Monitoring the vector-mediated movement of INSV into Arizona and secondary distribution

Collaborators:

PI: Stephanie Slinski, University of Arizona, Yuma Center of Excellence for Desert Agriculture
- Dr. Slinski will coordinate the laboratory procedures for detection.

Co-PI: Dr. Samuel Discua, University of Arizona, Department of Entomology
- Dr. Discua will manage the thrips testing and data collection.

Co-PI: John Palumbo, University of Arizona, Cooperative Extension
- Dr. Palumbo will collect thrips from transplants and manage outreach activities.

Background

In March 2021 the tospovirus *Impatiens necrotic spot virus* (INSV) was identified in lettuce fields in the Yuma growing region. The disease was most severe in the Tacna area but was found throughout the growing region. Disease incidence was less than 1% in most fields and did not significantly affect yield. However, if the virus can survive through the summer on non-lettuce hosts, lettuce growers may see disease earlier and higher disease incidence in the upcoming growing season. If the virus were to persist over the summer, weeds would be the primary reservoir. Several weeds commonly found in Arizona are known hosts for INSV including nettleleaf goosefoot (*Chenopodiastrum murale*), lambsquarters (*Chenopodium album*), sow thistle (*Sonchus sp*.), cheeseweed (*Malva parviflora*), and purslane (*Portulaca oleracea*). A survey was conducted in cooperation with Dr. Daniel Hasegawa (USDA-ARS) in 2021 to determine if INSV can be detected in weeds from late spring through the summer. Results from this survey suggested that INSV did not persist through the summer and a new introduction would be necessary for disease to occur in fall 2021.

This survey continued with funding from the AILRC from September 2021 to May 2022. INSV disease incidence was low within fields, on average around 1%, and INSV was not detected in weeds sampled until January. Four thousand weeds were collected for this project with thirty-four positive weeds identified.

Presently the survey is funded by the USDA Specialty Crop Block Grant Program (SCBGP) led by Dr. Samuel Discua. This survey is necessary to understand the dynamics of INSV in the growing region over time. For example, if disease incidence increases, the disease may persist in the environment and not require a new introduction for disease to occur in the fall.

An unfunded objective was added to the INSV monitoring project in September 2021, which resulted in important data on the spread of INSV to Arizona: thrips associated with lettuce and brassica transplants were tested. INSV was detected in thrips in late September and multiple detections occurred in romaine and brassica transplants through October. By early October 2021, infected lettuce could be found in fields of transplanted lettuce. The virus spread to

adjacent direct-seeded fields and INSV-infected lettuce could be found throughout the growing region by early spring.

This work helped to determine that INSV was introduced to Arizona with transplants. The results from the past year highlight the importance of monitoring thrips for INSV to detect and confirm a new introduction and to monitor the spread of the virus.

Objectives:

1. Track the movement of INSV from transplants into Arizona
    a. Thrips will be collected by Dr. Palumbo and analyzed by PCR. Results will be supplied to Dr. Palumbo for reporting.
2. Monitor the spread of viruliferous thrips in the AZ lettuce growing region
    a. Thrips will be collected by Dr. Discua and analyzed by PCR

Methods

Western flower thrips (WFT) were collected using a modified vacuum method that employed a ½ gallon cordless portable vacuum (DeWALT DCV517) fitted with cloth-screened 150 ml containers to capture and retain vacuumed thrips.

Thrips collection from Transplants. On each collection date, samples were collected from individual transplant trays of lettuce, cauliflower, and broccoli located either on tables in the nursery or from trays removed from bins in the field just prior to transplanting. Vacuuming was conducted by making 5 passes across each tray (lengthwise), slowly passing over the tray and contacting the upper 1/3 of the plants during the process. On each sample date, we sampled 160-200 trays of lettuce (40 plants / container from 4-5 locations in the nursery) and 80-120 trays of cauliflower/broccoli (40 plants / container from 2-3 locations).

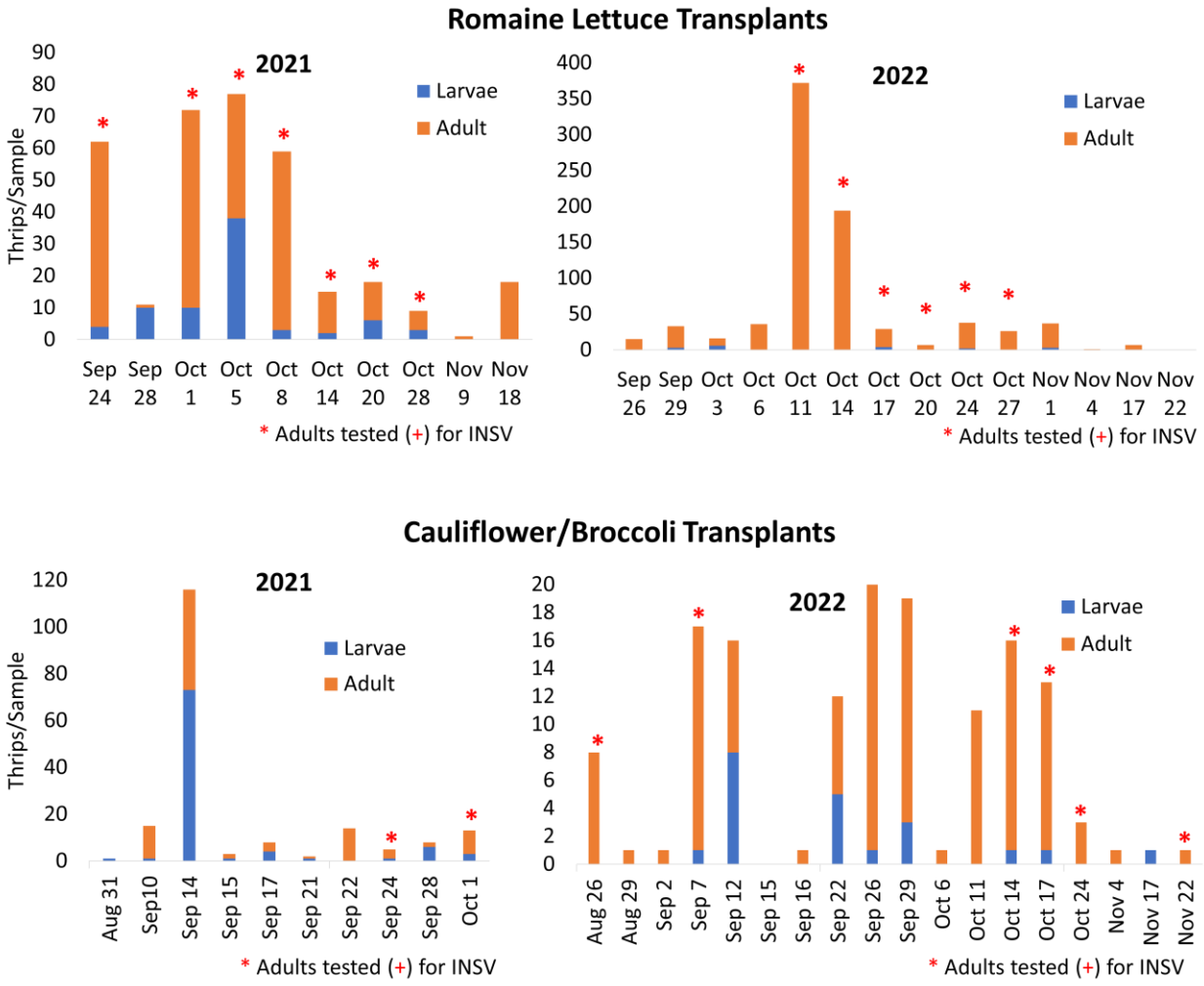
Thrips collection from weeds. On each vacuum sample, 10 separate weeds from each site were randomly sampled by vacuuming the flowers/terminal area of the plants for 3 seconds per plant. Representative weeds to the overall weed population from each site were sampled.

Sample processing. Containers with vacuumed WFT are then placed in ice chest and transported to the laboratory. Once in the lab, containers were placed in a refrigerator for a short period (no more than 8 hrs), after which adults and larvae were removed from vials, counted, and placed in 100 microliters (µl) of DNA/RNA Shield (Zymo Research) and stored in the freezer (-20 °C) until testing for INSV via RT-qPCR.

Results

Thrips collected from transplants. From August 2022 through November 2022, we collected 99 vacuum samples across 22 separate collection events. From 80 vacuum samples containing thrips, a total of 953 thrips (adults = 914, larvae = 39) were tested. Nearly a third of the samples (n = 26), contained INSV positive thrips (total = 641, adults = 615, larvae = 26). The first INSV positive thrips were found on August 26th, 2022, on brassica transplants. We found INSV positive thrips across 12 dates, from August through November (Figure 1).

Figure 1. Thrips collected from transplants originating from Salinas, CA.



Thrips collected from weeds. From August 2022 through May 2023, we sampled over 3,000 weeds and collected 295 vacuum samples over 28 different weekly collection events (dates). From the 242 vacuum samples containing thrips (82% of total), a total of 1,532 thrips (1,209 adults and 332 larvae) were

collected and tested. 120 vacuum samples, 46% of total, (adults = 681, larvae = 195, total = 876) contained INSV positive thrips (Table 1).

All positive thrips were collected from sites with or neighboring INSV infected lettuce (either present or that had INSV infected lettuce recently). INSV positive thrips were collected from 16 weeds/crops: bur clover, common purslane, desert horsepurselane, nettleleaf goosefoot, poverty weed, lesser sea spurrey, lambsquarters, cheeseweed, London rocket, sowthistle, onion, sea purslane, salt heliotrope, knotweed, saltbush (Atriplex), and palmer amaranth. INSV infected weeds were collected for most species that contained INSV infected thrips, except for sea purslane, salt heliotrope, knotweed, saltbush (Atriplex spp.), and palmer amaranth.

Table 1. Number of thrips and INSV status by sample collected from weeds per month.

Year	Month	Thrips collected	Samples tested	INSV Positive Samples	Percent INSV positive samples
2022	August	3	3	0	0%
	September	42	15	0	0%
	October	15	7	2	29%
	November	39	7	6	86%
	December	69	13	8	62%
2023	January	179	21	5	24%
	February	133	14	6	43%
	March	192	43	33	77%
	April	561	70	39	56%
	May	299	50	21	42%

The percentage of INSV samples with positive thrips was 49%. There were positive thrips collected from weeds all months except for August and September (Table 1). The number of samples testing positive for INSV does not indicate the number of positive thrips (or infection status) per sample, just the presence or absence of INSV, therefore the percentage of INSV infected thrips is likely much lower.

However, the percentage of INSV positive samples over time is an important finding because it indicates that INSV was likely not present in the thrips population at the beginning of the produce season. The first INSV infected lettuce collected in the field during the 2022-2023 produce season was found on October 13th, 2022, in the Tacna region on organic lettuce transplants originating from Salinas, CA. The first positive thrips sample collected from weeds was on October 5th, 2022, on a field neighboring the aforementioned INSV infected lettuce transplant field. In contrast, INSV positive thrips were collected on

brassica transplants on Aug 26th, 2022, almost six weeks before INSV symptomatic lettuce was found on the field or INSV positive thrips were collected on weeds.

The results from our thrips transplant sampling are significant because they conclusively showed that INSV entered the desert in fall of 2022 via lettuce and brassica transplants, similar to our 2021 findings. The results from our thrips transplant and weed sampling also support our conclusions from the INSV weed survey that INSV does not bridge the summer gap between seasons on weeds.

Continuation and Dissemination of project results:

- Results from this Project at the University of Arizona Entomology Department Seminar Series on Dec 2nd, 2022, in Tucson, AZ.

- On March 30th, 2023, a poster related to this project was presented at the University of Arizona ALVSCE Research Poster Forum. The poster was titled "Monitoring introduction and movement of Impatiens Necrotic Spot Virus infected thrips in Arizona". Approximately 150 people attended the event. Participants were primarily UA faculty, students, and cooperative extension personnel.

- On Apr 4th, 2023, an update from this work was presented at the 2023 Entomological Society of America Pacific Branch Meeting. The title of the talk was "Western Flower Thrips and Impatiens Necrotic Spot Virus Management in the Arizona Lettuce Growing Region". Approximately 40 people attended the talk. Participants at the event were primarily university faculty, extension personnel and chemical industry representatives.

- On Aug 23rd, 2023, results from this work were presented at the University of Arizona Yuma Fall IPM Workshop at a talk titled "INSV Host Weed Survey". Approximately 80 people attended the talk.

- A manuscript relating to this study is currently in preparation.
  Discua, S., Slinski, S. and Palumbo. 2024. Monitoring introduction and movement of Impatiens Necrotic Spot Virus infected thrips in Arizona. Journal of Entomological Science.

- A continuation project SCBGP23-30 "Survey of novel plant viruses in Arizona's produce growing region" studying weeds and crops as reservoirs of economically important viruses (including INSV) started in Jun 2023. This new project continues with much of our current work has the following objectives:
  1. Determine the role of transplants as sources of insect vectors of plant viruses in the desert. – Thrips testing from transplants is currently underway and initiated Sep 2023.
  2. Screen weeds and associated insects as vectors of economically important plant viruses in lettuce and melons. Thrips sampling from weeds has continued since this project ended in Aug 2023 and will continue until the beginning of the 2024-2025 produce season.