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Association of Tomato Chlorosis Virus Complicates the Management of Tomato Yellow Leaf Curl Virus in Cultivated Tomato (*Solanum lycopersicum*) in the Southern United States

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Abstract: Tomato (*Solanum lycopersicum* L.) production in the USA has been severely impacted by the tomato yellow leaf curl virus (TYLCV). Furthermore, a complex association of whitefly-transmitted TYLCV (genus, begomovirus) and tomato chlorosis virus (ToCV, genus, crinivirus) were recently identified in tomato. Several tomato cultivars were developed and commercialized with intermediate resistance (IR) against TYLCV-IL (Israel), the predominant strain of TYLCV found in Georgia, USA. TYLCV-resistant cultivars were tested in open field conditions against multiple whitefly-transmitted viruses in Georgia under natural disease pressure during the fall of 2022. The area under disease progress curve (AUDPC) over time showed a steady increase in disease severity among all cultivars. Further analysis of infected samples using high throughput sequencing (HTS) and quantitative PCR (qPCR) revealed the presence of TYLCV and ToCV in symptomatic upper and lower leaves, respectively. Moreover, the presence of both viruses in upper and lower leaves was determined. A mixed infection of both viruses, TYLCV and ToCV, resulted in severe disease development which may enhance the commercial tomato plants to break resistance and lead to decreased fruit quality and marketable yields.

Keywords: TYLCV; breaking resistance; whitefly-transmitted viruses; HTS; virus copy number

Citation: Kumar, M.; Kavalappara, S.R.; McAvoy, T.; Hutton, S.; Simmons, A.M.; Bag, S. Association of Tomato Chlorosis Virus Complicates the Management of Tomato Yellow Leaf Curl Virus in Cultivated Tomato (*Solanum lycopersicum*) in the Southern United States. *Horticulturae* **2023**, *9*, 948. https://doi.org/10.3390/horticulturae9080948

Academic Editors: Rafael José Carvalho Mendes, Leandro Pereira Dias, Renato Lopes Gil and Fernando Tavares

Received: 18 July 2023
Revised: 8 August 2023
Accepted: 15 August 2023
Published: 21 August 2023

1. Introduction

Tomato yellow leaf curl disease (TYLCD) caused by the tomato yellow leaf curl virus (TYLCV) and a group of related begomoviruses threatens tomato (*Solanum lycopersicum* L.) production worldwide. The most widespread begomovirus isolates associated with TYLCD are those of the type strain of the *Tomato yellow leaf curl virus* species, known as Israel (TYLCV-IL) [1]. It is responsible for reduced fruit production and substantial yield loss (up to 100%) in susceptible tomato cultivars worldwide [2,3]. Tomato plants infected with TYLCV show upward leaf curling, leaf yellowing, reduced leaf size, and stunted growth with decreased fruit yield (Figure 1) [3]. These symptoms are more prominent in the upper canopy of the plant. TYLCV has a circular, single-stranded DNA (ssDNA) genome and is 2.7−2.9 kb in size. The genome is composed of six-seven open reading frames (ORFs) which encode essential proteins required for virus replication, movement, suppression of RNA silencing, and transmission [4,5]. TYLCV is transmitted by whitefly (*Bemisia tabaci*) in a persistent and circulative manner [6,7]. TYLCV infects at least 16 families of plants including important food crops such as *Capsicum* (*Capsicum* spp.), bean (*Phaseolus vulgaris*), squash (*Cucurbita* spp.), tomato (*Solanum lycopersicum*), and several other non-cultivated host plants worldwide [8].

Figure 1. Symptoms observed on tomato plants infected with tomato yellow leaf curl disease caused by tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV) in Georgia, USA. Symptoms observed are (**A**,**B**) upward curling of leaves, yellow margin; (**C**) dwarfism; and (**D**) leaf curling on the upper foliage and interveinal chlorosis of lower leaves.

In the USA, the occurrence of TYLCV was first reported in 1997 in Florida infecting pepper (*Capsicum annuum*) and tomato [9] and shortly thereafter in Georgia [10,11]. TYLCV is commonly present in northern Florida and southern Georgia in the late summer and early fall season and causes a serious threat to commercial tomato production. TYLCV-IL is the only begomovirus associated with the tomato yellow leaf curl disease in the Southern USA [12]. Begomoviruses can be transmitted efficiently by a single viruliferous whitefly with a minimum acquisition accesses period (AAP) of 24 h [13]. In the past decade, several management strategies have been utilized to minimize losses due to TYLCV in tomato crops [14,15]. The introduction of resistance genes into a cultivated tomato species from several wild, tetraploid domesticated tomatoes (*S. lycopersicum*) has been the most successful approach to manage this disease [16]. Resistance to TYLCD was first found in several wild relatives of tomato, and six TYLCV resistance genes (*Ty-1*–*Ty-6*) have been identified [16]. Resistance against TYLCV has been incorporated into cultivated tomato and has been proven to be effective in managing this virus [17]. In this study, seven commercial tomato cultivars carrying different combinations of *Ty* genes were evaluated for resistance under natural disease incidence in South Georgia.

2. Materials and Methods

2.1. Plant Materials

In the fall of 2022, seven commercially available slicer tomato cultivars with different combinations of *Ty* genes were evaluated under natural incidence in Tift County (Horticulture farm, University of Georgia, Tifton, GA, USA) and a commercial field in Colquitt County, Georgia, USA (Table 1). These cultivars have intermediate resistance against TYLCV (https://www.vegetables.cornell.edu/pest-management/disease-factsheets/disease-resistant-vegetable-varieties/disease-resistant-tomato-varieties/) (Accessed on 1 July 2023). Tomato seedlings were grown in an insect-free greenhouse and were transplanted 30 days after germination. Ten plants of each variety were transplanted on the first week of August 2022 in four replications in Tift and Colquitt Counties. In Grady County, six cultivars were transplanted with a similar layout. The plots were randomized at each location and maintained as per standard Georgia tomato production guidelines [18].

Table 1. Mean and standard error (SE) of cumulative symptom severity measured as area under symptom progress curve (AUSPC) of tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV) symptomatic tomato varieties.

Sl. No.	Tomato Cultivar	Source Used ≠	*Ty*-Gene	AUSPC (Mean) €	Standard Error	Multiple Mean Comparison ©
1	Grand Marshall	Sakata Seeds, Morgan Hill, CA, USA	*Ty3* and *Ty6*	1593.75	242.03	a
2	STM 2255	Sakata Seeds, Morgan Hill, CA, USA	*Ty3* and *Ty6*	2018.75	184.67	a
3	Red Snapper	Sakata Seeds, Morgan Hill, CA, USA	*Ty3* and *Ty6*	1868.75	149.09	a
4	Camaro	Sakata Seeds, Morgan Hill, CA, USA	*Ty3* and *Ty6*	1850.00	200.78	a
5	Varsity	Syngenta Vegetable Seeds, Greensboro, NC, USA	*Ty1*	2231.25	187.47	a
6	Jolene	Bejo Seeds, Inc. Oceano, CA, USA	*Ty3* and *Ty6*	1700.00	195.52	a
7	Myrtle	Bayer Crop Science, Creve Coeur, MO, USA	none	2431.25	79.30	a
8	SkyWay 687 £	Enza Zaden, Enkhuizen, Netherlands	*Ty3* and *Ty6*	225.0	159.10	a
9	HM 8148 £	HM Clause, Halls, NY	*Ty3* and *Ty6*	112.50	64.95	a
10	Saybrook £	Bayer Crop Science, USA	none	ND *	ND *	a

© Mean comparisons of 10 individual plants with four replications (10 × 4 = 40 plants) in each cultivar were performed using the ANOVA statistical test followed by multiple mean comparisons with Tukey's (HSD) test using XLSTAT, 2023. € AUSPC is calculated using mean of 10 individual plants with four replications (10 × 4 = 40 plants), measured at 0, 15, 30, 45, and 60 days post transplantation. AUSPC was performed as described earlier by Simko et al. [19]. * Not determined. £ These varieties were only used in Grady County. ≠ *Solanum lycopersicum cv.*

2.2. Disease Observation

Research symptom severity was visually observed, and disease incidence was monitored every two weeks after transplantation. A disease severity scale ranging from 1 to 5 was utilized, where 1 = no symptoms, 2 = very mild yellowing, 3 = mild yellowing, downward/upward leaf curling, 4 = severe yellowing, leaf rolling and yellowing (in lower leaves), leaf chlorosis, and 5 = severe leaf curling and stunted growth. Symptom progression was recorded and area under the symptom progress curve (AUSPC) was calculated using the formula:

$$\text{AUSPC} = \sum_{i=1}^{n} \left(y_i + y_{i+1}/2\right)\left(t_{i+1} - t_i\right)$$

where, n = total number of observations from all seven cultivars with four replications of each, y_i = disease incidence score measured until the i^{th} observation, and t = number of days at the i^{th} observation (Table 1) [19].

2.3. Sample Collections

Symptomatic leaves were collected from the five plants of each cultivar from each replication at 45 days and 60 days post transplanting (DPT); the surface was washed with distilled water and stored in −80 °C until further analysis. A second leaflet from the top of the plant was collected to detect and quantify TYLCV, while lower leaves were collected to quantify tomato chlorosis virus (ToCV).

2.4. Virus Cloning and Sequencing

To ensure specificity of the amplicons generated by the primers (Table 2), the amplicons were gel-purified and cloned into pJET1.2 blunt ended vector (Thermo Scientific, Waltham, MA, USA). Recombinant clones for each virus were screened using restriction digestion and confirmed by Sanger sequencing (Azenta Life Sciences, Burlington, MA, USA). Because there is very little information about ToCV isolates from Georgia, symptomatic tomato samples collected from the University of Georgia Research Farm in Tifton in 2021 were subjected to high throughput sequencing (HTS). The small RNAs (sRNA) generated were mapped against the reference sequences of ToCV (NCBI Reference Sequence NC_007340 and NC_007341) and TYLCV (NCBI Reference Sequence NC_004005), and the consensus sequence of viruses were retrieved using CLC Genomics Workbench v23.0.2 (Qiagen, Redwood City, CA, USA) as described earlier [20].

Table 2. Primers used for the detection and quantification of tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV).

Primer Name	Used for	Sequence 5′–3′	T_m (°C)	Amplicon Size (bp)	Reference
ToCV-258F	qPCR	GTCTGTTCCGGCTGATTACAAGT	60	74	[21]
ToCV-331R	qPCR	AATTGAAACCCAAAGAGGAACAAA			
TYLC-C2-For	qPCR	GCAGTGATGAGTTCCCCTGT	65	102	[22]
TYLC-C2-Rev	qPCR	CCAATAAGGCGTAAGCGTGT			
ToCV-172 (F)	PCR	GCTTCCGAAACTCCGTCTTG	60	439	[21]
ToCV-610 (R)	PCR	TGTCGAAAGTACCGCCACC			
ToCV-RdRP	PCR	GCACCCTGATTGGTTCTAAAC	60	643	[23]
Solanaceae reverse primer	PCR	GTGTTBGAYAACCAWGTGTT			

2.5. Virus Quantification

Samples were homogenized in 4 M Guanidine thiocyanate buffer (pH 5.0) by mechanical disruption in Bead Mill 24 Homogenizer (Thermo Fisher Scientific, Waltham, MA, USA). Total nucleic acid (TNA) was extracted from leaf tissues by magnetic bead technology using the MagMAX 96 viral RNA kit in the KingFisher Flex Purification System (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions, excluding the

DNase treatment using a protocol described earlier. A detailed description of the protocol was published earlier [20]. For virus quantification, 100 ng of TNA was used to prepare complementary DNA (cDNA) using Superscript III (200 U/µL) reverse-transcriptase (RT) following the manufacturer's instructions. qPCR was performed using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) with gene specific primers (10 µM) in CFX96 Touch Deep Well Real-Time PCR detection system (Bio-Rad, Hercules, CA, USA). Five microliters of initial cDNA were used as a template for qPCR assay to quantify the presence of viruses [24,25]. The primer pair TYLC-C2-For and TYLC-C2-Rev were used for amplifying 102 bp corresponding to the TYLCV transcriptional activator (TrAP) protein gene (Table 2). The cycling conditions were 98 °C for 2 min, followed by 40 cycles of 15 sec at 98 °C, 20 sec at 65 °C, with an increment of 65–95 °C with an increment ramp of 0.5 °C per sec [22]. The primer pair of ToCV-258F and ToCV-331R that amplifies 74 bp of amplicon corresponding to the coat protein region was used to quantify ToCV. The cycling parameter for ToCV was 3 min at 98 °C, followed by 40 cycles of 10 s at 98 °C and 30 s at 60 °C, followed by the melt curve analysis with an increment of 65−95 °C with an increment ramp of 0.5 °C per sec. Melt curve analysis was performed at the end of each cycle to determine specificity of the qPCR assay using CFX Maestro software (Bio-Rad, Hercules, CA, USA).

The amplified TYLCV-C2 and ToCV-CP fragments were cloned in a pJET1.2 blunt ended vector (Thermo Scientific, Waltham, MA, USA) and used as standards to estimate virus copy number as previously described [12,26]. The initial DNA concentration of the plasmid was measured on Nanodrop (Thermo Scientific, Waltham, MA, USA) in ng/µL. The standard curve graph was prepared using Ct values obtained from eight ten-fold serial dilutions of cloned plasmid containing TYLCV-C2 and ToCV-CP fragments in triplicate. The cycle threshold (Ct) values from test samples were compared with the standards to estimate copies of TYLCV or ToCV present in the samples.

2.6. Statistical Analysis

In this study, statistical analyses were carried out using the GraphPad Prism tool (https://www.graphpad.com) (accessed on 1 December 2022) and the XLSTAT 2023 software (https://www.xlstat.com/en) (accessed on 27 February 2023). The effect of virus copy numbers at 45 and 60 days on lower and upper leaves on seven different varieties were analyzed using ANOVA followed by multiple mean comparisons with Tukey's (HSD) test using significant p values of 0 < ***< 0.001 < ** < 0.01 < * < 0.05 and a confidence interval of 95% [27] (Supplementary Materials Tables S1 and S2).

3. Results and Discussion

TYLCD is a serious threat to tomato production worldwide. Since the TYLCV was first discovered in Florida in the late 1990s, viral epidemics have posed a major threat to tomato production in south-eastern USA. [28,29]. During the last few decades, several tomato lines have been used to develop resistance against TYLCV through various breeding programs around the world [16]. Among the six known Ty resistance genes, all show dominant or incompletely dominant resistance except Ty-5, which is recessive in nature [30]. The Ty-1 gene contains an allele of a gene that encodes for RNA-dependent RNA polymerase (RDRP) [31] and increases the transcriptional silencing of the virus gene to confer resistance against TYLCV. It is the most commonly used gene for breeding resistance in tomato against this virus. In cultivation areas where multiple virus strains are endemic, resistance with a narrow spectrum conferred by a single gene is expected to be the least effective [32]. However, resistance is not absolute in the genotypes carrying resistant genes against TYLCV, and they typically display milder symptoms, suffer reduced yield losses, and accumulate lower levels of the virus compared to the susceptible genotypes [22].

Cultivars carrying resistance genes against TYLCV are commercially available in the USA. Seven such cultivars with different combinations of resistance genes were evaluated under field conditions in different locations in South Georgia during the fall

of 2022; this is when whiteflies and TYLCV incidences were the highest during each year. The different resistance genes present in the cultivars are summarized in Table 1. Symptom severity was measured until 60 DPT and the area under symptom progress curve (AUSPC) was calculated. In Grady County, the symptoms of TYLCD started after 30 DPT, whereas the symptoms started appearing after 45 DPT in Colquitt County (Supplementary Figures S1 and S2). The symptoms were more severe in Tift County as compared to Colquitt and Grady Counties. Due to the lower disease incidence, cultivars evaluated in Grady County expressed less severe symptoms. However, in Tift County after 45 DPT, all seven cultivars evaluated had greater than a 50% incidence of TYLCD, and by 60 DPT, there was 100% TYLCD incidence (Figure 2). The high incidence of the disease in these genotypes was not unexpected because they are not immune and get systemically infected. However, all test lines carrying resistance genes showed severe symptoms typical of those produced on susceptible cultivars, contrary to the milder symptoms expected. At the Tift County site, the susceptible cultivar Myrtle had the largest AUSPC among the cultivar tested (mean = 2431.25); however, other cultivars also had very high AUSPC with Varsity (mean = 2231.25) and STM 2255 (mean = 2018.75) ranking behind Myrtle at 45 DPT (Table 1).

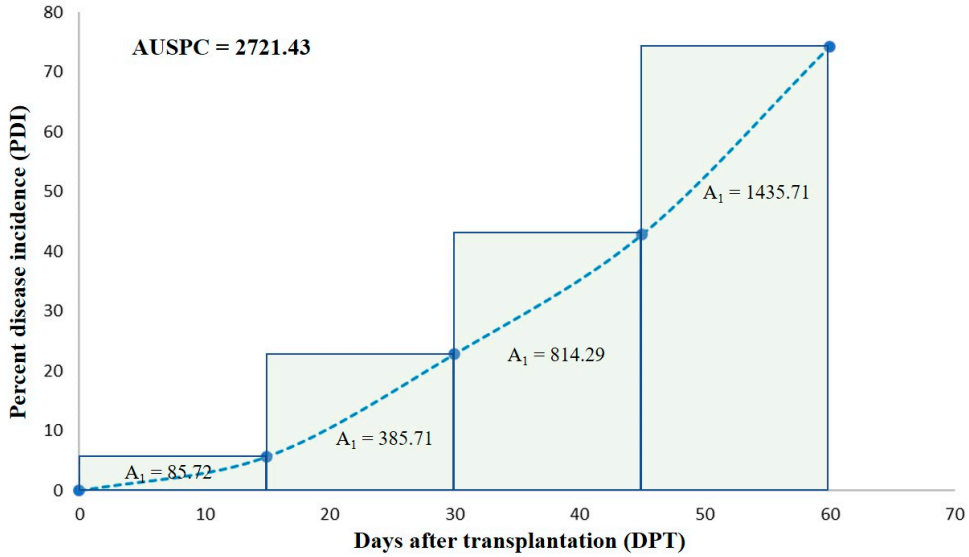

Figure 2. Graphical representation of area under the symptom progress curve (AUSPC) over time: Ten plants for each cultivar were transplanted in four replications at Tift County. The data were collected at every 15 days of time intervals. A rating was given from a 0-to-5-point scale, based on symptoms progression. The blue color dashed line indicates the symptom progress curve. A blue rectangular box is used to calculate AUSPC based on the disease severity recorder on each plot at 0, 15, 30, 45 and 60 days of intervals. The *y*-axis represents the percent disease incidence (PDI) whereas the x-axis shows days after transplantation.

Even though the cultivars tested had lower AUSPC compared to the susceptible cultivar Myrtle, there was clearly no significant difference observed between the AUSPC among them (Table 1). However, in Grady County, disease pressure was very low; therefore, AUSPC for the cultivar Saybrook was not determined. The other two remaining cultivars, SkyWay687 (mean = 225.0) and HM8148 (mean = 112.50), displayed the least symptoms appearance under the natural conditions (Table 1).

The titer of TYLCV was estimated in infected tomato leaves from all cultivars at 45 and 60 DPT. The susceptible cultivar Myrtle supported significantly higher TYLCV counts compared to the test cultivars at 60 DPT in all infected plant samples (Figure 3A). In the susceptible cultivar Myrtle, the virus copy number on the upper leaves was 2.13×10^5, which is relatively higher than that of other tomato cultivars such as Grand Marshall (4.34×10^4), STM2255 (4.09×10^4), Red Snapper (9.27×10^4), Camaro (6.11×10^4), Varsity (2.36×10^4),

and Jolene (6.61×10^4) at 60 DPT. Additionally, a significant increase in viral count has been observed in Myrtle (Pr > Diff = 0.036, p value = 0.001–0.01) and STM2255 (Pr > Diff = 0.021, p value = 0.001–0.01) over time when comparing 45 vs. 60 DPT (Figure 3A). Therefore, a significantly lower level of TYLCV in the test cultivars compared to those in Myrtle indicates that they have some degree of resistance to TYLCV. In contrast, as described in earlier sections, there was no significant difference in either symptom severity or AUSPC (Table 1) between Myrtle and the test cultivars. This indicated that there is some contributing factor to the symptoms observed other than TYLCV.

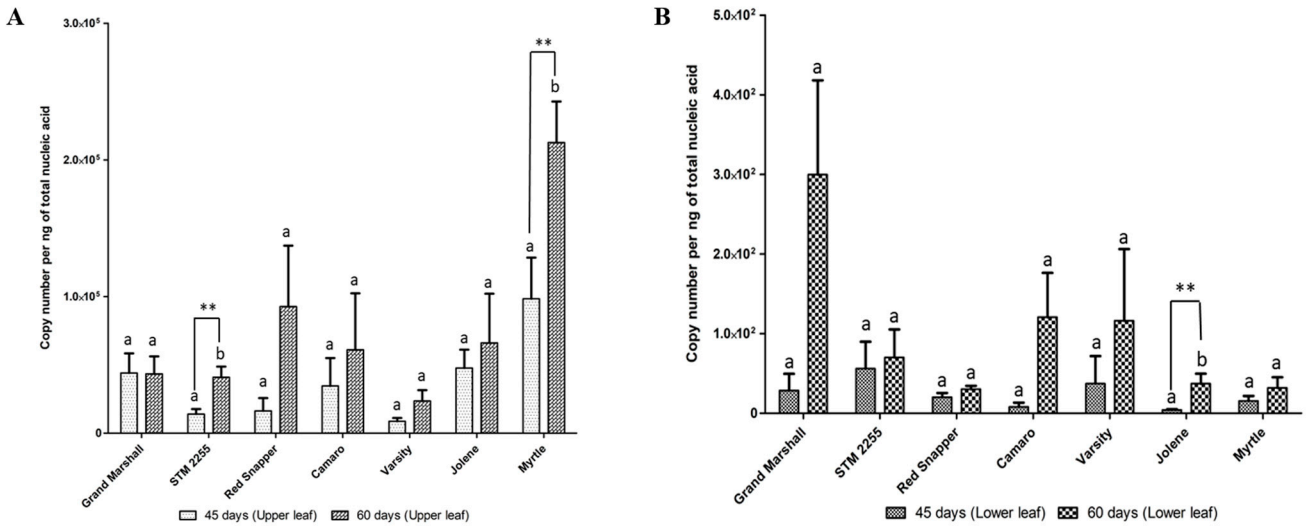

Figure 3. Quantification (qPCR) of virus copy number in symptomatic tomato plants with tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV) at 45 and 60 DPT. TYLCV copy numbers were quantified in the latter from the top symptomatic tissues (**A**), while for ToCV (**B**) it was in the lower foliage from pooled samples from at least five plants. The graphs represent an average of four replicates from each cultivar. The *y*-axis represents virus copy number per ng of total nucleic acid; the *x*-axis shows tomato cultivars used in this study. Statistical significance was calculated using ANOVA followed by multiple mean comparisons with Tukey's (HSD) test/analysis of differences between the categories with a confidence interval of 95% using XLSTAT, 2023. ** indicates *p* value 0.001–0.01. Significant differences are indicated using different lower-case letters.

Different strains that are able to break down the resistance offered by some of these genes have been reported. For example, an isolate of the mild strain of TYLCV is able to overcome Ty-2-mediated resistance [33]. However, a comprehensive study conducted recently has concluded that the predominant isolate present in the Southern USA (Florida and Georgia) is TYLCV-IL and no other TYLCV isolates infecting susceptible/resistant tomato varieties were identified [12]. Coinfection of TYLCV with betasatellite has also been shown to subvert Ty-1-mediated responses and showed resistance break in tomato [34]. In addition, total sRNAs sequenced from a TYLCD sample collected in 2021 were also searched for the presence of betasatellite in silico but betasatellite could not be detected. We also probed the sRNA sequences for the presence of other viruses. Apart from TYLCV, we also detected ToCV (Figure 4). This virus belongs to the genus Crinivirus; family, Closteroviridae and has a bipartite, positive-sense, and single-stranded (ssRNA) genome [35]. Typical symptoms of ToCV include interveinal yellowing of leaves, leaf thickening, leaf rolling, and infected leaves that are brittle and crispy on the lower canopy of the plant. Often, these symptoms are misdiagnosed as abiotic stress including nutritional deficiencies or heat stress [36]. ToCV is transmitted by a number of whitefly species including B. tabaci, Middle East–Asia Minor 1 (MEAM1; formerly biotype B), Mediterranean (MED; formerly biotype Q), and New World (formerly biotype A) [35], which also transmit TYLCV. In Georgia, ToCV was first reported in field-grown tomato in research trials conducted in 2009 and

2010 [37]. However, there had been no further reports of the incidence or spread of ToCV in the region.

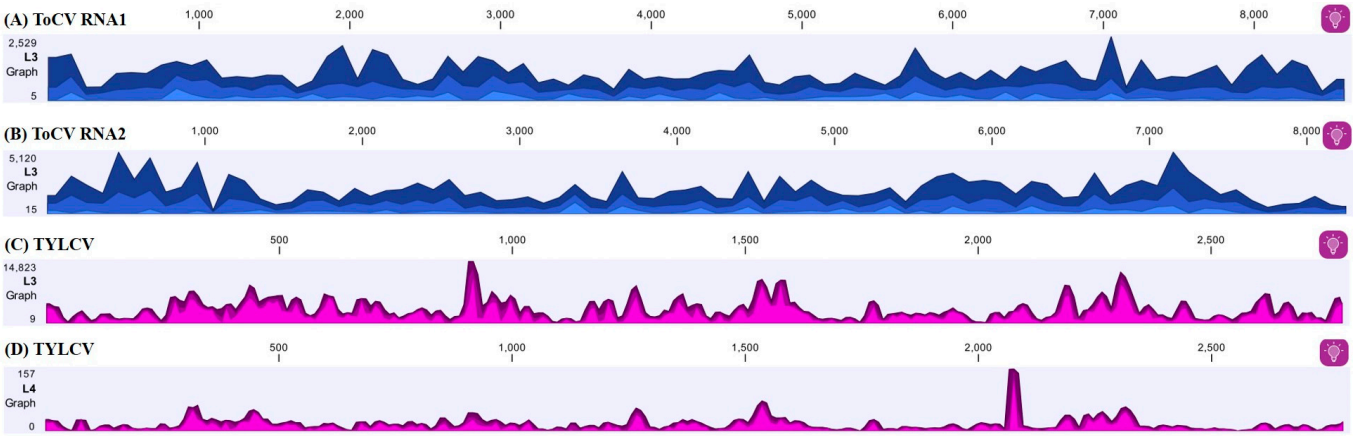

Figure 4. Mixed infection of ToCV with TYLCV in tomato collected from the field in Georgia: Read coverage map of tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV) detected by HTS. The data were analyzed using CLC Genomics workbench v23.0.2. The colored heatmap shows the coverage with number of reads. Read coverage map of TYLCV (**A**,**B**) and ToCV (**C**,**D**) virus genome detected by HTS of small RNAs of symptomatic plants. The *y*-axis represents the number of reads matched with reference genomes (GenBank Accession number NC_004005 for TYLCV. Similarly, NC_007340 for RNA1 and NC_007341 for RNA2 of ToCV).

We noticed that the plants also displayed prominent interveinal chlorosis on the lower leaves which is similar to the symptoms caused by criniviruses. Lower (older) leaves showed abnormal yellowing, similar to crinivirus ToCV-like symptoms. In addition, the upper (younger) leaves had more prominent TYLCV-like symptoms such as upward leaf curling, leaf yellowing with chlorosis, reduced leaf size, and stunted plant growth. These samples were analyzed for the presence of TYLCV (Figure 3A) and ToCV (Figure 3B) using qPCR assays.

ToCV was mixed infected with TYLCV and detected in all the tomato cultivars at 60 DPT. The presence of ToCV at significant levels in the symptomatic plants mix infected with TYLCV might boost the breakdown of TYLCV-mediated resistance/tolerance in tomato plants. We also observed that the symptomatic tomato cultivars such as STM2255, Red Snapper, Camaro, and Jolene had a decreased TYLCV virus copy number after 45 DPT (Supplementary Materials Figure S3A). Interestingly, the lower leaves of symptomatic tomato varieties show differential response against ToCV under natural infection. ToCV virus copy number was higher in the lower symptomatic tissues among the cultivars (Figure 3B). In lower leaves, Grand Marshall had the highest (3.00×10^2) virus copy number in comparison to STM2255 (7.01×10^1), Red Snapper (3.04×10^1), Camaro (1.21×10^2), Varsity (1.16×10^2), and Jolene (3.72×10^1). However, only Jolene had a significant increase in viral count (Pr > Diff = 0.040, p value = 0.001−0.01) at 60 DPT as compared to 45 DPT (Supplementary Materials Figure S3B).

Detection of ToCV on all samples tested and in three different counties supports that ToCV is now widely prevalent in Georgia (Figure 3 and Supplementary Materials Figure S4). Co-infection of ToCV and TYLCV in tomato has been recently reported in China [38] and Spain [39] as well. During co-infection of TYLCV and ToCV, there is an increase in cathepsin B and promotion of ToCV transmission by Bemisia tabaci MED [40], which might explain its widespread prevalence. In addition, tomato plants co-infected with TYLCV and ToCV showed more severe symptoms at late stages compared to single infections [39]. Mixed infection resulted in increased accumulation of both the viruses in tomato plants and induced severe symptoms, resulting in decreased plant height and weight [38]. Therefore,

mixed infection in tomato cultivars could contribute to a long-term breakdown of resistance in Ty-gene-harboring plants.

It is known that the participation of ToCV during mixed infection with tomato spotted wilt orthotospovirus (TSWV) can compromise the TSWV resistance in tomato [41]. Resistance conferred by Ty-1 is also compromised by co-infection with cucumber mosaic virus infection [42]. A recent report suggests that mixed infections with an isolate of tomato chlorosis virus (ToCV), as an ssRNA virus also transmitted by B. tabaci emerging worldwide in tomato crops, enhance the breakdown of the TYLCD-tolerance provided by the Ty-1 gene either with TYLCV-IL IS76-like or canonical TYLCV-IL isolates [43]. It was observed that symptom severity had increased in Ty-1 tomato plants after virus infection. This suggests that tomato plants infected with either canonical or non-canonical (IS-76-like) TYLCV-IL along with ToCV advances the breakdown of resistance in plants. In our study, similar results were observed in natural field conditions. We have observed very high viral load and symptoms in tomato varieties harbouring Ty-1 or Ty-3 and Ty-6 genes. We anticipate a similar condition might be favoring the ToCV and TYLCV to overcome the resistance in these tomato plants. Further investigation is needed to determine the role of ToCV in TYLCV-mediated resistance breakdown and the potential chance of the emergence of a new viral strain in intermediate or resistance tomato varieties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9080948/s1, Figure S1: Area under the disease progress curve (AUDPC) over time at Grady County; Figure S2: Area under the disease progress curve (AUDPC) over time at Colquitt County; Figure S3: Quantification (qPCR) of virus copy number in symptomatic tomato plants with tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV) at 45 and 60 DPT; Figure S4: Quantification (qPCR) of virus copy number in symptomatic tomato plants with tomato yellow leaf curl virus (TYLCV) and tomato chlorosis virus (ToCV) at Grady and Colquitt Counties at 45 and 60 DPT; Table S1: Statistics analysis for TYLCV (45 days vs. 60 days) performed by XLSTAT using ANOVA and Tukey's (HSD) test with 95% of confidence interval; Table S2: Statistics analysis for ToCV (45 days vs. 60 days) performed by XLSTAT using ANOVA and Tukey's (HSD) test with 95% of confidence interval.

Author Contributions: Conceptualization, M.K., T.M. and S.B.; methodology, M.K., T.M. and S.B.; formal analysis, M.K. and S.R.K.; investigation, M.K. and S.B.; resources, M.K., T.M. and S.B.; data curation, M.K., T.M., S.H. and S.B.; writing-original draft preparation, M.K.; writing-review and editing, M.K., S.R.K., T.M., S.H., A.M.S. and S.B.; supervision, S.B. and T.M.; project administration, S.B.; funding acquisition, S.B. and A.M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This study was partially supported by the USDA-HATCH grant awarded to S.B., number 1020319, Georgia Fruit and Vegetable Growers Association, and the USDA, ARS-UGA Cooperative Agreement, project number 58-6080-9-006. The funders had no role in the study design, data collection and analysis, the decision to publish or the manuscript preparation.

Data Availability Statement: Not applicable.

Acknowledgments: The authors acknowledge the UGA-ANR County extension coordinator Ty Nicholas Torrance (Colquitt County) and Cale Cloud (Grady County) for their support for field experiments. The authors also appreciate anonymous reviewers for their constructive criticism and suggestions on the manuscript. The mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA or University of Georgia.

Conflicts of Interest: The authors declare no conflict of interest.

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