Project Report: Virus surveillance in peppers for resistance-breaking (RB) strains of tomato spotted wilt virus (TSWV) and assessment of the susceptibility of peppers to emerging tomato-infecting tobamoviruses

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Overall objective:

To monitor, characterize and assess the potential threat of pepper resistance-breaking (RB) TSWV strains and other emergent viruses to pepper production in California

Specific Objectives:

1. Virus surveillance in peppers in 2023 with emphasis on RB TSWV strains.

We are fortunatey to be collaborating with Matt and Kenlee of to conduct survey of pepper fields in key growing area of California for plants with symptoms of virus infection. In addition, we have our excellent Farm Advisor PCA Network looking for symptoms of virus disease in vegetable and other crops. In this way, we feel that we have a 'finger on the pulse of the virus disease situation in peppers in California in a given year. Unfortunately, given the increasingly long-distance spread and emergence of new or previously unimpotant viruses, this surveillence needs to be done on annually.

Low incidence of virus disease in pepper crops in California in 2023. In 2023, the incidence of virus diseases of pepper was very low. This was likley associated with the cool wet weather conditions in 2023 that delayed planting and as well as appearance of insect vectors of plant viruses, especially beet leafhoppers and thrips. Thus, in the North beet leafhoppers were absent as was curly top disease an the pressure also was relatively low in Fresno and Kern counties. Thus, we received no pepper plants with curly top symptoms in 2023. With the 2024 season also shaping up as cool and wet, we might predict low curly top pressure again.

Spotted wilt was common in plants of resistant processing tomato varieties but not pepper fields of resistant varieties. Thrips populations were delayed and began to appear in late May/early June in the North and spotted wilt symptoms began to appear in resistant processing tomato varieties and these were confirmed as RB tomato strains. However, we did not hear of spotted wilt in plants of resistant pepper varieties and only received 2 spotted wilt samples of a resistant variety from a commercial field in 2023 (samples of cv. Josh found by Matt). Thus, disease pressure by RB tomato TSWV was much higher than disease pressure of RB pepper TSWV strains in 2023. This is typical of what we see in California.

Emergence of the new super RB (SRB) TSWV isolates in California. Typically, RB tomato TSWV strains do not overcome the *Tsw* gene in pepper and RB pepper strains do not overcome the *Sw-5b* gene in tomato (Table 1). In 2019, we identified RB TSWV isolates that overcome both resistance genes and these were referred to as super RB strains (SRB, Table 1). Thus, TSWV isolates recovered from resistant pepper or tomato varieties must undergo multiple tests, including a pathogenicity test on a known resistant variety for RB pepper TSWV, and confirmation of the presence of the resistance gene in the plant sample (Table 1).

Table 1. Major resistance-breaking (RB) tomato spotted wilt strains in California and their infection of differential species/varieties

	Pepper	Pepper	Tomato	Tomato	Tobacco
TSWV strain	**Susceptible**	**Resistant *Tsw* gene**	**Susceptible**	**Resistant *Sw-5* gene**	***N. benthamiana***
Wild-type	+	-	+	-	+
RB pepper	+	+	+	-	+
RB tomato	+	-	+	+	+
Super RB	+	+	+	+	+

Another SRB hotspot in California: Woodland in 2023. In 2022, we identified a hotspot of RB pepper TSWV in Gilroy, CA and many of the isolates were the SRB TSWV strain. In 2023, an experimental trial to assess breeding materials for resistance to naturally occurring RB pepper TSWV was established in a commercial field in Woodland, CA (Yolo County) identified by our collaborator Matt. We conducted two field surveys of this trial, which included numerous resistant materials, including the known resistant varieties Josh and Huntington. We observed spotted wilt symptoms on numerous plants of Huntington and Josh growing in this plot, which indicated infection with RB pepper TSWV. Samples were collected from plants of resistant varieties in this field plot and were received from three other fields in Yolo County for a total of 30 potential RB pepper TSWV isolates tested. The results of these tests are presented in Table 2.

Table 2. Isolates, test results and properties of TSWV isolates from pepper received/collected in 2023.

Location	Number of Samples	Resistant from field	Analyses carried out in la Lab				
			Tsw gene	TSWV-immuno strip	RT-PCR for tomato TSWV-RB	RT-PCR for NSs gene	Reaction on pepper cv. Huntington
Yolo	30	24 (R)	19(+), 1(NT)	5(+), 1(-), 13(NT)	12(+), 7(-), 1(NT)	17(+), 2(-), 1(NT)	14 (RB), 1(WT), 2 (NT), 3*,
			4(-)	4(NT)	4(+)	4(+)	1(RB), 1(WT), 1*, 1**
		6 (S)	6(-)	6(NT)	5(+), 1(-)	6(+)	3(RB), 2(WT), 1*

NT: Not tested; R: Resistant; S: Susceptible; *Isolates that did not infect *Nicotiana benthamiana* and ** Isolates that did not infect pepper cv. Huntington nor cv. California Wonder.

Of the 30 samples, six were from susceptible varieties that tested negative for the *Tsw* gene and 5 were RB tomato (positive in RT-PCR test) and three also infected and caused symptoms in the resistant cv. Huntington (Table 2). These results are consistent with the predominance of RB tomato strains in Yolo County and (ii) indicate that three isolates from susceptible peppers were SRB and (iii) that susceptible pepper cultivars can harbor RB tomato and pepper isolates.

The remaining 24 isolates were received/collected from plants of resistant varieties in the fields in Yolo County. Four samples tested negative for the *Tsw* gene, indicating these were not resistant plants (possibly did not received the gene or were a contaminant). For the other 20 samples one could not be tested and 19 were postive for the *Tsw* gene and the virus was recovered from these (Table 2). Of these 19 isolates, 12 were RB tomato (based on results of RT-PCR tests). Fifteen of these isolates were inoculated onto susceptible and resistant pepper plants and 14 were RB pepper (infected and caused symptoms in susceptible and resistant pepper varieties, Table 2). These results (i) confirmed 14/24 samples of resistant peppers with spotted wilt were infected with RB pepper TSWV; (ii) that most of these (12) alsdo were RB tomato positive, meaning these are SRB isolates; and (iii) that some isolates did not infect *N. benthamiana* (5 isolates), which is used to increase the virus before inoculating pepper and in one case infected *N. benthamiana* but not neither variety of pepper. Thus, these results reveal the genetic diversity and flexibility of this virus, and the challenges of using the biological indicator for identification of RB pepper isolates.

Thus, the RB pepper TSWV outbreaks in fields in Wooldland in 2023 were mostly caused by SRB isolates, revealing another hotspot of this strain, which breaks both the *Sw-5b* and *Tsw* resistance genes. Previous hotspots were identified in Gilroy (2022) and in Merced (2021). This indicates the continued spread of the SRB strain, which has likley been facilitated by the capacity to infect and increase in the widely grown resistant tomato plants. It will be important to determine if SRB strains become predominant and to continue to search for sources of resistance to RB pepper isolates/strains..

2. Evaluate the response of peppers to the emerging tomato-infecting tobamoviruses ToBRFV and ToMMV.

The emerging tobamoviruses tomato mottle mosaic virus (ToMMV) and possibly tomato brown rugose fruit virus (ToBRFV) are established in Southern California. These viruses have been reported to infect pepper and, thus, could be a problem for pepper production. However, the information available in the literature regarding the interaction of these viruses and pepper is incomplete and we are conducting these experiments to gain a better understanding of the potential threat these viruses pose to pepper production.

In 2023, we focused on obtaining pepper germplasm without any L gene for tobamovirus resistance as well as germplasm having each of the L_1-L_4 genes. We have made good progress on obtaining these genotypes and have conducted mechanical transmission experiments of these genotypes with ToBRFV. The results of these experiments are presented in Table 3. First, we have been able to achieve high rates of

infection of pepper with ToBRFV via our mechanical inoculation method. Second, ToSRV induced severe symptoms in the L_0 genotype, showing that the virus can systemically infect pepper and cause severe symptoms. Third, most genotypes with one of the L genes (known or suspected) responded with a hypersensitive reaction in the inoculated leaves and leaf drop, thereby conferring strong resistance to the plant. In the case of Scotch Bonnet, which should possess the L_3 gene, the failure to observe the resistance response (plants become systemically infected and developed severe symptoms) may indicate that the gene is not present. We are addressing this by using markers for the L genes and obtaining other sources of Scotch Bonnet seeds.

Thus, our results so far indicate that ToBRFV can infect and cause symptoms in pepper, but that the widely deployed L genes for resistance to tobamoviruses confers a strong HR-associated resistance to ToBRFV infection. This would also suggest that ToBRFV does not pose a threat to pepper production in California, unless genotypes without L genes are grown.

Table 3. Preliminary results of mechanical inoculations on pepper genotypes with the tomato brown fruit rugose virus (ToBRFV)

Gene	**Specie/Variety**	**# number of symptomatic plants/# of inoculated plants**	**Symptomatology**
L0	corno di toro	7/7*	severe systemic necrosis
L2-b	*Capsicum baccatum*	9/9	hypersensitivity reaction (local necrotic lesions) and leaf drop
L2	Tobasco	7/7	hypersensity reaction (local necrotic lesions) and leaf drop
L3	Scotch Bonnet	5/5	severe stunting and systemic necrosis
L?	Huntington	12/12	hypersensity reaction (local necrotic lesions) and leaf drop
L?	Sweet Cal Wonder	6/6	hypersensity reaction (local necrotic lesions) and leaf drop

* Some of these plants showed recovery phenotype after 10-12 d post inoculation

Key takeaways from 2023 research:

1. There was a very low incidence of pepper virus diseases in 2023, which may have been partly due to the cool wet winter and early spring weather negatively impacting insect vectors (beet leafhoppers and thrips)

2. No curly top samples in 2023

3. A hotspot of RB pepper TSWV was identified in Woodland (Yolo County) in 2023 and most of the isolates were identified as the new super RB (SRB) that overcomes the Tsw gene in pepper and the Sw-5 gene in tomato

4. This is the third such hotspot: Merced, Monterey (Gilroy) and Yolo counties

5. ToBRFV infects and causes severe symptoms in the Lo pepper genotype, but the L genes for tobamovirus resistance in pepper are providing strong resistance of ToBRFV in most genotypes