



## Short Communication

# Identification and distribution of aphid vectors spreading Citrus tristeza virus in Darjeeling hills and Dooars of India



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## ABSTRACT

Darjeeling hills and Dooars of West Bengal (India) are well known for production of mandarin orange and lime. *Citrus tristeza virus* (CTV) is wide spread in this region. Role of insect vectors in spreading CTV in this region has not been studied so far. Therefore, a study on identification of insect vectors along with their temporal and spatial distribution was undertaken. Five aphid species were identified from citrus orchards of Darjeeling and Dooars viz. *Toxoptera citricida*, *T. aurantii*, *Aphis gossypii*, *Myzus persicae* and *Brachycaudus helichrysi*. *T. citricida* was found predominant in the orchards of lower altitude and was responsible for maximum spread of CTV. *T. aurantii* was dominant in the citrus orchards at high altitude (>500 m). Incidence of CTV was higher in the orchards where *T. citricida* was present either alone or with other species. Under caged conditions, *T. citricida* was more efficient to transmit CTV than the other aphid species. Occurrences of all aphid species were highly influenced by the advent of new flushes.

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## Introduction

Citrus is cultivated in almost every state of India despite the diversity of soil, climate and topography (Chenulu and Ahlawat, 1993). Total area under citrus in India is 0.62 million hectare producing 4.79 million tonnes (Jagtap et al., 2013). The Darjeeling hill in India is well known for production of mandarin orange with a yearly production over 36.45 thousand tonnes from 3.7 thousand hectare area (Chattopadhyay and Roy, 2011). The foothills of Darjeeling and the floodplains popularly known as 'Dooars', comprise the major producing area of kagzi (*Citrus aurantifolia*) and Assamese lime (*Citrus assamensis*). Citrus is reported to be the host of several aphid species (Barbagallo, 1966; Blackman et al., 1984; Komazaki, 1981; NietNafria et al., 1984; Viggiani, 1988; Yokomi et al., 1992). Globally nineteen aphid species have been recorded to harbour citrus plants so far (Blackman and Eastop 2000) having regional variation in composition and occurrence. In India, ten aphid species colonize on citrus (Chakrabarti and Sarkar, 2001; Raychaudhuri et al., 1983). Only five of these viz. *Toxoptera citricida*, *Toxoptera aurantii*, *Aphis gossypii*, *Aphis spiraecola* and *Myzus persicae* are important having ability to transmit different strains of citrus tristeza virus (CTV) in a semi-persistent manner (Ahlawat and Raychaudhuri, 1988; Costa and Grant, 1951; Norman and Grant, 1956; Sasaki, 1974; Varma et al.,

1960). CTV is now widespread in this region causing severe loss in production (Ghosh et al., 2014; Mukhopadhyay et al., 1986). Earlier studies on CTV from this region were focused on molecular diagnosis of CTV, host resistance, sequencing of the complete genome, characterisation of isolate and distribution of CTV (Biswas, 2008; Biswas et al., 2012; Ghosh et al., 2014a,b). There were no evidence on the identity, spatial distribution and temporal occurrence of the vector responsible for spread of CTV in this region. The present study was undertaken to identify the vector species co-existing with CTV along with their temporal occurrence and distribution in citrus orchards of Darjeeling hills and Dooars.

## Methodology

## Collection of aphids and plant samples

Extensive survey was conducted in citrus orchards of Darjeeling hills and Dooars region during 2012–2014. Samples were collected during late winter to spring (February–March) and late summer to early rainy season (June–July) when new flushes of citrus appeared. Place of sampling, altitude, citrus species, time of collections are mentioned in Table 1. Bark and mid-rib parts from representative citrus plants were sampled randomly from each orchard. Samples packed in plastic bags, were taken into the laboratory and processed immediately. Aphids were collected from randomly selected citrus plants of each orchard and carried to laboratory in glass vials for further study.

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**Table 1**  
Distribution of CTV and its vector in citrus orchards of Darjeeling and Dooars region.

Place	Altitude (m)	Citrus species	Time of collection	% infection by DIBA (n)	Aphid species identified
Pedong	1300	mandarin	spring, rainy	55 (20)	<i>T. aurantii</i>
Mahakaldara	1300	mandarin	spring, rainy	90 (20)	<i>T. aurantii</i> , <i>T. citricida</i>
Kashyem	1300	mandarin, pomelo	spring, rainy	30 (10)	<i>T. aurantii</i>
Mirik	1300	mandarin	Spring	28 (25)	<i>T. aurantii</i>
Kolbung	1200	mandarin	Spring	50 (10)	<i>T. aurantii</i>
St Joseph, Kalimpong	1180	mandarin	spring, rainy	100 (8)	<i>T. aurantii</i> , <i>T. citricida</i>
UBKV, Kalimpong	1150	mandarin	spring, rainy	90 (20)	<i>T. aurantii</i> , <i>T. citricida</i>
Latpanchar	1100	mandarin	spring	0 (5)	<i>T. aurantii</i>
Bhage	1100	mandarin, pomelo	spring	41.67 (12)	<i>T. aurantii</i>
Mansong	1100	mandarin	spring, rainy	50 (20)	<i>T. aurantii</i> , <i>A. gossypii</i>
Kamjer	1100	mandarin	spring	32.86 (21)	<i>T. aurantii</i>
Ichebasty	1050	mandarin, pomelo	spring, rainy	64.29 (14)	<i>T. aurantii</i> , <i>M. persicae</i> , <i>B. helichrysi</i>
Posher basty	1000	mandarin, pomelo	spring, rainy	66.67 (9)	<i>T. aurantii</i> , <i>M. persicae</i> , <i>A. gossypii</i>
Yagdhe	1000	mandarin	spring	42.86 (7)	<i>T. aurantii</i>
Peshok	900	mandarin, pomelo	spring, rainy	60 (15)	<i>T. aurantii</i>
Bijanbari	900	mandarin	spring	90 (30)	<i>T. aurantii</i> , <i>T. citricida</i>
Bingbong	900	mandarin	spring	73.33 (15)	<i>T. aurantii</i>
Soriang	900	mandarin	spring, rainy	20 (10)	<i>T. aurantii</i>
Mangmaya	800	mandarin	spring, rainy	85.12 (27)	<i>T. aurantii</i> , <i>T. citricida</i> , <i>A. gossypii</i>
Takling	800	mandarin	spring	14.29 (14)	<i>T. aurantii</i>
Jholung	500	mandarin	rainy	85.71 (7)	<i>T. citricida</i>
Jaldhaka	400	mandarin	rainy	80 (5)	<i>T. citricida</i>
Samsing	400	mandarin	rainy	80 (5)	<i>T. citricida</i>
Tista valley	400	mandarin	spring, rainy	100 (11)	<i>T. citricida</i> , <i>T. aurantii</i>
Mal bazar	180	Assam lime	spring, rainy	100 (7)	<i>T. citricida</i>
Matigara	130	kagzi	spring, rainy	100 (8)	<i>T. citricida</i>
Mohitnagar	100	Assam lime, kagzi	spring, rainy	83.33 (30)	<i>T. citricida</i>
Kumar gram	100	kagzi	spring, rainy	100 (4)	<i>T. citricida</i>
Alipurduar	100	kagzi	spring	80 (5)	<i>T. citricida</i>
Daukimari	80	Assam lime	spring, rainy	86.67 (15)	<i>T. citricida</i> , <i>M. persicae</i>
Nathua	80	kagzi, Assam lime	spring, rainy	82.61 (23)	<i>T. citricida</i> , <i>M. persicae</i> , <i>A. gossypii</i>
IARI, Kalimpong#	1200	mandarin, Assam lime, kagzi, rough lemon, Rangpur lime, trifoliolate, pomelo, kumquat, mosambi	spring, rainy	86.46 (226)	<i>T. aurantii</i> , <i>T. citricida</i> , <i>A. gossypii</i> , <i>M. persicae</i> , <i>B. helichrysi</i>

n = total number of samples tested. # institute research farm.

#### Identification of aphid species

All the collections of adult apterous and alate were examined. Specimens were cleared and mounted on microscope slides following the methods described by Footitt and Maw (2000). Identifications of the aphids were undertaken based on morphological characters using keys formulated by Blackman and Eastop (2000).

#### Diagnosis of plant samples and detection of CTV by DIBA

All plant samples were tested for presence of CTV through dot-immunobinding assay (DIBA) following standard protocol (Rocha-Peña et al., 1991). Polyclonal antiserum and respective conjugate (Art. No. 151572, Bioreba, Switzerland) were used at 1:5000 and 1:10000 dilutions, respectively. CTV positive and negative plants maintained in the glasshouse of IARI, Regional Station, Kalimpong were used as control. Spread of CTV was expressed by percent infection of CTV in a particular site. Percent CTV infection was determined by calculating the number of plants found CTV positive in DIBA test out of total plants sampled.

#### Transmission of CTV by aphid species

Based on morphological identity of aphids, mature, apterous, non-viruliferous clonal *T. citricida*, *T. aurantii*, *A. gossypii*, *M. persicae* and *B. helichrysi* were released separately onto mandarin plants infected with Kpg3 isolate of CTV. After the acquisition of 24 hours, ten aphids per plant were released on healthy mandarin orange plants within insect proof cages following the methods laid by Zhou et al. (2011). Forty five mandarin plants were taken for each aphid species. After 24 hours of feeding on healthy plants, aphids were killed by spraying

imidacloprid. After four months, all the plants were tested for CTV by DIBA as described above.

#### Seasonal abundance of CTV vectors

Populations of identified aphid species on different citrus hosts were monitored at experimental field of IARI, Regional Station, Kalimpong (Darjeeling) during 2012–14. The weekly aphid population was recorded by randomly selecting 40 plants each time from the same field. Representative aphids were collected from each colony for identification of species. Number of aphids in each colony was counted from apical 10 cm twig by visual method (Agarwala and Bhattacharya, 1994; Dewar et al., 1982). Weekly populations of different aphid species were recorded separately for the aforesaid period to explore the temporal fluctuation of aphid populations. The means and variances of aphid populations over time were calculated using SAS version 9.3. Weather data of this period were collected from Automatic Weather Station of UBKV, RRS, Hill zone, Kalimpong (Darjeeling).

## Results

#### Identification and distribution of aphid species in Citrus

Five distinct aphid species viz. *Toxoptera citricida*, *T. aurantii*, *Aphis gossypii*, *Myzus persicae* and *Brachycaudas helichrysi* were identified from all the samples collected from Darjeeling and Dooars region (Table 1, Fig. 1). Findings revealed that *T. citricida* was predominant in the Kagzi and Assam lime orchards of the Dooars. Very little or no incidence of *T. aurantii* was recorded in areas of low altitude specifically in Dooars region (Table 1). *T. citricida* was more abundant, irrespective of season, in the lower altitudes below 500 m. The CTV recorded 80% to 100% incidence in the orchards of Dooars where population of

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