2023-2024 CALIFORNIA LEAFY GREENS RESEARCH PROGRAM
ANNUAL REPORT

Project Title: Novel approaches for understanding thrips dispersal using gut content analysis

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Abstract
Western flower thrips (*Frankliniella occidentalis*) is a ubiquitous and polyphagous insect pest that causes cosmetic damage in lettuce and is the primary vector for impatiens necrotic spot virus (INSV). INSV has emerged as a major problem for lettuce production in the Salinas Valley and has recently been identified in desert lettuce-producing regions in southern California and Arizona. Both western flower thrips and INSV have an extensive host range that includes hundreds of cultivated and uncultivated plants, creating unique management challenges within the landscape of the Salinas Valley, where over a hundred specialty crops are grown in addition to lettuce. Therefore, having a greater understanding of how other crops and habitats may influence the occurrence of INSV outbreaks in lettuce would be beneficial for devising management strategies. Specifically, identifying plants that thrips have fed on prior to lettuce infestations can provide new knowledge on the patterns of thrips dispersal between lettuce crops and the surrounding environment. Molecular gut content analysis is a method used to identify plants that insect vectors had previously fed upon, and to infer the insects' landscape level dispersals based on their dietary history. Gut content analysis has been developed and used extensively to study landscape movements of phloem-feeding insects, but it is unknown whether this method can be used to study thrips. The current work had two objectives: 1) determine if molecular gut content analysis protocols are amenable for thrips, and 2) apply gut analysis protocols to thrips collected from lettuce to identify previous plant hosts in which the insects fed on. Here, we determined that molecular gut content analysis can be applied to as few as a single thrips. We also describe our ongoing efforts to determine the length of the dietary history of thrips, and how this information can be applied to infer patterns of dispersal under field conditions.

Immediate Objectives

1. Apply and optimize molecular gut content analysis protocols for thrips.
   Deliverables: Provide a proof-of-concept to implement the protocols for thrips.
2. Perform gut content analysis on thrips sampled from lettuce crops.
   Deliverables: Provide dietary history profiles for thrips collected from lettuce to infer their landscape origins and patterns of dispersal.

Procedures

Objective 1: Apply and optimize molecular gut content analysis protocols for thrips.

To test the protocols, western flower thrips from laboratory reared colonies with a known dietary history (i.e., green beans and lettuce) were used. Thrips reared on green beans in the laboratory at USDA-ARS in Salinas, CA were transferred to lettuce and allowed to feed for either 1, 3, or 7 days. This served as a reference for interpreting the dietary history profiles as thrips transition from a known previous host (beans) to lettuce. Thrips were surface sterilized as described previously (Cooper et al. 2019) and DNA was extracted from single thrips, or groups of 5 and 10 thrips. Ten samples for each treatment were conducted for a total of 90 samples used for molecular gut content analysis (**Figure 1** and **Table 1**). Due to the high susceptibility to PCR contaminations, especially for insects that harbor low quantities of plant DNA within their guts, the described set of studies using known dietary histories (i.e., green beans and lettuce) were performed to estimate the threat of PCR contamination on gut content analysis of thrips under field settings.

A second experiment (Objective 1b) was conducted to further characterize thrips feeding on green beans and lettuce. Thrips were reared on green beans and, 1) immediately processed for gut content analysis, 2) transferred to lettuce potted in soil and allowed to feed for 1 day, 3) transferred to lettuce in water and allowed to feed for 1 day, 4) killed in the freezer and then mixed in soil. All thrips were collected in batches of 10 into ethanol and processed for gut content analysis (**Table 2**).

The plant genes, ITS and trnF, were targeted for PCR amplification using universal primers (Taberlet et al. 1991, Chen et al 2010) that included barcode sequences to label individual samples. PCR products were purified and combined into a single vial and submitted for sequencing using a PacBio Sequel platform at the Washington State University Laboratory for Biotechnology and Bioanalysis using methods described previously (Cooper et al. 2022). The sequences were grouped into operational taxonomic units (OTUs) of sequences that share at least 95% sequence identity. Each OTU was then be identified using the BLAST analysis tool of NCBI. The data was used to determine whether gut content analysis could be applied to single thrips, or whether thrips needed to be processed in groups (5 thrips or 10 thrips, or more).

Objective 2: Perform gut content analysis on thrips sampled from lettuce crops.

Thrips were collected from lettuce in 36 commercial fields in Salinas, Chualar, Gonzales, and Soledad from 3/7/23 – 10/19/23. Fields were managed conventionally or organically and were primarily romaine or iceberg types of lettuce. Thrips were collected randomly from 5 locations in each field at ~40-50 days after planting. Lettuce plants were washed, and thrips were collected

using a sieve and transferred to ethanol until processing for gut content analysis. At the time of sampling, observations of the adjacent crops and landscape were recorded for each field and used for reference once gut content analysis had been performed. Preparation of thrips, sequencing, and analysis are described in Objective 1.

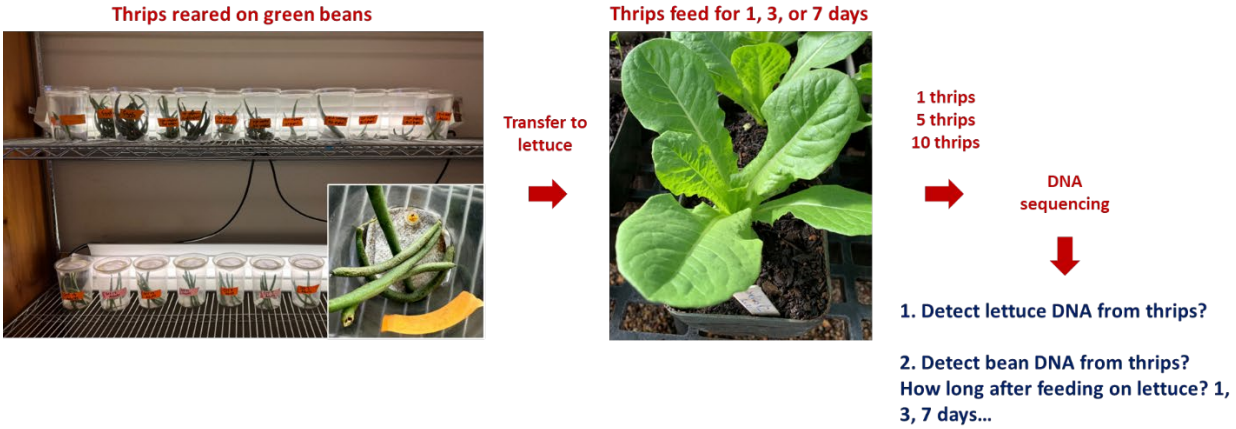

Figure 1. Overview of workflow to characterize gut content analysis on thrips. Thrips reared on green beans at the USDA in Salinas, CA were transferred to lettuce potted in soil and allowed to feed for 1, 3, or 7 days. Thrips were then processed individually or in groups before DNA sequencing.

Treatment	No. thrips	Days on primary host (Bean)	Days on secondary host (Lettuce)	Samples
1	1	Reared	1	10
2	1	Reared	3	10
3	1	Reared	7	10
4	5	Reared	1	10
5	5	Reared	3	10
6	5	Reared	7	10
7	10	Reared	1	10
8	10	Reared	3	10
9	10	Reared	7	10
Total				**90**

Table 1. List of treatments for Objective 1a. In all treatments, western flower thrips were reared on green beans in the laboratory at the USDA and transferred to lettuce that was potted in soil in a greenhouse. Thrips were allowed to feed for either 1, 3, or 7 and then collected as individual insects, or in groups of 5 or 10. This work provides insight on the number of thrips needed to conduct gut content analysis and determines the length of dietary history in thrips.

Treatment	No. thrips	Days on primary host (Bean)	Days on secondary host	Samples
1	10	Reared	0	10
2	10	Reared	1 (lettuce in soil)	10
3	10	Reared	1 (hydroponic lettuce)	10
4	10	Dead	Mixed in soil	10
Total				**40**

Table 2. List of treatments for Objective 1b. Western flower thrips reared on green beans were 1) immediately processed for gut content analysis, 2) transferred to lettuce potted in soil and allowed to feed for 1 day, 3) transferred to lettuce in water and allowed to feed for 1 day, 4) killed in the freezer, mixed in soil, and then processed for gut content analysis.

Results

To determine the feasibility of using molecular gut content analysis on thrips, a set of experiments were conducted where thrips were fed on a previously known host (beans) and then transferred to feed on a new host (lettuce). In all experiments, genetic signatures of lettuce were consistently detected from thrips that were transferred from beans to lettuce. Importantly, lettuce was detected from as few as a single thrips that fed on lettuce for one day. This provides evidence that the input DNA quality and quantity was sufficient to conduct gut content analysis on a single thrips (**Figure 2**). The consistent amplification of lettuce using both PCR primer sets to the ITS and trnF genes also confirmed that thrips were feeding on the host lettuce in the experiments (**Figure 2**). Surprisingly, in all treatments, the genetic signatures of the previously known host (beans) were not detected in thrips, which may suggest a possible short signal lifespan in the insect. Numerous other plant taxa were identified from the sequence analysis, including genetic signatures belonging to spinach, squash, tomato, tree species, and more. However, in nearly all cases, non-lettuce plant taxa were only detected by one of the PCR primers targeting the ITS or trnF genes, but rarely, did a sample amplify with both primers (**Figure 2**). Considering thrips utilize a rasping and sucking feeding mechanism, we anticipate that the genetic material of plant cells with nuclear origin (ITS gene) and chloroplast origin (trnF gene) should both be consumed, which was only the case for lettuce (**Figure 2**). Therefore, it remains uncertain if the signals belonging to other plants were truly ingested or reflected possible contamination.

To address this issue, we then conducted a follow-up experiment where thrips were 1) immediately processed for gut content analysis after feeding on beans, 2) allowed to feed on lettuce that was grown in soil (same as original experiment), 3) allowed to feed on lettuce grown hydroponically, or 4) killed and mixed with soil (to test the thrips surface sterilization protocols) (**Table 2**). The experiments have been conducted and we are currently waiting on the sequencing data.

Reared on bean →		1 day on lettuce			3 days on lettuce			7 days on lettuce		
No. thrips	Gene	Lactuca	Spinach	Squash	Lactuca	Spinach	Squash	Lactuca	Spinach	Squash
1	ITS	100%	85%	42%	67%	0%	17%	50%	0%	33%
	trnF	100%	0%	0%	100%	0%	0%	80%	0%	0%
5	ITS	88%	63%	0%	100%	0%	0%	86%	14%	0%
	trnF	100%	0%	0%	100%	0%	0%	100%	0%	0%
10	ITS	100	25%	0%	80%	20%	0%	80%	20%	0%
	trnF	100	0%	0%	88%	0%	0%	88%	0%	0%

Figure 2. Percentage of thrips samples with reads to Lactuca, Spinach, and Squash. Universal primers to the nuclear ITS gene or the chloroplast trnF gene were used to PCR amplify DNA extracted from single, or groups of 5 or 10 thrips.

Discussion

Both western flower thrips and INSV have a large host range that includes hundreds of plants, many of which, can grow year-round, and thus, creating unique management challenges within the landscape of the Salinas Valley. Capturing the history of which plants thrips have fed on prior to infestation of lettuce crops would provide a greater understanding of the dispersal patterns and the distance at which thrips travel between food sources. To fill this gap in knowledge, we sought to develop a proof-of-concept for using molecular gut content analysis (GCA), which has been used previously to identify a nearly complete dietary history of psyllids and leafhoppers (Cooper et al., 2019, 2022). Gut content analysis has been especially useful in tracking the landscape-level movements of insects, and for pinpointing the weedy sources of insect vectors (Reyes Corral et al., 2021a, 2021b, Cooper et al., 2022a, 2022b).

Our initial studies using western flower thrips that were reared on a known host (beans), followed by transferring to a second host (lettuce), revealed that GCA could be used on as few as a single thrips, allowing us to obtain a high level of resolution when tracking the movement of thrips collected from the environment. While we consistently detected the genetic signatures of lettuce, we were unable to detect the signatures of previously fed on hosts (beans) in our initial trials. Based on this unexpected outcome, we suspect that thrips diets may have a relatively short signal lifespan, but further studies are underway to ensure that the ITS and trnF PCR primers are sensitive enough to beans and other plants within the Phaseolus genus.

We also detected some unexpected plant signatures (squash, tomato, spinach, others), which may point to the possibility that thrips were feeding in the soil. In our studies, lettuce plants were potted in soil that has a high amount of organic material, so it is possible that trace amounts of other crops and plant species were present for thrips to feed on. However, for all the non-lettuce plants that were detected, majority of them were only detected by one of the plant primers (ITS or trnF) that were used for PCR amplification and sequencing. When considering the plant taxa that were amplified using both primers, lettuce was the only host that was

consistently detected. Therefore, it remains uncertain whether thrips can feed on organic soil matter, but we are conducting some additional studies to address this possibility. The outcomes of the work could have implications for a feeding behavior of thrips that we had not previously considered and raises additional questions about similar behaviors in field conditions and their natural environment.

Additional studies are ongoing to confirm the length of the dietary history of thrips, in addition to analyzing the dietary history of thrips that were collected from lettuce fields during the 2023 season. We are also continuing to collect thrips in 2024 using the same described methods. If successful, the data will provide a better understanding of thrips dispersal between lettuce crops and the diverse landscape of the Salinas Valley. The ability to track landscape-level movement of thrips vectors will provide valuable information for identifying drivers of infestations and INSV outbreaks in lettuce crops.