OPEN ACCESS Freely available online

Journal of Citrus Pathology

Recently Accepted

BRIEF REPORT

Survey estimates of the incidence and diversity of *Citrus tristeza virus* in California.

RK Yokomi[1*] and S Hajeri[2]

[1]U.S. Department of Agriculture, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, 9611 S. Riverbend Avenue, Parlier, CA 93648, USA

[2]Citrus Pest Detection Program, Central California Tristeza Eradication Agency, 22847 Road 140

Tulare, CA 93274, USA

*Correspondence to: ray.yokomi@usda.gov

Citation: Yokomi, R., & Hajeri, S. (2023). Survey estimates of the incidence and diversity of Citrus tristeza virus in California. Journal of Citrus Pathology, 10. http://dx.doi.org/10.5070/C410159011 Retrieved from https://escholarship.org/uc/item/6cf9n2dq

Abstract:

Surveys were conducted to assess the status of *Citrus tristeza virus* (CTV) in California. Orchard surveys in central California during 2009 to 2013 estimated CTV incidence from 0.05% to 2.9%. Similar surveys conducted in Ventura, Riverside, San Bernardino, and San Diego Counties in 2020-22 estimated CTV incidence from 6.3% to 34.9%, while CTV was rarely found in the other five counties surveyed. T30 comprised over 95% of CTV detected alone or in mixtures with other strains in southern California and constituted 59% of 550 CTV accessions maintained in Tulare, California. VT, RB, and S1 genotypes were also found but T36 was rarely detected. No evidence of CTV-induced economic damage was noted except for occasional CTV quick decline on sour orange rootstock.

Key words. CTV, epidemiology, genotypes, strains, commercial citrus

Introduction

Citrus tristeza virus (CTV) is a graft-transmissible virus and spread naturally by aphid vectors. CTV occurs in nearly all citrus-growing regions in the world (Moreno et al. 2008). CTV causes two principal diseases: 1) tristeza quick decline (QD) of citrus grown on sour orange rootstock; 2) CTV stem pitting (SP) in scions regardless of rootstock. Commercial citrus production began in California in the early twentieth century in the Los Angeles Basin with growers planting citrus on sour orange rootstock. CTV in California was first noted when trees began suffering from QD as early as 1939 (Wallace 1978). This catastrophic collapse of sour-rooted citrus was due a CTV-induced necrosis at the budunion which results in girdling and rapid death of the tree. It is probable that this virus was introduced into California from infected propagations from other states or abroad and spread to other citrus trees by aphid vectors (Wang et al. 2013). In California, the cotton or melon aphid, *Aphis gossypii*, is the principal vector of CTV (Roistacher et al. 1984). Due to the widespread occurrence of QD and high land values in southern California, most of the new citrus planting were relocated to central California. Growers planted citrus on CTV resistant or tolerant rootstocks such a Troyer citrange or other *Poncirus trifoliata* hybrids (Wang et al. 2013). During this period, the UC Citrus Clonal Protection Program (CCPP), Department of Plant Pathology and Microbiology, University of California, Riverside was created and provided pathogen-free citrus budwood (Vidalakis et al. 2010) to citrus nurseries. The nursery would increase the clean budwood and propagate new citrus trees by bud grafting onto CTV tolerant rootstocks, hence, providing growers with healthy trees. The California Department of Food and Agriculture (CDFA), Sacramento, California, working with the citrus growers and nurserymen, also established the State Interior Quarantine 3407 (CDFA 2011) which required registration and annual testing of budwood source trees to be CTV-

free. In addition, the Central California Tristeza Eradication Agency (CCTEA), Tulare, California was created by a Joint-Powers Agreement between Citrus Pest Control Districts (PCD) and tasked to conduct annual surveys of citrus orchards in Kern, Tulare, and Fresno Counties and test trees for CTV infection. When CTV infected trees were found, the CCTEA would assist in the eradication of these trees (Polek 2010). In 1996, due to grower push back, the CCTEA stopped mandatory surveys in Tulare and West Fresno PCDs and adopted testing in the remaining PCDs by the hierarchical subsampling (HS) method (Gottwald and Hughes 2000). In addition, the CCTEA converted antisera from a universal CTV polyclonal antibody to MCA13. This monoclonal antibody reacts to known virulent exotic CTV strains but not with non-QD isolates (Permar et al. 1990). Trees infected with MCA13-reactive CTV are eradicated in participating PCDs (Barnier et al. 2010).

Through these programs and the cooperation of growers and citrus nurseries, CTV causes little significant economic damage in California (O'Connell et al. 2010; Yokomi et al. 2020) except for occasional occurrences of QD of citrus planted on sour orange rootstock. To investigate the present status of CTV in California, we conducted surveys of CTV in commercial citrus orchards in all major citrus-growing counties in California to estimate the incidence and determine the genotypes of CTV present.

Methods and Materials

Scope of the sampled area. The total commercial citrus acreage in California is 108,838 HA (NASS 2022). The surveyed area included commercial citrus grown from Monterey County in the north to San Diego and Imperial Counties in the south (Fig. 1).

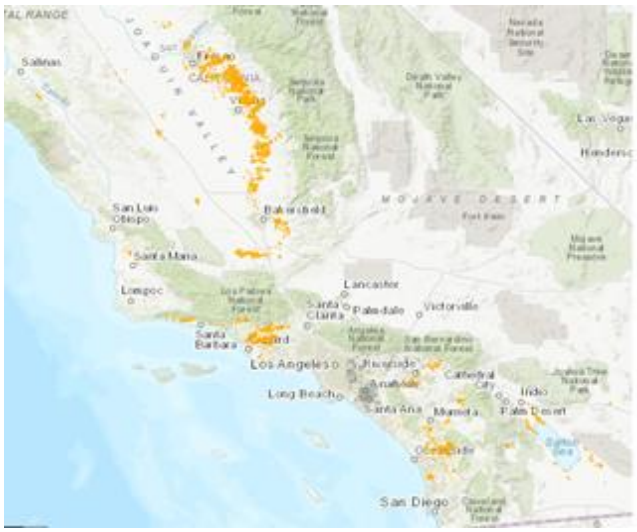


Fig. 1. Commercial citrus growing regions in California. Orange indicates citrus production areas. Map obtained from https://ucanr.edu/sites/ACP/Distribution_of_ACP_in_California/.

The total citrus area in the counties of Tulare, Kern, and Fresno totals 82,926 HA and constitutes 68.2% of the total California citrus plantings (Fig. 2).

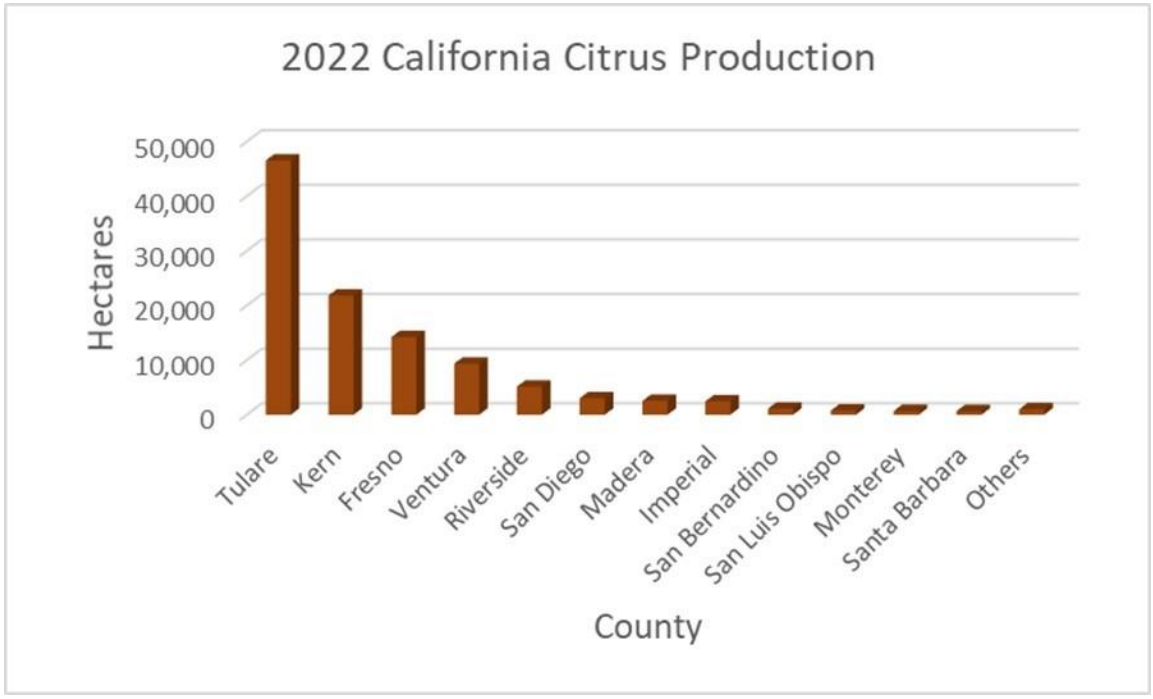


Fig. 2. Relative hectares of commercial citrus production per county in California. Area in Statewide citrus production totals 109,106 HA. Others = Counties of Orange, Glenn,

Stanislaus, Butte, Placer, Kings, and Yolo with comparatively small citrus plantings (NASS 2022).

CTV data collected by the CCTEA from PCDs in central California from 2009 to 2013 was used in this study and, therefore, were not duplicated. The new survey in this report was conducted in 2020-22 and included nine major citrus-producing counties in California (Imperial, San Bernardino, Riverside, San Diego, Ventura, Santa Barbara, San Luis Obispo, Monterey, and Madera) with a total of 34,528 HA of citrus. This constitutes 28.5% of the total citrus plantings in the State. Therefore, this report represents 96.8% of the citrus acreage in California.

CTV survey 2020-22. The sample area and number of samples collected using the Hierarchical Subsamples (HS) method for this survey is shown in Table 1.

Table 1. Estimated incidence of *Citrus tristeza virus* (CTV) in commercial citrus orchards from hierarchical subsample (HS) surveys conducted in 2020-2022 from the major citrus-growing counties of California excluding those in the San Joaquin Valley Citrus Pest Control Districts.

County	County acreage (HA)	Sample date	No. orchards sampled	No. HS samples	No. trees sampled	Hectares sampled	% area sampled	No. HS samples with CTV	Estimated % CTV
Monterey	539	11/13/2020	5	189	756	73	13.6	0	0
San Luis Obispo	952	3/5/2021	3	96	384	16	1.7	0	0
Madera	2,676	8/18/2021	5	240	960	149	5.6	0	0
Imperial	2,747	5/10/2021	5	240	960	47	1.7	0	0
San Bernardino	1,362	6/7/2021	3	144	576	20	1.5	112	31.34
Riverside	8,477	6/8/2021	13	735	2,940	106	1.2	275	11.96
San Diego	5,522	6/8/2021	8	480	1,920	46	0.8	394	34.94
Ventura	10,614	10/12/2021	15	954	3,816	146	1.4	217	6.25
Santa Barbara	4,640	6/16/2022	7	336	1,344	15	0.9	12	0.91
Totals	34,528		64	3,414	13,656	616	1.8	1,010	8.40

In general, the number of samples collected per county was proportional to its total citrus acreage; the larger the acreage in a county, the larger was the number of samples taken. Each orchard sampled was at least 4 HA in size but, more often, 8 HA or larger and distributed in different locations of the county. CTV titer in the tree canopy is temperature sensitive, favoring mild spring and fall seasons in California (Polek 2010). This is also ideal for young flush growth where CTV replication is readily detectable (Dodds et al. 1987). The surveys covered all major citrus-growing regions of California: 1) interior; 2) coastal-Intermediate; 3) San Joaquin Valley; and Desert (Yokomi et al. 2010a). Collection dates were selected, as best as possible, on the best temperature for CTV replication and new flush growth per region (Fig. 3).

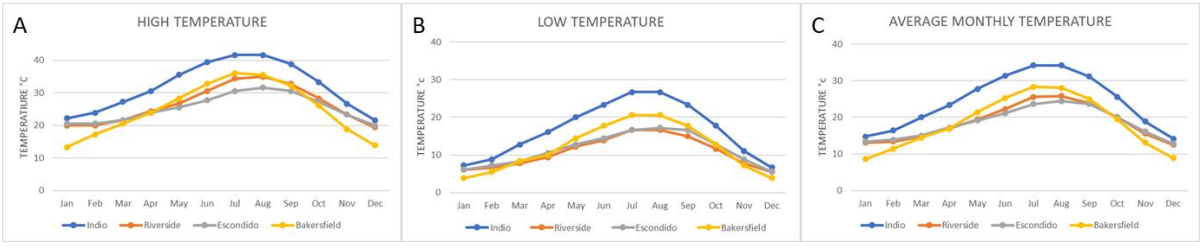


Fig. 3. Monthly average temperatures from California citrus-growing regions. A. Average high temperatures; B. Average low temperatures; C. Average monthly temperatures. Regions are represented by Indio (desert) (blue); Riverside (inland) (red); Escondido (coastal intermediate) (grey); and Bakersfield (San Joaquin Valley) (yellow). Temperatures calculated from: https://www.usclimatedata.com/climate/california/united-states/3174.

Additionally, an effort was made to sample cultivars prevalent in each county. A total of 64 HS samples were collected from an orchard, but when only smaller orchards were available, 48 HS samples were collected. Table 1 also includes the date and number of samples collected. On average, the survey covered approximately 1.8% of the citrus acreage per county.

Sample collection. For HS sampling, each orchard was partitioned into groups of sixteen 4-tree quadrats and only one quadrat of each 4-quadrat group was sampled. This resulted in sampling of 25% of the orchard or sample area. Four leaves per tree were collected and pooled with leaves from the other three trees for a total of 16 leaves. Petioles were excised and placed in a paper envelope. Envelopes with plant tissue were placed in a zip lock bag filled with desiccant, double bagged and stored in an ice chest and transported to the laboratory (Garnsey et al. 1996). Since CTV detected from a pooled sample could result from one or more trees in the sample, the formula developed by Gottwald and Hughes (2000) was used to calculate an estimate of the overall infection level of the area sampled. Thus, it was not necessary to resample and test individual trees of a HS (quadrat sample) to estimate CTV incidence.

CCTEA surveys. The CCTEA CTV surveys from 2009-2013 used the HS method where 25% of the citrus acreage were sampled per year from participating PCDs of Kern, Tulare, and Fresno counties with a total of 65,559 HA of citrus (Fig. 4).

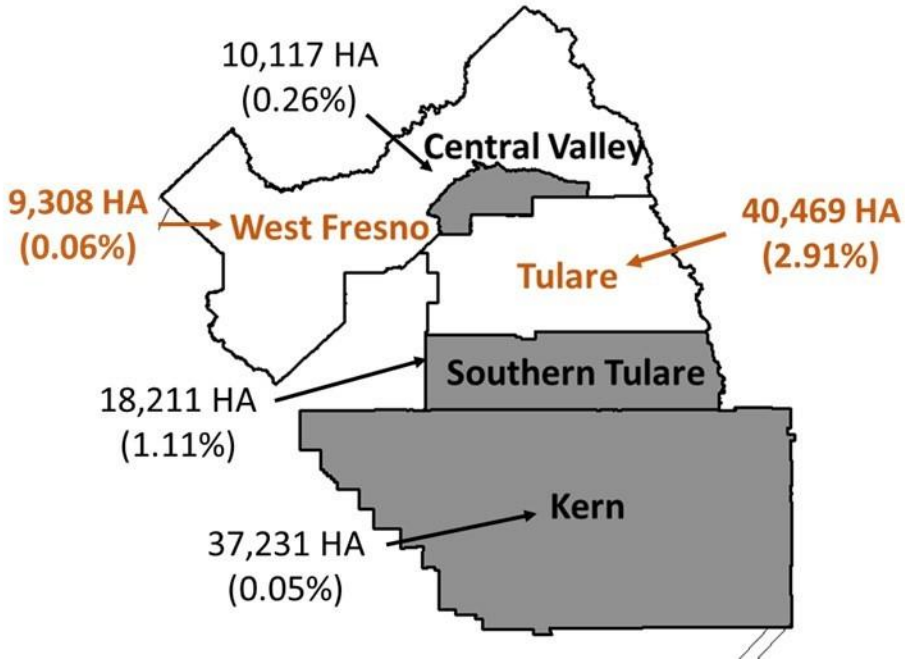


Fig. 4. Citrus Pest Control Districts in central California. Grey = Joint Powers Districts (JPDs) with a total of 64,750 citrus HA; white = non-JPD districts with a total of 140,000 citrus HA and estimated citrus tristeza virus incidence per district in parenthesis. In this map, one citrus HA = 41 trees.

Each year, a different acreage of the PCD was sampled, therefore, over a 4-year period, 100% of the PCD orchards were sampled. Additionally, HS samples collected and tested by the CCTEA under contract from non-participating PCD of Tulare and a 1.6 km radius around the Lindcove Research and Extension Center (LREC) which totals 51,051 HA was sampled and data included in this report.

CTV detection and strain differentiation. CTV was detected by Indirect Double-Antibody-Sandwich Enzyme-Linked Immunosorbent Assay (I-DAS ELISA). CTV polyclonal antibodies raised in rabbit and chicken along with MCA13 monoclonal antibodies were used in these tests (Permar et al. 1990; Polek 2010; Maheshwari et al. 2017). Microtiter and qPCR plates were coated with trapping antibody (rabbit anti-CTV antibody) for ELISA detection with MCA13 and for Immuno-Capture of CTV for detection by Real Time Reverse Transcription Polymerase Chain Reaction (IC-RT-qPCR); and for broad spectrum CTV detection by ELISA, chicken anti-CTV antibody with rabbit anti-CTV polyclonal antibodies were used. Desiccated citrus petioles were homogenized in an extraction buffer (EB) (1X PBS-T and 2% PVP) using a Geno/Grinder 2010 homogenizer (SPEX SamplePrep). Each extract was transferred to two-wells each on three CTV antibody-coated 96-well ELISA/qPCR plates, one for broad-spectrum CTV detection, the second for MCA13 reactive CTV detection, and the third meant for strain differentiation using IC-RT-qPCR. Every ELISA plate had three types of controls: extraction buffer, healthy plant material, and known CTV-positive plant material. Microtiter/qPCR plates were incubated overnight at 4 °C. After

three washes with PBS-T (1X PBS buffer and 0.05% tween-20), the microtiter wells were incubated with detection antibodies at 37 °C for 2 h. The plates were washed with PBS-T and alkaline phosphatase conjugated antibody (Sigma Aldrich, St. Louis, USA) was added and incubated for 2 h at 37 °C. Finally, the plates were washed three times with PBS-T and p-nitro phenyl phosphate substrate (0.5 mg/ml) was added. The absorbance was measured at 405 nm after 1 h incubation using microplate reader (EMax by Molecular Device). Based on the ELISA reaction, selected positive wells from duplicated qPCR plates were chosen for IC-RT-qPCR. qPCR plates were washed as described for microtiter plates. cDNA was made using CTV specific reverse primers and SuperScript™ IV Reverse Transcriptase kit (Thermo Fisher Scientific) following manufacturer instructions. The CTV strain differentiating primers and probes used in this survey have been described previously (Yokomi et al. 2010b, 2017, 2018; Selvaraj et al. 2019).

CCTEA *in planta* CTV collection. CCTEA has been surveying commercial citrus orchards for over 50 years to detect and remove CTV-infected trees. Prior to eradication of CTV-infected trees, budwood and leaves was collected from representative infected trees and graft-inoculated into Madam Vinous sweet orange to propagate the virus isolate for biological indexing in the greenhouse. Over the years, over 550 CTV isolates were collected and maintained by the CCTEA. This *in planta* collection represents a snapshot of CTV strains in central California. Polyclonal (broad-spectrum) and monoclonal (MCA13) ELISA tests were conducted side-by-side only during survey periods of 2009 to 2013, hence, Table 3 represents data collected only during this survey period.

Results

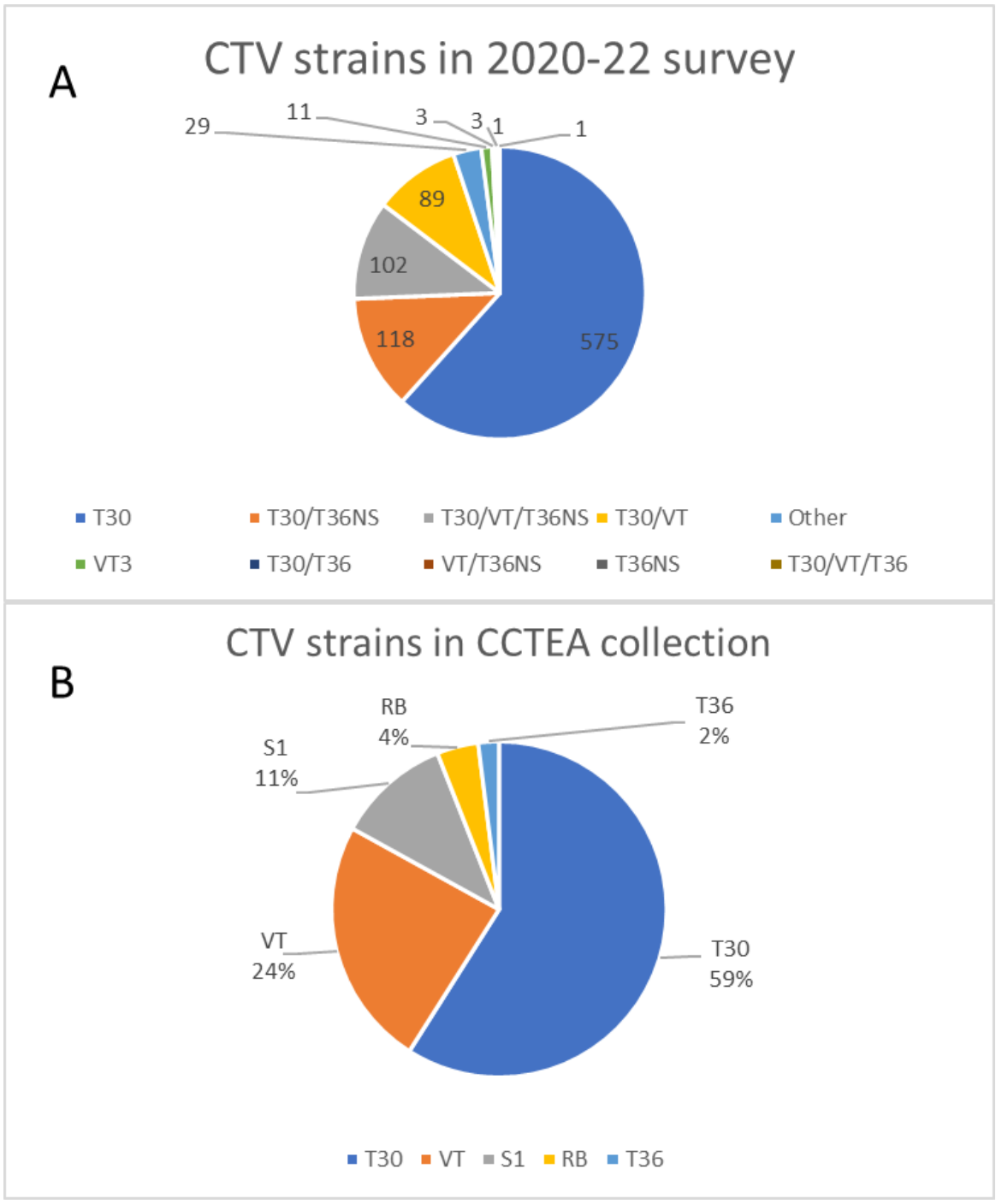
CTV survey. Table 1 show results of the 2020-22 CTV survey from the counties of Ventura, Riverside, San Diego, Madera, Imperial, San Bernardino, San Luis Obispo, Santa Barbara, and Monterey. A total of 3,414 HS samples were collected from a sample area of 616 HA and included 64 orchards and 13,656 trees. These samples were collected from 1.8% of the citrus but covered a wide area in each county. There were 1,010 HS samples positive for CTV for an estimated CTV incidence from the nine counties of 8.4%. Specifically, the estimated CTV incidence was highest in San Diego and San Bernardino counties at 34.9% and 31.3%, respectively. CTV estimates in Riverside and Ventura were lower at 11% and 6.3%, respectively. Santa Barbara County had a low level of CTV at 0.9% and no CTV was detected from Madera, Imperial, San Luis Obispo, and Monterey County samples. The highest level of CTV was found in sweet orange at 34.6%, grapefruit at 5.9%, mandarin at 2.4%, lemon at 0.1%, and no CTV was found in Minneola or sweet lime cultivars sampled (Table 2). HS samples with MCA13 reactivity were rarely encountered.

198 **Table 2.** Estimated incidence of *Citrus tristeza virus* (CTV) per cultivar from hierarchical
199 subsample (HS) surveys conducted in 2020-2022 from Ventura, Riverside, San Diego, San
200 Bernardino Counties. CTV was not detected from surveyed citrus orchards in Imperial,
201 Madera, San Luis Obispo, Santa Barbara, and Monterey Counties.

Cultivar	No. HS samples	Hectares sampled	No. HS with CTV	Estimated CTV incidence
Lemon	1,451	231	7	0.12
Orange	1,052	161	859	34.55
Grapefruit	560	71	120	5.85
Mandarin	256	101	24	2.43
Minneola	48	12	0	0
Sweet lime	48	32	0	0
Overall totals	3,415	608	1,010	8.40

202

203 CTV genotype T30 was the predominant strain detected at 61.6% alone or 95.2% in mixtures
204 with other genotypes (Fig. 5A). The T36NS group was present in mixtures with other
205 genotypes in 24% of the CTV detected. The T36NS group contains the S1 and RB genotypes
206 but strain differentiation of this group was not included in this study. The VT genotype was
207 present as mixtures with other strains in 20% of the CTV detected. T36 comprised only 0.4%
208 of the CTV detected. Interestingly, 3.1% of the CTV detected did not fall in any of the
209 genotype categories. The non-T30 genotype samples which typically should react with
210 MCA13 did not react.



211

212 **Fig. 5.** *Citrus tristeza virus* (CTV) genotypes detected in California. A. 2020-2022 survey.
213 Number of hierarchical subsamples (HS) infected with CTV per genotype from Ventura,
214 Riverside, San Diego, San Bernardino Counties. CTV was not detected from surveyed citrus
215 orchards in Imperial, Madera, San Luis Obispo, Santa Barbara, and Monterey Counties. **B.**
216 Central California Tristeza Eradication Agency (CCTEA) collection. Proportion CTV

genotypes from representative isolates in the CTV collection acquired from the 1960s to 2018 in central California.

CTV in Central California Citrus PCDs. Surveys conducted from 2009 to 2013 from participating PCDs show an overall low incidence of CTV (Table 3A).

Table 3. Estimates of *Citrus tristeza virus* (CTV) incidence in central California based on hierarchical subsamples (HS) collected from 2009-2013 by the Central California Tristeza Eradication Agency (CCTEA). A. Data includes 100% of the orchards from participating Pest Control Districts (PCD). B. Data from non-participating PCDs growers that requested hierarchical subsample (HS) for CTV. Data includes Enzyme-Linked Immunosorbent Assays (ELISA) with antisera for universal and MCA13 CTV.

PCD	Hectares	No. HS samples	CTV universal antisera		MCA13 antisera		
			No. CTV positive	Estimated CTV %	MCA13 positive	Estimated MCA13 positive	No. MCA13 trees removed
A. **Participating**							
Central Valley	10,117	177,596	1,806	0.26	4	0.0008	6
Southern Tulare	18,211	217,089	1,214	1.11	12	0.0013	20
Kern	37,231	591,115	9,441	0.05	4	0.0003	6
Totals	65,559	985,800	12,461	0.32	20	0.0005	32
B. **Non-participating**							
West Fresno	9,308	11,673	28	0.06	1	0.0021	0
Tulare	40,469	130,359	14,506	2.91	84	0.0161	366
LREC 1.6 km	1,275	51,822	4,833	2.42	22	0.0106	53
Totals	51,051	193,854	19,367	0.32	107	0.0005	419

Specifically, estimated CTV incidence was 1.11%, 0.26%, and 0.05% from Southern Tulare, Central Valley, and Kern PCDs, respectively. The combined estimate for CTV incidence in these three districts was 0.32%. Additional surveys conducted in orchards from non-participating Joint Powers PCDs (Table 3B) showed a higher CTV incidence of 2.9%, 2.4%

and 0.06% in Tulare, LREC 1-mile survey, and West Fresno PCDs, respectively. Fig. 3 contrasts citrus acreage and CTV estimated incidence in joint powers participating versus non-participating PCDs. Table 3 includes data for MCA13 and shows MCA13-reactive CTV are rare in the PCDs. These data indicates that T30 genotype is the predominant strain in the PCD. However, the CCTEA maintains representative and unique isolates of CTV *in planta* collected from Central California (Polek 2010) from the 1960s to 2018. 531 isolates from the CCTEA collection were tested by strain-discriminating primers/probes in Real time RT-PCR. Results indicated that the major genotype was T30 (59%), followed by VT (24%), S1 (11%), RB (4%), and T36 (2%) (Fig. 5B).

Discussion

The CTV surveys in this report provides an estimation of CTV levels and distribution in the major citrus growing regions of the state. These data show CTV incidence in California is highest in the interior region which includes western Riverside, and San Bernadino counties and inland portions of San Diego, and coastal Ventura counties and reflects the long history of CTV in southern California. Because of the higher levels of CTV in these regions, no quarantine actions except use of new propagations free of CTV are practiced here. This is in contrast with the low CTV incidence in central California which is geographically separated from southern California by the Tehachapi Mountains and the Tejon Pass at an elevation of 1,220 m, regulatory and survey efforts with grower support has managed CTV by eradication (Polek 2010). CTV incidence was negligible in the desert region of Imperial and Coachella Valley likely due to the hot temperatures that are detrimental for CTV replication and no CTV was detected from new shoot growth in May when this survey was conducted.

T30 was found to be the predominant genotype of CTV in all surveys in this report. Yokomi and DeBorde (2005) have shown that CTV T30 genotypes are efficiently transmitted by the cotton aphid. The VT genotype is present in central and southern California. This is of concern since some VT strains are associated with virulent stem pitting of citrus scions regardless of rootstocks (Moreno et al. 2008). However, no stem pitting was observed when stems were peeled from infected trees even if flush was showing seedling yellows-like symptoms. Also notable was the presence of S1 and RB genotypes described as mild strains from California (Yokomi et al. 2017, 2018). However, these genotypes have been associated with more severe symptoms elsewhere (Harper et al. 2010; Qin et al. 2023). CTV incidence was ~36% in sweet orange which typically maintains high CTV levels and is a favorable host for aphid vectors. Incidence was ~6% in grapefruit which is likely due to lower aphid populations and low and erratic levels of CTV titer. Cultivars like Minneola, mandarins and lemons have rapid shoot growth. This, coupled with hot summer maximum temperatures (Fig. 3A), resulted in low levels of virus not readily detectable by ELISA or IC-RT-qPCR from HS samples when CTV incidence is low. Although this survey did not include aphid surveys, aphids were rarely encountered during the CTV surveys. With no evidence of stem pitting, the only CTV damage detected was an occasional CTV-QD on sour orange rootstock. CTV strains remained asymptomatic on citrus grown on tolerant rootstock. As CTV incidence and genotype complexity increases, however, CTV genetic diversity is expected to increase due to mixtures, mutations, or recombination (Harper 2013). Fortunately, the efficient CTV vector, *Toxoptera citricida*, the brown citrus aphid, does not occur in California. If this aphid becomes established in California, CTV strains will likely become more virulent as has been the case in Florida. Therefore, continued annual orchard surveys to monitor CTV by the CCTEA and removal of trees infected with MCA13-positive strains will slow the buildup of CTV strain mixtures. Currently, CTV remains at below economic

threshold levels due to the absence of virulent stem pitting strains and generally low aphid levels due in part to insecticidal use by grower to prevent establishment of the Asian citrus psyllid (ACP). The pesticides used for ACP control are also efficacious against aphid vectors of CTV.

References

Barnier, J., Grafton-Cardwell, B., and Polek, M. 2010. *Citrus tristeza virus* (CTV): Then and now. Citrograph 1(6) Nov/Dec: 16-23. CDFA. 2011. Section 3407 Interior Citrus Tristeza Virus Quarantine. California Department of Food and Agriculture Plant Quarantine Manual 404.1 http://pi.cdfa.ca.gov/pqm/manual/pdf/404.pdf

Dodds, J.A., Jarupat, T. Lee, J.G. and Roistacher C.N. 1987. Effects of strain, host, time of harvest, and virus concentration on double-stranded RNA analysis of citrus tristeza virus. Phytopathology 77:442-447.

Garnsey, S.M., Gottwald, T.R., and Borbón, J.C. 1996. Rapid dissemination of mild isolates of citrus tristeza virus following introduction of *Toxoptera citricida* in the Dominican Republic. Pages 92-103 in: Proc. 13th Conf. Int. Organ. Citrus Virol. IOCV, Riverside, CA.

Gottwald, T.R., and Hughes, G. 2000. A new survey method for citrus tristeza virus disease assessment. pp. 77-87. In: da Graça, J.V.; Lee, R.F.; Yokomi, R.K. (eds.). Proc. 14th International Organization of Citrus Virologists IOCV, Riverside, California.

Harper, S.J. 2013. *Citrus tristeza virus*: evolution of complex and varied genotypic groups. Front Microbiol 4:93. https://doi.org/10.3389/fmicb.2013.00093.

Harper, S.J., Dawson, T.E., and Pearson, M.N. 2010. Isolates of *Citrus tristeza virus* that overcome *Poncirus trifoliata* resistance comprise a novel strain. Arch. Virol. 155, 471–480.

Maheshwari, Y. Selvaraj, V, Hajeri, S., Ramadugu, C., Keremane, M.L., and Yokomi, R.K. 2017. On-site detection of Citrus tristeza virus (CTV) by lateral flow immunoassay using polyclonal antisera derived from virions produced by a recombinant CTV. Phytoparasitica doi 10.1007/s12600-017-0591-0.

Moreno, P., Ambros, S., Albiach-Marti, M.R., Guerri, J., and Peña, L. 2008. *Citrus tristeza virus*: a pathogen that changed the course of the citrus industry. Mol. Plant Path. 9: 251-68.

NASS, USDA. 2022. 2022 California Citrus Acreage Report. https://www.nass.usda.gov/Statistics_by_State/California/Publications/Specialty_and_Other_Releases/Citrus/index.php.

O'Connell, N.V., Seymore, A.D., Chaffe-Stengel, P., and Stengel, D.N. 2010. Economic impact of mild strains of Citrus tristeza virus in the San Joaquin Valley. Citrograph 1(6) Nov/Dec: 24-26.

Permar, T.A., Garnsey, S.M., Gumpf. D.J., and Lee, R.F. 1990. A monoclonal antibody that discriminates strains of *Citrus tristeza virus*. Phytopathology 80:224-228.

Polek, M. 2010. Eradication of tristeza in the Central Valley of California, pp.219-232. In: Karasev, A.V., and Hilf, M.E. (eds.) *Citrus tristeza virus* Complex and Tristeza Diseases, APS Press, St. Paul, MN.

Qin, Y., Lui, Y., Zhao, J., Hajeri, S., Wang J., Ye, W., Zhou, Y. (2023). Molecular and biological characterizations of a novel *Citrus tristeza virus* isolate that causes severe symptoms in Ziyangxiangcheng junos. Arch. Virol. 168(2):59. doi: 10.1007/s00705-022-05644-y.

Roistacher, C.N., Bar-Joseph, M, and Gumpf, D.J. 1984. Transmission of tristeza and seedling yellows tristeza by small populations of *Aphis gossypii*. Plant Dis. 68: 494-496.

Selvaraj, V., Maheshwari, Y., Hajeri, S., and Yokomi, R. 2019. A rapid detection tool for VT isolates of *Citrus tristeza virus* by immunocapture-reverse transcriptase loop-mediated isothermal amplification assay. PLoS ONE 14(9): e0222170. https://doi.org/10.1371/journal.pone.0222170.

Vidalakis, G., da Graça, J. V., Dixon, W.N., Ferrin, D., Kesinger, M., Krueger, R. R., Lee, R.F., Melzer, M.J., Olive, J., Polek, M., Sieburth, P. J., Williams, L, and Wright, G.C. 2010. Citrus quarantine, sanitary, and certification programs in the USA: Prevention of introduction and distribution of citrus diseases. Part 2. Certification schemes and national programs. Highlights of new California citrus nursery regulations. Citrograph 1:27-39.

Wallace, J.M. 1978. Chapter 2. Virus and virus-like disease, p 67-184. In. Reuther, W., Calavan, E.C., and Carmen, G.E. (eds.). The Citrus Industry, Vol. IV. University of California, Div. Agric. Sci., Berkeley, California.

Wang, J., Bozan, O., Kwon, S. J., Dang, T., Rucker, T., Yokomi, R.K., Lee, R.F., Folimonova, S.Y., Krueger, *R.R., Bash, J., Greer, G., Diaz, J., Serna, R., and Vidalakis, G. 2013. Past and future of a century old Citrus tristeza virus* collection: a California citrus germplasm tale. Front. Microbiol.

Yokomi, R.K., and DeBorde, R. L. 2005. Incidence, transmissibility, and genotype analysis of *Citrus tristeza virus* (CTV) from CTV eradicative and non-eradicative districts in central California. Plant Dis. 89:859-866.

Yokomi, R. K., Polek, M. and Gumpf, D.J. 2010. Transmission and spread of Citrus tristeza virus in Central California, pp. 151-165. Karasev, A.V., and Hilf, M.E. (eds.) Citrus tristeza virus Complex and Tristeza Diseases, American Phytopathological Society Press, St. Paul, MN.

Yokomi, R. K., Saponari, M., and Sieburth, P. J. 2010. Rapid differentiation and identification of potential severe strains of *Citrus tristeza virus* by real-time reverse transcription-polymerase chain reaction assays. Phytopathology 100:319-327.

Yokomi, R.K, Selvaraj, V., Maheshwari, Y., Saponari, M., Giampetruzzi, A., Chiumenti M., and Hajeri, S. 2017. Identification and characterization of *Citrus tristeza virus* isolates Breaking Resistance in trifoliate orange in California. Phytopathology 107:901-908.

Yokomi, R., Selvaraj, V., Maheshwari, Y., Chiumenti, M., Saponari, M., Giampetruzzi, A., Weng, Z., Xiong, Z. and Hajeri, S. 2018. Molecular and biological characterization of a novel mild strain of citrus tristeza virus in California. Arch. Virol. 163(7):1795-1804.

Yokomi, R., Sisterson, M., and Hajeri, S. 2020. Spread of citrus tristeza virus in abatement and non-abatement citrus orchards in central California. Plant Disease 104:1925-1931.