Research Abstract for the
California Leafy Greens Research Board
April 2009 to March 2010

Project Title: Investigation of Tospovirus Outbreaks in California Lettuce

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Summary

Tospoviruses are plant-infecting viruses that are vectored by thrips. These viruses are extremely important because they are widespread throughout the world, are notoriously difficult to manage, and can cause significant losses for many crops. Two common tospoviruses in California are *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV). Historically, TSWV has been found occasionally in California lettuce but was never a significant economic factor; INSV had not been reported on lettuce in California. However, beginning in 2006 and continuing through 2009, significant and damaging cases of INSV were found on numerous romaine, greenleaf, redleaf, butter, and iceberg plantings in Monterey and San Benito counties. TSWV was also increasing in incidence in coastal lettuce. In the San Joaquin Valley, thus far only TSWV infection has been confirmed on lettuce.

Molecular evidence indicates that the coastal INSV outbreaks are caused by a typical strain of INSV that does not appear to differ significantly from INSV strains previously characterized from ornamental or other hosts. Therefore, the lettuce INSV problem is not caused by a novel INSV strain or new tospovirus. A two year survey indicated that the vast majority of thrips present in diseased lettuce fields are western flower thrips (WFT). An RT-PCR assay was developed by the Gilbertson lab that allows for detection of INSV in thrips. This assay could be a useful tool for monitoring for the virus. Field surveys conducted in 2007 and 2008 failed to reveal a widespread weed or alternate host candidate that could act as a reservoir and source of INSV. However, the summer 2009 and winter 2010 surveys revealed that cheeseweed (*Malva parviflora*) and shepherd's purse (*Capsella bursa-pastoris*) weeds were widely infected; such weeds were collected on ranches having a history of INSV outbreaks. It is notable that infected weeds appear symptomless and therefore do not give visual indications of being reservoirs of INSV.

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Introduction:

Tospoviruses are plant-infecting viruses that are vectored by thrips. These viruses are extremely important because they are widespread throughout the world, are notoriously difficult to manage, and can cause significant losses for many crops. Perhaps the two most familiar tospoviruses encountered in California are *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV). TSWV is common throughout California, can infect a great number of plants (several hundred vegetables, ornamentals, and weeds), and is found throughout the state. INSV also infects many plants but is more often found infecting ornamental and not vegetable crops.

Historically, TSWV has been found occasionally in California lettuce but was never a significant economic factor. Prior to 2006, INSV had not been reported as a pathogen on lettuce. However, in 2006 in coastal California we confirmed INSV on lettuce for the first time. The disease caused significant losses in some fields in 2007 and 2008. In 2009 the disease occurred again in the Salinas Valley, though problems did not become evident until later (August) in the growing season and disease incidence was lower. During these past few years, TSWV continues to be a rare occurrence on the coast. However, TSWV occurs in varying degrees in Huron and other regions in Fresno County.

Objectives:

1. Monitor lettuce fields to follow INSV and TSWV disease development.

2. Identify and quantify thrips vectors in lettuce/Test thrips for presence of INSV.

3. Search for alternate hosts and sources of INSV inoculum.

Procedures:

1. Monitor lettuce fields: In 2009 we monitored the Salinas Valley for INSV outbreaks on lettuce. Monitoring consisted of bi-weekly surveys on ranches that have a history of INSV problems. We also kept in communication with growers and pest control advisors who have encountered INSV in the past. Symptomatic samples were tested with the immunostrip quick test.

For selected fields we surveyed disease incidence by assessing the number of symptomatic plants in 5 locations: the four corners and the center of the field. At each location, the number of plants with tospovirus symptoms was visually determined in randomly chosen planted strips of 200 feet long by 5 beds wide. Overall incidence of tospovirus symptoms was then calculated. Selected plants with tospovirus symptoms were tested for INSV and TSWV by using immunostrip quick tests. Throughout the season, we also provided diagnostic services to growers and pest control advisors by testing submitted samples for INSV and TSWV; these diagnostic samples provided additional information on the extent of the tospovirus outbreak in the coastal area. In the San Joaquin Valley, Tom Turini monitored lettuce fields for TSWV developments. Any tospovirus-like symptoms were tested for TSWV and INSV with immunostrip quick tests.

2. Identify and quantify thrips vectors/ Test thrips for presence of INSV: Following methods developed by Bill Chaney and others, we monitored thrips populations with a leaf washing method (Chaney, 2006). For each field, 25 symptomatic and 25 healthy plants (5 replications of 5 plants each) were cut, bagged, and taken back to the UCCE lab in Salinas. Each plant was cut apart and every leaf held under running water to dislodge all thrips. The thrips were recovered by filtering the wash water through a fine mesh screen. Thrips were removed from the mesh screen and stored in alcohol.

Collected thrips were sorted into adults and juveniles and counted. Representative adult thrips (75 thrips each from healthy and diseased plants) for each field were removed, cleared by soaking overnight in sodium hydroxide, and mounted in Hoyer's solution for examination with a compound microscope. Thrips prepared in this way were identified to species using standard taxonomic features (Hoddle et al. 2004; Hoddle. 2008). Other thrips were maintained in alcohol and sent to the Gilbertson lab for testing for tospoviruses. The thrips were vacuum dried and then the RNA was extracted from the dried thrips with a Qiagen RNAeasy kit. The extracted RNA was used for RT-PCR tests using the INSV N gene-specific primers.

3. Search for alternate hosts: Because INSV disease incidence was low in 2009 there were few

fields available for weed and alternate host surveys. We therefore selected one ranch on which significant INSV was found in 2009. Surveys included weed species found in the diseased lettuce field as well as weeds and other plants from surrounding fields, roadsides, reservoirs, and other weedy sites. Each sample consisted of a composite collection from five plants of one species. Samples were placed in a cooler and transported to the UCCE lab in Salinas. These bulked plant samples were tested using immunostrip quick tests for INSV and TSWV. Representative samples were also sent to the Gilbertson lab for testing with RT-PCR and INSV N gene-specific primers.

Results:

1. Monitor lettuce fields: In contrast to previous seasons when INSV occurred on lettuce as early as April and May, significant INSV was not observed in the Salinas Valley in 2009 until late July and early August. Disease incidence was lower overall, and fewer fields had disease incidence greater than 1%. For our survey, one field in June and two fields in August were evaluated for disease incidence and thrips species. For these three fields, disease incidence ranged from 1 to 5% (Table 1). Plants in these survey fields tested positive for INSV using immunostrip quick tests. TSWV was not detected in these fields.

In the Fresno County lettuce production areas, TSWV was generally higher in fall 2009 but no TSWV was detected in the two fields monitored in spring 2010. In the eight fields monitored in fall 2009, incidence ranged from 0.3 to 5.5 %. Eighty-eight lettuce samples were tested from the monitored fields in fall and all tested negative for INSV and positive for TSWV. In the four Huron-area fields monitored, TSWV incidence in October harvested fields at the time of the last evaluation were 2 and 1.8 %, and November harvested fields reached 5.5 and 5 %. In the Five Points fields, incidence was 2.5 and 2.3% in October and November harvested fields, respectively. Incidence was substantially lower in the monitored fields west of Five Points near Interstate 5 at 0.3 and 1.5 %, with both of these fields harvested in very early November. In addition, samples received from pest control advisors in October (3 samples) and November (5 samples) all were positive for TSWV and negative for INSV. Therefore, it appears that at this time INSV has not yet been confirmed on lettuce in Fresno County.

Table 1. Field survey for INSV disease incidence and thrips populations in the Salinas Valley						
Site / lettuce type			mean number of thrips / 5 plant sample			
location / month	INSV incidence		adult	juvenile	total	% juvenile
EB romaine	0		91	236	327	72
(June, Salinas)	1.00%		92	294	386	76
NIv greenleaf	0		82	0	82	0
(Aug., Gonzales)	5.10%		82	4	86	5
D7A romaine	0		50	1	51	2
(Aug., Gonzales)	2.00%		38	0	38	0

2. Identify and quantify thrips vectors/ Test thrips for presence of INSV: For the three surveyed fields in the Salinas Valley, thrips populations varied greatly (Table 1). For lettuce plants showing symptoms of INSV infection, total thrips recovered ranged from 38 to 386 per 5-plant sample. For healthy plants collected from the same fields the recovered thrips ranged from 51 to 327 per 5-plant sample. The numbers of thrips recovered from healthy and INSV-infected plants did not differ significantly.

Because only the juvenile stage of thrips can acquire tospoviruses, we separated all collected thrips into juvenile and adult stages (Table 1). However, the numbers of juveniles recovered from healthy and INSV-infected plants did not differ significantly. Significant numbers (76 from INSV plants, 72 from healthy plants) of juveniles were collected only from the Salinas romaine that was sampled in June.

For each coastal field, representative thrips from healthy and INSV-infected lettuce samples were examined microscopically to determine species. The majority of the thrips, in all fields and from both healthy and diseased plants, were western flower thrips (WFT) (*Frankliniella occidentalis*) (Table 2). This species made up between 84 and 99% of the recovered thrips. A small number of onion thrips (*Thrips tabaci*) and a few individuals of other species were identified from lettuce (Table 2). There were no significant differences between species identified from healthy or INSV-infected lettuce plants.

Huron area lettuce infected with TSWV was also collected and washed to collect thrips. For these samples, 95% of the thrips were WFT (Table 2).

Table 2. Thrips species from lettuce fields with tospovirus disease.

		Species of thrips recovered from lettuce*		
Site / Month	Lettuce type	West. Flower	Onion	Other**
EB (Salinas)	romaine	84%	13%	3%
June				
Nlv (Gonzales)	greenleaf	93%	4%	3%
August				
D7A (Gonzales)	romaine	99%	0%	1%
August				
TT (Huron)	iceberg	95%	0%	5%
March				

*Western flower thrips = Frankliniella occidentalis; onion thrips = Thrips tabaci.

** Thrips australis, Frankliniella insularis, Frankliniella minuta, Ankothrips sp., Chirothrips sp., Aeolothrips sp.

Testing thrips for infection with INSV by RT-PCR: We collected thrips from INSV-positive and INSV-negative iceberg lettuce, organic lettuce, and INSV-negative alyssum samples in fall 2009. These thrips were tested for INSV by the RT-PCR test. In contrast to the results with the thrips we tested in summer, the thrips that were collected in fall were negative for INSV infection. The finding that the these thrips were negative for INSV when tested by the sensitive RT-PCR test may indicate that a low proportion of the insects were actually infected with the virus, even though some were collected from lettuce known to be infected with INSV. This is also consistent with fact that the overall incidence of INSV was relatively low. Thus, it is not clear whether we can use the RT-PCR test as a predictor of the potential for INSV outbreaks in lettuce.

3. Search for alternate hosts: A priority of this project was to identify the source of INSV and determine where thrips were picking up the pathogen that they subsequently vectored to lettuce in the Salinas Valley. Weed and alternate host surveys in 2007 and 2008 found only a very few INSV-infested weeds (London rocket, silverleaf nightshade) and no other suspect plants or crops. However, more progress was made in this past season.

A 2009 weed survey was conducted around a ranch near Soledad that had a history of INSV outbreaks on lettuce. We collected weeds three times during the summer for a total of 102 samples. Tests indicated that a number of cheeseweed (*Malva parviflora*) and shepherd's purse (*Capsella bursa-pastoris*) weeds were positive for INSV (Table 3).

We returned to this Soledad ranch during the winter (February) of 2010 and conducted another weed survey. During this winter collection, we added a second Soledad ranch that also had a history of INSV in lettuce. A total of 104 samples were collected and cheeseweed and shepherd's

purse again tested positive for INSV (Table 3).

Also in February 2010 we collected six composite samples of cheeseweed plants from a vineyard adjacent to the second Soledad location. Many of the cheeseweed plants tested positive for INSV (Table 3). It is important to note that for all weed collections, INSV-infected cheeseweed and shepherd's purse appeared symptomless.

In addition to testing the plants for infection by INSV using the immunostrip method, a number of weed species also were tested for INSV with the more sensitive RT-PCR test. As shown in Table 4, we confirmed the infection of cheeseweed (*Malva* spp.) and shepherd's purse with INSV and that these were symptomless infections. We also detected INSV infection in 4 other weed species (Table 4) and these were symptomless infections: fiddleneck, pineapple weed, summer mustard, nettleleaf goosefoot.

Therefore, weeds can serve as reservoirs for INSV in the Salinas Valley and it is likely that these weeds, particularly cheeseweed and shepherd's purse, are serving as sources of the INSV outbreaks in lettuce. Unfortunately, these weeds do not show symptoms of INSV infection, so growers and PCAs will not be able to tell if these weeds, if present, are acting as virus reservoirs.

In Fresno County, weed surveys conducted by Tom Turini, to date, have not found INSV in plants in that lettuce producing region. However, weeds in Fresno County have been found to be infected with *Tomato spotted wilt virus*.

Table 3. Salinas Valley weed and alternate host survey in 2009-2010: immunostrip tests

Summer survey			
Location	Timing	Sample size	+Results for INSV
1 ranch with INSV outbreak on lettuce	3 sample dates (summer 2009)	102 weeds	+ cheeseweed + shepherdspurse
Winter survey			
Location	Timing	Sample size	+Results for INSV
2 ranches with summer INSV outbreaks on lettuce	1 sample date (winter 2010)	104 weeds	+ cheeseweed + shepherdspurse
1 vineyard next to field with history of INSV on lettuce	1 sample date (winter 2010)	6 composite samples of cheeseweed only	+ cheeseweed

Table 4. Results of testing weeds collected in the fall 2009 and winter 2010 for the presence of *Impatiens necrotic spot virus* (INSV) by the reverse-transcriptase (RT) PCR test.

Samples	Number tested	Positive for INSV
Burr clover	3	0
Fiddleneck	3	1
Filaree	26	0
Groundsel	2	0
Pineapple weed	2	2
Summer mustard	2	1
London rocket	2	0
Nettle leaf goosefoot	1	1
Sowthistle	1	0
Radish	1	0
Mustard	1	0
Plantain	1	0
Cheeseweed	98	30
Shepherd's purse	46	19

Alternate crop hosts for INSV:

At the UC Cooperative Extension facility in Salinas, Koike operates a diagnostic lab for growers and PCAs. During the past season, submitted basil, fava bean, pepper, and radicchio samples exhibited virus-like symptoms. Tests confirmed that these plants were infected with INSV, which appear to be new reports for California. Previous work by USDA researchers had earlier confirmed spinach as another newly reported host of INSV in the coastal area of California.

In summer 2009, pepper and basil samples were confirmed INSV-positive by RT-PCR. Sequencing results indicate that N gene, which was PCR-amplified from those infected hosts, was 99-100% identical compared to the sequence of lettuce-infecting INSV isolates, 98% identical to INSV from The Netherlands and the US, and 97% identical to an INSV isolate from Japan.

Discussion:

For the past several years, outbreaks of tospovirus diseases have been occurring in California. Lettuce in the coastal region has been affected primarily by INSV, though TSWV is occasionally found infecting lettuce. In the San Joaquin Valley, thus far only TSWV has been confirmed infecting lettuce. Molecular evidence indicates that the coastal INSV outbreaks are caused by a typical strain of INSV that does not appear to differ significantly from INSV strains previously

characterized from ornamental or other hosts. Therefore, the lettuce INSV problem is not caused by a novel INSV strain or new tospovirus. The vast majority of thrips present in diseased lettuce fields are western flower thrips, which is consistent with previous research that indicates WFT is the main vector of INSV and also of TSWV in California. An RT-PCR assay was developed that allows for detection of INSV in thrips. Field surveys in summer 2009 and winter 2010 indicated that cheeseweed and shepherd's purse weeds may be reservoirs of INSV in the coast. Both weeds, when infected, do not show disease symptoms. In coastal California, INSV is being found in additional crop hosts such as basil, fava bean, pepper, radicchio, and spinach. We anticipate that INSV will be a long term concern for lettuce production in the Salinas Valley and that the severity will depend on thrips populations and prevalence of INSV-infected crops and weeds.

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