

1 **Recently Accepted**

2

3 **BRIEF REPORT**

4

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

6

7 RK Yokomi^{1*} and S Hajeri²

8

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

11 ²Citrus Pest Detection Program, Central California Tristeza Eradication Agency, 22847 Road
12 140

13 Tulare, CA 93274, USA

14 *Correspondence to: ray.yokomi@usda.gov

15

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

20

21 **Abstract:**

22 Surveys were conducted to assess the status of *Citrus tristeza virus* (CTV) in California.

23 Orchard surveys in central California during 2009 to 2013 estimated CTV incidence from

24 0.05% to 2.9%. Similar surveys conducted in Ventura, Riverside, San Bernardino, and San

25 Diego Counties in 2020-22 estimated CTV incidence from 6.3% to 34.9%, while CTV was

26 rarely found in the other five counties surveyed. T30 comprised over 95% of CTV detected

27 alone or in mixtures with other strains in southern California and constituted 59% of 550

28 CTV accessions maintained in Tulare, California. VT, RB, and S1 genotypes were also found

29 but T36 was rarely detected. No evidence of CTV-induced economic damage was noted

30 except for occasional CTV quick decline on sour orange rootstock.

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

32 Introduction

33 *Citrus tristeza virus* (CTV) is a graft-transmissible virus and spread naturally by aphid
34 vectors. CTV occurs in nearly all citrus-growing regions in the world (Moreno et al. 2008).
35 CTV causes two principal diseases: 1) tristeza quick decline (QD) of citrus grown on sour
36 orange rootstock; 2) CTV stem pitting (SP) in scions regardless of rootstock. Commercial
37 citrus production began in California in the early twentieth century in the Los Angeles Basin
38 with growers planting citrus on sour orange rootstock. CTV in California was first noted
39 when trees began suffering from QD as early as 1939 (Wallace 1978). This catastrophic
40 collapse of sour-rooted citrus was due a CTV-induced necrosis at the budunion which results
41 in girdling and rapid death of the tree. It is probable that this virus was introduced into
42 California from infected propagations from other states or abroad and spread to other citrus
43 trees by aphid vectors (Wang et al. 2013). In California, the cotton or melon aphid, *Aphis*
44 *gossypii*, is the principal vector of CTV (Roistacher et al. 1984). Due to the widespread
45 occurrence of QD and high land values in southern California, most of the new citrus
46 planting were relocated to central California. Growers planted citrus on CTV resistant or
47 tolerant rootstocks such a Troyer citrange or other *Poncirus trifoliata* hybrids (Wang et al.
48 2013). During this period, the UC Citrus Clonal Protection Program (CCPP), Department of
49 Plant Pathology and Microbiology, University of California, Riverside was created and
50 provided pathogen-free citrus budwood (Vidalakis et al. 2010) to citrus nurseries. The
51 nursery would increase the clean budwood and propagate new citrus trees by bud grafting
52 onto CTV tolerant rootstocks, hence, providing growers with healthy trees. The California
53 Department of Food and Agriculture (CDFA), Sacramento, California, working with the
54 citrus growers and nurserymen, also established the State Interior Quarantine 3407 (CDFA
55 2011) which required registration and annual testing of budwood source trees to be CTV-

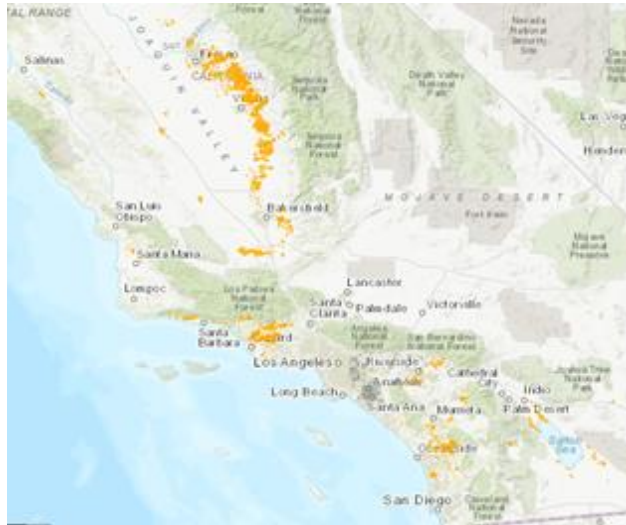
56 free. In addition, the Central California Tristeza Eradication Agency (CCTEA), Tulare,
57 California was created by a Joint-Powers Agreement between Citrus Pest Control Districts
58 (PCD) and tasked to conduct annual surveys of citrus orchards in Kern, Tulare, and Fresno
59 Counties and test trees for CTV infection. When CTV infected trees were found, the CCTEA
60 would assist in the eradication of these trees (Polek 2010). In 1996, due to grower push back,
61 the CCTEA stopped mandatory surveys in Tulare and West Fresno PCDs and adopted testing
62 in the remaining PCDs by the hierarchical subsampling (HS) method (Gottwald and Hughes
63 2000). In addition, the CCTEA converted antisera from a universal CTV polyclonal antibody
64 to MCA13. This monoclonal antibody reacts to known virulent exotic CTV strains but not
65 with non-QD isolates (Permar et al. 1990). Trees infected with MCA13-reactive CTV are
66 eradicated in participating PCDs (Barnier et al. 2010).

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

73

74 **Methods and Materials**

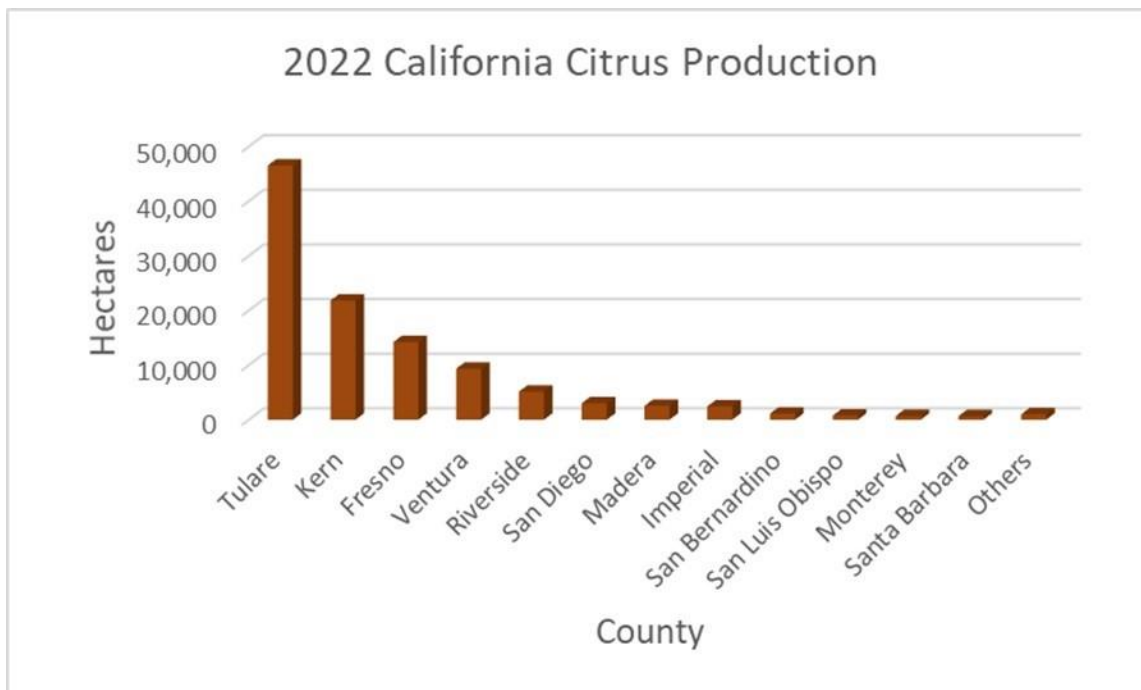
75 **Scope of the sampled area.** The total commercial citrus acreage in California is 108,838 HA
76 (NASS 2022). The surveyed area included commercial citrus grown from Monterey County
77 in the north to San Diego and Imperial Counties in the south (Fig. 1).



78

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

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



83

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

86 Stanislaus, Butte, Placer, Kings, and Yolo with comparatively small citrus plantings (NASS
87 2022).

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

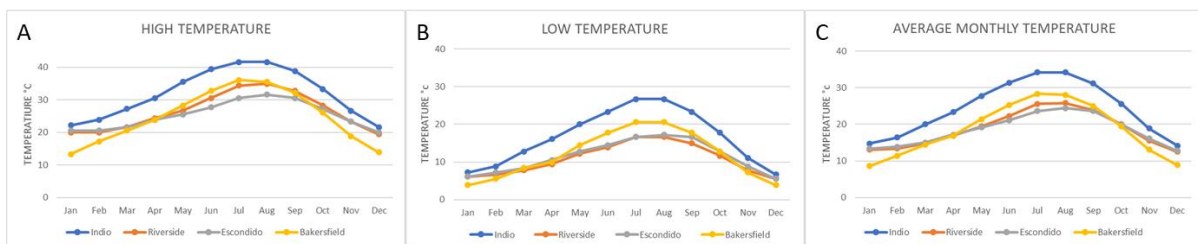
95 **CTV survey 2020-22.** The sample area and number of samples collected using the
96 Hierarchical Subsamples (HS) method for this survey is shown in Table 1.

97 **Table 1.** Estimated incidence of *Citrus tristeza virus* (CTV) in commercial citrus orchards
98 from hierarchical subsample (HS) surveys conducted in 2020-2022 from the major citrus-
99 growing counties of California excluding those in the San Joaquin Valley Citrus Pest Control
100 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

101

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

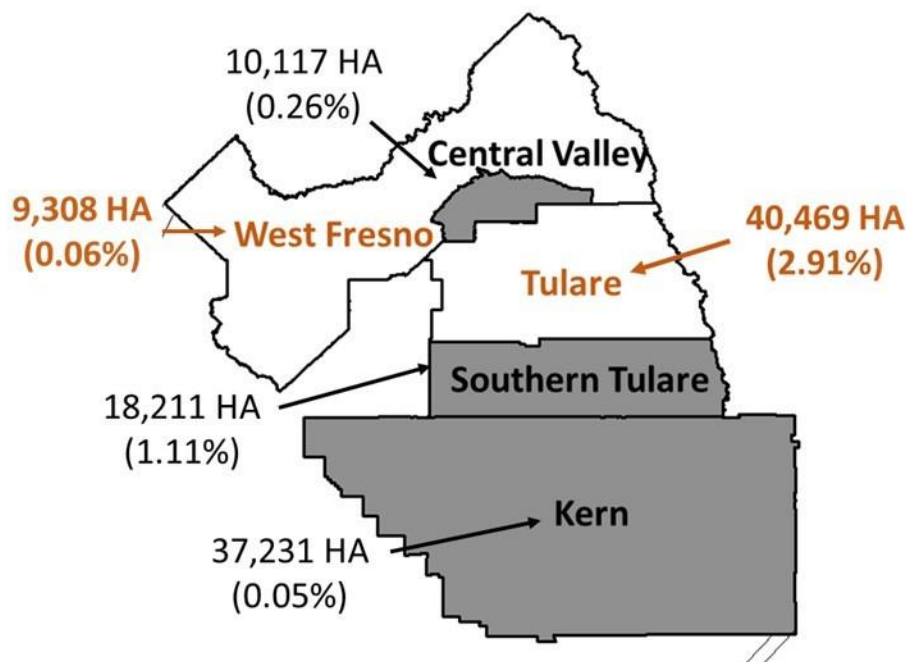


112
 113 **Fig. 3.** Monthly average temperatures from California citrus-growing regions. A. Average
 114 high temperatures; B. Average low temperatures; C. Average monthly temperatures. Regions
 115 are represented by Indio (desert) (blue); Riverside (inland) (red); Escondido (coastal
 116 intermediate) (grey); and Bakersfield (San Joaquin Valley) (yellow). Temperatures calculated
 117 from: <https://www.usclimatedata.com/climate/california/united-states/3174>.

118 Additionally, an effort was made to sample cultivars prevalent in each county. A total of 64
 119 HS samples were collected from an orchard, but when only smaller orchards were available,
 120 48 HS samples were collected. Table 1 also includes the date and number of samples
 121 collected. On average, the survey covered approximately 1.8% of the citrus acreage per
 122 county.

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

134 **CCTEA surveys.** The CCTEA CTV surveys from 2009-2013 used the HS method where
 135 25% of the citrus acreage were sampled per year from participating PCDs of Kern, Tulare,
 136 and Fresno counties with a total of 65,559 HA of citrus (Fig. 4).



137

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

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

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

162 three washes with PBS-T (1X PBS buffer and 0.05% tween-20), the microtiter wells were
163 incubated with detection antibodies at 37 °C for 2 h. The plates were washed with PBS-T and
164 alkaline phosphatase conjugated antibody (Sigma Aldrich, St. Louis, USA) was added and
165 incubated for 2 h at 37 °C. Finally, the plates were washed three times with PBS-T and p-
166 nitro phenyl phosphate substrate (0.5 mg/ml) was added. The absorbance was measured at
167 405 nm after 1 h incubation using microplate reader (EMax by Molecular Device). Based on
168 the ELISA reaction, selected positive wells from duplicated qPCR plates were chosen for IC-
169 RT-qPCR. qPCR plates were washed as described for microtiter plates. cDNA was made
170 using CTV specific reverse primers and SuperScript™ IV Reverse Transcriptase kit (Thermo
171 Fisher Scientific) following manufacturer instructions. The CTV strain differentiating
172 primers and probes used in this survey have been described previously (Yokomi et al. 2010b,
173 2017, 2018; Selvaraj et al. 2019).

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

183 **Results**

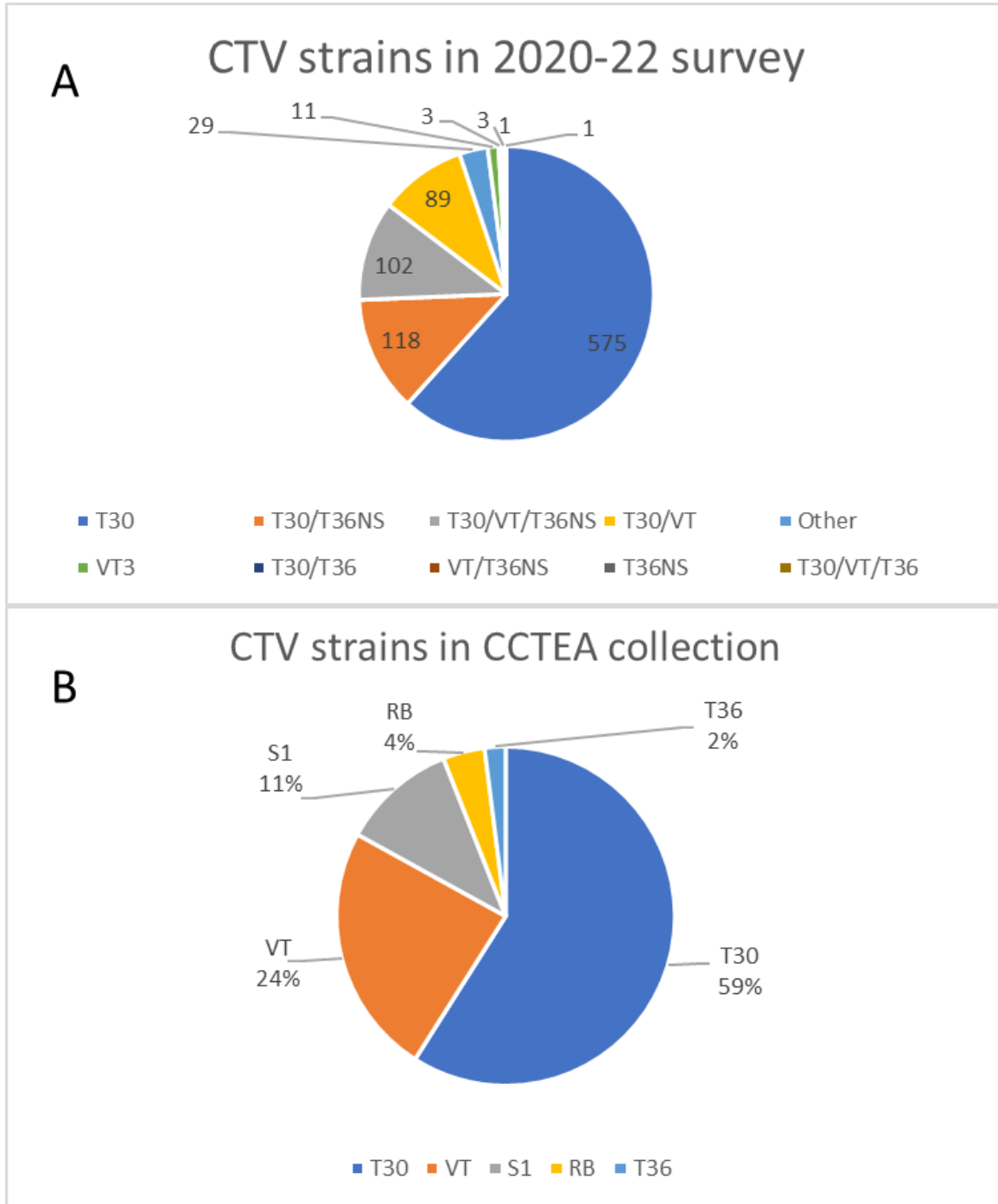
184 **CTV survey.** Table 1 show results of the 2020-22 CTV survey from the counties of Ventura,
185 Riverside, San Diego, Madera, Imperial, San Bernardino, San Luis Obispo, Santa Barbara,
186 and Monterey. A total of 3,414 HS samples were collected from a sample area of 616 HA and
187 included 64 orchards and 13,656 trees. These samples were collected from 1.8% of the citrus
188 but covered a wide area in each county. There were 1,010 HS samples positive for CTV for
189 an estimated CTV incidence from the nine counties of 8.4%. Specifically, the estimated CTV
190 incidence was highest in San Diego and San Bernardino counties at 34.9% and 31.3%,
191 respectively. CTV estimates in Riverside and Ventura were lower at 11% and 6.3%,
192 respectively. Santa Barbara County had a low level of CTV at 0.9% and no CTV was
193 detected from Madera, Imperial, San Luis Obispo, and Monterey County samples. The
194 highest level of CTV was found in sweet orange at 34.6%, grapefruit at 5.9%, mandarin at
195 2.4%, lemon at 0.1%, and no CTV was found in Minneola or sweet lime cultivars sampled
196 (Table 2). HS samples with MCA13 reactivity were rarely encountered.

197

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

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

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

221 **Table 3.** Estimates of *Citrus tristeza virus* (CTV) incidence in central California based on
 222 hierarchical subsamples (HS) collected from 2009-2013 by the Central California Tristeza
 223 Eradication Agency (CCTEA). A. Data includes 100% of the orchards from participating
 224 Pest Control Districts (PCD). B. Data from non-participating PCDs growers that requested
 225 hierarchical subsample (HS) for CTV. Data includes Enzyme-Linked Immunosorbent Assays
 226 (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

227
 228 Specifically, estimated CTV incidence was 1.11%, 0.26%, and 0.05% from Southern Tulare,
 229 Central Valley, and Kern PCDs, respectively. The combined estimate for CTV incidence in
 230 these three districts was 0.32%. Additional surveys conducted in orchards from non-
 231 participating Joint Powers PCDs (Table 3B) showed a higher CTV incidence of 2.9%, 2.4%

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

241 242 Discussion

243 The CTV surveys in this report provides an estimation of CTV levels and distribution in the
244 major citrus growing regions of the state. These data show CTV incidence in California is
245 highest in the interior region which includes western Riverside, and San Bernadino counties
246 and inland portions of San Diego, and coastal Ventura counties and reflects the long history
247 of CTV in southern California. Because of the higher levels of CTV in these regions, no
248 quarantine actions except use of new propagations free of CTV are practiced here. This is in
249 contrast with the low CTV incidence in central California which is geographically separated
250 from southern California by the Tehachapi Mountains and the Tejon Pass at an elevation of
251 1,220 m, regulatory and survey efforts with grower support has managed CTV by
252 eradication (Polek 2010). CTV incidence was negligible in the desert region of Imperial and
253 Coachella Valley likely due to the hot temperatures that are detrimental for CTV replication
254 and no CTV was detected from new shoot growth in May when this survey was conducted.

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

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

284

285 References

286

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

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

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

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

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

303 Harper, S.J., Dawson, T.E., and Pearson, M.N. 2010. Isolates of *Citrus tristeza virus* that
304 overcome *Poncirus trifoliata* resistance comprise a novel strain. *Arch. Virol.* 155,
305 471–480.

306 Maheshwari, Y. Selvaraj, V, Hajeri, S., Ramadugu, C., Keremane, M.L., and Yokomi, R.K.
307 2017. On-site detection of Citrus tristeza virus (CTV) by lateral flow immunoassay
308 using polyclonal antisera derived from virions produced by a recombinant CTV.
309 *Phytoparasitica* [doi 10.1007/s12600-017-0591-0](https://doi.org/10.1007/s12600-017-0591-0).

310 Moreno, P., Ambros, S., Albiach-Marti, M.R., Guerri, J., and Peña, L. 2008. *Citrus tristeza*
311 *virus*: a pathogen that changed the course of the citrus industry. *Mol. Plant Path.* 9:
312 251-68.

313 NASS, USDA. 2022. 2022 California Citrus Acreage Report.
314 https://www.nass.usda.gov/Statistics_by_State/California/Publications/Specialty_and
315 [Other_Releases/Citrus/index.php](https://www.nass.usda.gov/Statistics_by_State/California/Publications/Specialty_and/Other_Releases/Citrus/index.php).

- 316 O'Connell, N.V., Seymore, A.D., Chaffe-Stengel, P., and Stengel, D.N. 2010. Economic
317 impact of mild strains of Citrus tristeza virus in the San Joaquin Valley. Citrograph
318 1(6) Nov/Dec: 24-26.
- 319 Permar, T.A., Garnsey, S.M., Gumpf, D.J., and Lee, R.F. 1990. A monoclonal antibody that
320 discriminates strains of *Citrus tristeza virus*. Phytopathology 80:224-228.
- 321 Polek, M. 2010. Eradication of tristeza in the Central Valley of California, pp.219-232. In:
322 Karasev, A.V., and Hilf, M.E. (eds.) *Citrus tristeza virus* Complex and Tristeza
323 Diseases, APS Press, St. Paul, MN.
- 324 Qin, Y., Lui, Y., Zhao, J., Hajeri, S., Wang J., Ye, W., Zhou, Y. (2023). Molecular and
325 biological characterizations of a novel *Citrus tristeza virus* isolate that causes severe
326 symptoms in Ziyangxiangcheng junos. Arch. Virol. 168(2):59. [doi: 10.1007/s00705-
327 022-05644-y](https://doi.org/10.1007/s00705-022-05644-y).
- 328 Roistacher, C.N., Bar-Joseph, M, and Gumpf, D.J. 1984. Transmission of tristeza and
329 seedling yellows tristeza by small populations of *Aphis gossypii*. Plant Dis. 68: 494-
330 496.
- 331 Selvaraj, V., Maheshwari, Y., Hajeri, S., and Yokomi, R. 2019. A rapid detection tool for VT
332 isolates of *Citrus tristeza virus* by immunocapture-reverse transcriptase loop-
333 mediated isothermal amplification assay. PLoS ONE 14(9): e0222170.
334 <https://doi.org/10.1371/journal.pone.0222170>.
- 335 Vidalakis, G., da Graça, J. V., Dixon, W.N., Ferrin, D., Kesinger, M., Krueger, R. R., Lee,
336 R.F., Melzer, M.J., Olive, J., Polek, M., Sieburth, P. J., Williams, L, and Wright, G.C.
337 2010. Citrus quarantine, sanitary, and certification programs in the USA: Prevention
338 of introduction and distribution of citrus diseases. Part 2. Certification schemes and
339 national programs. Highlights of new California citrus nursery regulations. Citrograph
340 1:27-39.
- 341 Wallace, J.M. 1978. Chapter 2. Virus and virus-like disease, p 67-184. In. Reuther, W.,
342 Calavan, E.C., and Carmen, G.E. (eds.). The Citrus Industry, Vol. IV. University of
343 California, Div. Agric. Sci., Berkeley, California.
- 344 Wang, J., Bozan, O., Kwon, S. J., Dang, T., Rucker, T., Yokomi, R.K., Lee, R.F.,
345 Folimonova, S.Y., Krueger, R.R., Bash, J., Greer, G., Diaz, J., Serna, R., and
346 Vidalakis, G. 2013. Past and future of a century old *Citrus tristeza virus* collection: a
347 California citrus germplasm tale. Front. Microbiol.
- 348 Yokomi, R.K., and DeBorde, R. L. 2005. Incidence, transmissibility, and genotype analysis
349 of *Citrus tristeza virus* (CTV) from CTV eradicated and non-eradicated districts in
350 central California. Plant Dis. 89:859-866.
- 351 Yokomi, R. K., Polek, M. and Gumpf, D.J. 2010. Transmission and spread of Citrus tristeza
352 virus in Central California, pp. 151-165. Karasev, A.V., and Hilf, M.E. (eds.) Citrus
353 tristeza virus Complex and Tristeza Diseases, American Phytopathological Society
354 Press, St. Paul, MN.
- 355 Yokomi, R. K., Saponari, M., and Sieburth, P. J. 2010. Rapid differentiation and
356 identification of potential severe strains of *Citrus tristeza virus* by real-time reverse
357 transcription-polymerase chain reaction assays. Phytopathology 100:319-327.

- 358 Yokomi, R.K, Selvaraj, V., Maheshwari, Y., Saponari, M., Giampetruzzi, A., Chiumenti M.,
359 and Hajeri, S. 2017. Identification and characterization of *Citrus tristeza virus* isolates
360 Breaking Resistance in trifoliolate orange in California. *Phytopathology* 107:901-908.
- 361 Yokomi, R., Selvaraj, V., Maheshwari, Y., Chiumenti, M., Saponari, M., Giampetruzzi, A.,
362 Weng, Z., Xiong, Z. and Hajeri, S. 2018. Molecular and biological characterization of
363 a novel mild strain of citrus tristeza virus in California. *Arch. Virol.* 163(7):1795-
364 1804.
- 365 Yokomi, R., Sisterson, M., and Hajeri, S. 2020. Spread of citrus tristeza virus in abatement
366 and non-abatement citrus orchards in central California. *Plant Disease* 104:1925-
367 1931.