Project Report: Continued surveillance and characterization of pepper resistance-breaking strains of *Tomato spotted wilt virus* (TSWV) in the Central Valley of California and screening for sources of resistance

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Background

In general, spotted wilt disease of pepper caused by *Tomato spotted wilt virus* (TSWV) can cause substantial economic losses to pepper production worldwide, including California. In nature, this virus is transmitted by various species of thrips, but mostly the western flower thrips, (*Frankliniella occidentalis*) in California. Although thrips feeding can cause direct damage to pepper, transmission of TSWV and other tospoviruses to pepper results in the greatest economic loss.

A very effective and environmentally friendly means of managing tomato spotted wilt in pepper (and tomato) is through the planting of resistant varieties. In the case of pepper, these are varieties with the dominant *Tsw* gene. Studies have revealed that the nonstructural NSs protein, encoded by the small (S) RNA segment, is the TSWV effector that is recognized and triggers the resistant response in peppers with the *Tsw* gene. However, **resistance-breaking (RB) strains** of TSWV that overcome the resistance conferred by the *Tsw* gene in pepper have been reported from a number of geographical locations, including Europe (Hungary, Italy and Spain), the Middle East (Israel), Australia, South America (Argentina) and South Korea. This has occurred in the Central Valley of California, where a **tomato RB TSWV strain** able to overcome the *Sw-5* resistance gene appeared in 2016. This strain is now prevalent in Fresno, Kings and Merced Counties.

With the increasing cultivation of Tsw varieties in California, it was not totally unexpected when **we identified pepper RB TSWV in Yolo County in 2017.** This virus

isolate (RB-TSWV-CA-P-1) came from mature red fruits of a Tsw variety that were showing ringspots and blotches, typical of spotted wilt. Following mechanical inoculation, this caused severe spotted wilt symptoms in plants of cv. Huntington (the standard test for pepper RB TSWV). Moreover, **the continued detection of pepper RB TSWV strains in multiple locations in California (Fresno, Merced, San Joaquin, Santa Clara and Yolo Counties) in 2018 and 2019 indicates that pepper RB TSWV has been persisting** in between growing seasons. Thus far, these pepper RB strains seem to **appear late in the season, but can cause economic loss in mature red peppers**.

Pepper RB TSWV strains are recognized when 'typical' spotted wilt symptoms appear on a known resistant variety (e.g., cv. Huntington and Badger). To validate a pepper RB isolate, **it is necessary to confirm the presence of the *Tsw* gene in the pepper sample** with the spotted wilt symptoms, and to **show the isolate causes spotted wilt symptoms in a known resistant variety following mechanical inoculation.**

Here, it is important to point out that the Califoria **tomato RB-TSWV strain** that appeared in 2016 and breaks the *Sw-5* gene of tomato, infects and causes spotted wilt in pepper, but does not break the *Tsw* gene (i.e., does not infected cv. Huntington). Conversely, the **pepper RB TSWV isolated** from Tsw pepper fruit in 2017 (e.g., RB-TSWV-CA-P-1) does infect tomato plants, but does not break the *Sw-5* gene.

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	+	-	+	-	+
Pepper RB	+	+	+	-	+
Tomato RB	+	-	+	+	+
Super RB	+	+	+	+	+

However, there are notable difference between the emergence of pepper RB-TSWV and tomato RB-TSWV strains in Central California. **First**, the tomato RB-TSWV strain has become the predominant strain infecting processing tomatoes in Central California, whereas pepper RB-TSWV typically appears late in the season and to date it has not emerged as the predominant strain of TSWV infecting peppers in California. **Second**, resistance to TSWV in pepper and tomato is conferred by two different resistance genes: the *Tsw* gene in pepper and the *Sw-5* gene in tomato. **Third,** the viral gene product or **effector** that is recognized by these TSWV resistance genes is different: **the *Tsw* gene of pepper recognizes the NSs protein**, a viral suppressor of gene silencing, and ***Sw-5* gene recognizes the NSm protein**, which is the viral movement protein. Finally, in the case of the NSm protein of the tomato RB-TSWV, the tyrosine (Y) amino acid residue at

position 118 is a marker for all tomato RB-TSWV strains, whereas no such diagnostic amino acid marker has been identified in the NSs of the pepper RB-TSWV strains.

In 2019 we identified a **super RB (SRB) strain** that breaks the resistance of the *Tsw* gene in pepper and the *Sw-5* gene in tomato (Table 1). This strains has been detected in Fresno and Yolo Counties If this strain is able to become more prevalent in processing tomato, it could emerge as a more important problem.

Thus, pepper RB-TSWV strains have emerged and become established in Central California. Though **not as economically important as the tomato RB strains,** there are **multiple factors that may favor emergence of pepper-infecting super RB strains.** Therefore, it is important to assess the potential for these pepper RB TSWV strains to become a greater economic problem and to identify sources of resistance to RB strains.

Because of the limited funding available in 2021, we focused on virus diagnostics with pepper samples received in 2021 and identifying potential outbreaks of pepper RB strains, especially the super RB strain.

Overall Objectives:

The overall objectives of this project are to 1) identification and management of viruses that pose a threat to pepper production in California and 2) emphasize the emergene of resistance-breaking strains of TSWV.

Specific Objectives:

1. Virus diagnostics in pepper in 2021

Curly top. Pepper plants infected with beet curly top virus (BCTV) are stunted, leaves are upcurled and light green-yellow and fruits are small and premature. Samples with curly top-like symptoms were received from Fresno and Woodland, CA and a location in Nevada (Table 2). All of these samples were positive for BCTV infection with the multiplex PCR test, and there was an equal mixture of mild and severe strains. The incidence of curly top in these fields was low and did not cause economic loss. It is notable that the leaves of the Fresno samples showed pale yellow blotches, which is not typical and may be due to mixed infection with alfalfa mosaic virus.

Table 2. Results for samples with curly top-like symptoms received in 2021

Sample	Location	Multiplex PCR	Severe/Mild
Go-1 N-31 (21-564)	Nevada	++	Mild
Go-1 N-1-1 (21-566)	Nevada	+++	Mild
Go-2 (21-598)	Woodland	++	Severe
Fr1-1 (21-516)	Fresno	+++	Severe
Fr1-2 (21-517)	Fresno	+++	Severe
Fr1-3 (21-518)	Fresno	++	Mild

Spotted wilt. In 2021, we received samples with spotted wilt symptoms from **four locations:** Gilroy, Oregon, Woodland and Merced and **these included susceptible (Sus) and resistant (Res) varieties.** All of these had spotted wilt symptoms of the leaves or fruits and were all postive **with the immunostrip test** indicating infection with TSWV. The samples from Woodland and Oregon (homeowner) were from susceptible varieties and are considered to be non-resistance breaking.

Table 3. Results for samples with tomato spotted wilt symptoms received in 2021

Sample	Location	Res/Susc	Immunostrip	Tsw gene	RT-PCR Tomato RB	RT-PCR NSs
TS 21-597	Woodland	Sus	+	NT	NT	+
TS 21-599	Woodland	Sus	+	NT	NT	+
TS 21-600	Woodland	Sus	+	NT	NT	+
TS 21-601	Woodland	Sus	+	NT	NT	+
TS 21-602	Gilroy	Res	+	-	NT	+
TS 21-603	Gilroy	Res	+	-	NT	+
TS 21-614	Oregon	Sus	+	NT	NT	+
TS 21-619	Merced	Res-H	+	+	+	+
TS 21-620	Merced	Res-H	+	+	+	+
TS 21-621-L	Merced	Res-H	+	+	+	+
TS 21-623	Merced	Res-H	+	+	+	+
TS 21-624	Merced	Res-S	+	+	+	+
TS 21-625	Merced	Res-S	+	+	+	+
TS 21-626	Merced	Res-S	+	+	+	+
TS 21-621-F	Merced	Res-H	+	+	+	+
TS 21-622-F	Merced	Res-H	+	+	+	+

For the samples from resistant varities, we obtained different results. For the isolates from blotchy symptomatic fruits taken from two Huntington plants, the *Tsw* gene was not detected in these plants. Furthermore, when these isolates were mechanically inoculated onto susceptible (Cal Wonder) and resistant (Huntington) pepper plants, only

the plants of the susceptible variety developed tomato spotted wilt symptoms. Thus, we concluded that these plants likely failed to receive the *Tsw* gene and that local non-RB wild-type strains were able infect these plants.

Finally, we received sampled from a number of **late-planted fields of the resistant varieties Huntington and Sycamore,** both of which have the *Tsw* gene. These plants were at the green fruit stage and samples included shoots and fruits. These plants showed symptoms of **necrostic spots and mosaic of leaves, tip die-back of shoots and fruits with blotches and striking necrotic lesions** (Fig. 1). The necrotic symptoms of the fruits and shoots was more severe for cv. Sycamore, whereas cv. Huntington developed mosaic symtoms in addition to necrosis.

Fig. 1. Fruit symptoms in cv. Sycamore infected with SB-TSWV

All these samples were **postive with the TSWV immunostrip test** and negative with the potyvirus, TMV and CMV immunostrips, indicating TSWV infection. Most of the plants (leaves or fruits) **tested postive for the *Tsw* gene** (Table 3). The NSs fragment was amplified by RT-PCR from 9 TSWV isolates associated with this outbreak. To **confirm these were really pepper RB isolates**, three isolates (21-622F, 624 and 625) were mechanically inoculated onto plants of cvs. Cal Wonder and Huntington, and **plants of both cultivars developed symptoms, indicating that these are pepper RB isolates.**

To test if these were SRB TSWV isolates, we performed the RT-PCR test for tomato RB TSWV and **all of the 9 isolates tested positive**. Together, these results indicated that this late-season spotted wilt outbreak in these fields of resistant peppers was caused by the SB TSWV strain. This is particularly concerning because, if this strain becomes established, it could build-up in the widely plants Sw-5 processing tomato varieties, thereby increasing spotted wilt pressure on resistant varieties.

2. Determine the genetic diversity of pepper RB TSWV strains

The TSWV 'effector' (the protein of the virus recognized by the product of the resistance gene that triggers resistance) for the *Tsw* gene is the **NSs protein**, which is an anti-plant defense factor that suppresses gene silencing. In our 2018 project, we determined that the sequence of the NSs gene of the RB-TSWV-P-CA-1 isolate from Yolo County was more similar to sequences of other TSWV isolates from California, and that it lacked an obvious amino acid change associated with the pepper RB phenotype, including strains from other geographical regions, e.g., Europe. This suggests that the pepper RB strains in California likely emerged locally rather than being introduced. This is also consistent with TSWV not being seed-transmitted in pepper. Results of phylogenetic analyses with NSs sequences of isolates collected in 2018 and 2019 revealed that **pepper RB TSWV variants from Central California are not genetically homogeneous**, indicating multiple emergence events in California. Furthermore, our analysis of the aligned NSs amino acid sequences of non-RB and RB variants has still not revealed an amino acid motif(s) specific to pepper-RB-TSWV variants and from which a pepper RB TSWV rapid diagnostic test could be generated.

In 2021, we were able to obtain and characterize a diverse set of TSWV isolates from non-resistant and resistant varieties**. From these the NSs sequences were amplified and sequenced for 16 isolates, 7 from non-resistant varieties and 9 from resistant varieties (Table 2)**. The sequences of the SRB TSWV isolates associated with the outbreak in Merced should be an important addition to the database.

With the updated database and additional sequence added from GenBank contributions of other researchers, we will continue to search for amino acid motifs that are common to pepper RB TSWV strains, and that could be used to develop a rapid molecular test for pepper RB variants, such as is available for tomato RB TSWV. This would be very desirable as the current method to confirm pepper RB TSWV strains is by mechanical inoculation of the resistant pepper variety, e.g., Huntington.