



Natural host range and thrips transmission of capsicum chlorosis virus in Australia

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Abstract

The orthotospovirus, capsicum chlorosis virus (CaCV) was shown to be common and widespread in the weed host *Ageratum conyzoides* in eastern coastal regions of Queensland, Australia with up to 92% of plants infected. This is the first report of *A. conyzoides* being an important host of CaCV in Australia. CaCV was also found as natural infections of *Arachis hypogaea* (peanut), *Ananas comosus* (pineapple), *Sonchus oleraceus*, *Tagetes minuta* and *Emilia sonchifolia*. This is the first report of CaCV infecting pineapple and being associated with severe disease symptoms. *Thrips palmi*, *Frankliniella schultzei* and *Microcephalothrips abdominalis* were shown to transmit CaCV while no transmission was achieved using *F. occidentalis*.

Keywords Host range · Epidemiology · ELISA · RT-PCR

Introduction

Members of the orthotospoviruses (order *Bunyvirales*, family *Tospoviridae*, genus *Orthotospovirus*) (Adams et al. 2017) have a global distribution and are among the most economically damaging group of plant viruses affecting a wide range of agricultural and ornamental crops (Oliver and Whitfield 2016; Parrella et al. 2003). There are 18 recognised orthotospovirus species (ICTV 2018) and at least a further 7 proposed species (King et al. 2012; Zhou et al. 2011). The orthotospoviruses are transmitted by thrips (Order *Thysanoptera*) in a persistent circulative manner (Ullman et al. 1997). There are over 7500 species of thrips but only 15 thrips species have been reported as vectors of orthotospoviruses (Jones 2005; Riley et al. 2011; Rotenberg et al. 2015; Zhou and Tzanetakis 2013).

Four orthotospoviruses have been reported from Australia, three recognised species, tomato spotted wilt virus (TSWV) iris yellow spot virus (IYSV), capsicum chlorosis

virus (CaCV), and an uncharacterised species from a native orchid (Persley et al. 2006). In Australia, CaCV has been reported from crops in tropical and subtropical vegetable production regions of Queensland and New South Wales and also from Kununurra in Western Australia (Jones and Sharman 2005; Persley et al. 2006). CaCV has also been reported from India (Kunkaliker et al. 2007), Thailand (Premachandra et al. 2005), China (Chen et al. 2007b), Taiwan (Chen et al. 2007a), Iran (Bayat et al. 2018), United States of America (Hawaii; Melzer et al. 2014), Greece (Orfanidou et al. 2018) and Japan (Chiaki et al. 2019). Persley et al. (2006) reported natural infections of CaCV in Australia in bell capsicum (*Capsicum annuum*), tomato (*Solanum lycopersicum*), peanut (*Arachis hypogaea*) and a Hoya species. However, no common weed species which may act as a source of infection near to affected crops were found to be infected with CaCV and no further hosts of CaCV have been reported from Australia.

To date, three thrips species have been reported to be vectors of CaCV. *Ceratothripoides claratris* was shown to be an efficient vector of CaCV-AIT strain in Thailand glasshouse tomatoes (Premachandra et al. 2005). However, based on genome sequence analysis, the AIT-Thailand strain of CaCV may be a distinct orthotospovirus species compared to CaCV from Australia (Widana Gamage et al. 2015) and *C. claratris* has not been reported from Australia (Mound 2005). *Thrips palmi* and *Frankliniella schultzei* were reported to be vectors of CaCV in Australia based on unpublished data

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(Persley et al. 2006) and were not included as confirmed vectors of CaCV in a review of orthospovirus vector species by Riley et al. (2011).

In this study we investigated the natural host range of CaCV in Australia to determine important alternative hosts that may act as a source of inoculum moving into nearby susceptible crops. We also present thrips transmission studies for CaCV in Australia.

Methods

Virus isolates and field surveys

All isolate numbers refer to samples stored in the Queensland Department of Agriculture and Fisheries plant virus collection. All virus isolates used for transmission tests were collected from capsicum crops at Gatton, Childers, Bundaberg and Bowen, Queensland, except for CaCV transmitted directly from thrips taken off *Ageratum conyzoides* from Bundaberg. Capsicum (*Capsicum annuum*) cv. Yolo Wonder plants were used for maintaining isolates and for test plants used in transmission tests. Virus isolates were sap-inoculated to capsicum grown in a glasshouse until severe systemic symptoms developed and were then used as a virus source in transmission tests.

Potential alternative hosts of CaCV were initially collected from close to crops affected by CaCV. Samples with virus-like symptoms were targeted but random samples were also collected. Initial results indicated that *Ageratum sp.* may be a common host for CaCV but no obvious symptoms were observed on infected plants. As such, randomly collected plants were collected from ageratum populations at sites either close to crops with CaCV symptoms or in locations away from horticultural cropping where ageratum was prolific such as in and nearby to paddocks used for sugar cane production (Table 1). From each site, ageratum plants were tested by ELISA or PCR as described below. A selection of collected ageratum plants were identified to species either by the Queensland herbarium or by PCR amplification and direct sequencing of the partial maturase K gene using primers matK472Fa (CCCATCCATCTRGAAATCTTRGTTTC) and matK1248Ra (CCACTRTRATAATGAGAAAGATTCTG) modified from Yu et al. (2011). PCR was done using 1 unit native Taq DNA polymerase (Thermo Fisher), 1.75 mM MgCl₂, 200 mM dNTPs, 200 nM of each primer and 1 µL of total nucleic acid extract prepared as described by Sharman and Thomas (2013) in a 25 µL reaction volume. Cycling parameters were: 95 °C for 1 min, then 35 cycles of 95 °C for 15 s, 62 °C for 20 s, 50 °C for 10 s, and 72 °C for 45 s, followed by a final extension of 72 °C for 3 min.

Thrips colonies

Healthy colonies of *T. palmi* and *F. occidentalis* were established from vegetable crops at Gatton. A healthy colony of *F. schultzei* (yellow form) was established from snakeweed (*Stachytarpheta jamacensis*) at Bowen. *T. palmi* was maintained on plants of *Cucumis sativus* while *F. occidentalis* and *F. schultzei* were maintained on plants of *Phaseolus vulgaris* cv. Bountiful. The identity of the three species was confirmed by entomologists Mr. John Donaldson, Ms. Desley Tree or Ms. Bronwyn Walsh, Department of Agriculture and Fisheries, Queensland (DAFQ).

Thrips transmission tests

Virus transmissions were done by transferring at least 100 healthy thrips into a cage containing a pot of 4–6 CaCV-infected capsicum plants along with one or more pots of healthy capsicum test plants. Cages were made from thrips-proof mesh with openings of 106 µm. The infected and healthy plants were not touching and the thrips were allowed to feed, multiply and disperse between plants in the cage for 12–35 days.

Transmission tests were also done using *Microcephalothrips abdominalis* collected from *A. conyzoides* from Bundaberg (site 6 and site 7, Table 1). The *A. conyzoides* plants, from which the *M. abdominalis* were collected, were positive by orthospovirus serogroup IV ELISA as described below. At least 5 thrips per plant were transferred onto two pots of healthy capsicum (10 plants total) for thrips from site 6. There were limited thrips from site 7 and a total of 10 were transferred to four healthy capsicum plants.

Following the access period for infective thrips, test plants were then sprayed with systemic insecticide Imidacloprid at 0.125 g/L, removed from the cage and grown in a glasshouse to monitor symptom development over a period of at least four weeks. The glasshouse was sprayed at regular intervals for insects and monitored for the presence of thrips, of which none were found. Symptomatic and non-symptomatic plants were then tested by ELISA as described below.

Orthospovirus serogroup IV ELISA

ELISA tests were performed for orthospovirus serogroup IV using Agdia reagent set for watermelon silver mottle virus (WSMoV) and groundnut bud necrosis virus (GBNV) (catalogue number SRA61500). CaCV cross reacts with this reagent set and is currently the only serogroup IV species reported from Australia so the use of serogroup IV ELISA is effective for its diagnosis. However, we also tested representative samples by CaCV-specific PCR as described below to confirm that other serogroup IV species were not present. Samples were extracted at 0.1 g per 1 ml of PBS-Tween

Table 1 Collection sites of *A. conyzoides* and diagnostic results for samples tested

Collection Date	Site #	Collection location ^a	Nearest town	Land use ^b	ELISA Positive / total plants ^c	Voucher Isolate ^d
1/12/2006	1 ^e	-25.0027, 152.3583	Bundaberg	Capsicum crop	36/39	1995 *
1/03/2007	2	-25.0988, 152.2749	Childers	Peanut crop	23/60	2017 * ^f
1/03/2007	3	-24.9533, 152.4056	Bundaberg	Sugar cane	11/22	2016 * ^f
15/01/2008	4	-21.1585, 149.0512	Walkerston	Sugar cane	20/30	2137 * ^f
10/06/2009	5		Yeppoon	Pineapple crop	3/4	2403 ^f
8/09/2009	6 ^e	-24.8337, 152.3031	Bundaberg	Sweet potato crop	5/23	2460 ^f
27/02/2019	7	-24.9441, 152.3905	Bundaberg	Sugar cane	9/12	5533 *
28/02/2019	8 ^e	-25.5672, 152.7464	Maryborough	Sugar cane	11/20	5553

^a Where available, location is shown as degrees latitude and longitude

^b Samples were collected within or directly adjacent to crops as listed

^c Orthotospovirus serogroup IV ELISA used

^d At least one voucher isolate from each location was confirmed using CaCV-specific PCR to confirm the orthotospovirus serogroup IV ELISA positives were for CaCV. Those PCR positive samples shown with an asterisk (*) were also directly sequenced to confirm identity to published sequences (see text for sequence analysis)

^e From field collected *A. conyzoides* from Bundaberg in 2006 (site 1), thrips identified included 16 individuals of *F. occidentalis* and 10 *M. abdominalis*. From *A. conyzoides* from Bundaberg in 2009 (site 6) there were 9 *F. occidentalis* and 17 *M. abdominalis*. From *A. conyzoides* from Maryborough in 2019 (site 8), 6 thrips were identified, all were *M. abdominalis*

^f Maturase K sequence used to confirm host species as *A. conyzoides*

containing 2% polyvinylpyrrolidone with assays carried out in Nunc Maxisorb microtitre plates with reaction volumes of 100 µl in duplicate. Coating antibody and conjugate were diluted 1:500 in PBS-T. Overnight incubations were at 5 °C and all other incubations at room temperature for 2–3 h. Samples were considered positive when their A410_{nm} absorbance values exceeded three times the mean of A410_{nm} values of appropriate healthy controls.

CaCV RT-PCR

Total nucleic acid extracts, cDNA synthesis and PCR were done as described by Sharman and Thomas (2013) using PCR primers CaCV.NPF and CaCV.NPR (Jones and Sharman 2005) to amplify the N-gene region using the following cycling parameters, 95 °C for 1 min, then 35 cycles of 95 °C for 15 s, 56 °C for 30 s and 72 °C for 45 s, followed by a final extension of 72 °C for 3 min. PCR products were sequenced directly by the Australian Genome Research Facility and nucleotide sequences aligned using the MUSCLE algorithm included in the MEGA7 software package (Kumar et al. 2016).

Results

Thrips transmission tests demonstrated that *T. palmi*, and *F. schultzei* were able to transmit CaCV between capsicum plants (Table 2). Field collected *M. abdominalis* was able to transmit CaCV from *A. conyzoides* to capsicum plants in separate tests in 2009 and 2019. In two separate tests, *F. occidentalis* was not able to transmit CaCV. Thrips were

identified from field collected *A. conyzoides* from three sites from Bundaberg and Maryborough between 2006 to 2019 (Table 1).

Ageratum samples collected from Giru, Ayr, Proserpine and Bundaberg were identified by the Queensland Herbarium as *A. conyzoides*. Partial maturase K gene sequence was also determined from five additional ageratum samples representative of the geographical range of collections (Table 1) and compared to reference sequences on GenBank. All five shared 100% nt identity with each other and with an *A. conyzoides* voucher specimen from China (GenBank accession HM989755) and had 7 nt differences to *A. houstonianum* voucher specimen from Australia (AF151434) over a 673 bp region. There were no published sequences of *A. conyzoides* from Australia for comparison. This indicates that all collections of ageratum during this study were *A. conyzoides*.

We found CaCV to be common in populations of *A. conyzoides* from many locations across Queensland from a range of environments (Table 1). No obvious symptoms were observed on infected *A. conyzoides*. For each site surveyed in Table 1, at least one representative sample of *A. conyzoides* was selected from the ELISA-positive samples and confirmed by CaCV-specific PCR. The incidence of CaCV in *A. conyzoides* was variable but was up to 92% (site 1, Table 1). At site 1, the *A. conyzoides* was upwind of a crop of capsicum, cv. Red Jewel displaying symptoms of CaCV infection. Closest to the *A. conyzoides*, the disease incidence of CaCV in the capsicum crop was 17% and dropped to 11% at 100 m and 7% at 220 m downwind. A high incidence of CaCV in *A. conyzoides* also occurred at sites with no

Table 2 Transmission tests of capsicum chlorosis virus by different thrips species

Thrips species	Virus source (isolate and host plants)	Number of thrips used	Inoculation access period (days)	Positive test plants from total tested ^b
<i>T. palmi</i>	CaCV-1492 ^a on capsicum	>100	18	3/10
<i>F. schultzei</i>	CaCV-1862 and – 1861 on capsicum	>200	14	11/16
<i>F. occidentalis</i>	CaCV-1607 and – 1491 on capsicum	>150	12	0/12
<i>F. occidentalis</i>	CaCV-1628 on capsicum	>150	35	0/8
<i>M. abdominalis</i>	CaCV on ageratum	>50	34	5/10 ^c
		10	7	1/4 ^d

^a Department of Agriculture and Fisheries plant virus isolate number

^b Infection of capsicum test plants confirmed by ELISA using serogroup IV antibodies

^c *M. abdominalis* used directly from infected *A. conyzoides* from Site 6 (Table 1)

^d *M. abdominalis* used directly from infected *A. conyzoides* from Site 7 (Table 1)

susceptible crops nearby, such as at site 4 (Table 1) where the incidence of CaCV in *A. conyzoides* was 66%.

We confirmed CaCV by CaCV-specific PCR in field collected samples of *Sonchus oleraceus* (isolate 1996) and *Tagetes minuta* (isolate 1997) from Bundaberg, in *Emilia sonchifolia* (isolate 1905) from Childers, in peanut from Childers (isolates 2015, Fig. 1) and Maryborough (isolate 5532), and in *Ananas comosus* (pineapple) from Yeppoon (isolate 2407) and Yandina (isolate 5290, Fig. 2). The CaCV-infected peanut and pineapple samples were from locations with CaCV-infected *A. conyzoides* adjacent to the crops (data not shown). CaCV-infected peanuts displayed symptoms of chlorotic spots and rings and terminal necrosis (Fig. 1) similar to that described for the orthotospovirus groundnut bud necrosis virus (Reddy et al. 1991). Symptoms on infected pineapple plants included some concentric chlorotic rings and sunken lesions developing into extensive areas of necrosis and terminal death of the crown (Fig. 2). Infected pineapple fruit displayed large areas of necrosis on the side of the fruit.

CaCV was confirmed by sequencing the partial N-gene from five samples of *A. conyzoides* (Table 1), capsicum from Bundaberg (isolate 1993), peanuts from Childers (isolate 2015) and Maryborough (isolate 5532), and *Sonchus oleraceus* from Bundaberg (isolate 1996). All CaCV isolates sequenced shared between 95 and 99% nt identity over a 744 bp region. From the CaCV isolates sequenced, isolates 2016 and 2137, both from *A. conyzoides* from Bundaberg and Walkerston respectively, had the most divergent partial N-gene sequences. By BLAST analysis (Zhang et al. 2000), isolate 2016 shared highest nt identity (98.6%) with CaCV from Queensland (accession KM589495), and isolate 2137 shared highest nt identity (99.6%) with another CaCV isolate from Queensland (AY879105).

CaCV was also isolated from three field samples collected from Thailand in 2007. Isolates Q1517 (from *Praxelis clematidea*) and Q1535 (from *A. conyzoides*)

were from Khon Kean and isolate Q1571 (from *A. conyzoides*) was from Kamphaeng Sean. CaCV was confirmed by orthotospovirus serogroup IV ELISA and CaCV-specific PCR. The partial N gene was sequenced from isolates Q1517 and Q1535 and shared greater than 99% nt identity with CaCV isolated from peanut from the same region of Thailand (accession DQ022745) but only about 90% nt identity with the CaCV-AIT strain. This indicates that the Thailand CaCV isolates in our study were not members of the CaCV-AIT strain. The Khon Kean samples of *P. clematidea* and *A. conyzoides* were collected from next to a crop of tomato with numerous plants displaying orthotospovirus-like symptoms and two tomato samples (isolates Q1515 and Q1516) were also positive for CaCV by PCR. Tomato isolate Q1516 shared 100% nt identity with isolates Q1517 and Q1535 from *P. clematidea* and *A. conyzoides*.



Fig. 1 CaCV isolate 2015 from peanut displaying chlorosis, chlorotic spots and rings, and terminal necrosis



Fig. 2 CaCV isolate 5290 symptoms on pineapple showing chlorotic rings and spreading necrotic lesions

Discussion

This is the first report of data demonstrating CaCV transmission by *T. palmi*, *F. schultzei* and *M. abdominalis* from Australia. Prior to this study, data for only one thrips species, *Ceratothripoides claratis* was reported for transmission of CaCV (Premachandra et al. 2005). *T. palmi* has previously been reported to be a vector of two other serogroup IV orthospovirus species occurring in Asia, GBNV and WSMoV (Reddy et al. 1992; Yeh et al. 1992). Prior to our study, only 2 of the 15 thrips species reported as vectors of orthospovirus (Riley et al. 2011; Jones 2005; Rotenberg et al. 2015; Zhou and Tzanetakis 2013) were from genera other than *Thrips* or *Frankliniella*. Our finding of *M. abdominalis* as a vector of CaCV is only the third reported orthospovirus vector species outside of the genera *Thrips* or *Frankliniella*.

The transmission of CaCV by *T. palmi* and *F. schultzei* under experimental conditions is in general agreement with thrips trapping studies over several years in capsicum crops in southern and northern Queensland (Abbott 2002; Persley, Sharman and Clift, unpublished data; Walsh 2004). Both *F. schultzei* and *T. palmi* were regularly recorded from traps located within crops at Bundaberg, when CaCV was the dominant orthospovirus detected in capsicum crops. Although *T. palmi* has a restricted distribution in the capsicum production areas of north Queensland, *F. schultzei* has been an important pest of solanaceous crops in the region for several decades (Abbott 2002). These new records of transmission for CaCV will be an important addition to any management program of orthospovirus disease in Australia. Transmission studies with infective thrips collected from affected crops may be necessary to confirm these associations in a similar manner as done for *M. abdominalis* from *A. conyzoides* in this study.

To the best of our knowledge, this is the first report of *M. abdominalis* as an orthospovirus vector and justifies a

more rigorous investigation of its role in the epidemiology of CaCV. *M. abdominalis* is commonly found to be the most abundant thrips species in ageratum species while the other two thrips species shown to be vectors of CaCV, *T. palmi* or *F. schultzei*, are rarely, if ever found on ageratum. Greber et al. (1991) found that virtually all thrips in *Ageratum houstonianum* flowers were *M. abdominalis* and this remained the predominant thrips species from nearby collections of *A. houstonianum* over several months of monitoring. In contrast, only one of the 248 thrips records from capsicum in the DAFQ insect collection is *M. abdominalis*. From the 33 records of thrips from *Ageratum sp.* in the DAFQ insect collection, there are no records of *T. palmi* or *F. schultzei*. Even though we found *F. occidentalis* to be relatively common from *A. conyzoides*, our transmission tests indicate this thrips species is not a vector of CaCV. Hence, it appears *M. abdominalis* may play an important role in the primary spread of CaCV from infected *A. conyzoides* into nearby crops and *F. schultzei* may be important for secondary spread of CaCV within crops such as capsicum or tomato. *T. tabaci* was not tested as a vector in this study. However, it makes up 8% of the 248 thrips records from capsicum in Queensland (DAFQ insect collection). It was observed in high numbers in association with CaCV disease outbreaks in Iran and was detected in thrips by RT-PCR but thrips transmission tests for CaCV have not yet been reported for this species (Bayat et al. 2018). It would be useful to determine if *T. tabaci* is a potential vector species for CaCV.

During the revision process of our manuscript, a report by Chiaki et al. (2019) has been published for the transmission of a Japanese isolate of CaCV using *T. palmi* and the non-transmission of CaCV using *F. occidentalis*, *F. intonsa* and *T. tabaci*. These findings support our data for *T. palmi* and *F. occidentalis* and also indicates that *T. tabaci* may not be an important vector of CaCV.

We found CaCV to have a widespread distribution in *A. conyzoides* populations in eastern coastal areas of Queensland, sometimes occurring at high incidence in this host regardless of nearby land use, indicating that *A. conyzoides* plays an important role in the persistence of CaCV in this region. Two species of ageratum occur in Queensland, *A. conyzoides* and *A. houstonianum*. However, *A. houstonianum* is mostly restricted to south east Queensland and all plants identified from the areas that we surveyed were *A. conyzoides*. We often found that crops such as capsicum, peanut and pineapple adjacent to CaCV-infected *A. conyzoides*, were also infected with CaCV. We also found CaCV in *A. conyzoides* from two locations in Thailand, over 400 km apart. This may indicate *A. conyzoides* is a key alternative host in many locations in Australia and possibly also in south east Asia. *A. conyzoides* has previously been reported as a major alternative host of another serogroup IV

orthospovirus in India, groundnut bud necrosis virus, and its efficient thrips vector, *F. schultzei*. Greater than 50% of *A. conyzoides* plants from the field were found to be infected with what was assumed to be TSWV at the time (Reddy et al. 1983) but was later characterised as groundnut bud necrosis virus (Reddy et al. 1992).

To the best of our knowledge, this is the first report of CaCV infecting the weeds *Ageratum conyzoides*, *Emilia sonchifolia*, *Praxelis clematidea*, *Sonchus oleraceus* and *Tagetes minuta*. It is also the first report of CaCV infecting *Ananas comosus* (pineapple). Yellow spot disease in pineapple has been reported from Hawaii and Australia and has been assumed to be caused by tomato spotted wilt virus (Rohrbach and Johnson 2003; Sakimura 1940; Cooke et al. 2009) but to best of our knowledge it has not yet been confirmed by molecular methods. The symptoms we observed on CaCV-infected pineapple are similar to the original descriptions of yellow spot disease from Hawaii (Linford 1932; Illingworth 1931) and may provide some clues to the aetiology of this disease from other locations. The pineapple samples we tested were collected from crops that had weedy areas nearby infested with ageratum (Col Scott pers. comm.).

The close association that we found of CaCV, *A. conyzoides* and *M. abdominalis* has similarities to another virus/ host/ vector interaction. *Ageratum houstonianum* has also been shown to be a key alternative host for a thrips-transmitted ilarvirus, ageratum latent virus (AgLV; Greber et al. 1991; Sharman 2015). Greber (1991) found that up to 50% of *A. houstonianum* plants were infected with the virus, previously thought to be tobacco streak virus, and leading to disease epidemics in adjacent tobacco crops. Interestingly, some pineapple production areas in south-east Queensland now affected by CaCV are in the same locations that previously supported tobacco crops which were affected by AgLV. These observations suggest the surrounding ageratum populations have been an important reservoir for both of these thrips-transmitted viruses from different virus families.

Crop species known to host CaCV such as tomato and capsicum, and CaCV-vector species such as *F. schultzei* are present in southern production regions in Victoria and South Australia. However, CaCV has not been reported from these production areas. We propose from our data that the distribution of CaCV may be determined by the distribution of key alternative hosts such as *A. conyzoides* which is largely restricted to eastern coastal areas of Queensland and northern New South Wales. The control of weedy areas of *A. conyzoides* close to susceptible crops may play an important role in minimising risk of CaCV disease outbreaks.

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