Project Title: **Monitoring of thrips/*Tomato spotted wilt virus* (TSWV) in California peppers and the development of a regional IPM strategy for reducing the incidence and severity of TSWV (2013).**

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Summary

Western flower thrips (*Frankliniella occidentalis*) population densities and *Tomato spotted wilt virus* (TSWV) incidence in pepper fields in the Central Valley of California were monitored to improve understanding of disease development and to allow development of an effective strategy for disease management. Field monitoring for thrips and TSWV was conducted in representative direct-seeded and transplanted fields in San Joaquin County (SJC) and Solano County. Our results revealed low populations of thrips (10-350 thrips/yellow sticky card/two weeks) on peppers in Solano County in March until late May. However, by early June, populations increased and remained high (>500-2000 thrips/yellow sticky card/two weeks) through harvest. In SJC, peppers were not planted until early May. Thus, high thrips populations were detected throughout the season, until dropping to low levels in October. Consistent with these results, the first detection of TSWV in pepper plants was 1 May in Solano County and 24 May in SJC. TSWV was eventually detected in all eight monitored fields; however, the overall incidence was relatively low (<4%) through harvest. Incidence of TSWV in early-planted pepper fields was less (<1%) than in late-planted fields (3-4%). Winter and spring weed surveys revealed very low levels of TSWV infection in weeds (~2% infection). A notable exception was rough-seeded buttercup (*Ranunculus muricatus*) in SJC and Solano County where this weed was identified as a potentially important reservoir of TSWV. Large numbers of buttercup weeds showed virus disease symptoms and high rates (85%) of TSWV infection were detected in these weeds in 9/17 walnut orchards surveyed. This is an important potential reservoir host for TSWV in peppers. During the 2013 growing season, the web site for the thrips phenology (degree-day) model was made available for growers, and was regularly updated to provide thrips population projections for each surveyed county. This model, developed for processing tomato production in the Central Valley, accurately predicted the timing of adult thrips generations (~80% accuracy) in monitored pepper fields. Thus, we believe that this model can be used as a reliable predictor of when thrips populations begin to increase in pepper fields in the spring, and when it is best to apply thrips management strategies. Additionally, we started to work on development of a TSWV Risk Index (TRI), for predicting potential losses due to TSWV in pepper fields in the Central Valley. Based on gathered information for each monitored field, the TRI for all monitored fields in 2013 was predicted to be high or moderate, and did not correlate well with observed TSWV incidences. Thus, this model needs to be modified further and tested again until proven to be reliable for pepper fields. An integrated pest management (IPM) strategy for thrips and TSWV management in pepper is proposed and we believe that this IPM strategy can be highly effective at reducing disease incidence, particularly if followed regionally.

Objectives
The objectives of this study were 1) to conduct surveys of selected pepper fields in the Central Valley to gain insight into when and from where thrips and TSWV enters into commercial fields, 2) to assess the applicability of our degree-day model and TSWV risk index, which were developed and validated for processing tomatoes, to predict thrips populations and TSWV in peppers, and 3) to develop an IPM strategy for thrips and TSWV for the Central Valley peppers.

Materials and Methods
Thrips monitoring in representative fields. Table 1 lists the pepper fields (8) that were monitored for thrips and TSWV in 2013. Yellow sticky cards were placed at the four corners of each field, just above the canopy. The cards were changed biweekly beginning in March and up to harvest (August-October). Population densities were estimated by counting thrips on yellow sticky cards in the laboratory with a dissecting microscope at 2X magnification. Thrips were identified to species at 40X magnification.

TSWV incidence and detection. Percent TSWV incidence in pepper fields was determined by visually examining plants at the four locations in each field. At each location, all plants in 10 yards (meters) of each of 5 randomly selected rows (each separated by 5 rows) were examined. An overall incidence of tomato spotted wilt at each site of the field (four per field) was calculated (presented as number of infected plants per 100 row feet and % incidence). Disease incidence was assessed biweekly and selected plants tested with ImmunoStrips (AgDia) and RT-PCR (with N gene-specific primers) to confirm TSWV infection.

Assessment the applicability of our degree-day model and TSWV risk index, which were developed and validated for processing tomatoes, to predict thrips populations and TSWV in peppers. The phenology model is based on a "degree day' model that utilizes the effects of weather (i.e., temperature) on thrips biology. Currently, this model generates the degree-day accumulations for each year from January 1 to October 31 in a map format (heat availability). We compared results of this model with actual thrips population data collected from monitored fields in San Joaquin and Solano Counties in 2013. To do this, weather stations were selected from these counties, and weather parameters were used to map climate conditions for each county. We then used this data and the phenology model to predict appearance of thrips generations in a particular area in 2013. The predicted data were compared with actual thrips population dynamics that were documented in the pepper fields surveyed in 2013.

The previously developed TSWV risk index (TRI) for processing tomatoes of California was used to predict TSWV in pepper fields in the Central Valley. To obtain a risk index value for a particular field, point values were assigned to critical components of tomato production practices according to their relative influence on spotted wilt incidence. For establishing the TRI we have emphasized factors that play important role on disease development in processing tomatoes (i.e., tomato variety, planting time and location, proximity to the known TSWV and thrips resources etc.), based on results of our research. This TRI was applied to our monitored pepper fields to determine whether the values for pepper fields (low, moderate and high risk) accurately reflected TSWV incidence in these fields.

Results

Field Monitoring: In 2013, field monitoring for thrips and TSWV was initiated in direct-seeded pepper fields on 1 March in Solano County and on 24 May in transplanted peppers in SJC. Overall thrips population in early planted pepper fields (i.e., Solano County) was initially low (10-350 thrips/yellow sticky card/two weeks) during March-May, but populations increased significantly by 1 June and high thrips populations (>500-2000 thrips/yellow sticky card/two weeks) were observed throughout the season in these fields through harvest (Fig. 1). In SJC peppers, high thrips populations were detected in early June, fluctuated through July-August, and peaked in September (Fig. 2). Thrips populations gradually declined through mid-October (Fig. 2). The low thrips populations detected in peppers during March-May in Solano County were not expected because in parallel surveys conducted in processing tomatoes in the same area revealed higher thrips populations than peppers (Fig. 1). This may reflect insecticide management for thrips early in the season in pepper fields. Furthermore, overall thrips populations in processing tomatoes in Solano and neighboring counties were found to be much higher than in SJC in 2013; however, this trend was not observed among monitored pepper fields in SJC vs. in Solano County where thrips populations in these counties were comparable (Fig. 3). All thrips captured in the fields were identified as western flower thrips. Female thrips populations were usually three fold higher than male thrips populations, a trend that we observed in processing tomato surveys in 2007-2013 in the Central Valley of California.

Tomato spotted wilt virus (TSWV) incidence: In 2013, the first detection of TSWV in a pepper field was on 1 May in a direct seeded pepper field in Solano County. TSWV was not detected until 24 May in monitored fields in SJC. TSWV was eventually detected in all 8 monitored fields. However, the overall incidence of TSWV in pepper fields in Solano County was very low (<1-3%, Table 1). Similarly, TSWV incidence in SJC peppers was also very low (<1-4%, Table 1). In two relatively late-planted pepper fields, one in Solano and another in SJC, TSWV was present at higher incidences (3-4%) than in early-planted pepper fields (<1%). This trend is similar to that observed in processing tomatoes, in which the incidence of TSWV is lower in early-planted compared with late-planted fields. Overall, we do not believe that TSWV caused economic losses in any of the monitored pepper fields in 2013.

In 2013, *Alfalfa mosaic virus* (AMV, up to 70% incidence) and curly top virus (CTV, up to 15% incidence) were more common in pepper fields than TSWV. AMV was also detected in some weed samples collected in and around monitored fields before peppers were established. Together with high populations of aphids observed in alfalfa early in the season, especially in Solano County, it is likely that AMV was moved into pepper fields from surrounding alfalfa and AMV-infected weeds. Although AMV reached in high incidences in some pepper fields, most plants appeared to 'recover' from initial disease symptoms (i.e., bright-yellow mosaics on leaves) later in the season and AMV infection did not seem to result in substantial damage or yield loss. Curly top virus was mainly observed in SJC peppers and incidences reached up to 15% in one late-planted field. Otherwise curly top incidence was sporadic and was <1% in monitored fields.

Additionally, very low (sporadic and <1%) levels of other pepper-infecting viruses including *Cucumber mosaic virus* and *Tobacco etch virus* as well as sporadic or patchy areas with fungal/bacterial diseases including Fusarium wilt, Phytophthora blight and bacterial spots diseases were detected in some of the monitored fields.

Survey of potential hosts for TSWV: In conjunction with our ongoing thrips/TSWV project in processing tomato, we continued our efforts to identify reservoir hosts of TSWV for peppers/tomatoes before, during and after the 2013growing season. We focused our efforts around pepper fields that were monitored in SJC and Solano County, and we collected numerous weeds from these areas in the winter and spring before peppers and tomatoes were established.

Bridge and Cover crops: Fall crops (i.e., lettuce and radicchio) that are good bridge hosts for TSWV and thrips were not found in our surveyed areas during fall/winter seasons in 2013. Early in 2013, we monitored two fava bean (cover crop) fields in Yolo County; however, low thrips populations and no TSWV infection were detected in these fields.

Weeds: In 2013, weeds were collected from surveyed areas and tested for TSWV (Table 2). Both in SJC and northern counties (Solano, Yolo, Colusa, Sutter and Sacramento Counties), weeds were abundant along roads, levees, fallow fields and some orchards. With the exception of buttercups (a new TSWV weed host, see below), most weeds collected before and during 2013 pepper/tomato growing season were symptomless and tested negative for TSWV (with immunostrips or PCR). A small number of weeds with symptoms (necrosis and thrips-feeding damage) were infected with TSWV. However, the overall incidence of TSWV infection in weeds was very low (a total of 12 TSWV-infected weeds detected/435 tested; overall incidence 2%). Some of the common weeds that TSWV were detected in were nightshade, pineapple weed, sowthistle, Malva and Datura (Table 2). This was similar to results of previous surveys that were conducted in the Central Valley during 2007-2013.

A new potentially important TSWV weed host identified in San Joaquin and northern counties: In our 2013 weed surveys, we identified a new weed host of TSWV: rough-seeded buttercup (*Ranunculus muricatus*). Buttercup is a low-growing biennial plant that produces round leaves and yellow flowers. When infected with TSWV, the leaves develop mosaic and mottling symptoms. TSWV-infected buttercup plants were found in large numbers in and around walnut orchards in SJC (3 of 8 orchards) and northern counties (6 of 9 orchards). TSWV infection rates in patches of buttercups in these orchards ranged from 10-100% (a total of 128 TSWV-infected buttercups were detected/149 samples tested, for an overall incidence of 85%; Table 2). Since the beginning of our ongoing processing tomato survey project in 2007, buttercup is the only weed species with such a high rate of TSWV infection in the Central Valley.

Interestingly, buttercup has become more common in California recent years, although it is not yet a widespread weed problem. It grows well in wet soils, such as low spots in orchards or at the end of rows where water might accumulate. It does not appear to become established in pepper or tomato fields. However, it has also been found in wheat fields and in other orchards (e.g., cherry and almond). Buttercup is a biennial weed, so it would germinate with fall/winter rains and survive through the summer and into the following spring when it would flower and set seed. Because it is biennial, infected plants can carry the virus over multiple years and serve as an inoculum source for peppers. Thus, in 2014, we hope to continue to survey for the presence of TSWV-infected buttercups to further determine the importance of this TSWV inoculum source.

Assessment of the applicability of our degree-day model and TSWV risk index, which were developed and validated for processing tomatoes, to predict thrips populations and TSWV in peppers: In 2011, we developed a predictive tool to assist growers to determine when thrips populations begin to increase. This phenology model is based on a 'degree day' model that uses

the understanding of the effects of weather (temperatures) on thrips biology. Currently, this model generates the degree-day accumulations for each year from January 1 to October 31 in a map format (heat availability). We tested and validated this model with monitored processing tomato fields in Fresno, Kings, Merced, Yolo and Colusa Counties from 2009-2013. To do this, we selected weather stations from these counties and used weather parameters to map climate conditions for each county. We then used this data and the phenology model to predict appearance of thrips generations in a particular area and year. The predicted data were then compared with actual thrips populations that were documented during our surveys in 2009-2013. After testing and validating its accuracy (>80%) in processing tomatoes, this model was extended and made available for growers in San Joaquin County in 2013. The degree-day model can be accessed via its webpage:
http://ucanr.edu/sites/TSWVfieldriskindex/Thrips_Population_Projections/

In terms of pepper fields monitored in 2013, the model predicted eight thrips generations during the growing season (March-October). We reasoned that after emergence of each thrips generation there should be some degree of population increase in the fields. Thus, we looked at the generation times predicted by the model, and compared with actual population dynamics determined from yellow sticky card data to assess whether there was an increase in thrips populations after each generation and whether predicted and actual values were in agreement. The model predicted the timing of the generations with overall accuracy of 80% for both surveyed counties. However, in some cases, thrips generations predicted by the model were early or late, compared with actual thrips population counts. In most of these cases, these differences were in small or acceptable windows of time (± 2 days off). Nevertheless, these initial results are very promising and suggest this predictive model could be used to predict thrips build-up in pepper fields and for making thrips management decisions. We will propose to continue to fine-tune the phenology model and test it again for peppers in the 2014 growing season.

In 2011, we also developed a second predictive tool; the TSWV risk index (TRI), for California processing tomatoes as a tool to help growers calculate the relative level of risk for TSWV development in a given field based upon a variety of factors known to influence disease development. We assigned points (a relative numeric weight) to each factor so that an overall level of risk can be estimated. In the TRI the higher total points a field receives indicates higher levels of risk. The TRI can be accessed via its webpage:
http://ucanr.edu/sites/TSWVfieldriskindex/Field_Risk_Index/

Based on our extensive investigations of TSWV in processing tomatoes in California, we identified a number of factors that are important for TSWV development and management in processing tomatoes. These factors are tomato variety, planting time, planting density and method, proximity to known bridge crops and thrips sources, insecticide usage and weed situation. However, it is not clear if these same factors are important for TSWV development in peppers. Therefore, we initially evaluated the current TRI with monitored pepper fields in 2013 by using the factors that were found to be important for tomato-TSWV system.

We have now gathered most of the information about each monitored pepper field from growers, and we have calculated the risk index for each field and have compared this with the known levels of TSWV in each field. Based on the current TRI, the fields were assigned high and moderate risk (Table 1). When these TRI values were compared with the actual TSWV incidences in 2013 growing season for each monitored field, we did not find a good correlation.

In other words, the current TRI did not identify reliable risk categories for our monitored pepper fields. Although, many of the factors used in the current TRI for tomatoes are considered to be important for peppers, it is clear that other factors maybe involved. Thus, more pepper surveys need to be conducted in order to assess the applicability of the current TRI for TSWV prediction in peppers. This will allow us to modify or change the factors and/or their proposed point values so that the TRI can be developed as a tool for helping growers determine if TSWV poses a risk in a given pepper field. We will propose to continue to fine-tune the current TRI and test it again in the 2014 growing season.

Development of an IPM strategy for thrips and TSWV in peppers: By using the information generated in this project, we have developed the following IPM program for TSWV and thrips in peppers in the Central Valley of California. We believe that implementation of this package (all or in part) will help reducing TSWV to levels where economic losses can be substantially reduced.

Before planting
i) evaluate planting location/time of planting-this will involve determining proximity to potential inoculum sources during the time of planting (if possible avoid hot spots, planting near fields with bridge crops and weedy orchards or late planting dates).
ii) use TSWV- and thrips-free transplants
iii) plant TSWV resistant varieties (possessing the Tsw gene)-these are available, but may not be necessary if other practices are followed. At least, resistant cultivars should be used in hot-spot areas or in late-planted fields that will be established near early-planted pepper or tomato fields in which TSWV infections have already been identified.
iv) implement weed management-maintain weed control in and around pepper fields and especially in fallow fields and orchards, as some weeds are TSWV hosts, such as rough-seeded buttercup. If weeds are allowed to grow in fallow fields, they can amplify thrips and TSWV and serve as inoculum sources for peppers.

During the season
i) monitor fields for thrips with yellow sticky cards or use the predictive phenology (degree-day) model to estimate when thrips populations begin to increase.
ii) manage thrips with insecticides at early stages of crop development and when thrips populations begin to increase.
iii) rotate insecticides to minimize development of insecticide resistance in thrips.
iv) monitor fields for TSWV and remove infected plants early in development (<30 days old) and when percent infection is low (<5%)
v) implement weed management-maintain effective weed control in and around pepper fields.

After harvest
i) promptly remove and destroy plants after harvest
ii) avoid planting bridge crops that are thrips/TSWV reservoirs or monitor for and control thrips and TSWV in these crops
iii) control weeds/volunteers in fallow fields, non-cropped or idle land near next years pepper fields

Table 1. Pepper fields monitored in 2013: Locations, TSWV incidence and TSWV Risk Index (TRI) values.

Monitored Fields in 2013

	Solano County		**TSWV %**	**TRI**
DX	Dixon Rd., Dixon (Direct Seeded)		<1	high
ML	Miller Rd., Davis (Direct Seeded)		<1	moderate
RB	Robben Rd., Dixon (Direct Seeded)		<3	high
EG	Eggert Rd., Davis (Transplanted)	Red	<1	high
MW	Midway Rd., Dixon (Transplanted)		<1	moderate
	San Joaquin County			
FC	Fairchild Rd., Linden (Transplanted)		<4	high
CO	Concord Ave., Brentwood (Transplanted)	Red	<1	moderate
CL	Collier Rd., Clements (Transplanted)		<1	high

Red: The crops were harvested late after fruits were reddened.

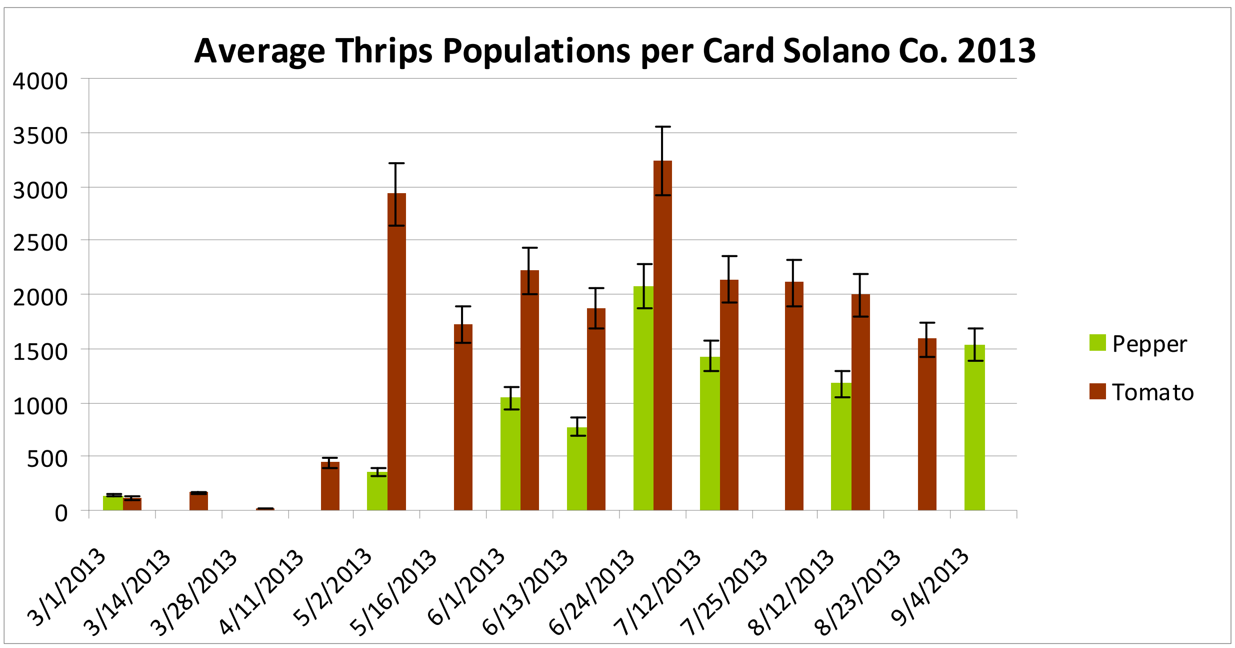

Fig. 1. Average thrips counts per yellow sticky card in monitored pepper and tomato fields in Solano County in 2013.

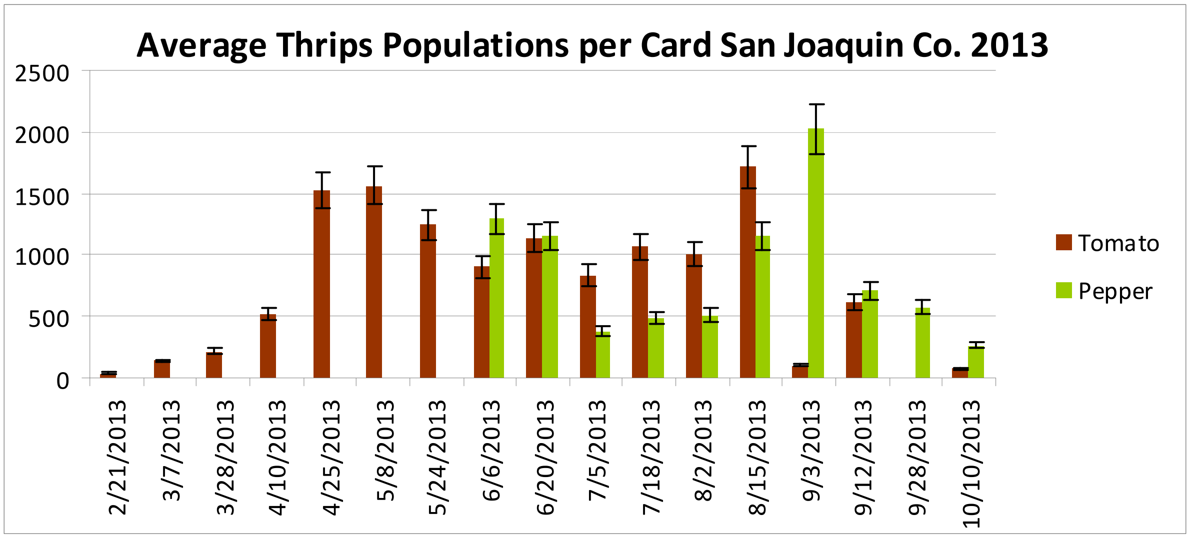

Fig. 2. Average thrips counts per yellow sticky card in monitored pepper and tomato fields in San Joaquin County in 2013.

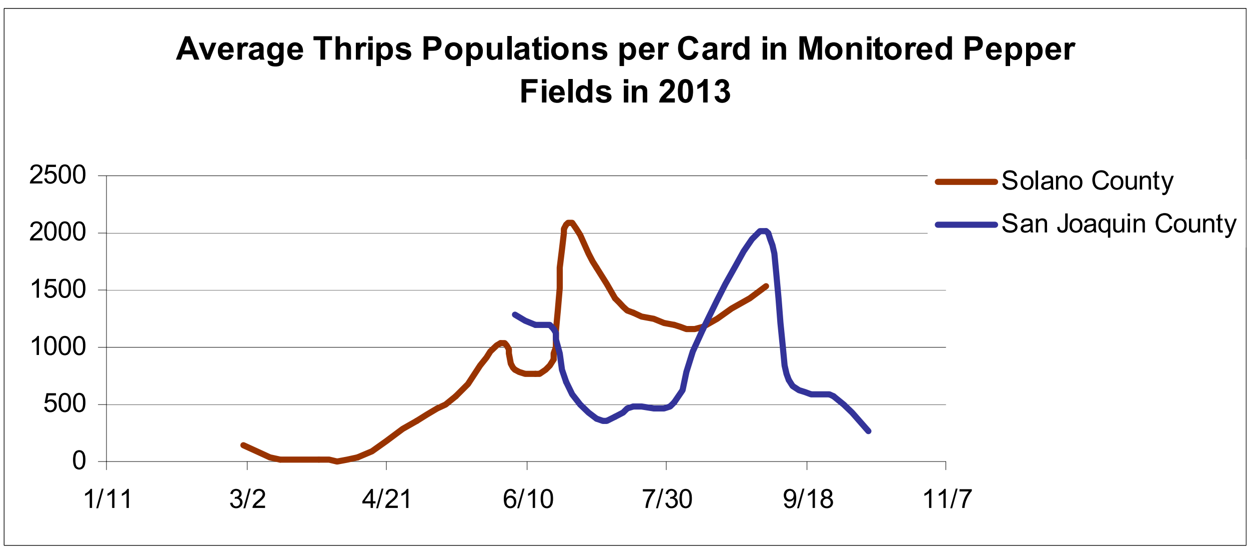

Fig. 3. Average thrips counts per yellow sticky card in monitored pepper fields in Solano and San Joaquin Counties in 2013.

Table 2. Result of weed surveys for TSWV in 2013.

Weed [a]	**Tested (+)**	**Weed** [a]	**Tested (+)**
Nightshade	10 **(1)**	Curly dock	3 (0)
Bindweed	22 (0)	**Malva**	135 **(5)**
Filaree	42 (0)	**Datura**	1 **(1)**
Pineapple weed	4 **(1)**	Monocots	9 (0)
Sowthistle	34 **(4)**	Shepherd's purse	15 (0)
Prickly lettuce	22 (0)	Fiddler neck	3 (0)
London rocket	15 (0)	Pigweed	4 (0)
Buckhorn Plantain	8 (0)	Turkey mullein	5 (0)
Lamb quarters	17 (0)	Groundsel	3(0)
Poison hemlock	26 (0)	Tree tobacco	12 (0)
Pennywort	5 (0)	Nettle	4 (0)
Rough-seeded Buttercup	**149 (128)**	Bermuda buttercup	18 (0)
Wild radish and Mustard	34 (0)	Other common weeds	28 (0)

(+), number of plants tested positive for TSWV by immunostrips and RT-PCR.
a, Total weed samples from all counties surveyed in 2013