2017-18 ANNUAL REPORT

Title: Completion of a flyer describing integrated pest management (IPM) of thrips/*Tomato spotted wilt virus* (TSWV) in pepper in California and surveys of fields pepper for emergence of resistance breaking strains

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Background

Tomato spotted wilt virus (TSWV) is one of the most important diseases of pepper crops worldwide, including California. This virus is transmitted by various species of thrips, but in peppers in California it is mostly the western flower thrips (*Frankliniella occidentalis*). Thrips affect pepper production by causing direct damage to pepper fruit through feeding damage and by spreading TSWV. In conventional production, thrips can be managed with insecticides, but this is difficult due to lack of effective insecticides and difficulties targeting thrips. The most effective use of insecticides for thrips management requires accurate timing of sprays, usually in advance of peak populations, which can limit feeding damage and TSWV transmission.

Another means of managing tomato spotted wilt in pepper is through the planting of resistant varieties carrying the dominant *Tsw* gene. All commercially available resistant pepper varieties possess this gene. Pepper plants carrying this gene usually show necrotic local lesions on inoculated leaves, followed by premature abscission of these leaves. Studies have revealed that the nonstructural (NSs) protein of TSWV is the effector (virulence/avirulence) that triggers the susceptible/resistant pepper response. TSWV-resistance-breaking (RB) strains of pepper (P) have been identified that cause typical symptoms in *Capsicum* spp. from several countries, e.g., Italy, Spain, Argentina, Australia and Hungary. Furthermore, evidence has been presented that all TSWV-RB-P strains are associated with different mutations in the *Tsw*. For example, in Hungary, a single point mutation in the NSs gene of TSWV gene at the amino acid position 104 (T for A) was shown to be sufficient to overcome *Tsw*-gene-mediated resistance in pepper. However, in other geographic locations, different

amino acid mutations in the NSs gene of TSWV have been reported to be involved in overcoming the *Tsw* resistance gene.

Overall Objectives:

To improve our understanding of viruses that infect pepper in California, with an emphasis on the thrips-transmitted TSWV. The long-term objectives are to reduce the impact of thrips and thrips-vectored tospoviruses on pepper production in California, with reduced application of insecticides through an effective integrated pest management (IPM) program.

1. The flyer on thrips and TSWV describing the IPM program was completed and is presented.

The flyer is attached at the end of the report.

2. Survey pepper fields planted with varieties having the *Tsw* resistance gene to determine if there is any evidence of emergence of resistance-breaking strains in California

- **Detection of viruses in pepper samples**

We surveyed pepper fields with and without *Tsw* gene in the Central Valley and Central Coast of California to see if there is any evidence of the emergence of TSWV-RB-P strain(s). A total of 40 pepper samples with virus-like symptoms were collected in 2017 in five Counties: Fresno, Kern, Merced, San Joaquim and Yolo.

Total RNA was extracted and used in RT-PCR for the common viruses of pepper: *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Pepper mottle virus* (PepMoV) and TSWV. Most of the samples were infected with AMV (~37%). TSWV was detected in 22% of samples. CMV (~15%) and PepMoV (~15%) was also found infecting pepper plants (Table 1).

Table1: Detection of pepper-infecting viruses

COUNTY	TOTAL	CROP	AMV	CMV	PepMoV	TSWV
FRESNO	6	PEPPER	0	5	0	0
FRESNO	3	JALAPEÑO	0	0	0	2 (RB-T)
KERN	15	PEPPER	12	1	0	0
MERCED	4	PEPPER	0	0	4	0
SAN JOAQUIM	9	PEPPER	3	0	0	6 (WT-P)
YOLO	3	PEPPER	0	0	2	1 (RB-P)
TOTAL	40		15 (37%)	6 (15%)	6 (15%)	9 (22%)

* TSWV-resistance-breaking (RB) strain of tomato (T) (T-RB); TSWV-wild type (WT) strain of pepper (P) (WT-P); and TSWV-resistance-breaking (RB) strain of pepper (P) (RB-P).

Samples infected with TSWV came from six susceptible pepper, two susceptible jalapeño, and one resistant pepper plant. We identified three TSWV strains in those samples based on sequencing/inoculation of resistant pepper plants. All susceptible pepper plants were infected with TSWV-wild type (WT) strain of pepper (TSWV-WT-P). The two jalapeños plants were infected with TSWV-RB strain of tomato (T) (TSWV-RB-T). It is important to point out that this TSWV-RB-T strain that overcome the tomato resistant varieties (carrying *Sw5*) did not overcome the resistance of pepper varieties carrying the *Tsw* gene.

- **Resistance-breaking strain**

Of the nine TSWV isolates collected, one caused symptoms in the resistant pepper cultivar (carrying the *Tsw* gene). The other eight isolates only caused disease in susceptible varieties. This TSWV-RB-P strain was collected from a symptomatic fruit (mosaic and distortion) from a commercial field in Woodland, California (Yolo County). Immunostrip tests and PCR/sequencing of the NSs gene confirmed that all nine isolates were infected with TSWV (>99% identity); however, this did not indicate if these were resistance-breaking strains.

We made an alignment of the sequences with TSWV-RB-P strains from regions of the world, including California, to see if we can find the mutation(es) responsible for the appearance of TSWV-RB-P on pepper. However, there were a number of possible mutations (~8) that could be responsible for the TSWV-RB-P phenotype, so we could not determine this.

This putative California TSWV-RB-P strain was then used to inoculate resistant and susceptible sweet-pepper plants (~30 days after germination) in the greenhouse by mechanical (sap) inoculation. We did two inoculations with 10 plants per treatment. Ten days post-inoculation with the California TSWV-RB-P strain, both susceptible and resistant sweet-pepper varieties showed typical and severe TSWV symptoms (Figure 1B, 2B, 3B and 3E).

Figure 1: Resistant sweet pepper plants (carrying *Tsw* gene) mechanically (sap) inoculated with (A) buffer (negative control), (B) with TSWV-RB-P strain, and (C) with TSWV-WT-T strain (C).

A TSWV-WT-T strain was used as a negative control. It only caused symptoms in the susceptible pepper varieties (Figure 1C, 2C, 3C and 3F).

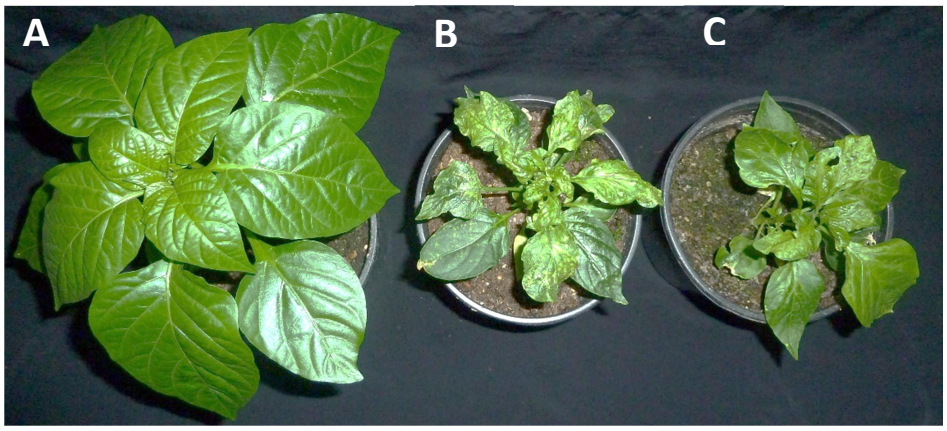

Figure 2: Susceptible sweet pepper plants mechanical (sap) inoculated with (A) buffer (negative control), with (B) TSWV-RB-P strain, and with (C) TSWV-WT-T strain.

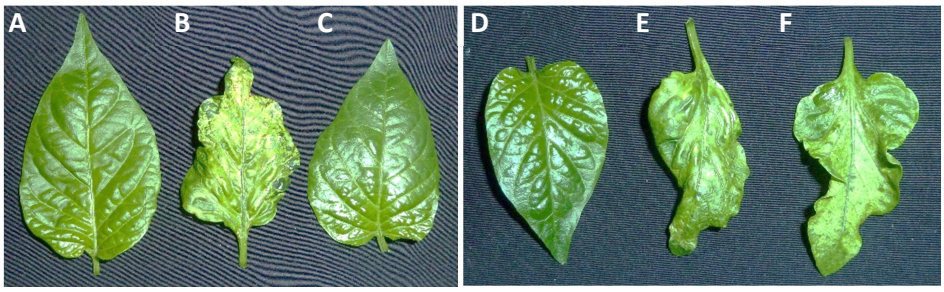

Figure 3: Close-up of resistant sweet pepper leaves mechanical (sap) inoculated with (A) buffer (negative control), (B) TSWV-P-RB strain, (C) and TSWV-T-WT strain; and close-up of susceptible sweet pepper leaves mechanical (sap) inoculated with (D) buffer (negative control), (E) TSWV-P-RB strain, and (F) TSWV-T-WT strain.

These results confirm the appearance of a TSWV-RB-P in resistant sweet-pepper varieties in Yolo County in 2017. This TSWV-RB-P strain was only detected in the fruit of one plant in Yolo County. It is not possible to say if this TSWV-RB-P strain will persistent overwinter and will infect pepper plants next season. In conclusion, because the most effective tool for growers to manage TSWV in pepper is plant resistant varieties, it is important to continue to monitor the incidence and distribution of the TSWV-RB-P strain on pepper fields in Central California.