N* gene of CaCV were CaCV-N-F2 and CaCV-N-R2 (Table 1).

To rule out the possibility of negative orthotospovirus test results due to poor RNA quality, the plant NADH dehydrogenase subunit 5 gene (*nad5*) was amplified using the primer pair NAD5-F/NAD5-R (Table 1), employing the same one-step RT-PCR protocol as for detection of TSWV and CaCV. This RT-PCR was done in parallel to the orthotospovirus RT-PCRs.

Thrips identification by DNA barcoding

To identify thrips to the species level, PCR was done using the C1-J-1751/C1-N-2191 (Simon et al. 1994; Lin et al. 2004) primer pair targeting the mitochondrial *COX1* gene. PCR was done using a MyTaq™ HS Red Mix (Meridian Bioscience) with reagent concentrations recommended by the manufacturer, and the thermocycling conditions were 95°C for 1 min and 40 cycles of 95°C for 15 s, 45°C for 15 s, and 72°C for 30 s. Amplicon sequencing was done using the same PCR primers.

Phylogenetic analyses

Orthotospovirus N protein sequences were clustered using the online server of CLUSS1 version 2.0 (Kelil et al. 2007a; 2007b; 2007c) with BLOSUM62 as the substitution matrix. The clustering results were visualised using FigTree 1.4 (Rambaut Lab 2018). CLUSS (Kelil et al. 2007a, 2007c) was used for the sequence clustering analysis as it conducts motif matching and is more sensitive to subtle differences among similar protein sequences compared to conventional phylogenetic tree reconstruction methods (e.g., maximum likelihood and distance-based methods like neighbor-joining) that treat each position in an alignment independently. CLUSS effectively groups sequences with similar patterns of sequence conservation.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-025-00325-5.

Additional file 1: Table S1. Sample details for the orthotospovirus isolates collected in this study. Samples from the same suburb/town with the same sample number before the hyphen (-) are collected from the same location. TSWV: tomato spotted wilt virus. CaCV: capsicum chlorosis virus

Additional file 2. Figure S1. A selection of symptoms observed on othotospovirus-infected spider lily leaves. a Hymenocallis littoralis infected with tomato spotted wilt virus (TSWV). b H. speciosa infected with TSWV. c H. littoralis infected with capsicum chlorosis virus (CaCV). d H. speciosa infected with CaCV

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Author contributions

HYC conceptualised the study, collected samples, generated, analysed, and interpreted the data, wrote the original draft, and finalised the manuscript. RGD, JET and ADWG collected plant samples and reviewed and edited the manuscript. Funding for this project was obtained by ADWG.

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Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

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Competing interests
The authors declare that they have no competing interests.

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