California Leafy Greens Research Program
Annual Research Report: April 1, 2019 – March 31, 2020

Project Title: Spatial and temporal patterns of Western flower thrips and INSV (Impatiens Necrotic Spot Virus) in lettuce fields in the Salinas Valley and impacts on plant health and productivity

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Cooperating Personnel: PCAs and stakeholders in the Salinas Valley

Abstract
Western flower thrips (*Frankiniella occidentalis*) and *Impatiens necrotic spot virus* (INSV) are current and economically relevant issues in lettuce production on the Central Coast. Since first reported in 2008, research on thrips and INSV has been intermittent. Due to limited chemistries that can effectively manage thrips, and a lack of genetic resistance to INSV in lettuce, there is a need to better understand the biology and ecology of thrips vectors and the epidemiology of INSV, such that the research can facilitate informed decisions in the context of IPM programs. The current study sought to understand, 1) The timing of thrips infestation and INSV infection in commercial romaine lettuce fields to assess the impact on plant health and market quality, 2) Assess the relationship between thrips pressure, feeding damage, and INSV incidence, and 3) The contribution of INSV to Lettuce Drop (Sclerotinia spp.) susceptibility and infection and document the incidence co-infections in romaine lettuce fields.

Objectives
Objective 1: Understand the relationship between thrips, as vectors, and INSV infection. Some of our research questions include: 1) How many thrips are required for INSV infection to occur in lettuce? 2) Does the number of thrips correlate with INSV infection and severity? 3) How much does wind contribute to adult thrips dispersal and observed patterns of INSV incidence? 4) How much does thrips pupae in the soil contribute to observed patterns of INSV incidence in new lettuce fields? To answer these questions, we will perform a combination of thrips and lettuce sampling, in-field severity scoring for INSV, thrips scoring damage, and lab-based diagnostics of INSV in both thrips and plant tissue using ELISA methods.

Objective 2: Monitor INSV symptoms progression through time. We will collect data from three different Romaine lettuce fields at the individual plant level. We would like to answer the question: How long does it take for a plant to show severe INSV symptoms? In our field studies, we will use a series of continuous evaluations that allow us to track the disease progression of individual plants over time to determine disease establishment and progression.

Objective 3: Understand the timing of INSV infection. We will conduct a series of greenhouse studies in which lettuce will be inoculated with INSV at different growth stages. We know from our current data that lettuce could be infected with INSV even before thinning! We would like to know what happens with those lettuces that get the INSV infection earlier in their life stages. Do symptoms of plants infected at early stages worsen over time, resulting in greater yield loss than plants infected at later stages of plant development? Is there a critical point in lettuce development in which thrips and INSV infection can occur, yet not affect the market value of the plant?

Objective 4: Understand the contribution of INSV to Lettuce Drop (Sclerotinia spp.) susceptibility and infection. Ongoing field observation revealed the presence of Lettuce Drop, which in some cases, co-infects lettuce plants with INSV. We would like to better understand the relationship between these two lettuce pathogens. Is INSV weakening the plant, and increasing susceptibility to Sclerotinia infection or vice versa? A series of growth chambers and greenhouse co-inoculation experiments will be conducted to tease apart the effects of these two pathogens on lettuce. These experiments will be complemented with molecular analyses to further confirm and characterize the potential interaction between the two lettuce pathogens.

Procedures

Selection of fields for thrips and INSV evaluations

Two commercial romaine fields on a single ranch in the Salinas Valley were chosen to be evaluated based on high levels of reported INSV incidence in the surrounding areas. The first field (referred to as F1) consisted of 40-in. beds of romaine, while the second field (referred to as F2) consisted of 80-in. beds of romaine. Both fields were ~10 acres in size and grown from August 8 to October 10 of 2019.

Field evaluations for INSV incidence

Twenty-five scouting locations were marked in each field in a format that consisted of 5 transects x 5 scouting locations per transect. At the center of each scouting location, 42 romaine lettuce plants were marked and used for weekly evaluations. In F1, the orientation of lettuce plants per scouting location consisted of 6 seed lines of lettuce across three 40-in. beds and 7 plants per row (42 plants per scouting location X 25 scouting locations = 1,050 plants evaluated) (**Figure 1A**). In F2, the orientation per scouting location consisted of 6 seed lines of lettuce across one 80-in. bed and 7 plants per seed line (42 plants per scouting location X 25 scouting locations = 1,050 plants evaluated) (**Figure 1B**). Each plant was evaluated in the field for INSV symptoms on a weekly basis beginning the week after thinning (week 3), and until the week before harvest (week 9). At week 9, plants in F1 were also evaluated for symptoms of co-infections between INSV and Sclerotinia spp. F1 was not harvested due to elevated INSV and Sclerotinia infections and/or other factors, and therefore week 9 was the last evaluation. For F2, plants were harvested several days after the week 9 evaluations and then evaluated again two days after harvest to assess the impact of INSV and Sclerotinia on market quality. At the end of evaluations, plants were grouped into three categories based on their final outcome: 1) Harvested, 2) Not harvested due to INSV and Sclerotinia mixed infections, and 3) Not harvested

due to INSV and/or other factors, such as plant size and shape, blemishes, food safety concerns, and other disease issues. INSV scores were averaged on a weekly basis to assess the progression of INSV incidence and development.

INSV severity scale for field evaluations

A numeric INSV severity scale ranging from 0-5 was developed to evaluate the initial occurrence and progression of disease symptoms: 0 = No INSV symptoms; 1 = Small and few brown necrotic spots with yellowing of leaves, sometimes stunting of new growth in the heart; 2 = Moderate levels of brown necrotic spots and yellowing of leaves, sometimes stunting of new growth in the heart; 3 = High levels of brown necrotic spots and yellowing occurring in multiple leaves, stunting; 4 = Obvious brown necrotic spots and yellowing occurring in multiple leaves including within the heart, stunting, distortion of leaves; 5 = Plant is dead (**Figure 2A**).

Assessing thrips abundance, feeding damage, and detecting INSV from thrips and lettuce plants using ELISA

Concurrent with the weekly field evaluations for INSV, a single lettuce plant was selected weekly from each transect. Each plant was adjacent to and south of the 42 plants that were evaluated weekly and was representative of INSV severity for the transect. Plants were brought back to the laboratory and evaluated for, 1) number of thrips per plant, 2) thrips damage, 3) ELISA values for INSV from thrips, and 4) ELISA values for INSV from lettuce plants. Every week, 25 new lettuce plants were selected for analysis, and continued for the duration of the field evaluations (week 3-9). For F1, these protocols were implemented at weeks 1 and 2 as well, which took place prior to thinning.

For each sample, lettuce plants were broken down into individual leaves, and thrips were counted and collected into a 1.5 mL tube using a paintbrush. To assess thrips feeding damage, the five oldest leaves from each plant were chosen for evaluation. For plants that were younger and did not have five leaves, the entire plant was used for analysis. A numeric scoring system was used to assess thrips feeding damage based on the number of scars present per leaf: 0 = No scars; 1 = 1-25 scars; 2 = 26-50 scars; 3 = 51-75 scars; 4 = 76-100 scars; 5 = >100 scars. The scores for all evaluated leaves were averaged and used as a final score for the sampled plant.

Detection of INSV in lettuce and thrips samples was accomplished using triple antibody sandwich-enzyme-linked immunosorbent assay (TAS-ELISA) with monoclonal antiserum (Agdia, Elkhart, IN). Manufacturer's protocols were followed with sample dilutions of 1:5 in the provided GEB buffer for the lettuce. For thrips samples, insects were processed in batch for each lettuce plant by macerating in a 1.5 mL tube with GEB buffer and a sterile needle and transferring all fragments to the test wells in the ELISA plate. Plates were incubated for 1 hr at room temperature, and absorbance readings were collected at 405 nm using a microplate reader. Samples that had values 2X greater than the negative control (uninfected lettuce and uninfected thrips) were considered positive for INSV.

Results

Documenting the spread and impact of INSV on market quality of romaine

Both fields were adjacent to and near romaine fields on the same ranch that were infected with INSV (estimated ≥25% incidence). In each field, a total of 1,050 romaine plants were marked at week 3 (post-thinning), which were equally distributed across 25 scouting locations spanning the entire ~10-acre field (**Figure 1A-B**). Each week until harvest (week 9), every plant was individually scored for INSV using a symptom severity scale (**Figure 2A**). Weekly evaluations revealed a rapid spread of INSV, likely due to older adjacent lettuce plantings that served as reservoirs for INSV and thrips populations (**Figure 2B**).

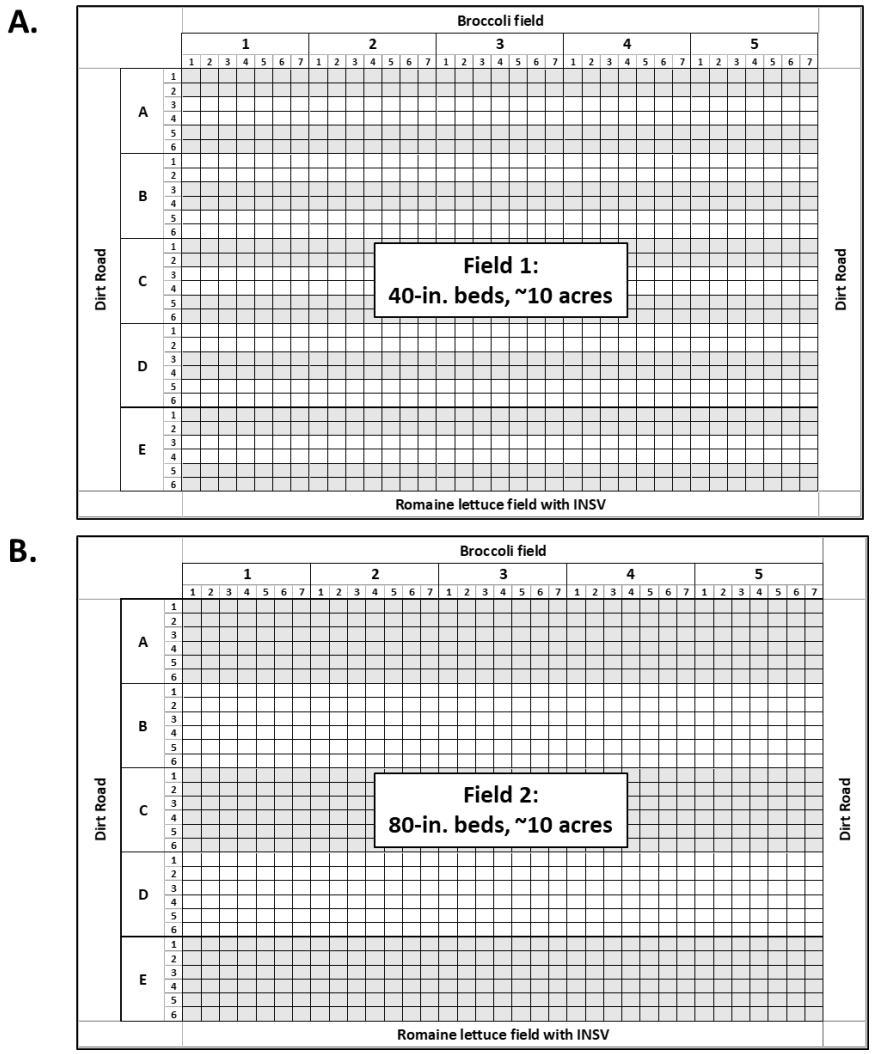


Figure 1. Layout of two romaine lettuce fields evaluated for thrips and INSV. Twenty-five scouting locations were created to monitor thrips and INSV infection (transects labeled from 'A' through 'E', and scouting locations within each transect labeled from 1 through 5). At the center of each scouting location, 42 lettuce plants were marked and scored for INSV severity on a weekly basis. Both fields were adjacent to older lettuce fields that had INSV present.

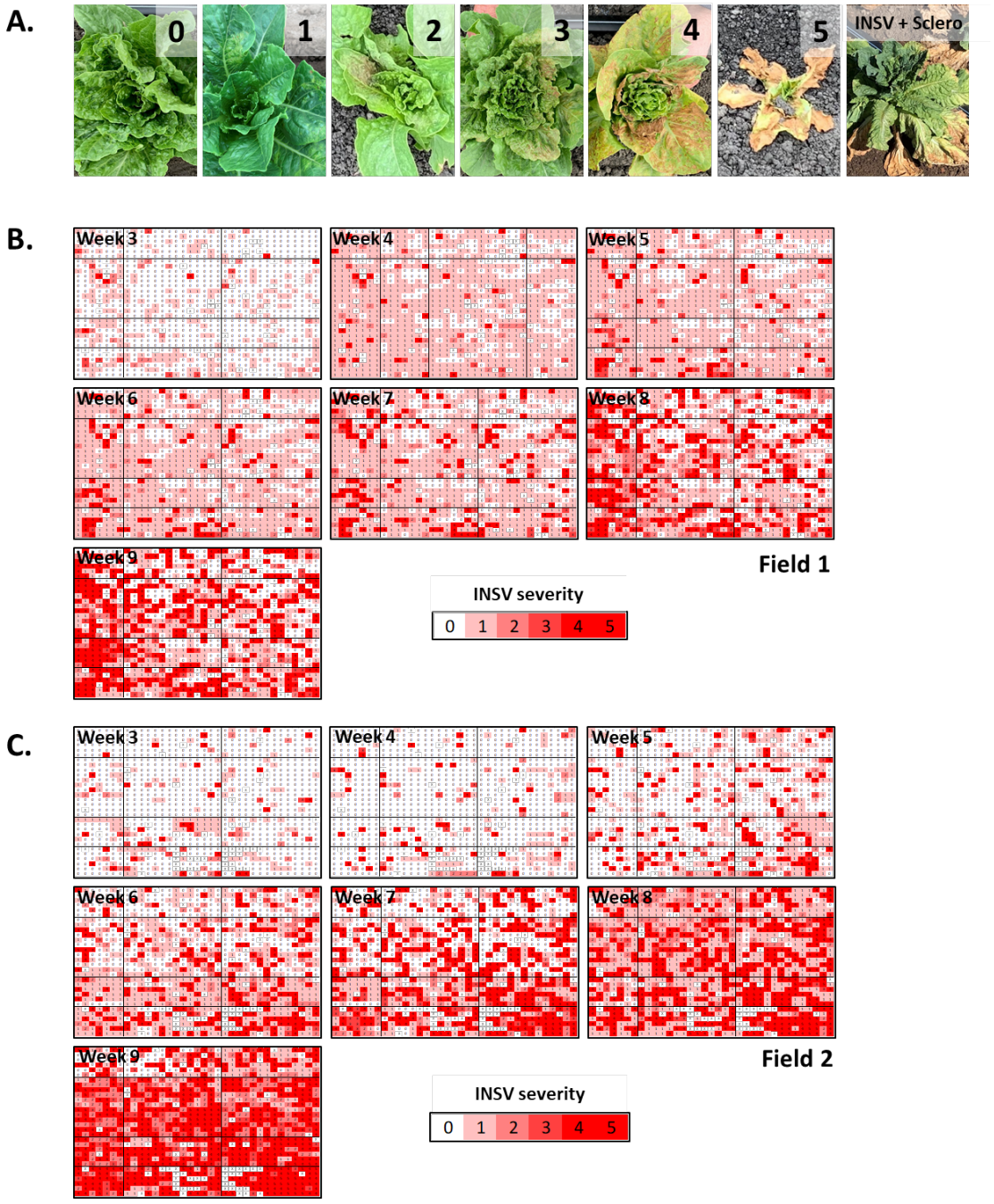


Figure 2. Weekly evaluations of lettuce plants capture the progression of INSV severity in two fields. A) Lettuce plants were evaluated weekly using an INSV severity scale from 0-5. Field 2 was also evaluated two days after harvest to document the presence of Sclerotinia spp. B) Field 1. C) Field 2.

For F1, a total of 1,000 plants were evaluated at week 9, with 37.9% of plants showing symptoms of Sclerotinia and had an average INSV score of 3.41 ± 0.06 (mean ± standard error),

while plants that did not show symptoms of Sclerotinia had an average INSV score of 1.26 ± 0.06 (**Figure 3A**). For F2, a total of 998 plants were evaluated at week 9. Among them, 27.6% of the plants were harvested and had an average INSV rating of 1.54 ± 0.06 at week 9, while 72.3% of the plants were not harvested. A total of 33.8% of the evaluated plants showed symptoms of Sclerotinia and an average INSV rating of 4.0 ± 0.03, while the remaining 38.4% of the plants that were not harvested had INSV scores of 3.47 ± 0.08 (**Figure 3B**).

INSV detection from thrips and lettuce plants using ELISA

During the weeks of field evaluations, lettuce plants were also sampled from each scouting location (25 plants/week) and tested for INSV using ELISA, along with the total number of thrips that were collected from each plant. F1 included sampling that occurred at weeks 1 and 2, which was prior to thinning the field. Weeks 1-3 revealed that 5-20% of lettuce in F1 already had INSV, despite the absence of thrips collected from those plants (**Figure 4A**). However, weeks 4-6 suggest a shift towards increasing thrips populations in the field, as well as an increase in plants that tested positive for INSV. Interestingly, thrips also tested positive for the virus, and in some cases, were collected from plants that tested negative for INSV, suggesting movement and potential transmission of INSV to healthy plants. At week 9, 83% of the plants and their associated thrips tested positive for INSV, with some instances where there were up to 58 thrips per head (**Figure 4A**). The same data was collected for F2 from weeks 3-9. Similar trends were observed, and at week 9, 76% of lettuce and their associated thrips both tested positive for INSV. However, thrips populations remained lower on average (**Figure 4B**). It is worth noting that we were able to detect INSV from a single thrips insect. Considering thrips were processed in batch for this study (rather than individual insects), we are not able to determine the percentage of thrips that were carriers of the virus.

Relationship between thrips populations, feeding damage, and ELISA values

Prior to testing the lettuce plants and thrips for INSV using ELISA (as described above), each plant was also assigned a thrips feeding damage score. The following variables were acquired as a result of this project from a total of 350 lettuce plants collected from F1 and F2: 1) Thrips feeding damage, 2) Number of thrips per lettuce plant, 3) ELISA values for INSV in thrips, and 4) ELISA values for INSV in lettuce. All four variables were correlated with one another, including the number of thrips on lettuce vs. INSV ELISA value in lettuce ($r=0.3049$, $p<0.0001$) and thrips damage on lettuce vs. INSV ELISA value in lettuce ($r=0.3820$, $p<0.0001$). Interestingly, there were numerous examples where lettuce plants tested positive for INSV but had low levels of thrips feeding damage (i.e., <5 scars for every five leaves) (**Figure 5**).

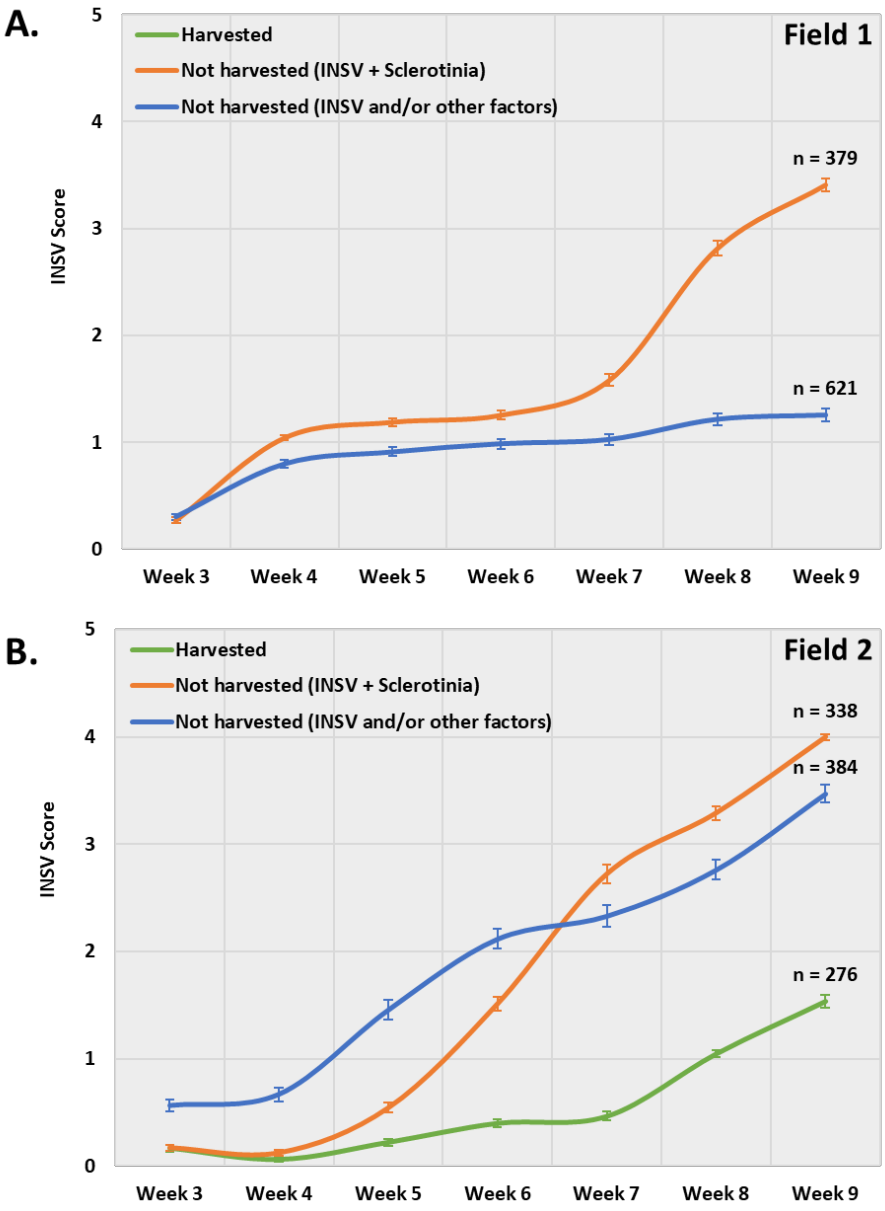


Figure 3. INSV scores for lettuce plants based on their market quality. INSV scores for individual lettuce plants were averaged for each week and grouped by their outcome: Harvested (green); Not harvested and showed symptoms of Sclerotinia infection (orange), Not harvested but did not show signs of Sclerotinia infection (blue). A) Field 1, which was not harvested, B) Field 2. Error bars represent standard error means.

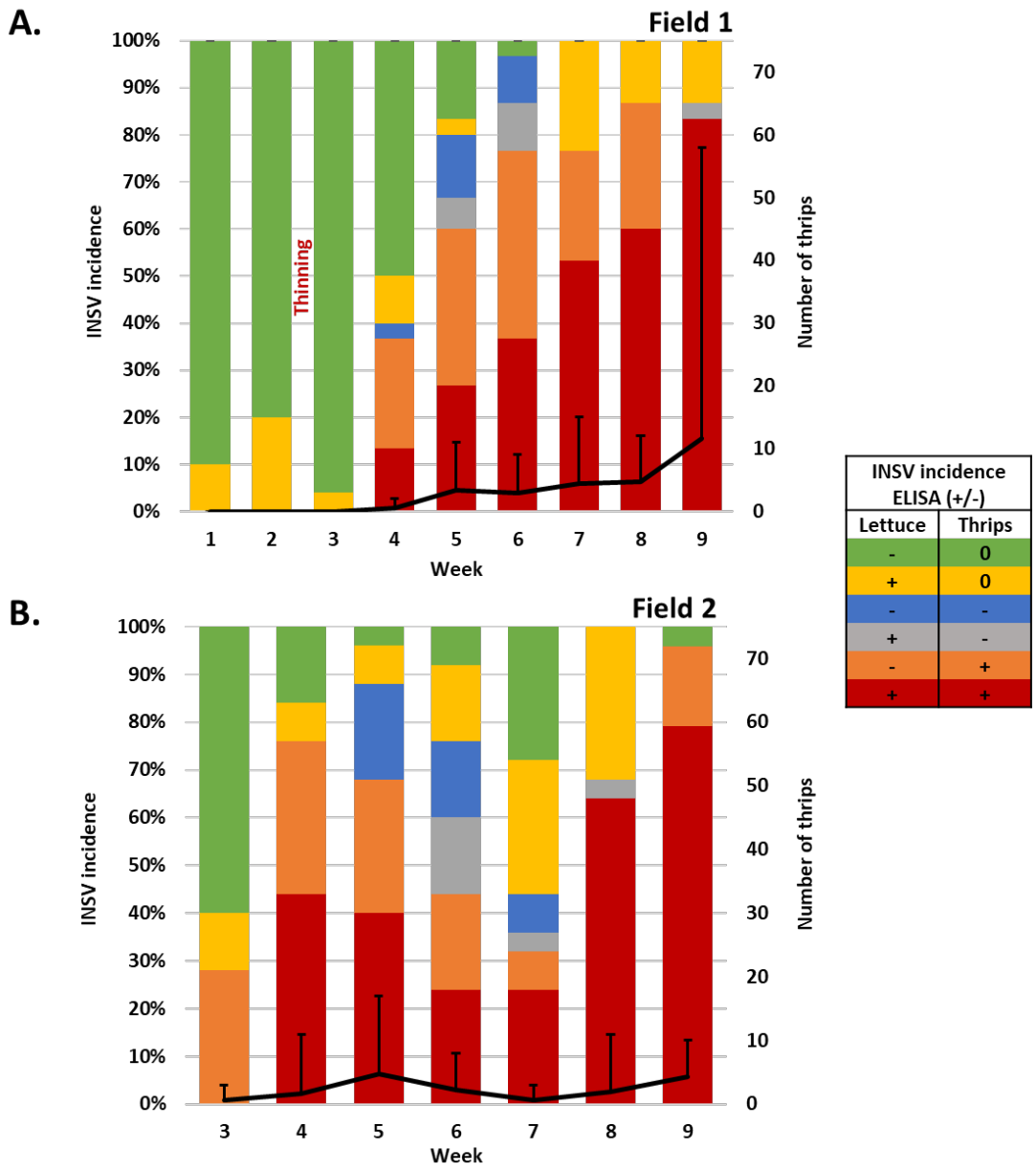
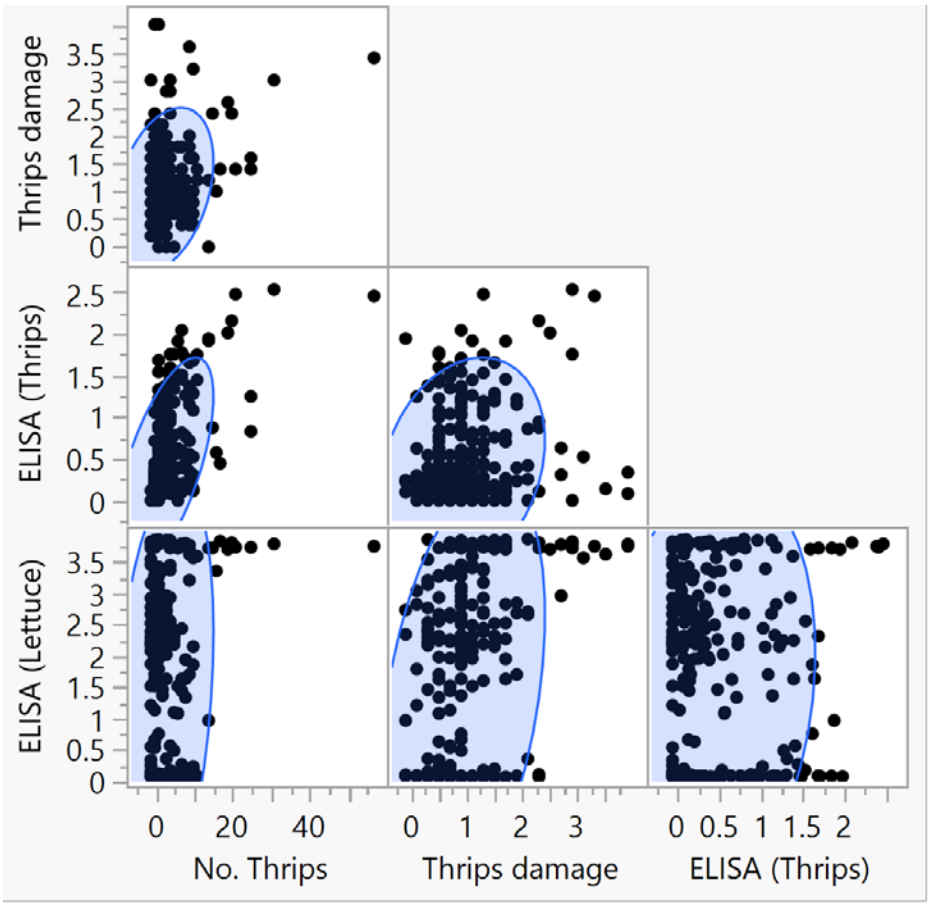


Figure 4. Detection of INSV from lettuce and associated thrips populations. Twenty-five lettuce plants were sampled weekly and tested for INSV using ELISA. Associated thrips from each of the lettuce samples were also counted and screened in batch for the presence of INSV using ELISA. Color bars indicate: Green = Lettuce negative for INSV, no thrips present; Yellow = Lettuce positive for INSV, no thrips present; Blue = Lettuce and thrips negative for INSV; Grey = Lettuce positive for INSV, thrips negative for INSV; Orange = Lettuce negative for INSV, thrips positive for INSV; Red = Lettuce and thrips positive for INSV. A) Field 1, which was assessed beginning at week 1 until week 9; B) Field 2, which was assessed from week 3 until week 9. The number of thrips indicates the mean number collected per lettuce plant per week; standard error bars indicate the upper range.



Correlation (r) values

	No. Thrips	Thrips damage	ELISA (Thrips)	ELISA (Lettuce)
No. Thrips	**1.0000**	0.3462	0.6442	0.3049
Thrips damage	0.3462	**1.0000**	0.2406	0.3820
ELISA (Thrips)	0.6442	0.2406	**1.0000**	0.2132
ELISA (Lettuce)	0.3049	0.3820	0.2132	**1.0000**

n = 350; P = <0.0001

Figure 5. Correlation analysis for four variables related to thrips and INSV. Four variables were analyzed: Number of thrips per lettuce plant, Scores for thrips feeding damage per lettuce plant, ELISA values for INSV in thrips collected from each lettuce plant, and ELISA values for INSV per lettuce plant. A total of 350 plants from Fields 1 and 2 were used for the analysis. Blue ellipses in each panel enclose 95% of the observations; P = <0.0001.

Discussion

The current research documents the rapid spread and development of INSV that can occur by transmission by western flower thrips in romaine lettuce fields and can have a significant impact on production and market quality of lettuce. Both experimental fields were planted towards the end of the planting season in 2019 and were located on the same ranch that reported moderate to high levels of INSV earlier that summer. Our observations revealed that the week prior to harvesting (week 9), over 75% of the lettuce was infected with INSV. In

addition, the thrips that were collected from those plants also tested positive for the virus, which poses an immediate threat to subsequently planted lettuce in adjacent fields. This phenomenon is supported by the data in **Figure 4A**, where INSV was detected in lettuce plants as early as the first week after planting. The data emphasizes the need to consider previous and subsequent lettuce plantings, such that the timing of thrips management should begin during the transition period in which thrips from older fields migrate to newly planted adjacent fields (i.e. after harvesting and disking of fields).

From the two fields that were evaluated, only 27% of lettuce from one field was harvested. Interestingly, those plants had an average INSV rating of 1.54, suggesting that lettuce plants showing mild symptoms of INSV can still possess market quality. Considering that INSV can take up to 10-14 days to develop symptoms in lettuce, it is likely that these plants were infected mid-late season, but due to the lack of strong INSV symptoms, still maintained market value. This data further emphasizes the need for thrips management early in the season to reduce crop loss due to INSV.

We also observed correlations between numerous variables related to thrips and INSV, which included the number of thrips per plant, thrips feeding damage per plant, and levels of INSV found in lettuce and associated thrips. The data suggests that while thrips pressure can be very low (based on the lack of thrips seen on a plant, or the lack of scarring due to feeding damage), INSV can still show strong disease symptoms in lettuce. Future studies will include determining the minimum thresholds for 1) the number of thrips and, 2) feeding times that are required for thrips to transmit INSV to lettuce.

Lastly, we observed that ~30-40% of the unharvested lettuce was co-infected with INSV and Sclerotinia, suggesting that this type of mixed infection had a large impact on the market quality in these fields. Cultures of thrips, INSV, and Sclerotinia have been established, and future studies will explore the interplay between the insect and pathogens to further understand the effects of this type of mixed infection on plant health and quality.

These studies highlight the timing and severity at which INSV can affect lettuce productivity and market quality and suggests that strategies to manage thrips and INSV can be improved by focusing on the timing at which thrips infestations may occur. This is particularly true during transition periods where older fields are being harvested and disked, and adjacent fields are being planted with new lettuce. Furthermore, although INSV infection is correlated with thrips pressure, infection can also occur under circumstances where pressure appears to be low.

Acknowledgements

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