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 (2014).**

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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 for a second year in 2014 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 Solano and San Joaquin Counties (SJC). Our results revealed relatively low populations of thrips (50-300 thrips/yellow sticky card/two weeks) on peppers in Solano County in March until mid-April. However, by early May, populations increased and remained high (>500-2500 thrips/yellow sticky card/two weeks) through harvest. High thrips populations fluctuated throughout the season, until dropping to low levels in October. In SJC, the monitored pepper field was not planted until early May. Thus, high thrips populations were detected throughout the season in this field, until dropping to low levels in October. The first detection of TSWV in pepper fields was in mid-April in Solano County, at or around the time when thrips populations increased. In 2014, TSWV was eventually detected in all eight monitored fields; however, the overall incidence was relatively low (1-2%) in early-planted pepper fields as well as *Tsw*-resistant peppers through harvest, similar to 2013. However, the incidence of TSWV in late-planted fields was higher (8-14%). Winter and spring weed surveys revealed very low levels of TSWV infection in weeds (~2% infection). The newly identified TSWV weed host, rough-seeded buttercup (*Ranunculus muricatus*), which is typically found in walnut orchards, was again widespread in walnut orchards and some plants were infected with TSWV. During the 2014 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 in 2013 and 2014. 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 continued to develop a TSWV Risk Index (TRI), for predicting potential losses due to TSWV in pepper fields in the Central Valley. Based on information gathered from each monitored field, the TRI for monitored fields in 2014 accurately predicted high, moderate or low risks, and correlated well with observed TSWV incidences. Thus, the TRI showed good promise predicting TSWV incidence, but needs to be tested further. An integrated pest management (IPM) strategy for thrips and TSWV management in pepper is proposed that should be 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 enter into commercial fields; 2) to assess the applicability of our degree-day model and TSWV risk index to predict thrips populations and TSWV in peppers, respectively; and 3) to develop an IPM strategy for peppers grown in the Central Valley.

Materials and Methods

Thrips monitoring in representative fields. Table 1 lists the 8 pepper fields that were monitored for thrips and TSWV in 2014. Yellow sticky cards were placed at the four corners of each field, just above the canopy. The cards were changed biweekly beginning in April 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 for TSWV symptoms. An overall incidence of tomato spotted wilt (and other viruses) 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. For other pepper-infecting viruses, RT-PCR or PCR with virus-specific primers was used to confirm the infection in plants.

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 2014. 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 from yellow sticky cards. The predicted data were compared with actual thrips population dynamics that were documented in the pepper fields surveyed in 2014.

The previously developed TSWV risk index (TRI) for processing tomatoes of California was used to predict TSWV incidence 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 pepper 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 pepper (i.e., pepper variety, planting time and location, proximity to the known TSWV and thrips sources 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 2014, field monitoring for thrips and TSWV was initiated in direct-seeded pepper fields in late-April in Solano County and in transplanted peppers in SJC. It is important to note here that in 2014, because there were very few pepper fields in SJC, we focused our monitoring efforts in Solano County. Overall thrips populations in early-planted pepper fields was initially low (50-300 thrips/yellow sticky card/two weeks) until late-April, when populations increased significantly by early-May and high thrips populations (>500-2500 thrips/yellow sticky card/two weeks) were observed throughout the season in all monitored fields through harvest (Fig. 1). This thrips population increase occurred about a month earlier than in 2013. In all monitored pepper fields, high thrips populations were detected in early May, and fluctuated through September (Fig. 1). Thrips populations gradually declined through the end of October (Fig. 1). Fluctuations observed in thrips populations during the 2014 growing season suggested that thrips management with insecticides in monitored peppers were effective, but that the effects only lasted a short period of time. Thus, after a drop in thrips populations, a rapid increase usually occurred in monitored fields. Therefore, we believe that if more precise timing of the thrips management in peppers were implemented based on thrips monitoring or our degree-day model, efficiency of the insecticide sprays could be more effective and delay appearance of TSWV.

All thrips captured in the pepper fields were identified as western flower thrips. Female thrips numbers were usually three-fold higher than males, a trend that we observed in processing tomato surveys in 2007-2014 in the Central Valley of California.

Tomato spotted wilt virus (TSWV) incidence: TSWV appeared in all monitored fields including those that were established with TSWV resistant peppers (varieties with the *Tsw* gene). However, we believe that TSWV caused minimal economic losses, particularly in some late-planted and/or red harvested monitored pepper fields in 2014.

In 2014, TSWV first appeared in pepper fields in mid-April at or around the time when thrips populations increased in Solano County. This was about a month earlier than in 2013, when the thrips population increase was in mid-May. This suggested a correlation between appearance of TSWV and the increase in thrips populations. In 2014, TSWV appeared in all 8 monitored pepper fields, including those with TSWV-resistant pepper varieties (with *Tsw*-gene). Overall TSWV incidences in early-planted monitored pepper fields remained relatively low (1-2%), similar to 2013 (Table 1). However, one monitored early-planted pepper field, which was planted adjacent to a processing tomato field with a high TSWV incidence (14%), had a TSWV infection rate that reached ~40% in one corner of the field (Table 1). Pepper plants showed severe symptoms of TSWV infection, including wilting and necrosis on leaves and stems and shoot dieback (Fig. 2). However, because the infection was relatively late and fruits were mature, TSWV did not seem to cause substantial economic losses in this field. Moreover, the detection of TSWV in TSWV-resistant pepper fields in Byron-Brentwood in SJC indicated that these production areas were under high TSWV disease pressure in 2014 and peppers without *Tsw*-gene could have been affected substantially. Thus, we believe that use of TSWV resistant varieties here prevented what could have been substantial economic losses in peppers.

In some late-planted green and red pepper fields monitored in 2014, TSWV incidences were higher (8-14%) and increased up to 14%-20% in parts of the fields by the end of the season

(Table 1). In some of these fields, TSWV came in late, probably via thrips from nearby processing tomato fields that were being harvested. However, even in these fields, damage from TSWV appeared to cause minimal economic loss, but clearly the potential for loss due to TSWV exists. 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.

In 2014, *Alfalfa mosaic virus* (AMV) was more widespread than TSWV (or any other disease) in monitored pepper fields, with incidences as high as 40-60% in some fields early in the season (Table 1). Interestingly, AMV did not seem to cause any visible damage to pepper fruits. Moreover, later in the season, most of the younger shoots that developed from these initially infected plants became symptomless and produced normal fruits. We believe that these pepper plants may be undergoing 'recovery' from AMV infection; this is a type of plant defense response (gene silencing) that specifically targets foreign nucleic acids (e.g., viruses).

In 2014, we also became involved in a virus disease problem in peppers in the Coachella Valley. Here, peppers plants were infected with AMV and *Beet curly top virus* (BCTV; Fig. 2), sometimes in mixed infection. In the case of mixed infection, symptoms were more severe than AMV infection alone, and plants with mixed infections either died or became stunted, and did not produce marketable fruits. In plants with these mixed infections we also did not observe recovery. Fortunately, the incidence of mixed infections was relatively low (~2%). In monitored fields in Solano and SJC, BCTV was observed in most monitored peppers but at low incidences ≤2% (Table 1).

Additionally, very low (sporadic and <1%) levels of other pepper-infecting viruses including a necrotic strain of AMV (the strain that usually causes necrosis on leaves, stems and fruits and did not show recovery), *Cucumber mosaic virus* (CMV) and *Tobacco etch virus* as well as phytoplasma and bacterial spots diseases were detected in some of the monitored fields (Fig. 2).

Key Findings: TSWV outbreaks are correlated with timing of thrips populations increase and field location (e.g., proximity to early-planted susceptible crops) and other viruses can impact pepper production.

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 2014 growing season. We focused our efforts around 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) or fava bean cover crop that are good bridge hosts for TSWV and thrips were not found in our surveyed areas during fall/winter seasons in 2014.

Weeds: In 2014, weeds were collected from surveyed areas and tested for TSWV (Table 2). In SJC, weeds were abundant along roadsides and levees, and in fallow fields and some orchards. Most weeds collected before and during the pepper growing season were symptomless and tested negative for TSWV (with immunostrips and/or PCR). A small number of weeds including rough-seeded buttercup, bindweed, sowthistle and prickly lettuce were infected with TSWV (Table 2). The overall incidence of TSWV infection in weeds was very low (~2% [9 TSWV-positive weeds/395 tested], Table 2). This was similar to results from previous years, and continues to indicate that weeds in the Central Valley of California are not extensively infected with TSWV.

In our 2014 surveys, particular attention was given to the newly identified weed host of TSWV, rough-seeded buttercup. We observed this weed in 13 of 17 walnut orchards that were surveyed. Most of the buttercups in these orchards did not show obvious disease symptoms. However, a relatively small number (~1%) showed virus-like symptoms including some that showed symptoms of TSWV infection (chlorosis, bronzing and necrosis) and others that showed necrotic spots. Because buttercups show symptoms upon infection with TSWV, only plants with symptoms (25 plants from 6 orchards) were tested for TSWV infection with immunostrips and PCR. Of these, only 5 plants tested positive for TSWV, and these came from walnut orchards that had been next to processing tomato fields that had TSWV outbreaks in 2013. Buttercups with necrotic spots that were negative for TSWV infection and this symptom was most likely due to chemical damage.

Key Findings: Some weeds, especially in fallow fields and orchards, may serve as initial TSWV inoculum sources if not managed before the start of growing season, and particular attention should be paid to rough-seeded buttercups in walnuts.

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: The phenology model is based on a 'degree day' model that uses the understanding of the effects of weather (temperatures) on thrips biology. In 2014, this model generated the degree-day accumulations from January 1 to October 31 in a map format (heat availability). We tested and validated this model with actual thrips numbers trapped on yellow sticky cards in monitored pepper fields in Solano and SJC in 2013 and 2014. 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 in peppers that were documented during our surveys in these years. After testing and validating its accuracy (>80%) in processing tomatoes and peppers, this model was extended and made available for growers in San Joaquin County in 2013 and 2014. 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 2014, the model predicted five thrips generations during the growing season (March-July). 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 these 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. In 2014, our degree-day model predicted generations of adult thrips in peppers with ~80% accuracy. Interestingly, for both years, the degree-day model targeted the 2nd thrips generations in early-planted peppers, and the 3rd generation in late-planted pepper fields for thrips control. However, in some cases, thrips generations predicted by the model were early or late, compared with actual thrips population counts, especially during the late-summer when thrips populations were very high (1,500-2,500 thrips/yellow sticky card/two weeks) in monitored peppers. However, these differences were in small or acceptable windows of time (± 2 days off), that occurred at the end of the growing season, and were negligible. Thus, these 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.

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. Thus, the higher total points a field receives indicates higher levels of risk for TSWV development. 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 was not clear if these same factors were important for TSWV development in peppers. Therefore, we initially evaluated the current TRI with monitored pepper fields in 2013 and 2014 by using the factors that were found to be important for tomato-TSWV system.

In fall 2014, we gathered most of the information about each monitored pepper field from growers, and we calculated the risk index for each field and compared this with the known levels of TSWV in each field. Based on the current pepper TRI, the fields were assigned high, moderate and low risk (Table 1 and 3). When these TRI values were compared with the actual TSWV incidences in 2014 growing season for each monitored field, we noticed a good correlation. In other words, the calculated TRI identified risk categories for our monitored pepper fields (Table 1). Although, many of the factors used in the current TRI for tomatoes (Table 3) are relevant to peppers, we feel that other factors may also be involved (i.e., red and/or late harvest of green peppers near processing tomatoes with TSWV infections being harvested). 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 are now in process of making the pepper TRI available to growers via a webpage and will propose to continue to fine-tune the current TRI and test it again in the 2015 growing season.

Key Findings: The thrips degree-day model accurately predicts appearance of thrips in pepper crops and has the potential to be a tool to time thrips control and reduce spread of TSWV. The pepper TRI can be used to predict TSWV threats in individual fields.

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 program (all or in part) will help reducing TSWV to levels where economic losses can be substantially reduced, particularly if followed regionally.

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 tomato or 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 (usually 2nd and/or 3rd thrips generations).
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

Key Finding: An IPM program for thrips and TSWV in pepper has been developed.

Table 1. Pepper fields monitored in 2014: Locations, TSWV and other virus incidence and TSWV Risk Index (TRI) values.

Monitored Pepper Fields in 2014						
Fields	**Solano County**		**TSWV %**	**TRI**	**Points** (see Table 3) [d]	**Other Virus Incidences**
HM	Hamel Ln., Davis	early-planted	2	Moderate	140	6% AMV and 1%BCTV
PH	Phillips Rd., Dixon	early-planted	2	Moderate	150	3% AMV and 1% BCTV
SI[a]	Sievers Rd., Dixon	early-planted	14	High	170	20 % AMV and 1% BCTV
TR[b]	Tremont Rd., Dixon	late-planted	8	High	200	5% AMV and 1% BCTV
BU	Bulkley Rd., Dixon	early-planted	2	Moderate	135	8% AMV and <1% CMV
HK	Hackman Rd., Dixon	early-planted	<2	Moderate	150	4% AMV
	San Joaquin County					
AR	Arch Rd., Stockton	early-planted	2	Moderate	125	2% BCTV
MC[c]	Marsh Creek Rd, Brentwood	early-planted	<1	Low	45	1% BCTV

[a] *This pepper field had ~40 TSWV infection in one corner of the field*
[b] *Part of this pepper field was kept for red pepper harvest*
[c] *This pepper was with TSWV resistant variety and monitoring of thrips was terminated early in the season, however, continued to monitor visually for TSWV incidence only*

[d] *Point values are calculated from factors listed in Table 3*

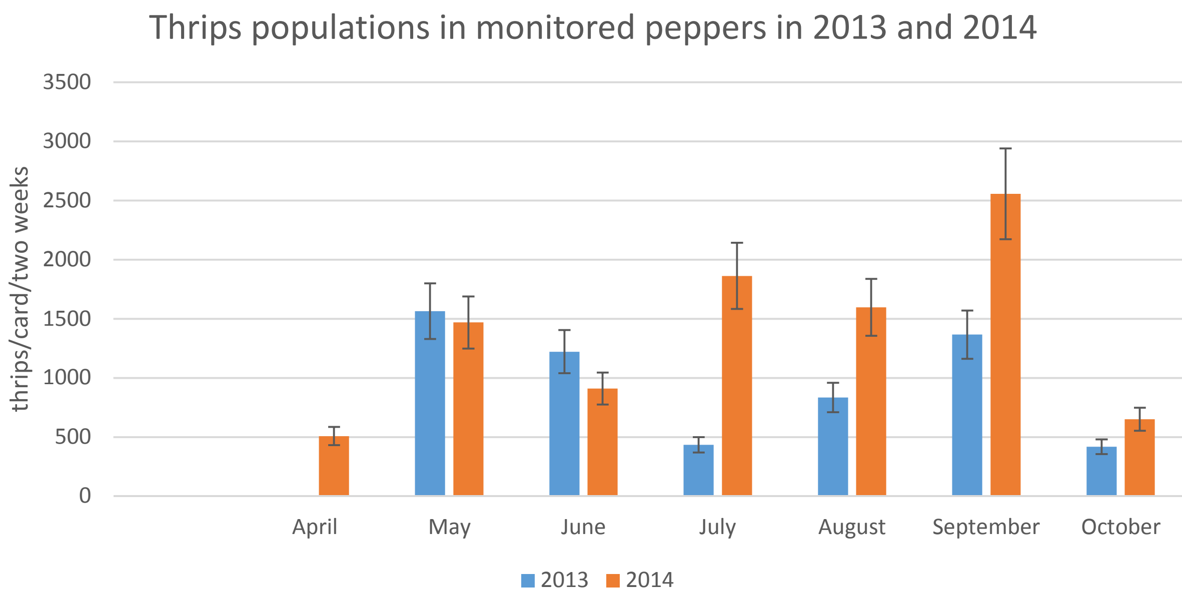

Figure. 1. Average thrips counts per yellow sticky card in monitored pepper fields in 2013 and 2014.

Figure. 2. Examples of some of the diseases and their characteristic symptoms that observed in monitored pepper fields in 2014. TSWV; *Tomato spotted wilt virus*, AMV; *Alfalfa mosaic virus*, BCTV; *Beet curly top virus*, CMV; *Cucumber mosaic virus*.

Table 2. Results of survey of weeds in Solano and San Joaquin Counties for TSWV infection in 2014.

Weed	Tested/ number(+)	Weed	Tested/ number (+)
Dandelion	5 (0)	Bur Clover	20 (0)
Prickly Lettuce	25 (**1**)	**Sowthistle**	45 (**2**)
Bindweed	15 (**1**)	Nettle	5 (0)
Vetch	5 (0)	Mustard	5 (0)
Chickweed	30 (0)	Groundsel	5 (0)
Knotweed	10 (0)	Cutleaf Geranium	5 (0)
Swine cress	10 (0)	Miner's Lettuce	15 (0)
London rocket	15 (0)	Henbit	5 (0)
Redmaids	15 (0)	Fiddleneck	5 (0)
Shephard's purse	15 (0)	Poison hemlock	15 (0)
Malva	25 (0)	Pineapple weed	15 (0)
Filaree	20 (0)	Chinese lantern	10 (0)
Rough-seeded Buttercup	25 (**5**)	Pigweed	10 (0)
Dandelion	5 (0)	Others	20 (0)
		Total :	**395 (9)**

(+)= number of plants that tested positive for TSWV by immunostrips and/or RT-PCR

Table 3. *Tomato spotted wilt virus* Risk Index (TRI) for peppers (2014).

Tomato spotted wilt virus Risk Index for Peppers-2014

Pepper Variety[1]	**Examples**	**Risk Index Points**
a,b,c	Very Highly suceptible varieties	50
d,e,f	Highly suceptible varieties	40
g,h,i	Suceptible varieties	30
j,k,l	Somehow suceptible varieties	20
m,n,o	Less suceptible varieties	10
p,q,r	with *Tsw* gene	-35
Planting Date[2]		
Prior to February 1	First planted fields in any given region	5
February 1-29	week or two later than first planted fields	10
March 1-15	week earlier than recommended period	15
March 16- April 31	Recommended period (Majority of fields)	20
May 1-20	week or two later than majority of fields	25
May 21- June 5	three weeks or more later planted from majority	30
After June 5	latest planted fields in a given region	35
Plant Population		
Less than 7000 plant per acre	single row	35
Between 7000 to 9000 plant per acre	double row	15
More than 9000 plants per acre	double row but more dense	5
Planting Method		
Direct seeded		10
Transplanted		5
Proximity to Known Bridge Crops		
adjacent	radicchio, lettuce, fava, weed/fallow field, pepper or tomato	25
less than 1 mile radius distance	(if TSWV confirmed add 20 more points)	15
1-2 mile radius distance	(if TSWV confirmed add 10 more points)	10
greater than 2 mile or None	(if TSWV confirmed add 5 more points)	5
Proximity to Thrips Source		
adjacent	wheat, pea, alfalfa or weedy patches etc.	20
less than 1 mile radius distance		15
1-2 mile radius distance		10
None		5
At-Plant Insecticide		
None		15
for other pests (+ thrips)		10
specifically for thrips		5
Weed situation/Herbicide use		
w/out herbicide but weedy	In-field ONLY weed population	15
w/out herbicide but not so weedy		10
w/out pre emergence herbicide or NO weed		5
Total Points (0-225)	**Risk of Losses Due to TSWV**	
Less than or equal to 95	**Low**	
Greater than 100 or equal to 150	**Moderate**	
Greater than 150	**High**	

[1] *Additional varieties will be included as data to support the assignment of an index value are available.*

[2] *In those years when the normal date of planting for the first pepper in an area is delayed due to inclement weather, these date ranges should be moved later by an equal amount.*