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RESEARCH ARTICLE

Natural host range, thrips and seed transmission of distinct *Tobacco streak virus* strains in Queensland, Australia

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Abstract

Diseases caused by *Tobacco streak virus* (TSV) have resulted in significant crop losses in sunflower and mung bean crops in Australia. Two genetically distinct strains from central Queensland, TSV-parthenium and TSV-crownbeard, have been previously described. They share only 81% total-genome nucleotide sequence identity and have distinct major alternative hosts, *Parthenium hysterophorus* (parthenium) and *Verbesina encelioides* (crownbeard). We developed and used strain-specific multiplex Polymerase chain reactions (PCRs) for the three RNA segments of TSV-parthenium and TSV-crownbeard to accurately characterise the strains naturally infecting 41 hosts species. Hosts included species from 11 plant families, including 12 species endemic to Australia. Results from field surveys and inoculation tests indicate that parthenium is a poor host of TSV-crownbeard. By contrast, crownbeard was both a natural host of, and experimentally infected by TSV-parthenium but this infection combination resulted in non-viable seed. These differences appear to be an effective biological barrier that largely restricts these two TSV strains to their respective major alternative hosts. TSV-crownbeard was seed transmitted from naturally infected crownbeard at a rate of between 5% and 50% and was closely associated with the geographical distribution of crownbeard in central Queensland. TSV-parthenium and TSV-crownbeard were also seed transmitted in experimentally infected ageratum (*Ageratum houstonianum*) at rates of up to 40% and 27%, respectively. The related subgroup 1 ilarvirus, Ageratum latent virus, was also seed transmitted at a rate of 18% in ageratum which is its major alternative host. Thrips species *Frankliniella schultzei* and *Microcephalothrips abdominalis* were commonly found in flowers of TSV-affected crops and nearby weed hosts. Both species readily transmitted TSV-parthenium and TSV-crownbeard. The results are discussed in terms of how two genetically and biologically distinct TSV strains have similar life cycle strategies in the same environment.

Introduction

Tobacco streak virus (TSV), the type member of the plant infecting Ilarviruses (family: *Bromoviridae*), has a wide host range (Brunt *et al.*, 1996), is pollen borne and transmitted by thrips (Sdoodee & Teakle, 1987; Prasada Rao *et al.*, 2003). Some strains of TSV have also been shown to be seed transmitted (Kaiser *et al.*, 1991; Sharman *et al.*, 2009). TSV has a single-stranded RNA genome, separated into three linear segments designated RNA-1 to -3 (King *et al.*, 2012), which are encapsidated separately in quasi-isometric to bacilliform virions.

TSV has been reported as the causal agent for major disease outbreaks in sunflower and mung bean in Australia (Sharman *et al.*, 2008), in oilseed and pulse crops in India (Prasada Rao *et al.*, 2000; Reddy *et al.*, 2002) and in soybean in Brazil (Almeida *et al.*, 2005) and the USA (Rabedeaux *et al.*, 2005). In Australia and India, parthenium weed (*Parthenium hysterophorus*) is the major alternative host of TSV strains that are closely associated with disease

outbreaks in nearby crops (Prasada Rao *et al.*, 2003; Sharman *et al.*, 2009). However, the TSV strains from the two countries are genetically distinct (Sharman & Thomas, 2013).

The subgroup 1 ilarviruses reported to date from Australia are three genetically distinct TSV strains, Ageratum latent virus (AgLV), and *Strawberry necrotic shock virus* (SNSV; Sharman *et al.*, 2011; Sharman & Thomas, 2013). AgLV and SNSV were originally described as strains of TSV in earlier work (Greber, 1979; Sdoodee, 1989), but we have shown these to be distinct viruses (Sharman & Thomas, 2013). The two most commonly found TSV strains in Australia that have been associated with disease outbreaks are referred to as TSV-parthenium and TSV-crownbeard. They have symptomless major alternative hosts of parthenium (*P. hysterophorus*) and crownbeard (*Verbesina encelioides*), respectively, they share only 81% total-genome nucleotide sequence identity and TSV-crownbeard reacts more strongly in a commercially available TSV enzyme-linked immunosorbent assay (ELISA) (Sharman & Thomas, 2013). Seed transmission of the TSV-parthenium strain occurs at rates of up to 48% in naturally infected parthenium and is likely to be a critical survival mechanism for the virus to survive drought conditions (Sharman *et al.*, 2009). While TSV-parthenium appears to be more important than TSV-crownbeard in disease outbreaks in sunflower crops (Sharman & Thomas, 2013) several aspects of the biology of these two TSV strains have not been reported.

In this article, we aim to fill the current knowledge gaps for aspects of the biology of the distinct TSV strains, TSV-parthenium and TSV-crownbeard. This includes describing their respective natural host ranges, the thrips species that transmit them and seed transmission. We also monitored for AgLV in central Queensland, a region previously unsurveyed for this virus. These results are discussed in terms of how these biological characteristics enable these distinct TSV strains to persist in the same environment and lead to disease epidemics in nearby susceptible crops.

Materials and methods

Virus isolates

We collected leaf material from a variety of plant species from many locations in central Queensland between 2006 and 2014, spanning a distance of about 750 km from Injune in the south to Alligator Creek in the north (Table 1). Samples were selected for indexing based on the presence of virus-like symptoms or randomly from locations close to high levels of typical TSV infection in susceptible crops. Observed symptoms varied depending on the host (Table 1) but often included chlorotic or necrotic line patterns, stem or terminal necrosis, stunting and leaf deformation.

We tested samples using TSV ELISA as previously described (Sharman *et al.*, 2009), and positive samples were tested using Polymerase chain reaction (PCR) as described below. The reference isolates previously used for complete genome characterisation (Sharman & Thomas, 2013), TSV-parthenium isolate-1973, TSV-crownbeard isolate-2334 and AgLV isolate-1998 were maintained in *Nicotiana tabacum* cv. Xanthi for further use as diagnostic controls and for additional biological studies. All isolate numbers refer to samples lyophilised and stored at −20°C in the Queensland Department of Agriculture and Fisheries plant-virus collection.

RNA segment-specific multiplex RT-PCRs for TSV strains

To design PCR primers (Table 2), we aligned previously published RNA-1 and -2 sequences (GenBank accessions listed in Sharman & Thomas (2013)) for TSV-WC, TSV-parthenium, TSV-crownbeard, AgLV, SNSV-MD and *Parietaria mottle virus* (PMoV) using the MUSCLE algorithm (Edgar, 2004). Regions that were either in common to both or specific to TSV-parthenium or TSV-crownbeard were selected visually. In doing so, primers for cDNA synthesis were designed to work for both TSV-parthenium and TSV-crownbeard (and other ilarvirus species) while strain-specific upstream primers were for use in PCRs.

Total nucleic acid extracts were prepared as previously described (Sharman & Thomas, 2013). To differentiate TSV-parthenium and TSV-crownbeard and to identify mixed infections and possible reassortments of RNA segments, we developed separate multiplex (MP) PCRs for RNA-1 and -2 to produce size-specific products for these two TSV strains. A MP-PCR for RNA-3 which detected the TSV strains and AgLV was used as previously described (Sharman & Thomas, 2013) except with the modified cDNA synthesis described here. SuperScript III reverse transcriptase (Life Technologies, Australia) was used to prepare cDNA essentially as per the manufacturer's instructions with the following modifications; a mix of 1 μM of each reverse primer TSVrep2769R (RNA-1), TSV2b2451R (RNA-2) and TSVRNA3.1982R (RNA-3) was used in a 10 µL reaction with the inclusion of 150 ng of acetylated bovine serum albumin (BSA; Life Technologies).

We used the resulting cDNA with TSV strain-specific forward primers for PCR (Table 2) with 1 unit native *Taq* DNA polymerase (Life Technologies), 1.75 mM $MgCl_2$, 200 mM dNTPs and 2 µL of cDNA template in a 25 µL reaction volume. Generic ramped annealing temperature cycling parameters were used for all PCRs, consisting of

Table 1 Natural host range of TSV-parthenium and TSV-crownbeard from surveys in central Queensland

Family	Species	Symptoms on Each Host Species	Isolate Number and TSV Strain; Parthenium (P) or Crownbeard (C)[a]	Month/Year of Collection	Nearest Locality
Amaranthaceae	*Amaranthus mitchellii*[b]	tn, sn, ld	2343 (P)	March 2009	Emerald
Apocynaceae	*Parsonsia* sp.[b]	crs, cll	2198 (P)	April 2008	Mt McLaren
Asteraceae	*Bidens pilosa*	Small ld, reddening	2201 (P)	April 2008	Clermont
	Carthamus tinctorius	cm, tn	2591 (P)	April 2010	Mt McLaren
	Conyza bonariensis	ld, ln, stunted	2520 (P), 2513 (P)	December 2009, November 2009	Arcturus, Emerald
	Eclipta prostrata[b]	Small ld	2521 (C)	December 2009	Arcturus
	Helianthus annuus	tn, sn, cm, cll, nll	1973 (P)[c], 1974 (P)[c], 2337 (C), 2341 (C), 2344 (P), 2580 (P), 5127 (P), 5128 (P), 5129 (P), 5140 (P), 5139 (P)	June 2006, June 2006, April 2009, April 2009, April 2009, March 2010, April 2014, April 2014, April 2014, May 2012, April 2013	Clermont, Clermont, Orion, Arcturus, Mt McLaren, Clermont, Clermont, Clermont, Capella, Mt McLaren, Clermont
	Lactuca serriola	chl	2610 (C)	May 2010	Arcturus
	Parthenium hysterophorus	None	2012 (P)[c], 2084 (P)[c], 2086 (P), 2087 (P)[c], 2103 (P)[c], 2105 (P)[c], 2139 (P), 2140 (P), 2514 (P), 2589 (P), 2590 (P), 5131 (P), 5132 (P), 5137 (P), 5138 (P)	February 2007, September 2007, September 2007, September 2007, October 2007, October 2007, January 2008, January 2008, November 2009, March 2010, March 2010, April 2014, April 2014, April 2014, April 2014	Mt McLaren, Orion, Tieri, Rubyvale, Collinsville, Alligator Ck, Nebo, Frankfield, Bauhinia, Mt McLaren, Gindie, Capella, Capella, Clermont, Clermont
	Senecio madagascariensis	None	2508 (P)	November 2009	Arcturus
	Sonchus oleraceus	ln, cm	2037 (P), 2511 (C)	April 2007, November 2009	Mt McLaren, Arcturus
	Tridax procumbens	None	3065 (C)	January 2012	Emerald
	Verbesina encelioides	None	2334 (C)[c], 2282 (C), 2338 (C), 2400 (C), 5141 (C), 5142 (P), 5143 (P + C), 5130 (P + C), 5133 (C), 5134 (C), 5135 (C), 5136 (C)	April 2008, November 2008, April 2009, June 2009, April 2010, April 2009, April 2009, April 2014, April 2014, April 2014, April 2014, April 2014	Emerald, Theodore, Orion, Gogango, Gindie, Emerald, Emerald, Arcturus, Comet, Comet, Arcturus, Arcturus
	Xanthium occidentale	ln, ld, nrs, nll, cm	2032 (P), 2102 (P), 2512 (P), 2524 (P), 834 (C), 835 (C)	April 2007, October 2007, November 2009, November 2009, August 1975, August 1975	Orion, Collinsville, Arcturus, Arcturus, Airdmillan, Airdmillan
Boraginaceae	*Trichodesma zeylanicum*[b]	None	2322 (P)	February 2009	Clermont
Commelinaceae	*Commelina benghalensis*	cm, tn	2615 (P)	May 2010	Arcturus
Fabaceae	*Alysicarpus muelleri*[b]	cm, clp	2323 (P)	February 2009	Clermont
	Arachis hypogaea	tn, cm, ld, crs	2165 (C) 2401 (C) 3098 (C) 2594 (P)	February 2008, May 2009, February 2012, April 2010	Emerald, Gogango, Emerald, Mt McLaren

Table 1 continued

Family	Species	Symptoms on Each Host Species	Isolate Number and TSV Strain; Parthenium (P) or Crownbeard (C)[a]	Month/Year of Collection	Nearest Locality
	Cajanus cajan	cll, nll, sn, crs	2143 (P)	January 2008	Clermont
	Cicer arietinum	tn, sn, wilting	1979 (P)[c], 2074 (P), 2075 (P)	August 2006, August 2007, August 2007	Clermont, Gindie, Gindie
	Crotalaria mitchellii subsp. *mitchellii* [b]	cm, crs	2199 (C)	April 2008	Emerald
	Glycine max	tn, mild cm	2200 (P), 2348 (P)	April 2008	Emerald
	Lupinus sp.	ld, tn	2592 (P)	April 2010	Mt McLaren
	Macroptilium lathyroides	cm, clp, cll	2163 (C)	February 2008	Emerald
	Phaseolus vulgaris	severe ld, cm	2346 (P)	April 2009	Mt McLaren
	Tephrosia sp. [b]	cll, crs	5126 (P)	February 2014	Mt McLaren
	Vicia faba	tn, cm	2347 (P)	April 2009	Mt McLaren
	Vigna radiata	tn, sn, nrs	2027 (P)[c], 2028 (P), 2342 (P), 2025 (P)	March 2007, March 2007, March 2009, March 2007	Orion, Dysart, Emerald, Arcturus
	Vigna radiata var. *sublobata* [b]	vn	2197 (P)	April 2008	Clermont
	Vigna unguiculata	nll, nrs	2345 (P)	April 2009	Mt McLaren
Malvaceae	*Abelmoschus ficulneus* [b]	chl.patches, clp	2036 (P), 2164 (P)	April 2007, February 2008	Mt McLaren, Emerald
	Gossypium hirsutum	Purple rs, spreading purple nll	2120 (P)[c], 2285 (P), 2399 (C), 2510 (C), 2735 (P)	November 2007, November 2008, June 2009, November 2009, January 2011	Emerald, Moura, Springsure, Theodore, Emerald
Phyllanthaceae	*Notoleptopus decaisnei* [b]	Mild clp, crs	2324 (P)	February 2009	Clermont
	Phyllanthus sp.	Mild clp, crs	2325 (P)	February 2009	Clermont
Solanaceae	*Capsicum annuum*	Mild cll	3107 (P)	April 2012	Clermont
	Datura ferox	ns, nrs	2326 (P)	February 2009	Clermont
	Datura leichhardtii	ld, ns, nrs	2035 (P)	April 2007	Mt McLaren
	Nicotiana megalosiphon subsp. *megalosiphon*[b]	ln, nlp	2593 (P)	April 2010	Capella
	Physalis lanceifolia	tn, sn, nrs	2034 (P)	April 2007	Mt McLaren
Tiliaceae	*Corchorus trilocularis*[b]	chl patches	2038[d]	April 2007	Mt McLaren
Verbenaceae	*Verbena bonariensis*	None	3063 (P)	January 2012	Arcturus

chl, chlorosis; clp, chlorotic line patterns; cm, chlorotic mottle; tn, tip necrosis; sn, stem necrosis; ld, leaf deformation; crs, chlorotic ringspots; cll, chlorotic local lesions; nll, necrotic local lesions; ns, necrotic spots; nrs, necrotic ringspots; nr, necrotic rings; rs, ring spots; nlp, necrotic line patterns; ln, leaf narrowing; vn, vein necrosis; ELISA, enzyme-linked immunosorbent assay; TSV, *Tobacco streak virus*.

[a]TSV strain determined by strain-specific MP-PCR for each RNA segment. TSV strain was confirmed as either TSV-parthenium (P) or TSV-crownbeard (C) for all three RNA segments.

[b]Plant species endemic to Australia.

[c]Partial or complete genome sequence has been derived for these isolates (Sharman *et al.*, 2008, 2009; Sharman & Thomas, 2013).

[d]Isolate-2038 failed in the MP-PCRs but had a TSV ELISA absorbance value of greater than 200 times the healthy control.

Table 2 PCR primers used in strain-specific MP-PCRs for RNA-1, -2 and -3 for identification of TSV-parthenium and TSV-crownbeard

Target RNA	Target TSV Strain	Primer Name	Sequence (5′ to 3′)	Final Concentration in PCR (nM)	Approximate PCR Product Size (bp)
RNA-1	TSV-parthenium	ParTSVrep2228F	CCCTCTGCACCCACTTCCGAA	200	540
	TSV-crownbeard	CrbTSVrep2420F	CTAGTCCCAACCTTCAAAATC	200	350
	Both strains	TSVrep2769R	GGAACTTGCTCKGTRTCACCAA	200	
RNA-2	TSV-parthenium	ParTSVpol1722F	GATAGTTTGATTGGATCGTTAAG	280	760
	TSV-crownbeard	CrbTSVpol2144F	GAGTTCCAAGGTTTGTATTCGT	200	300
	Both strains	TSV2b2451R	CCAGCACARTCAATGCAHTT	200	
RNA-3[a]	TSV-parthenium	CQTSVF	CCTACTCCAACCCTGATTA	300	920
	TSV-crownbeard	CrbTSVF	GCCCGTTTACCAGTACCAAT	80	570
	AgLV	SEQTSVF	CGCCATGTCTACTTCTAGGA	100	740
	TSV and AgLV	TSVRNA3.1982R	CCRCATCKCACACARGWATT	200	

[a]Primers and conditions for RNA-3 MP-PCR described by Sharman & Thomas (2013).

an initial denaturation of 95°C for 60 s, then 35 cycles of 95°C for 15 s, 62°C for 20 s, 56°C for 10 s and 72°C for 40 s, followed by a final extension of 72°C for 3 min.

Cross-infection studies of TSV strains and AgLV into major alternative hosts

The reference isolates maintained in *N. tabacum* cv. Xanthi, TSV-parthenium (-1973), TSV-crownbeard (-2334) and AgLV-1998 were manually inoculated using 0.1 M phosphate buffer and carborundum onto healthy seedlings of parthenium, crownbeard and ageratum (*Ageratum houstonianum*). Test plants were grown for 2–3 weeks before the newly emerging terminal growth was tested using TSV ELISA.

Seed transmission of TSV strains and AgLV in major alternative hosts and crop plants

In order to test for seed transmission of the TSV strains and AgLV from different hosts, we collected seed from either naturally infected plants or from plants inoculated with reference isolates. TSV-crownbeard transmission was tested from three naturally infected crownbeard plants (one of which was isolate-2334). TSV-parthenium transmission was tested from one plant each of naturally infected *Bidens pilosa* (isolate-2201) and *Conyza bonariensis* (isolate-2520), and multiple plants of infected sunflower and mung bean. All mother plants were tested using strain-specific PCR except for sunflower and mung bean mother plants which were tested using TSV ELISA prior to the PCR being available. The sunflower and mung bean mother plants were collected from the Clermont region where all other samples tested using PCR over several years have been TSV-parthenium with no TSV-crownbeard detected.

In order to determine whether seed transmission could occur with other virus–host combinations, we inoculated healthy plants of parthenium, crownbeard or ageratum with reference cultures as part of the cross-infection studies described above and collected seed from ELISA-positive plants. Test seed was grown in isolation of virus sources, glasshouses were routinely treated with insecticide, and no thrips were detected. Seedlings were tested using ELISA prior to flowering, generally within 3–4 weeks of germination.

Thrips surveys and transmission tests

The aim of this study was to determine which are the major thrips species associated with disease outbreaks caused by TSV-parthenium and TSV-crownbeard and to test whether these are capable of transmitting the two TSV strains. Between 2006 and 2011, we made a total of 35 collections of thrips from flowers of TSV-affected crops and nearby weed hosts from locations across central Queensland from Theodore in the south to Mt McLaren about 400 km to the northwest. Identifications were confirmed by Queensland Department of Agriculture, Fisheries and Forestry senior entomologist Desley Tree, and the species commonly collected from many locations were used to test their ability to transmit the TSV strains. *Frankliniella schultzei* and *Thrips tabaci* were established as live colonies in cages constructed with 106 μm thrips proof mesh. Because of the difficulties in establishing a culture, *Microcephalothrips abdominalis* were used as direct field collections.

Transmission test methods were similar to those described by Klose *et al.* (1996). Pollen was harvested from TSV-parthenium infected parthenium or TSV-crownbeard infected crownbeard and stored at 5°C for up to 6 months before being used in thrips transmission tests. The same batch of TSV-infected pollen was stored for more than 6 years at 5°C and used in manual inoculation to test longevity of the virus in pollen. The TSV strains present in each source of pollen

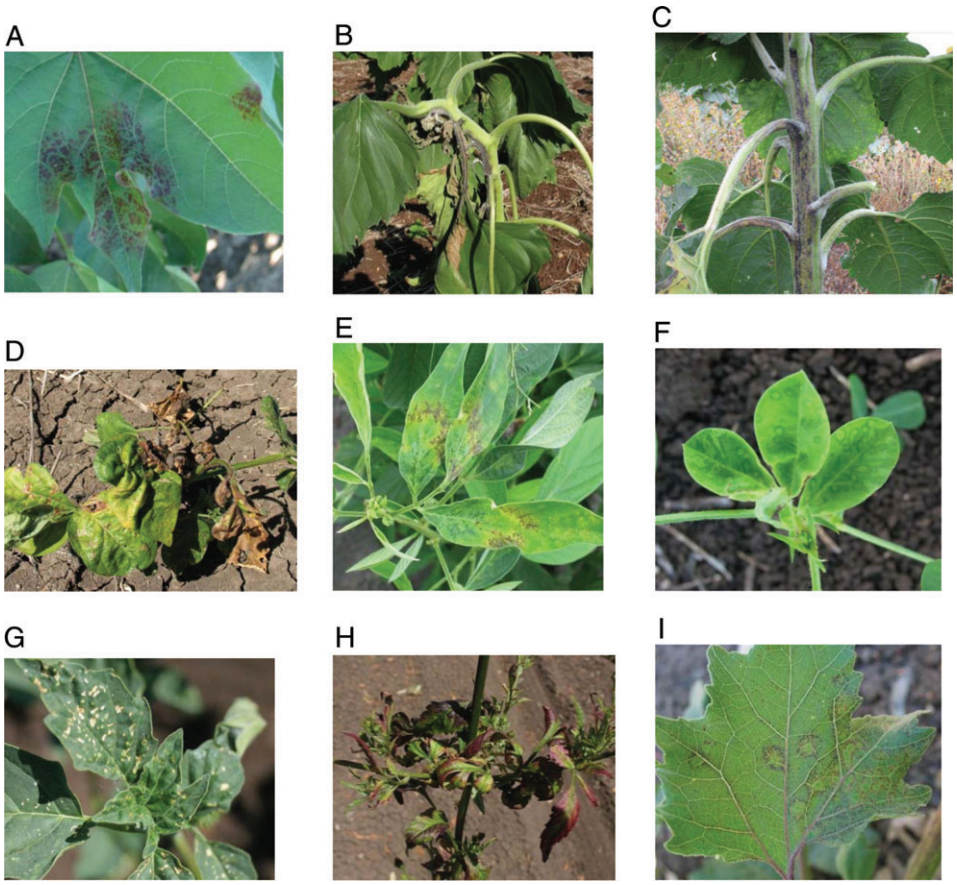

Figure 1 TSV symptoms on; (a) *Gossypium hirsutum*, isolate-2285; (b) *Helianthus annuus*, -5127 (TSV-parthenium) and (c) -2398 (TSV-crownbeard); (d); *Vigna radiata*, -2342; (e) *Cajanus cajan*, -2143; (f) *Arachis hypogaea*, -2594; (g) *Datura leichhardtii*, -2035; (h) *Bidens pilosa*, -2201; and (i) *Xanthium occidentale*, -2512. Isolate details and descriptions of host symptoms are given in Table 1.

was confirmed using PCR as described above. Thrips were mixed with TSV-infected pollen to cover the thrips bodies, and 6–10 thrips per plant were placed onto test plants. After 1–2 days of feeding access, thrips were killed with insecticide spray, and test plants were grown for 1–2 weeks before being assessed for symptoms and tested using TSV ELISA. Control plants included plants dusted with TSV-infected pollen but no thrips added, thrips added without pollen and neither treatment.

Results

Multiplex RT-PCRs for RNA segments and host-range studies

A diverse range of symptoms are described for the hosts listed in Table 1. We have previously illustrated symptoms on sunflower (isolate-1973), mung bean (isolate-2027), cotton (isolate-2120) and chickpea (isolate-1979) (Sharman *et al.*, 2008), and further images of TSV symptoms on a range of crop and weed hosts are shown in Fig. 1a–i.

The strain-specific MP-PCRs for each RNA segment worked very well for identification of TSV strains from a wide range of hosts (Table 1). Size-specific PCR products were produced for each TSV strain for the three RNA segments (Fig. 2). All tested samples had at least one complete set of RNA segments for either TSV-parthenium or TSV-crownbeard (Table 1).

From locations where both parthenium and crownbeard were growing, some samples had both complete sets of RNA segments or one complete set and one incomplete. From locations where either parthenium or crownbeard (but not both) were growing, the only TSV strain found in surrounding host species was the respective strain, TSV-parthenium or TSV-crownbeard. At these locations, testing for only one of the three RNA segments using strain-specific PCR would be adequate for identification of the strain present.

There were 41 naturally infected host species from 11 plant families, including 12 species endemic to Australia. Of the 41 species, 29 were infected with TSV-parthenium

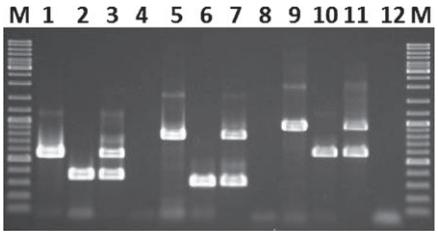

Figure 2 Electrophoresis gel (1.2% agarose in 0.5 × TBE) of MP-PCRs for RNA-1, -2 and -3. Lane 1 is TSV-parthenium isolate-1973; lane 2 is TSV-crownbeard isolate-2334; lane 3 is mixed isolate-5130; and lane 4 is PCR negative control (no-template). These samples are repeated for RNA-2 (lanes 5–8) and RNA-3 (lanes 9–12) MP-PCRs. Marker lanes (M) are GeneRuler DNA ladder mix (Catalogue # SM0332, Life Technologies). See Table 1 for isolate details.

only, 5 were infected with TSV-crownbeard only and 6 host species had individual plants with each TSV strain as separate infections. Crownbeard was the only species to have mixed infections of both TSV strains in some individual plants. One species, *Corchorus trilocularis* (isolate-2038), failed in the PCRs most likely because of high levels of polysaccharides, a known inhibitor of PCR reactions. However, this sample displayed chlorotic mottle symptoms and produced TSV ELISA absorbance values greater than 200 times those of the healthy controls. The RNA-3 MP-PCR detected the positive control used for AgLV (AgLV-1998; Sharman & Thomas, 2013), but AgLV was not detected in any samples tested in this study from central Queensland.

While results for a selection of parthenium and crownbeard samples are shown in Table 1, a total of 30 TSV ELISA-positive crownbeard samples were tested using PCR and gave positive results for all three RNA segments of one or both TSV strains. From locations where crownbeard was the dominant weed with very low numbers of parthenium, 11 crownbeard plants were TSV-crownbeard only, one had both strains but none were TSV-parthenium only. From locations where there were many of both weeds growing together, seven crownbeard plants were TSV-crownbeard only, nine had both strains and two were TSV-parthenium only. All 17 parthenium samples with complete PCR results for RNA segments were TSV-parthenium only. However, there were detections of one or two TSV-crownbeard RNA segments from four additional parthenium samples collected from locations where both weeds occurred.

Cross-infection studies of TSV strains and AgLV into major alternative hosts

The test hosts crownbeard and ageratum were readily infected by TSV-parthenium or TSV-crownbeard by manual inoculation (Table 3). However, parthenium was only readily infected by TSV-parthenium. PCR testing of 15 field samples of parthenium (Table 1) and inoculation results (Table 3) indicate parthenium is a poor host of TSV-crownbeard. AgLV did not infect parthenium but did infect crownbeard and ageratum via inoculation.

Table 3 Experimental cross-infection of TSV strains and AgLV into major alternative hosts

	Test Host and Number of Inoculated Test Plants Positive by TSV ELISA from Total Tested		
Virus Strain	Parthenium	Crownbeard	Ageratum
TSV-1973 (TSV-parthenium)	7/8, 3/6[a]	8/8	9/10, 3/4, 8/11
TSV-2334 (TSV-crownbeard)	1/18	8/8	8/12
AgLV-1998	0/11, 0/7	2/6	1/11, 3/12

ELISA, enzyme-linked immunosorbent assay; TSV, *Tobacco streak virus*.
[a]Numerator is number of plants positive by TSV ELISA and denominator is total tested. Results shown are from either single or multiple tests.

Some significant differences in symptoms where observed when tobacco (*N. tabacum* cv. Xanthi) was infected with the TSV strains or AgLV. Both TSV-parthenium and AgLV caused systemic symptoms similar to those illustrated by Costa & Carvalho, (1961) with deeply notched leaves and flower petals with a filament-like appendage not present in healthy flowers. TSV-crownbeard did not induce systemic notched leaves or affected flowers but slightly reduced and distorted leaves only.

Seed transmission of TSV strains and AgLV in major alternative hosts and crops

TSV-crownbeard was seed transmitted at relatively high rates from naturally infected crownbeard and from experimentally infected ageratum (Table 4) after up to 23 months storage at ambient room temperature and humidity. TSV-parthenium was seed transmitted from experimentally infected ageratum after up to 11 months storage. TSV-parthenium can readily infect crownbeard (Table 3), but all infected crownbeard plants had greatly reduced (shrivelled) seeds that were not viable. The weight of 100 crownbeard seeds was 196 mg from plants infected with TSV-crownbeard isolate-2334, and 44 mg from plants infected with TSV-parthenium isolate-1973. AgLV-1998 was seed transmitted from experimentally infected ageratum at a rate of 18% after more than 6 months storage.

TSV-parthenium was also seed transmitted at high rates from naturally infected *B. pilosa* and *C. bonariensis* (Table 4). The TSV-infected seedlings of *B. pilosa* and *C. bonariensis* were significantly stunted with narrowed leaves compared with the non-infected seedlings. TSV was not seed transmitted from naturally infected mother

Table 4 Test of seed transmission of TSV strains and AgLV in different hosts

	Number of Seedling Test Plants Positive by TSV ELISA from Total Tested				
Virus Strain	Parthenium	Crownbeard	Ageratum[a]	*Bidens pilosa*	Conyza
TSV-parthenium	24/50, 3/44[b]	No viable seed	1/13, 2/10, 0/4, 4/10	31/47[c]	8/30[c]
TSV-crownbeard	n/t[d]	6/12, 2/39, 6/21[e]	5/27, 3/11, 3/22	n/t	n/t
AgLV	n/t	n/t	5/27	n/t	n/t

ELISA, enzyme-linked immunosorbent assay; TSV, *Tobacco streak virus.*

[a]TSV-parthenium isolate-1973, TSV-crownbeard isolate-2334 or AgLV-1998 were used to infect ageratum plants (Table 3) from which seeds were collected and used in grow out tests of seed transmission. Results shown are of seedlings tested from either single or multiple mother plants.

[b]TSV-parthenium was previously shown to be seed transmitted in parthenium (Sharman *et al.*, 2009). The highest and lowest rates from six mother plants are shown.

[c]TSV isolates-2201 (*Bidens pilosa*) and -2520 (*Conyza bonariensis*) were the naturally infected mother plants for the seedlings tested and were shown to be positive for TSV-parthenium by PCR (Table 1).

[d]Not tested (n/t).

[e]Three naturally infected mother plants of crownbeard were confirmed as TSV-crownbeard by PCR and seedlings were tested for seed transmission. The reference isolate-2334 was one of the progeny from the third mother plant.

Table 5 The major thrips species collected from weeds and crop plants as a percentage of the total thrips collected from each

	Proportion of Thrips Collected from Different Hosts (%)			
Thrips Species	Parthenium (269)[a]	Crownbeard (132)	Sunflower (243)	Mung bean (82)
Frankliniella schultzei	41	17	45	54
Microcephalothrips abdominalis	49	76	37	0
Others	10	7	18	46

[a]Total number of individuals collected from each host shown in parentheses.

plants of sunflower or mung bean when 678 and 930 seedlings, respectively, were tested using ELISA.

Thrips surveys and transmission tests

We made 35 collections of thrips from sunflower (12), mung bean (2), parthenium (14) and crownbeard (7). From the 726 individuals collected, 44% were *M. abdominalis* and 40% were *F. schultzei*. These two species were dominant from almost all locations and hosts (Table 5). However, *Megalurothrips usitatus* accounted for 44% of the thrips collected from mung bean and *Tusothrips* sp. for 10% of thrips from sunflower. *M. usitatus* has been reported as an efficient TSV vector (Prasada Rao *et al.*, 2003), so it is possible that this species is involved in TSV transmission in mung beans.

We selected *F. schultzei* and *M. abdominalis* for transmission tests because they were the most numerous and commonly found species on the range of field hosts surveyed, and they have been previously shown to be vector species of another TSV strain and AgLV (Klose *et al.*, 1996). We also tested *T. tabaci* as a vector of the TSV strains because it was found in some field collections, and has been shown to be an efficient TSV vector species (Klose *et al.*, 1996).

F. schultzei, *M. abdominalis* and *T. tabaci* were efficient vectors of the TSV-parthenium strain (Table 6). TSV-crownbeard was also efficiently transmitted from crownbeard pollen to mung bean by *F. schultzei* in all 6 test plants, by *M. abdominalis* in all 11 plants, and by *T. tabaci* in 7 of 8 plants.

There was one positive plant for the thrips-only control treatment for TSV-parthenium transmission using *M. abdominalis* (Table 6). This is likely to be a false positive because of the use of *M. abdominalis* individuals collected directly from field samples of parthenium flowers where thrips may have been contaminated with TSV-infected pollen. *M. abdominalis* was unable to be cultured as was done for the other test species, leaving open the risk of collecting TSV-contaminated individuals.

Transmission was also attempted using TSV-parthenium pollen and an infestation of two-spotted mites (*Tetranychus urticae*) on mungbeans with no transmission to six test plants. TSV-parthenium infected pollen stored for more than 6 years at 5°C was still infective when manually inoculated to *Vigna unguiculata* (cowpea) with all three test plants displaying typical local and systemic symptoms of TSV infection.

Discussion

We report previously unknown biological characteristics such as host range, seed transmission and thrips transmission for two TSV strains from central Queensland, TSV-parthenium and TSV-crownbeard. A diverse

Table 6 Test of TSV-parthenium transmission using different thrips species

Treatment	Test Host	Number of Infected Plants from Total Tested Using Different Vector Species		
		Frankliniella schultzei	*Microcephalothrips abdominalis*	*Thrips tabaci*
Thrips + TSV-pollen	Sunflower	17/24	n/t	2/5, 4/5
	Mung bean	24/24	5/6, 10/10, 11/11	4/5, 5/9
TSV-pollen only	Sunflower	0/12	n/t	0/5
	Mung bean	0/12	n/t	0/5
Thrips only	Sunflower	0/12	n/t	0/5
	Mung bean	0/12	1/6, 0/12	0/5
Nil	Sunflower	0/6	n/t	n/t
	Mung bean	0/6	n/t	n/t

natural host range was identified for both TSV strains. TSV-parthenium had a wider natural host range over a larger geographical area in central Queensland compared to TSV-crownbeard. TSV-parthenium was very common in parthenium across most of its geographical range (Sharman *et al.*, 2009) but only infected other host species in locations where infected parthenium was growing. Similarly, TSV-crownbeard only infected hosts other than crownbeard in locations where infected crownbeard was growing. These results demonstrate the close association these two distinct TSV strains have with their respective major alternative hosts, parthenium and crownbeard. The exception to this was the TSV-crownbeard infected archived isolates of *Xanthium occidentale* (isolates 834 and 835) collected in 1975 from Ayr in north Queensland. It is unknown whether crownbeard was in the Ayr region at that time.

In a previous study of fewer samples, we did not find TSV-parthenium in crownbeard or TSV-crownbeard in parthenium (Sharman & Thomas, 2013). Now we have found that parthenium was a poor host of TSV-crownbeard in nature and through experimental inoculation. Conversely, we found that crownbeard was both a natural host of, and experimentally infected by TSV-parthenium. However, inoculations of crownbeard with one isolate of TSV-parthenium resulted in no viable seed. This could act as a biological barrier stopping TSV-parthenium persisting in crownbeard populations and provide parthenium with a biological advantage over crownbeard in locations where they are found together. A similar synergistic plant–virus interaction was also described by Malmstrom *et al.* (2005) who described a plant community shift in favour of virus-tolerant grass species over susceptible native grasses. There may be variation in the reaction of TSV-parthenium isolates infecting crownbeard and testing with further isolates would help to clarify if the effect on crownbeard seed is consistently observed.

Ageratum has been shown to be a natural host of TSV in India (Prasada Rao *et al.*, 2003) and was also indicated as a critical host of AgLV and thrips vectors that caused disease in tobacco crops in southeast Australia (Greber *et al.*, 1991). We did not detect AgLV from any samples from central Queensland, and ageratum is rarely recorded in this region. AgLV is most likely restricted to eastern coastal areas of Queensland and northern New South Wales where ageratum is often abundant (Klose, 1997; Sharman & Thomas, 2013).

There are similarities between the Indian TSV strain and TSV-parthenium from Australia in the disease epidemics they cause in sunflower and mung beans. Both have parthenium as their major alternative host. However, they are genetically distinct (Sharman & Thomas, 2013) and appear to have differences in host range and seed transmissibility. No seed transmission of TSV from India has been reported from several studies of crop plants and weeds including sunflower, groundnut (peanut), mung bean, soybean and parthenium (Prasada Rao *et al.*, 2003, 2009; Reddy *et al.*, 2007; Vemana & Jain, 2010). In contrast, we have demonstrated high rates of seed transmission from several *Asteraceae* species for TSV-parthenium, TSV-crownbeard and AgLV. Along with our previous record of TSV-parthenium transmission in parthenium seed (Sharman *et al.*, 2009), and to the best of our knowledge, these are the first records of TSV seed transmission in *Asteraceae* species. While TSV-parthenium is genetically closely related to a Brazilian strain of TSV (Almeida *et al.*, 2005; Sharman & Thomas, 2013), limited tests have been reported for the Brazilian strain and no seed transmission was found (Costa & Carvalho, 1961).

Similar to our observations for TSV in parthenium and crownbeard, several other disease outbreaks caused by TSV or AgLV have also been linked to TSV-infected *Asteraceae* species. They produce large amounts of pollen and can sustain high thrips populations. These include sunflower and parthenium in India (Prasada Rao *et al.*, 2003), *Ambrosia polystachia* in Brazil (Almeida & Corso, 1991) and *Ageratum houstonianum* in Australia (Greber *et al.*, 1991).

The genetically distinct TSV-parthenium and TSV-crownbeard share similar life cycle strategies that enable them to survive and persist in an environment that is often unpredictable and harsh. Inland regions of central Queensland typically have a dry tropical climate that often reduces alternative host populations to isolated patches. The high rates of seed transmission of these two TSV strains in their respective major alternative hosts enables them to remain dormant for up to several years (Sharman *et al.*, 2009). This enables them to rapidly re-establish and spread when conditions improve and is critical to the rapid development of TSV epidemics in this region.

TSV-parthenium and TSV-crownbeard were readily transmitted via infected pollen and three thrips species: *F. schultzei, M. abdominalis* and *T. tabaci*. *F. shultzei* and *M. abdominalis* play a critical role in facilitating the movement and transmission of TSV into susceptible crops via infected parthenium pollen. It is likely these thrips carry virus-infected pollen on their bodies in a similar manner to previously described for *Thrips imaginis*, another pollen feeding species (Kirk, 1984).

While all samples tested had a complete set of genome segments from either strain (TSV-parthenium or TSV-crownbeard), one or two additional segments from the other strain were also detected in some samples. Mixed infections provide an opportunity for recombination and reassortment of genetic material from multipartite viruses (Pressing & Reanney, 1984; Roossinck, 1997). Genetic exchange occurs with other *Bromoviridae* members (Codoñer & Elena, 2008) to potentially result in new species which may have quite different host range and pathogenicity characteristics. It is unclear if our observations of mixed RNA segments from TSV-parthenium and TSV-crownbeard indicate these strains are capable of complementing each other or if a new hybrid strain could arise from a reassortment of RNA segments. This could be clarified with further investigation of naturally occurring or experimentally induced mixed infections of these two strains.

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