Project title: Application of a degree-day model and risk index to predict development of thrips and *Tomato spotted wilt virus* (TSWV) and help implement an IPM program in California processing tomato fields

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Summary

The goal of this project is the continued development and implementation of a predictive thrips phenology (degree-day) model and *Tomato spotted wilt virus* (TSWV) risk index (TRI), and to complete our ongoing thrips and TSWV monitoring efforts in San Joaquin and Contra Costa Counties (hereafter referred as San Joaquin County or SJC). The long-term goal of this research has been to provide accurate and real-time information to growers about the population dynamics of thrips and development of TSWV infection to facilitate effective disease management with the integrated pest management (IPM) program developed through this project. In 2014, monitoring of tomato fields in San Joaquin County revealed similar thrips population dynamics as in 2013 in which thrips populations started to build-up in early April and rapidly increased to high population levels by early- to mid-May. Populations remained high through the summer, and began to decline in late summer to early fall (late August to early September). However, in 2014, the build-up of thrips populations began earlier than in 2013. TSWV was first detected in a monitored direct-seeded tomato field on 3 April in the Byron area in SJC. TSWV was eventually detected in all monitored fields, but overall incidences were low (<1-4%). Slightly higher incidences (up to 7%) were found in parts of two fields (the direct-seeded field in Byron and another field in the Tracy area) by early June, but economic losses were not experienced. Winter and spring weed surveys revealed very low levels of TSWV infection (~2%). In 2014, the newly identified TSWV weed host, rough-seeded buttercup (*Ranunculus muricatus*), was again widespread in walnut orchards and some plants were infected with TSWV. Laboratory experiments on the role of the soil-emerging adult thrips as an inoculum source for early season tomatoes revealed that thrips can stay dormant in soil for up to 8 weeks and that many of the emerging adult thrips (62-100%) transmitted TSWV. These results strongly suggest that adult thrips, emerging from soil, can be an early season TSWV inoculum source. 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 area. This model predicted the appearance of adult thrips generations with >80% accuracy. Thus, this model can be used as a predictor of when thrips populations begin to increase in the spring, and can help identify when to start implementing thrips management strategies (e.g., early- to mid-April in SJC in 2014). The prototype TSWV risk index (TRI) calculator was also made available on the web as well as on smartphone, tablet and computer-friendly interfaces. Growers were able to

submit required field information interactively to the TRI calculator and received a prompt response with the TRI value for their field (low, moderate or high risk) and recommendations on how to minimize TSWV risk. The TRI for the monitored fields in SJC was moderate, and this was consistent with observed disease levels. The IPM program for thrips and TSWV developed through this project can provide effective disease management, particularly if implemented on a regional level. We will continue to encourage the use of the grower-friendly thrips degree-day model and risk index, and publicize the IPM program for thrips and TSWV management.

Objectives

The objectives of this project in 2014 were to 1) conduct surveys of selected processing tomato fields in SJC to gain insight into when and from where thrips and TSWV enter into fields and to use this data to assess the reliability of our degree-day model to predict the appearance of thrips populations in 2014, 2) identify potential TSWV inoculum sources for processing tomatoes, 3) complete experiments assessing the role of soil-emerging adult thrips as an early season inoculum source for TSWV, 4) refine and validate a thrips phenology (degree-day) model and a TSWV risk assessment index, and 5) continue to develop and assess the IPM program for thrips and TSWV in the Central Valley.

Information on Materials and Methods can be found in our CTRI proposal for 2014 and are available upon request.

RESULTS

Field Monitoring: Our monitoring efforts for thrips and TSWV were initiated in selected processing tomato fields beginning 17 March 2014 in SJC. We monitored 5 processing tomato fields. All were established with transplants except one direct-seeded field in the Byron area. Table 1 lists the monitored fields and indicates the final TSWV incidence and calculated TSWV Risk Index (TRI) for each field.

Thrips populations determined on yellow sticky cards: In 2014, the build-up of thrips populations started in mid-March (200-350 thrips/card/two weeks), and populations rapidly increased in early-mid April in most monitored fields (Fig. 1). By early-May, large populations were present in all fields (>1000 thrips/card/two weeks). The build-up of thrips populations started earlier in 2014 (mid-March) than in 2013 (early April; however, a rapid population increase by early- to mid-May was observed in both years (Fig. 2). In 2014, after populations reached high levels in early- to mid-May (>1000 thrips/card/two weeks), populations fluctuated around these levels (900-2500 thrips/card/two week) until late August to early September (Figs. 1 and 2). By mid-October thrips populations had decreased considerably (<500 thrips/card/ two weeks).

In 2013 and 2014, a drop in thrips populations was detected during June-July in all monitored fields in SJC (Figs. 1, 2 and 3). This was not predicted based on our degree-day model; i.e., there was no evidence of a noticeable delay in thrips generations (Fig. 3). It is possible that the decrease in thrips population in June-July reflected the widespread implementation of thrips management (spraying) in monitored fields in early-June.

Key finding: Thrips population dynamics were similar in 2013 and 2014 in SJC and the timing of initial thrips sprays was recommended for early- to mid-April in 2014.

TSWV incidence: In 2014, TSWV was first detected in the direct-seeded field in the Byron area on 3 April (a week earlier than in 2013), but it was not detected until mid-May in the other monitored fields in eastern parts of SJC. As in previous years, TSWV was eventually detected in all monitored fields. The overall incidence of TSWV in the monitored fields was relatively low (<1-4%, Table 1). In two fields, one in Byron and another in Tracy, TSWV was present at higher incidences (7%) in a single corner of each field. Overall, we do not believe that TSWV caused economic losses in any of the monitored fields in 2014.

A number of other viruses were detected in the monitored fields including *Beet curly top virus* (see curly top report), *Alfalfa mosaic virus* (AMV, ~5% incidence), and *Tomato necrotic spot virus* (sporadic and <1%). High populations of aphids early in the season likely facilitated the movement of AMV out of weeds and alfalfa in to the processing tomato fields.

Key finding: Incidence of TSWV in processing tomatoes fields in SJC was low in 2014 and not economically important.

Survey for potential TSWV inoculum sources: We continued our efforts to identify TSWV inoculum sources before, during and after the processing tomato season. We focused our efforts around processing tomato fields that were monitored in SJC, and we collected weeds from these areas in the winter and spring before tomatoes were established.

Bridge crops: Fall crops (e.g., lettuce and radicchio) were not grown in the surveyed areas in SJC during fall/winter seasons in 2014. However, in 2014, spring-planted lettuce and radicchio, potential TSWV bridge crops, were grown in Fresno and Kings Counties. Surveys of these crops revealed low TSWV incidences in lettuce (1-3%), but much higher incidences (40%) in a radicchio field that was resprouting following harvest.

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 tomato growing season were symptomless and tested negative for TSWV (with immunostrips and/or PCR). A small number of weeds including 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 were negative for TSWV infection and this symptom was most likely due to chemical damage.

Key finding: Weeds were infected with TSWV at very low incidences (~2%) and rough-seeded buttercup was again identified as a potentially important TSWV weed host in California.

Assessment of the potential role of the soil-emerging adult thrips as inoculum sources of TSWV for early-planted tomatoes: We compared the emergence rates of viruliferous (virus-carrying) thrips and nonviruliferous thrips by incubating thrips pupae for up to 8 weeks in soil at 4°C. Our results showed that the emergence rate of nonviruliferous thrips declined over time from 50% (1 week), to 10% (5 weeks), to 4% after 8 weeks (Fig. 4). The emergence rate for viruliferous thrips was similar to that of nonviruliferous thrips. Here, the emergence rate declined from 50% (1 week), to 7% (5 weeks), to 3% after 8 weeks (Fig. 4).

Most importantly, we wanted to know whether the emerging viruliferous adult thrips could transmit TSWV to healthy plants. To this end, the viruliferous adult thrips that emerged from soil were tested with a leaf disc assay, and the presence of TSWV in these leaf discs was determined by ELISA. Our results showed that emerging viruliferous adult thrips, regardless of the period of time they were kept at 4°C, efficiently transmitted TSWV as infection was detected in 62-100% of leaf discs.

Thus, thrips pupae survived in soil at a simulated 4°C overwintering condition for up to 8 weeks, although survival decreased considerably after 4 weeks. The rate of emergence of adult thrips was similar for viruliferous and nonviruliferous thrips and, most importantly, **adult thrips emerging from pupae of viruliferous thrips remained viruliferous and efficiently transmitted TSWV.**

Next, we wanted to examine the emergence rate of viruliferous adult thrips under conditions that better approximated overwintering conditions in California. To find out the actual California overwintering temperatures, we looked at weather and soil temperature data from 2001-2010 for Fresno County. Here, we focused on winter temperatures from December to early February, which represents the period of time that thrips pupae would presumably be quiescent in the soil. We found that over this ten year period, the temperature in Fresno soils was consistently ~10°C in these winter months, and fluctuated little between day and night. Thus, we chose 10°C as our experimental temperature in order to mimic the natural overwintering conditions that thrips pupae would endure in California soil during the winter months. In all nine experimental replicates, our results showed that viruliferous adult thrips emerged from pupae maintained in soil at 10°C, with most of the emergence occurring in the third and fourth weeks of incubation (Fig. 5). The emergence rate of adult thrips from pupae of viruliferous thrips ranged from 35-58% with an average of 45% (Fig. 6). The emerging adult thrips were tested to determine if they were viruliferous and could transmit TSWV. Again, the leaf disc assays was used and TSWV infections were determined by ELISA. Our results showed that adult thrips that emerged from soil were viruliferous and efficiently transmitted TSWV (67-100%). In conclusion, up to 45% of the thrips pupae survived in soil at temperatures approximating overwintering conditions (10°C) in California, and emerging adult thrips were viruliferous and transmitted TSWV at high efficiency.

Key finding: Viruliferous adult thrips emerging from soil efficiently transmit TSWV and are likely to serve as a source of TSWV inoculum early in the season. This could explain how a single TSWV-infected plant could appear in the middle of a field early in the growing season.

Refinement and validation of the degree-day model and the risk index for thrips and tomato spotted wilt disease

In 2014, we ran thrips population projections for all 6 locations, i.e., Fresno, Kings, Merced, Yolo/Colusa, and the Western and Eastern SJC. The web site for the phenology (degree-day) model ran through November in 2014 for Fresno and through August for Kings, Merced, Western and Eastern San Joaquin and northern counties. We also regularly updated the web page to provide thrips population projections for each area. In 2014, real-time thrips population dynamics (from yellow sticky card counts) and phenology model projections were compared side by side for the SJC fields. In general, the phenology model continued to be ~80% accurate based upon correlating the projections of new generations with thrips population increases on yellow sticky cards in monitored fields (Fig. 3). In 2014, the model predicted 5 adult thrips generations through August. For example, in Western SJC, the generations were predicted for 12 March, 26 April, 26 May, 21 June and 14 July (Fig. 3). In 2014, the first generation was predicted to occur about three weeks earlier than in 2013 (5 April). The prediction for first generation in 2014 (12 March) coincided with the time that thrips population build-up was detected on yellow sticky cards in monitored fields (17 March), and was fully consistent with our observations that build-up of thrips populations in 2014 was earlier than in 2013 in SJC (Fig. 1, 2 and 3). Additionally, in 2014, we started tracking the usage of phenology model web site by following hits (visitor counts). The substantial increase in number of hits for these pages is evidence that more growers and PCAs are using our phenology model (Fig. 7).

In 2014, the TSWV risk index (TRI) calculator was available on the web for growers with smartphone, tablet and computer friendly interfaces. Growers would submit required field information interactively to the TRI calculator and then receive a prompt response from us with a TRI value for their field (low, moderate and high risk) and recommendations for TSWV management. After harvest of our monitored fields in SJC, we obtained all data needed to calculate the TRI for each field. We then correlated the calculated TRIs for these fields with actual TSWV incidences determined through our survey. All of our monitored fields in SJC in 2014 were given a moderate TRI (Table 1). TSWV incidences in these monitored fields were <1-4%, consistent with the predicted moderate TSWV risk by the TRI. We are now confident that the TRI is reliable and can be used to accurately predict the potential for TSWV in grower fields. We will make the TRI available for growers in the 2015 growing season.

Key finding: The thrips phenology (degree-day) model and TSWV TRI are useful tools that growers can use to help implement the IPM program for thrips and TSWV management in the Central Valley of California.

'Final' IPM program for thrips and TSWV in processing tomatoes in the Central Valley of California

By putting together all the information generated in this project, we have developed the following comprehensive IPM program for TSWV and thrips in processing tomatoes in the Central Valley of California. We believe that implementation of all or parts of this package allows for effective management of TSWV, and that this has been reflected in overall reduction of TSWV to manageable levels over the past 5 years. Clearly, the increasing availability of resistant varieties has provided a key tool for this program, but it is important to remember that this resistance can be overcome by resistance-breaking strains of the virus or high inoculum pressure with non-resistance breaking strains. Thus, growers should implement the complete IPM program to minimize TSWV outbreaks in resistant varieties.

Thrips/TSWV IPM Package

I- Before planting

i) determine the risk index for the field and plan your needs for TSWV management accordingly
ii) 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 or late planting dates).
iii) use TSWV- and thrips-free transplants
iv) plant TSWV resistant varieties (possessing the *Sw-5* gene)-these are now widely available, but may not be necessary if other practices are followed. Varieties without the *Sw-5* gene can also vary in susceptibility. Resistant cultivars should definitely be planted in hot-spot areas or in late-planted fields that will be established near early-planted fields in which TSWV infections have already been identified.
v) implement weed management-maintain weed control in and around tomato fields and especially in fallow fields and orchards, as some weeds are TSWV hosts, such as prickly lettuce, rough-seeded buttercup and sowthistle. If weeds are allowed to grow in

fallow fields, they can amplify thrips and TSWV and serve as inoculum sources for processing tomatoes.

II- 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 when thrips populations begin to increase (typically late March-early-mid-April).
iii) rotate insecticides to minimize development of insecticide resistance in thrips.
iv) monitor fields for TSWV and remove (rogue) infected plants early in development (up to 30-40 days after transplanting)
v) implement weed management-maintain effective weed control in and around tomato fields.

III- After harvest
i) promptly remove and destroy plants after harvest (typically done during mechanical harvest)
ii) avoid planting bridge crops that are thrips/TSWV reservoirs or monitor for and control thrips and TSWV in these crops (e.g., radicchio)
iii) control weeds/volunteers in fallow fields, non-cropped or idle land near next years tomato fields

Table 1. List of monitored processing tomato fields in San Joaquin and Contra Costa Counties in 2014: their locations, TSWV incidence and TSWV Risk Index (TRI) values.

Monitored Fields in 2014			
Field	**Location**	**TSWV %**	**TRI**
TR	Processing tomato on W Linne Rd, Tracy	3%	moderate
HM*	Processing tomato on Hoffman Ln, Byron	4%	moderate
MP	Processing tomato on E Mariposa Rd, Linden	1%	moderate
BD	Processing tomato on S Bird Rd, Tracy	4%	moderate
BR	Processing tomato on Levee Rd, Thorntorn	3%	moderate

* Direct-seeded processing tomato field

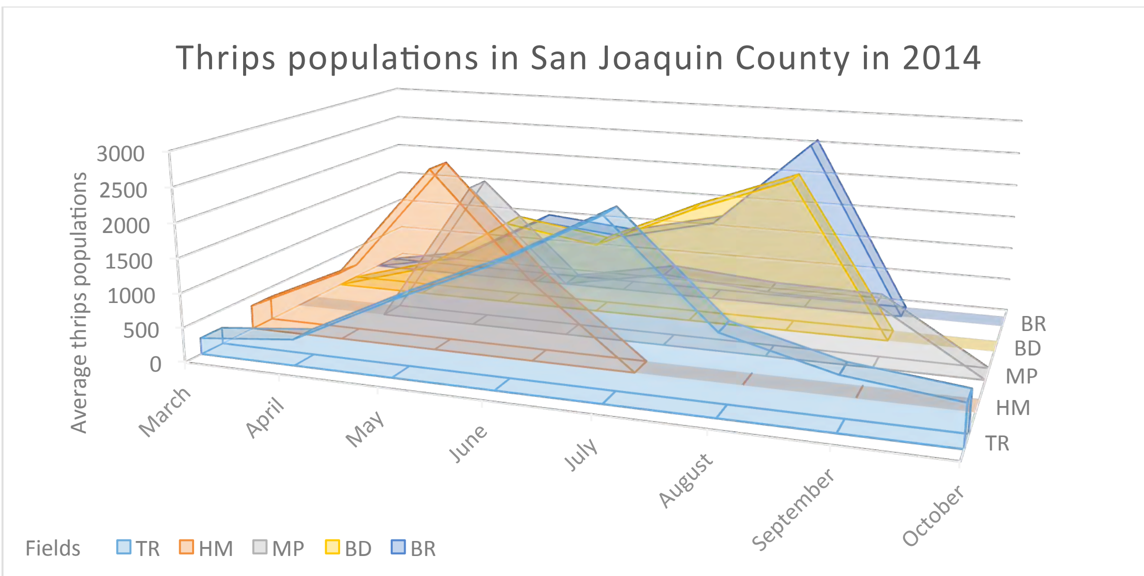

Fig. 1. Average thrips counts per yellow sticky card in monitored fields in San Joaquin County in 2014.

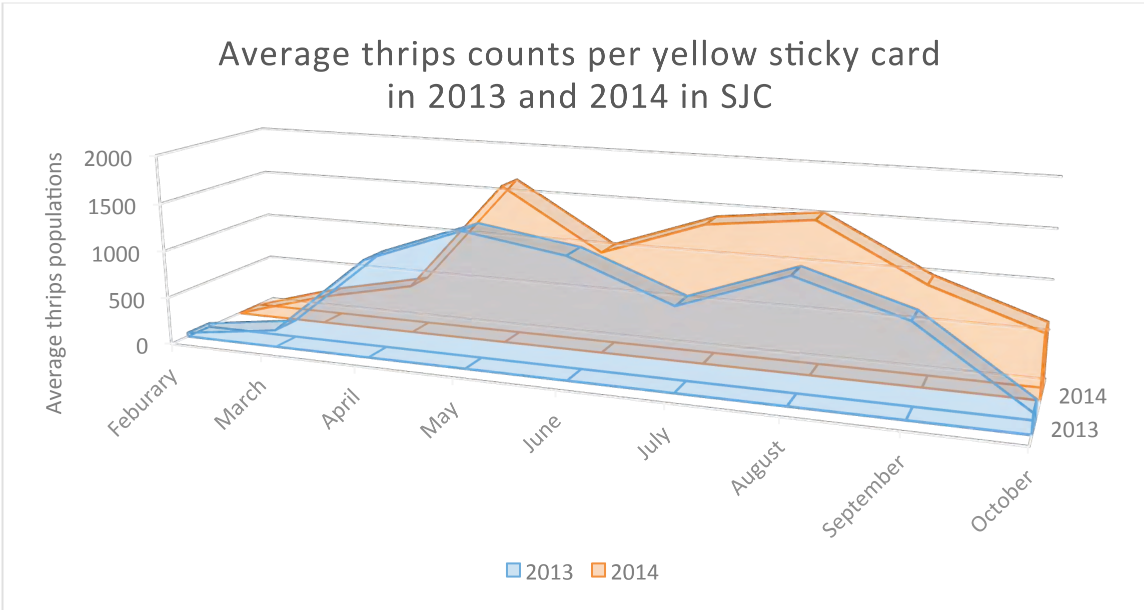

Fig. 2. Average thrips counts per yellow sticky card in monitored fields in 2013 and 2014 in San Joaquin County.

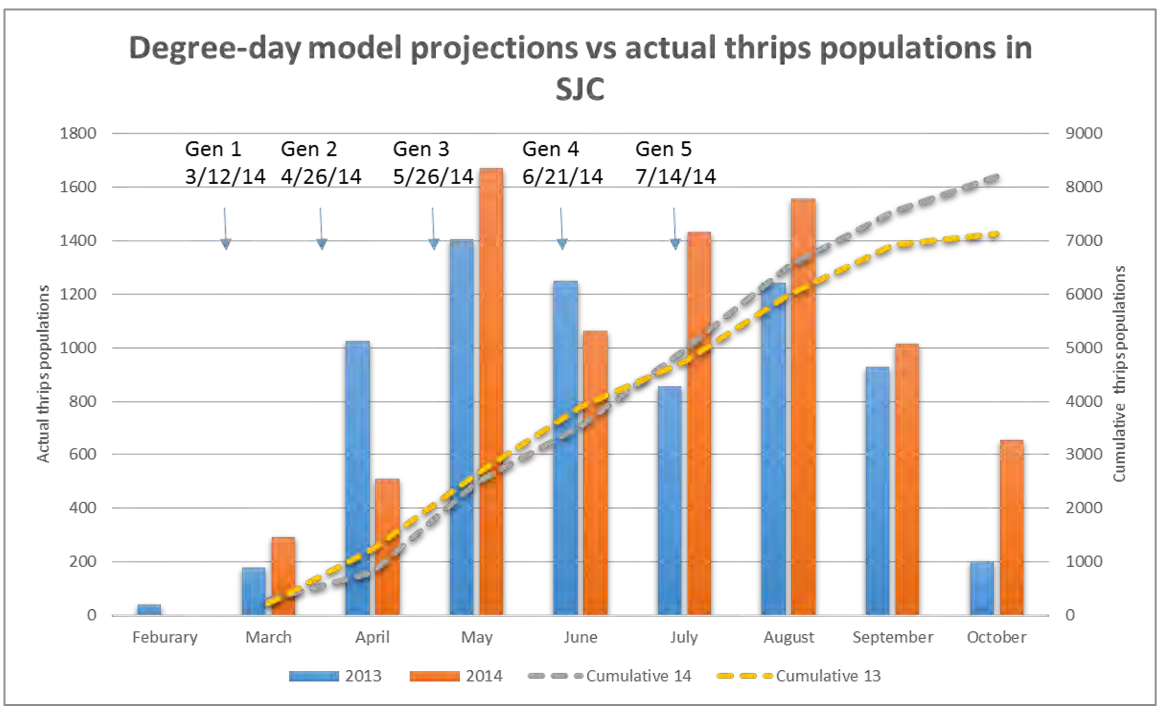

Fig. 3. Comparison of degree day model's projections with actual average thrips counts that were determined from yellow sticky cards in monitored fields in San Joaquin County in 2013 and 2014. Note that unexpected thrips population decrease 'drop' in fields in June-July in both years. Gen1-5= model's projected adult thrips generations and their dates.

Table 2. Results of survey of weeds in San Joaquin County 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

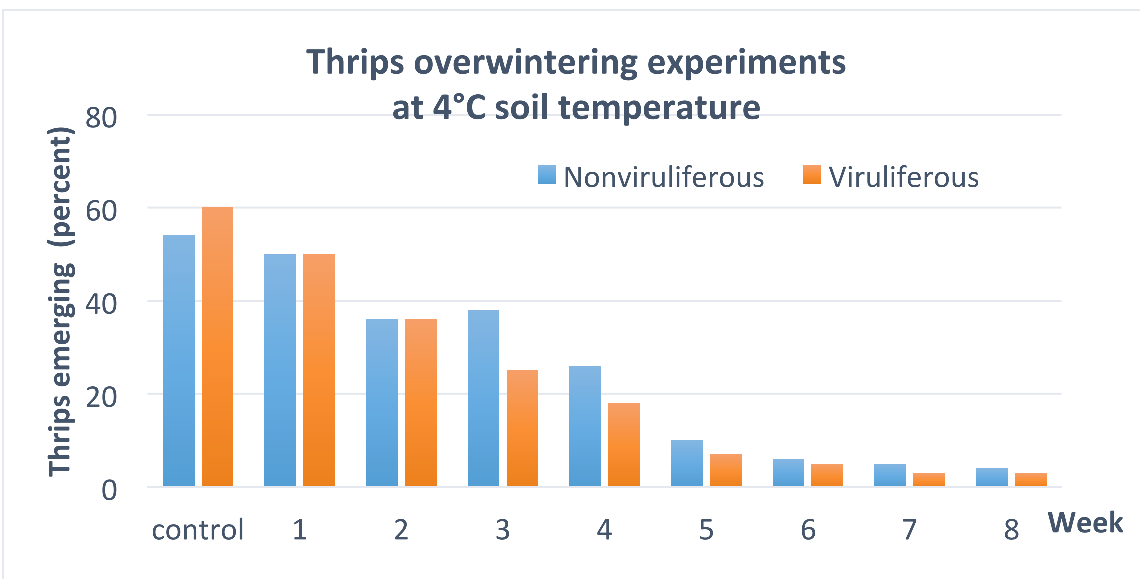

Fig. 4. Percentage of adult thrips emergence from pupae that underwent overwintering experiments that were conducted at 4°C soil temperature.

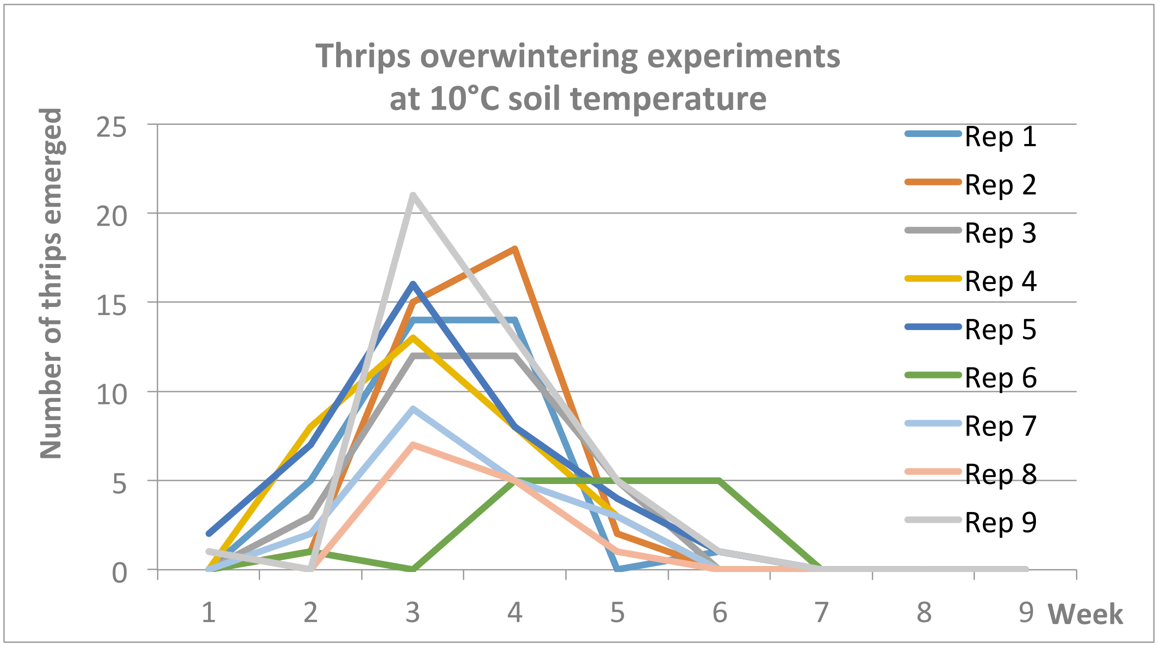

Fig. 5. The dynamics and time course of viruliferous adult thrips emerging from pupae in overwintering experiments conducted at a 10°C soil-temperature. Rep1-9: individual replicate numbers

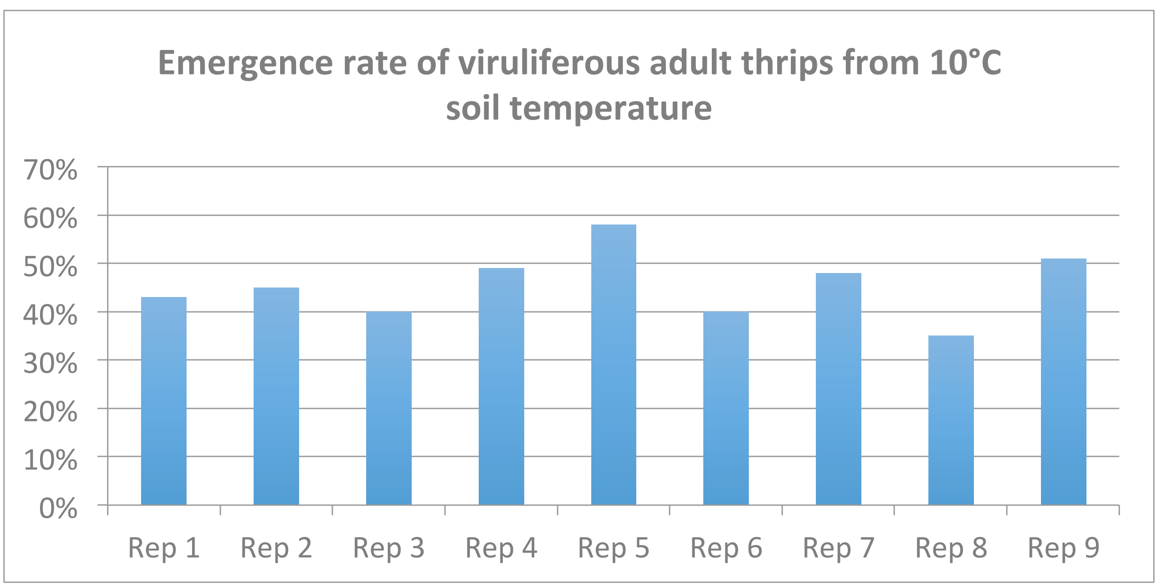

Fig. 6. The emergence rate of viruliferous adult thrips from overwintering experiments that were conducted at 10°C soil-temperature. Rep1-9: individual replicate numbers

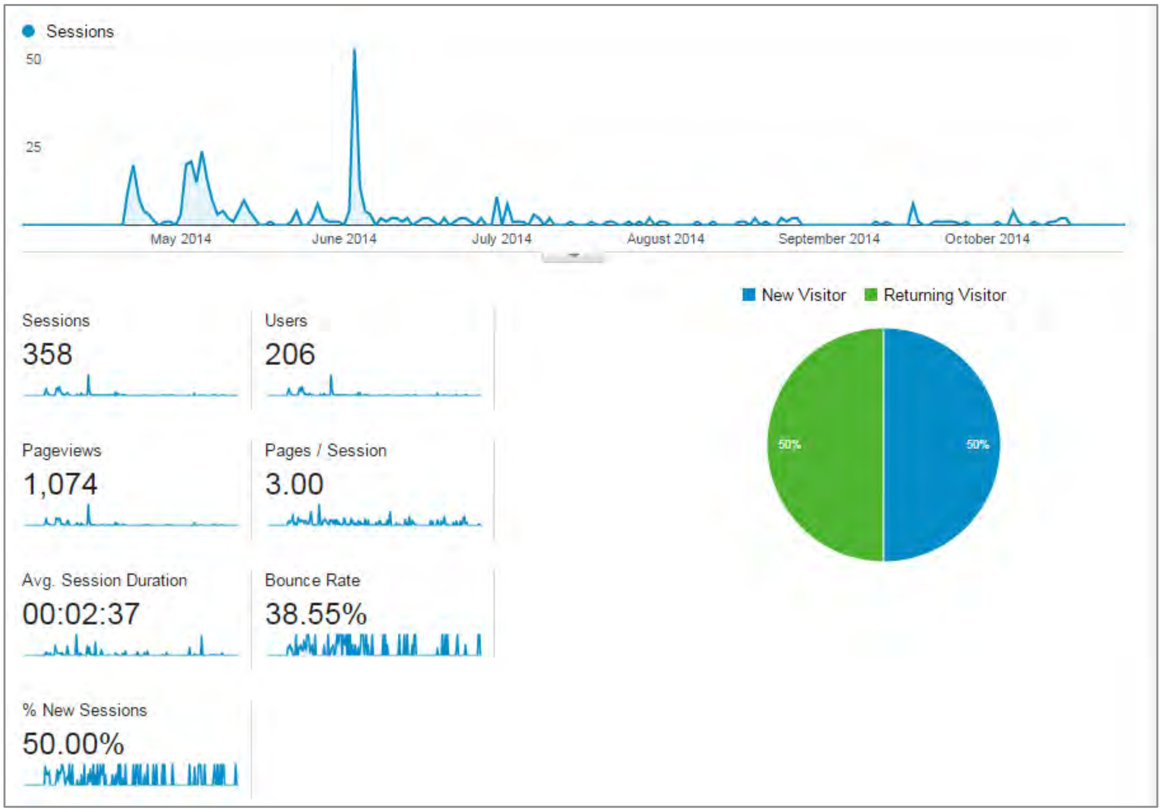

Fig. 7. The usage statistics of our web site for the phenology model as was followed by visitor counts. Note that over 50% of the users were identified as new users.