# 2021-2022 CALIFORNIA LEAFY GREENS RESEARCH PROGRAM
# ANNUAL REPORT

**Project Title:** Monitoring and cultural strategies to reduce the impact of thrips-transmitted INSV affecting lettuce on the Central Coast of CA


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**Abstract:**
Western flower thrips (*Frankliniella occidentalis*) is the primary insect vector for impatiens necrotic spot virus (INSV), a virus that caused widespread damage to lettuce in the Salinas Valley from 2019 to 2021. Only limited strategies exist for thrips management and there are no direct methods for managing INSV. Furthermore, the large host range of plant species that can support thrips populations and INSV creates additional management challenges, such that secondary hosts (i.e., weeds and non-lettuce crops) may serve as reservoirs for INSV inoculum, where thrips can potentially acquire the virus and subsequently transmit to neighboring lettuce fields. There is a need to further identify the regional hosts for INSV that builds on previous work we have conducted. Additional efforts to monitor and report on thrips populations are important for making management decisions, while determining if cultural strategies, such as roguing, could be a useful strategy for managing INSV infections in lettuce. Here, we report that thrips populations fluctuated normally at expected times during the 2021 season but have been

unusually higher at the beginning of the 2022 season. We provide further evidence for numerous plant species that can be infected with INSV using RT-PCR and Sanger sequencing methods. Finally, we discuss the results from four roguing trials that were conducted in commercial lettuce fields during the 2020 and 2021 seasons. The studies provide information on the biology of thrips vectors, plant hosts for INSV, and cultural strategies for managing the virus following infection in lettuce fields.

**Objectives:**

1. Valley-wide monitoring of thrips populations.
   *Deliverables:* Provide updates on thrips populations throughout the Salinas Valley via the Salinas Valley Agriculture website.
2. Expanding the host range of INSV in the Salinas Valley.
   *Deliverables:* Continue to identity cultivated and non-cultivated plants that can serve as hosts for INSV.
3. Assessment of roguing as a strategy to mitigate INSV infections in lettuce.
   *Deliverables:* Determine the effects of roguing INSV-infected lettuce as a cultural practice to reduce the incidence of virus infection.

**Procedures:**

Objective 1: Valley-wide monitoring of thrips populations. Double-sided yellow sticky cards were monitored in 21 locations from Castroville to King City. Cards were collected and replaced weekly. In the lab, each card was counted for the number of thrips, summarized, and presented in graphical format, keeping the locations of the traps disclosed. Data was also presented as an average number of thrips for all cards in the Salinas Valley, as well as separated into sub-locations**.** Graphs were sent to UCCE Monterey County to be posted on their website and were presented weekly at the INSV/Pythium Wilt Task Force meetings at the Grower-Shipper Association office.

Objective 2: Expanding the host range of INSV in the Salinas Valley. Weed sampling was conducted on INSV hosts that we previously identified in our top 10 list. Specifically, weeds were sampled from non-lettuce crops that are frequently grown in rotation with lettuce or nearby throughout the Salinas Valley (e.g., artichokes, broccoli, cauliflower, grapes). Additional samples were also tested for INSV based on raised concerns by the industry. For each plant sample, leaves were sampled and grouped together, and included a composite of older and newer leaves, as well as leaves that had discoloration or potential symptomatic areas. Flowers and any thrips present on the leaves were avoided in the sampling. All samples were processed in the lab at USDA, where 0.5 g of tissue from each plant was used to assess its INSV infection status using TAS-ELISA (Agdia). Assays were conducted in 96-well plates and absorbance values were read on a spectrophotometer. Samples that had absorbance values ($OD_{405nm}$) of 2.5X the value of healthy lettuce were considered positive for INSV. Furthermore, during this funding period, we worked towards validating the INSV infection status for several plant hosts using reverse-transcription PCR (RT-PCR), with special focus on species that we previously identified in our top 10 list. Total RNA was extracted from each sample using the RNeasy Plant Mini Kit

(Qiagen, Valencia, CA). RT-PCR was performed with OneStep Ahead RT-PCR Kit (Qiagen) with primers to the N gene of INSV S RNA (Accession KF745140.1; INSV F = CCAAATACTACTTTAACCGCAAGT; INSV R = ACACCCAAGACACAGGATTT). For samples that were infected with INSV, PCR reactions generated a single amplicon at 524 bp in size. PCR amplicons from various samples were also submitted for Sanger sequencing to verify the target region of the INSV genome.

<u>Objective 3: Assessment of roguing as a strategy to mitigate INSV infections in lettuce.</u> Fields of direct-seeded, drip-irrigated, romaine lettuce (planted in 40-inch beds) were identified based on reports of INSV infections from growers and PCAs. Approximately one month after planting, each field was scouted to identify areas exhibiting the highest INSV incidence. The boundaries of four plots were flagged, with each plot consisting of an area that was seven 40-in. beds wide and 30 feet long. A stand count was conducted for each plot, which had approximately the same number of plants exhibiting INSV symptoms. Each plot consisted of ~2,000 plants. Two of the plots receive the rogue treatment, where plants exhibiting INSV symptoms were bagged and removed from the field, while the remaining two plots were not rogued (untreated). Plots were evaluated for INSV symptoms weekly until the field was harvested. Roguing of INSV-infected plants occurred weekly for the first two weeks of the trial. Prior to harvest, a final evaluation was performed to determine the INSV incidence in the treatment plots (rogue treatment = total number of INSV symptomatic plants removed during the trial plus total number of INSV symptomatic plants present at harvest; untreated = total number of INSV symptomatic plants present at harvest). A total of three sticky cards were placed adjacent to each field to assess the number of thrips that entered the field on a weekly basis. The total number of thrips were counted on all three cards and reported as the average number of thrips that entered the field per week. Trials were conducted in a total of four fields: Aug – Oct 2020, May – Jul 2021, Jun – Aug 2021, and Aug – Oct 2021. Within each field, the number of INSV infected plants were averaged between the two plots for each treatment (rogue and untreated). Differences in the total incidence of INSV between the rogue treated and untreated plots in each field was reported with standard error values.

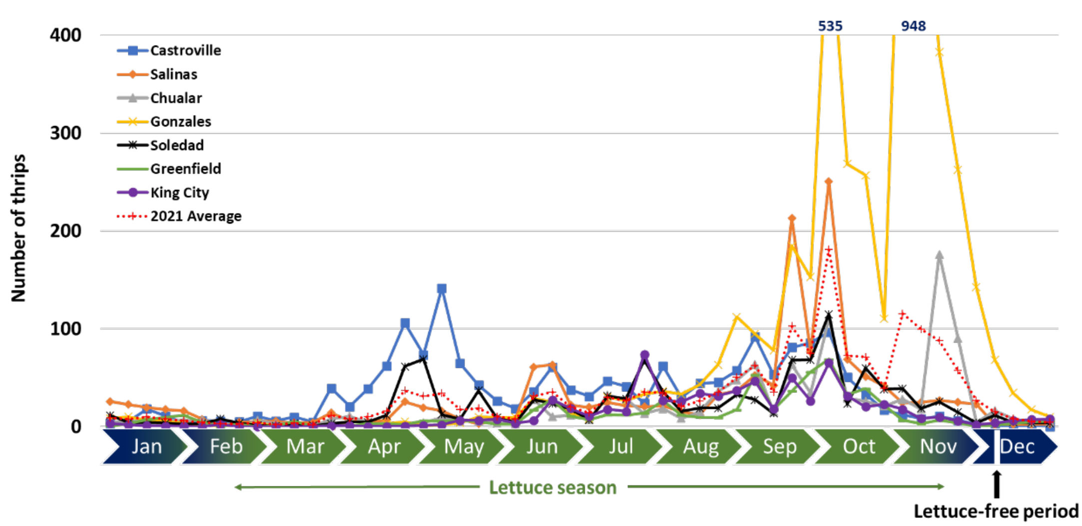
**Results:**

Thrips monitoring using sticky cards revealed similar trends in populations to the 2020 and 2019 season, such that populations increased as the season progressed. In 2021, the highest populations were recorded from September to November, specifically in the Gonzales area, where 948 thrips were recorded during the early part of November (**Figure 1A**). The lowest populations of thrips occurred during the off-season. However, during the early part of 2022, there were unusually high populations of thrips, compared to those recorded in previous years. Higher populations were recorded from all locations during the months of January and February (**Figure 1B and 1C**).

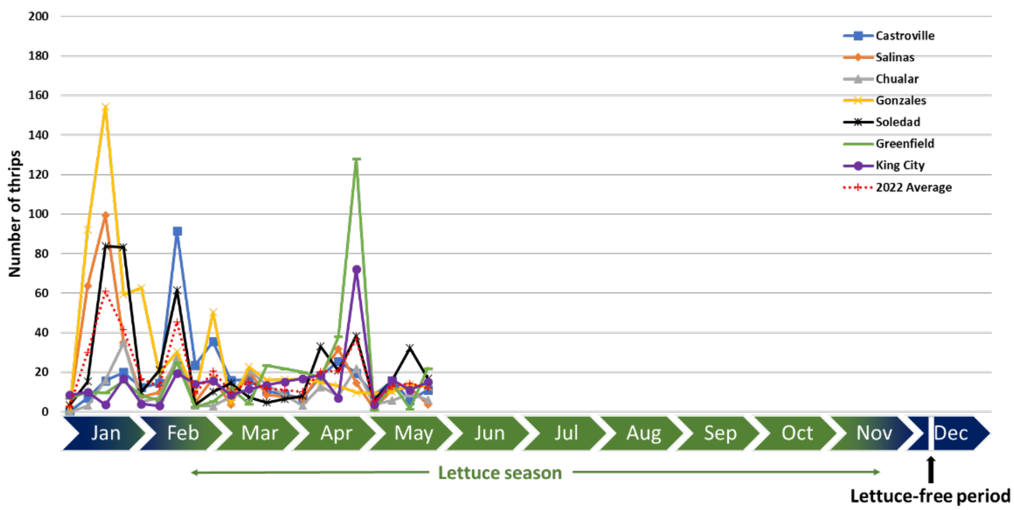
For the INSV host range studies, a total of 13 plant species that were identified as hosts for INSV using the ELISA method were validated using RT-PCR. Eight species from our top 10 list were validated, including annual sowthistle, common lambsquarter, purslane, field bindweed, shepherd’s purse, little mallow, burning nettle, and nettleleaf goosefoot. They were also confirmed using Sanger sequencing. Additional weed species that were validated by RT-PCR, but were not sequenced include curly dock, redroot pigweed, prickly lettuce, shortpod mustard, and knotweed (**Figure 2**). Sampling of weeds from broccoli, cauliflower, and artichoke fields, and vineyards indicated that several of our top 10 hosts, including little mallow, annual sowthistle, nettleleaf goosefoot, and common lambsquarter tested positive for INSV, highlighting the need to manage weeds in non-lettuce crops. Additional sampling identified peas and radicchio that tested positive for INSV using the ELISA method (**Table 1**).

In the roguing studies, a total of four field trials were conducted. All field trials began approximately one month after planting when the INSV incidence was less than 5% within the selected plots of each field. A total of ~8,000 plants were evaluated weekly for INSV symptoms in each of the four fields. The first three trials took place in Aug – Oct 2020, May – Jul 2021, and Jun – Aug 2021, and demonstrated that during the last evaluation, the number of plants showing INSV symptoms was lower in the rogued plots compared to the untreated plots (**Figure 3**). However, when the number of rogued plants were added to the total number of INSV-infected plants, there was not a difference in the overall INSV incidence between the two treatments. In all three fields, the final INSV incidence was 10-20% in the rogued and untreated plots. On average, the number of thrips that were captured on three sticky cards in each field was ~117 to 450 thrips per week (**Figure 3**). In the fourth field trial, which took place from Aug – Oct 2021, INSV incidence was below 5% at the beginning of the trial. During the last evaluation virus incidence had risen to 24% in the untreated plots, while the incidence in the rogued plots were at 14%. When the number of rogued plants were added to the final number of INSV-infected plants in the rogued treatment, the total incidence was 17%, which was still 7% lower than the plots that were not rogued. The number of thrips captured on three sticky cards in the field was ~186 per week (**Figure 3**).

**A**



**B**




**C**

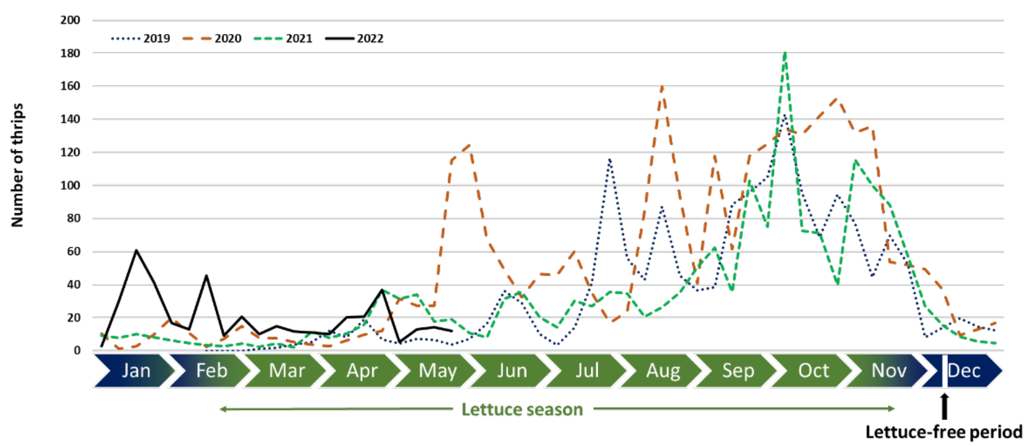



**Figure 1**. Thrips monitoring and reporting during the A) 2021 season, B) 2022 season, and C) average population data for 2019 – 2022. Data represents 21 sticky cards from Castroville to King City that were counted and replaced weekly. Data is reported as the number of thrips per card per week for each city.

**Host INSV Index = Avg $ELISA_{positive}$ x ($N_{positive}$/$N_{total}$)**

| Common name | Avg. ELISA Abs. | INSV positive | Total samples | % INSV | Host INSV Index | Validation: Immunostrip | Validation: RT-PCR | Validation: Seq. (partial) |
|---|---|---|---|---|---|---|---|---|
| Lettuce* | 3.061 | 100 | 100 | 100.0 | 3.061 | ✓ | ✓ | ✓ |
| Chickweed* | 3.210 | 26 | 50 | 52.0 | 1.669 | ✓ | | |
| Hairy fleabane | 1.730 | 26 | 93 | 28.0 | 0.484 | ✓ | | |
| Annual sowthistle | 1.721 | 64 | 295 | 21.7 | 0.373 | ✓ | ✓ | ✓ |
| Common lambsquarter | 1.211 | 15 | 54 | 27.8 | 0.336 | | ✓ | ✓ |
| Purslane | 1.724 | 13 | 75 | 17.3 | 0.299 | ✓ | ✓ | ✓ |
| Curly dock | 1.508 | 9 | 46 | 19.6 | 0.295 | ✓ | ✓ | ✓ |
| Field bindweed | 1.137 | 26 | 118 | 22.0 | 0.251 | ✓ | ✓ | ✓ |
| Shepherd's purse | 2.557 | 11 | 116 | 9.5 | 0.242 | | ✓ | |
| Little mallow | 1.066 | 101 | 544 | 18.6 | 0.198 | ✓ | ✓ | ✓ |
| Mare's tail | 1.720 | 15 | 157 | 9.6 | 0.164 | | | |
| Bristly oxtongue | 1.073 | 8 | 54 | 14.8 | 0.159 | | | |
| Burning nettle | 1.020 | 9 | 59 | 15.3 | 0.156 | | ✓ | ✓ |
| Redroot pigweed | 1.592 | 2 | 30 | 6.7 | 0.106 | | ✓ | |
| Nettleleaf goosefoot | 0.605 | 34 | 214 | 15.9 | 0.096 | ✓ | ✓ | |
| Prickly lettuce | 0.705 | 3 | 48 | 6.3 | 0.044 | ✓ | ✓ | ✓ |
| Shortpod mustard | 1.594 | 10 | 406 | 2.5 | 0.039 | ✓ | ✓ | |
| Swine cress | 0.644 | 1 | 30 | 3.3 | 0.021 | | | |
| Artichoke | 0.261 | 1 | 30 | 3.3 | 0.009 | | | |
| Wild arugula | 0.291 | 1 | 39 | 2.6 | 0.007 | | | |
| Bull mallow | 0.946 | 1 | 131 | 0.8 | 0.007 | | | |
| Alkali mallow | 0.249 | 0 | 31 | 0.0 | 0.000 | | | |
| Iceplant | 0.249 | 0 | 91 | 0.0 | 0.000 | | | |
| Field mustard | 0.249 | 0 | 32 | 0.0 | 0.000 | | | |
| Plantain | 0.249 | 0 | 31 | 0.0 | 0.000 | | | |

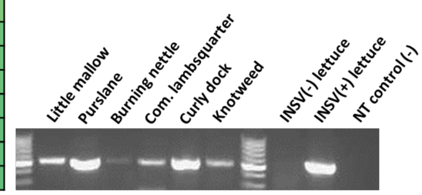



**Figure 2**. RT-PCR validation and Sanger sequencing to confirm the presence of INSV in sampled plant hosts. Using the previous index system that was established during the 2020-2021 season, additional sampling was conducted to validate the presence of INSV. Studies are continuing to validate the hosts that have not yet been tested using RT-PCR. Representative agarose gel is presented (bottom right) to highlight the presence of a specific 524 bp PCR amplicon representing the target region of the INSV genome.

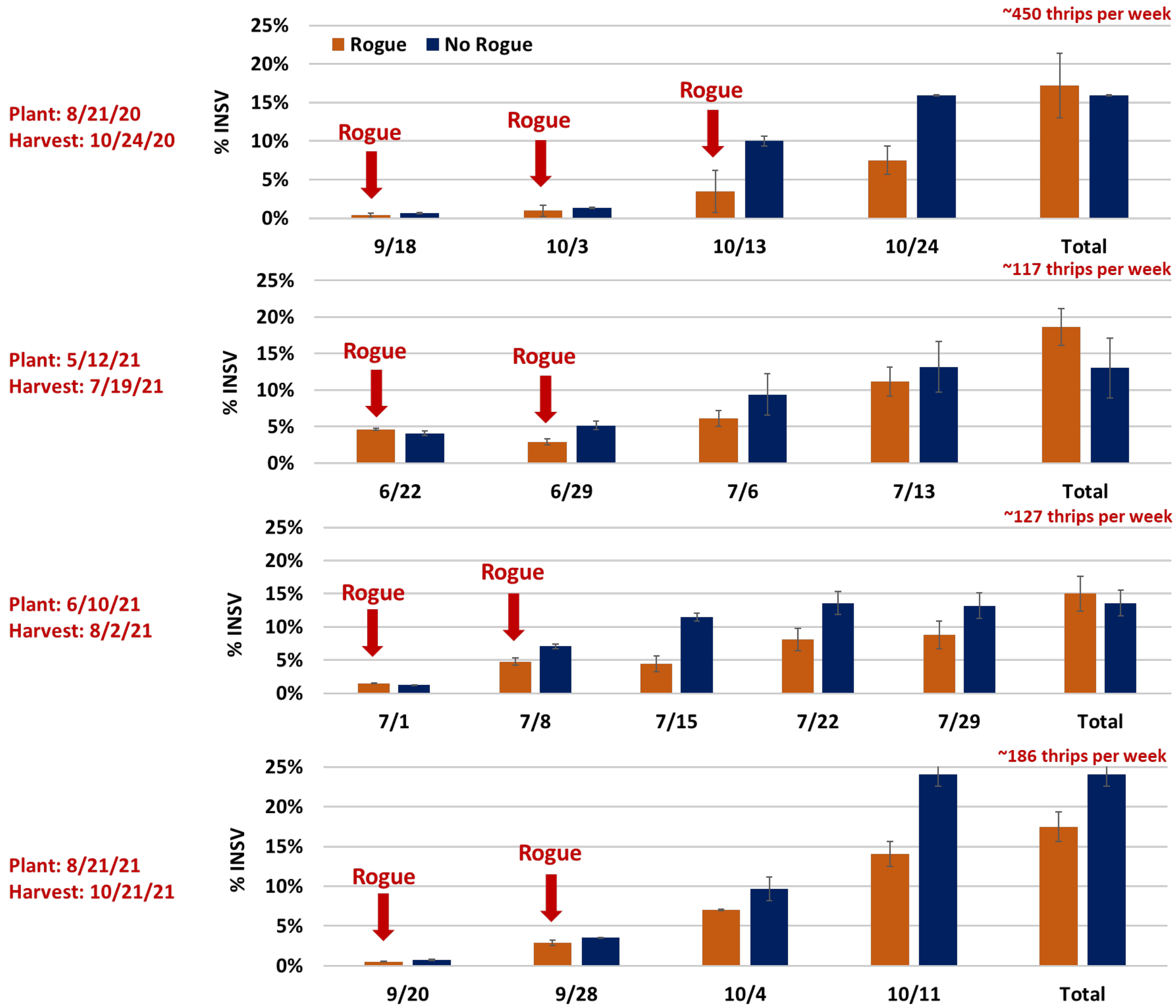



**Figure 3. Roguing trials from four commercial fields of direct-seeded, drip-irrigated, conventional romaine.** In each field, four plots were identified that had similar levels of INSV infection at ~1 month after planting. Each plot was seven 40-in. beds wide by 30 feet long, with two plots receiving the roguing treatment and two plots left untreated. Dates indicate the times that the plots were evaluated and % INSV is the average virus incidence observed between the two plots of the same treatment in each field. The total incidence represents the total number of plants that were infected with INSV at the end of the trial, which included the number of plants that were removed from the field in the rogued treatments. Number of thrips is the total number counted on three sticky cards and averaged across weeks. Cards were placed on the borders of the field and in proximity to the plots that were evaluated.

| TOP 10 HOSTS | INSV positive / Total | % INSV positive |
|---|---|---|
| ***Perennial Artichoke (2 fields)*** | | |
| Common chickweed* | 26/50 | 52% |
| Swinecress | 1/30 | 3% |
| ***Broccoli (2 fields)*** | | |
| Annual sowthistle | 1/20 | 5% |
| Nettleleaf goosefoot | 1/20 | 5% |
| ***Cauliflower (1 field)*** | | |
| Annual sowthistle | 1/20 | 5% |
| Nettleleaf goosefoot | 0/20 | 0% |
| Common lambsquarter | 0/20 | 0% |
| ***Vineyard (1 field)*** | | |
| Little mallow | 17/44 | 38% |
| Annual sowthistle | 23/37 | 62% |
| Nettleleaf goosefoot | 32/42 | 76% |

| Other crops | INSV positive / Total | % INSV positive |
|---|---|---|
| Black mustard | 0/60 | 0% |
| Russian knapweed | 0/56 | 0% |
| Beans | 0/42 | 0% |
| Peas | 8/46 | 17% |
| Sunflower | 1/32 | 3% |
| Radicchio* | 22/22 | 100% |

**Table 1**. Additional sampling of non-lettuce crops and weeds from non-lettuce crop fields during the 2021 season. All samples were tested using the ELISA method.

**Discussion:**

INSV is difficult to manage due to its obligate transmission by small and mobile thrips vectors. Both thrips and INSV can be supported by a large range of host plants, which includes many crops that are grown in the Salinas Valley. Therefore, efforts to improve the management of INSV must focus on monitoring thrips populations year-round, identifying important plant species that can serve as hosts for INSV, and identifying in-field strategies to reduce the spread of the virus. The data presented here addresses all three of these aspects to enhance our understanding and management of thrips and INSV.

Monitoring of thrips during the 2021 lettuce season revealed similar patterns in population fluctuations compared to 2020 and 2019, such that populations increased during summer and warmer times of the year. The increase in populations also coincided with increased INSV reporting and incidence in lettuce crops (GSA, personal communication). This phenomenon further emphasizes the need to manage thrips early in the growing season as populations remain low to minimize the acquisition and transmission of INSV to subsequent crops later in the summer months. During January and February of 2022, thrips populations have been higher than in previous years, possibly due to the warmer temperatures experienced during those months. The higher populations were consistent across all locations, suggesting synchronous emergence and/or dispersal of adult thrips. Future studies to develop phenology models using degree days and developmental characteristics could improve the prediction of thrips emergence patterns, particularly during the cooler winter and spring seasons. Monitoring efforts will continue throughout the 2022 lettuce season, while efforts to improve the efficiency of sharing thrips data with the industry is underway.

We also validated numerous plant species as being hosts for INSV using RT-PCR and Sanger sequencing. This list included 8 out of the top 10 plant species that were identified in last year's surveys using ELISA and is important to ensure that each of the species can be infected by INSV. The different forms of testing plants using serological techniques (ELISA) and genetic techniques (PCR) is equivalent to relevant testing strategies implemented for COVID-19 (i.e., rapid tests and PCR tests). The remaining two plant species that need to be validated by RT-PCR (Mare's tail and hairy fleabane) are in progress.

The current studies also identified INSV-infected weeds that were sampled from artichoke, lettuce, and cauliflower fields, as well as a vineyard. The long crop cycle for broccoli and cauliflower, particularly during the winter months can be problematic if weeds are not managed and may serve as reservoirs for INSV during the lettuce offseason. This can be challenging due to technical and economic limitations in managing weeds in each of the crops as the plants mature. Further efforts to improve awareness of weed management during the winter months and within vineyards were conducted in the form of several articles posted to the Salinas Valley Agriculture Blog with co-authors Richard Smith and Larry Bettiga.

It is important to note that although a plant can test positive for INSV, it must also be a reproductive host for the western flower thrips for virus acquisition and transmission to occur. This is because transmission can only occur if INSV is first acquired during the early larval stages of the thrips life cycle, and therefore, transmission can only occur if eggs are laid into existing INSV-infected tissue, for which the larvae can develop and acquire the virus. These studies are currently underway and supported by a CDFA grant that was awarded in 2021.

Roguing infected plants is a practice that has been described in other cropping systems, including tomatoes that are infected with tomato spotted wilt virus (TSWV) in the Central Valley of CA. However, it is unclear if similar strategies could be beneficial to crops that have shorter growth cycles. The studies conducted occurred in four commercial romaine fields, where plants that were exhibiting INSV symptoms were bagged and removed from the field. The studies demonstrated that in one field, roguing appeared to have an effect in reducing the incidence of INSV by 7% when conducted once a week for two consecutive weeks. However, in 3 of the trials, removing INSV-infected plants did not influence the total incidence of INSV within the field at the time of harvest. For all four trials, the number of thrips that were captured on sticky cards were quite high, suggesting that thrips infestations from outside of the field was consistently high. Under these circumstances, it was difficult to assess the effects that roguing had on secondary spread of INSV within the field, due to the possibility of consistent primary infections occurring from thrips that were migrating into the field. Overall, it is unclear why one of the field trials appeared to have a benefit from roguing, despite the high number of thrips that were migrating into the field. It is likely that individual chemical regimes, timing of applications, and other farming practices could have influenced the overall outcomes of the studies and should be considered in the future.

Understanding the factors that influence thrips and INSV epidemiology is critical to developing a diverse and sustainable integrated pest management (IPM) program. Factors must consider the movement of the vector and the host range for the pathogen across space and time, as well as consideration of production practices that may influence thrips and INSV incidence. The large, diverse, and dynamic cropping culture of the Salinas Valley continues to present a challenging backdrop to understand the epidemiology of the thrips-INSV complex. However, the studies presented here contribute to the diversification of our IPM toolbox to manage thrips and

INSV affecting lettuce production on the Central Coast, and serves as a model for other major lettuce growing regions where INSV has been recently detected, including the desert growing regions of CA and AZ.