2022-2023 CALIFORNIA LEAFY GREENS RESEARCH PROGRAM
RESEARCH PROPOSAL

Project Title: Developing RNA interference (RNAi) technology to manage thrips and viruses in lettuce

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Abstract:
Western flower thrips (*Frankliniella occidentalis*) is an insect pest that creates cosmetic issues in lettuce and is the primary insect vector for *Impatiens necrotic spot virus* (INSV). INSV has become an increasing problem in lettuce production in Monterey County, and in March 2021, was also detected in lettuce grown in Imperial and Riverside Counties, as well as the neighboring Yuma and Tacna regions in Arizona. Only limited strategies exist for thrips management due to the lack of efficacious chemistries, as well as increasingly tighter restrictions on maximum residue limits, particularly those set by export markets. There are also no direct methods for managing INSV, due to the absence of genetic-based resistant varieties available to the industry. This has created a need for new management strategies. RNA interference (RNAi) technology is an emerging strategy that has massive potential for the agriculture sector and has been demonstrated to be effective as a biopesticide for crop protection against insect pests and pathogens. RNAi is a natural process that results in gene silencing in insects, plants, and humans, and it's use as a biopesticide can be applied using methods that avoids genetic engineering of plants. Furthermore, RNAi-based biopesticides can be tailored to be species-specific and are completely degradable in the environment, providing unique advantages over many conventional insecticides. With previous support from the California Leafy Greens Research Program, we have successfully designed and synthesized double stranded RNAs (dsRNAs; the active triggers of RNAi) to target western flower thrips and INSV. This support also enabled our capacity to test larger volumes of dsRNA. We have demonstrated that dsRNA targeting INSV can be absorbed through the roots, move systemically throughout the plant, and persist for at least 30 days. We have also generated preliminary results that suggest the dsRNAs are having suppressive effects

on INSV development. The current proposal has one objective that will focus on continuing efficacy experiments to better understand the potential for RNAi as a novel IPM strategy for managing thrips and INSV in CA lettuce.

Immediate Objectives:

1. Efficacy of RNAi technology using non-GMO strategies to manage INSV.
   Deliverables: Work towards a proof-of-concept for using RNAi technology as a strategy for managing thrips and INSV in lettuce.

Procedures:

Objective 1: Efficacy of RNAi technology using non-GMO strategies to manage INSV. A field trial at the Spence Research Farm was conducted from 7/19/23 to 9/27/23. Romaine (var. Parris Island Cos) was direct seeded in 40-inch beds, sprinkler irrigated for 21 days and drip irrigated for the remainder of the trial. Synthesized dsRNAs targeting INSV were manually applied to the crown of individual plants beginning at 23 days and continued once a week for four consecutive weeks. DsRNA was applied at a rate of 500 ug per plant in 1 ml of water. The dsRNA treatment and untreated blocks were replicated eight times in the field. Evaluations were conducted on alternating weeks to assess, 1) visual symptoms of INSV infection using a 0-5 symptom severity scale (0=no symptoms, 5=dead) on five plants per replicate per week, 2) testing a single plant (leaves only) per replicate per week for INSV infection using TAS-ELISA, 3) testing a single plant (leaves, crown, and roots) per replicate per week for presence of dsRNA using RT-PCR. A total of four evaluations were made, with a final evaluation at 65 days to calculate the above ground wet biomass of three lettuce plants from each treatment and replicate. All field activities and evaluations are included in **Table 1.**

2023		Activity	Days	Notes	Data collected*
Wed	7/19	Planting (direct-seeded)	0		
Fri	7/21	First water	2		
Thu	8/10	Thinning	22		
Fri	8/11	RNAi drench (1)	23	500 ug dsRNA / plant in 442 ul	
Wed	8/16	Drip tape	28		
Fri	8/18	Post-trt eval (1)	30		1, 2, 3
Fri	8/18	RNAi drench (2)	30	500 ug dsRNA / plant in 1 ml	
Fri	8/25	Post-trt eval (2)	37		1, 2, 3
Fri	8/25	RNAi drench (3)	37	500 ug dsRNA / plant in 1 ml	
Fri	9/1	Post-trt eval (3)	44		1, 2, 3
Fri	9/1	RNAi drench (4)	44	500 ug dsRNA / plant in 1 ml	
Fri	9/8	Post-trt eval (4)	51		1, 2, 3
Fri	9/22	Post-trt eval (5)	65		1, 2, 3, 4
	9/27	Crop terminated	70		

Table 1. Schedule of activities for RNAi field trial. The trial was conducted at the Spence Research farm at USDA-ARS. *Data collected: 1 = In-field INSV symptom severity, 2 = Plant collections for INSV testing using TAS-ELISA, 3 = Plant collections for dsRNA detection using RT-qPCR, 4 = Above ground wet biomass.

Results:

The first application of dsRNA targeting INSV was applied one day after thinning to the base of the plant to allow root absorption. Each plant that received the dsRNA treatment, received a total of 500 ug of dsRNA (**Figure 1**). 7 days after dsRNA treatment (30 days after germination), dsRNA was detected in plants that received the dsRNA treatment, including all plant parts that were tested using RT-PCR (roots, crown, and leaves). Further studies are currently underway to determine if the dsRNA persisted through subsequent applications as the plants matured.

Overall, very low incidence of INSV was observed and detected in the field trial and was estimated to have ~1% disease incidence. Visual evaluation data did not reveal any differences in the INSV symptom severity, while the virus was not detected in any of the plants that were tested using ELISA (data not shown). To determine if dsRNA influenced plant growth, the aboveground wet biomass was calculated for each plant. DsRNA did not have a significant effect on the weight of lettuce plants (**Figure 2**).

Figure 1. Romaine lettuce 23 days after germination when the first dsRNA application was made. For each plant, 500 ug dsRNA was applied to the soil at the base of the plant.

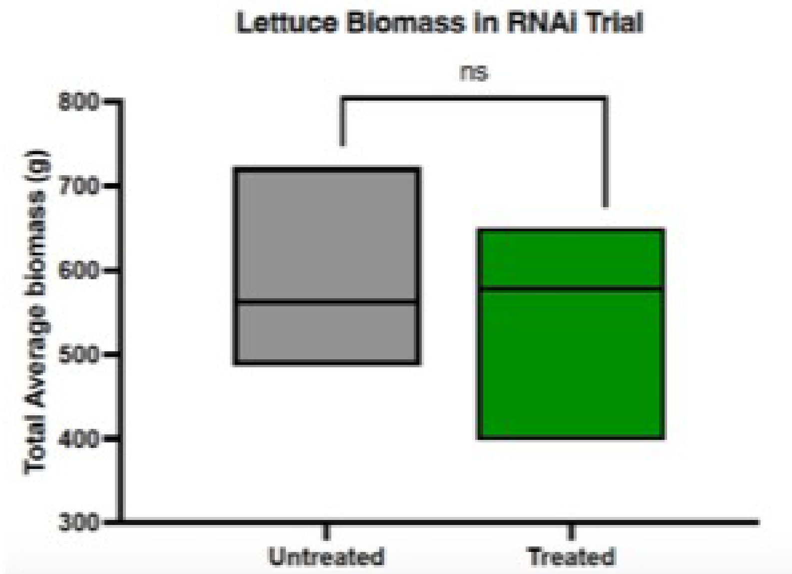

Figure 2. Wet biomass of aboveground tissue of lettuce. The weight of 24 plants was averaged per treatment; $p < 0.05$.

Discussion:
RNA interference technology (RNAi) has numerous applications for crop protection and improvement. RNAi is a natural process in plants, insects, and humans that results in gene silencing. In this process, the formation of double stranded RNAs (dsRNAs) leads to the degradation of complementary mRNA, resulting in reduced expression for that gene. Identifying regions within a gene that are unique to a particular organism can be used to design specific dsRNAs. Using this knowledge, we can design dsRNAs to target key genes of the INSV genome. This proposal explored the development of RNAi technology for managing INSV affecting lettuce, and for the first time, was tested in a field trial at the Spence research farm.

Here, we demonstrated that dsRNAs can be successfully absorbed into the root systems of lettuce plants under field conditions, and furthermore, moves systemically throughout the plant and can be recovered from leaf tissue for at least 7 days after application. While natural infections of INSV in the field remained low for the trial, and we were not able to assess any differences in INSV infection in the dsRNA treatments, we did determine that repeated applications of dsRNA at high concentrations did not affect the overall growth and biomass of the lettuce plants. Further studies are required to fully understand if the dsRNAs have suppressive effects on INSV infection and will be pursued in the current year. If there is a level of protection that is conferred by the introduction of dsRNA, it would be important to understand how long protection against INSV lasts. It is expected that this may be limited by the stability and longevity of the introduced dsRNAs.

The long-term goal of this project is to establish an RNAi-based tool that can effectively manage thrips and INSV using non-GMO methods and fits into the model of lettuce production practices in the Salinas Valley. While this proposal specifically focuses on the goals that are equivalent to early Phase 1 development of a new product, there are subsequent objectives that will need to be addressed. If efficacy studies continue to show promise, one future objective will be to optimize a delivery strategy for the dsRNAs at the field level – a topic that is highly popular and constantly changing as new technologies are emerging to enhance the stability and delivery of dsRNAs. As these technologies continue to advance and the cost to produce dsRNAs at field-scale levels continue to become cheaper, the implementation of RNAi technologies for integrated pest management will arrive.

Several promising responses by the EPA have favored the use of RNAi in plant protection for the management of insect pests. In June 2017, the first RNAi product was registered for the management of corn rootworm, https://www.epa.gov/pesticide-registration/epa-registers-innovative-tool-control-corn-rootworm. Additionally, in September of 2023, the EPA opened a public comment period for the registration of the first RNAi pesticide targeting Colorado potato beetle, https://www.epa.gov/pesticides/epa-opens-public-comment-period-proposal-register-novel-pesticide-technology-potato. Both of these actions are promising for the future of RNAi technology and its use for pest management across numerous cropping systems.