Project title
Tackling the triple threat of thrips, pythium, and impatiens necrotic spot virus using plant elicitors and soil amendments with biological control activity

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Abstract
Lettuce crops in CA are increasingly subject to infections by plant viruses, particularly those vectored by thrips. For lettuce grown in Monterey County (60% of CA production), managing thrips and the thrips-transmitted virus, impatiens necrotic spot virus (INSV) has become one of the top challenges facing growers. Our prior work shows that economically damaging levels of infection routinely occur at vector densities well below those causing damage to crops through direct feeding. In field trials, young plants with just two-to-three true leaves are rapidly colonized by thrips. We also found that some insecticides marketed for thrips control are ineffective (Radiant SC) and that it takes multiple applications of the mainstay product (Lannate SP) to see measurable reductions in thrips numbers. Our prior work also revealed a damaging synergy between INSV and the causal agent of Pythium wilt (*Pythium uncinulatum*). Plants with INSV infection show more severe symptoms of Pythium wilt, leading to further losses. Integrated disease management strategies are needed to reduce the frequency of virus infections, attenuate virus impacts on yields, and to disrupt the INSV-*Pythium* synergy. One way to achieve this is by improving the innate immune response of existing lettuce cultivars. Immunity priming agents that activate different defense systems are commercially available, but remain underexplored as tools for virus management, especially in lettuce. Additionally, there is potential to address both soil pathogen and viral pathogen challenges using soil amendments with immunity priming capabilities. Our prior CLRGP-funded work identified two commercial compounds which, when combined, show efficacy in preventing and attenuating INSV infections. In parallel work, we have begun studying the use of soil amendments produced by insect decomposers as priming agents and substrates for soil microbes with disease-suppressive traits. In the proposed project, we will carry out further testing of these materials for disease management through laboratory, greenhouse, and field experiments. We will quantify efficacy against INSV, effects on thrips

interactions with plants, efficacy against Pythium wilt, and effects of materials on populations of *Pythium*-suppressive bacteria. This work will provide timely recommendations for use of priming agents in integrated disease management efforts for INSV and Pythium wilt.

Procedures

We designed greenhouse and field experiments to test the efficacy of different soil-applied products for plant priming against INSV. The selected materials and approaches were based on our observations that early applications of elicitors are critical for INSV mitigation, and our observations of synergies between INSV and pythium.

1. Evaluate efficacy of soil-applied materials with plant priming and/or biocontrol-enhancing capabilities.

In the greenhouse, using romaine lettuce, we used previously employed methods (CLGRP grant FY 2021) to evaluate select soil-applied elicitor treatments and doses for efficacy against INSV infection and INSV-caused disease symptoms. Partner experiments explored efficacy against Pythium (co-PI Dundore-Arias). Treatments included the following applied to the soil:

1. 1:1 Actigard + Regalia (active ingredients = acibenzolar-*S*-methyl [Actigard] and extract of *Reynoutria sacchalinensis* [Regalia]), applied as 20mL of working solution to the soil.

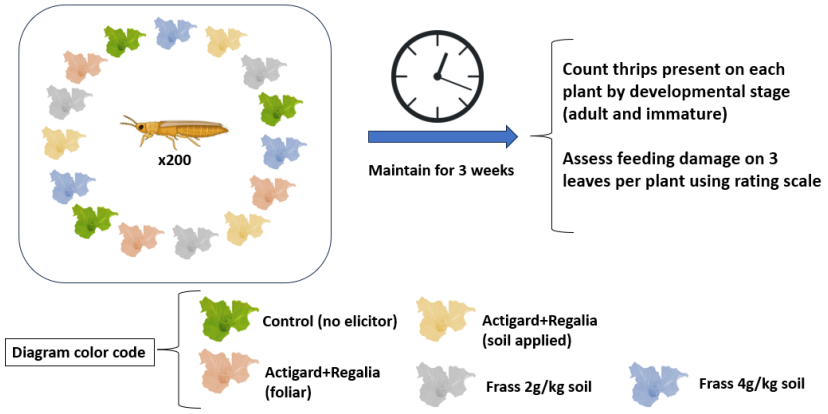
2. Soil IPM plus (active ingredient = chitosan), applied as 20mL of working solution to the soil.

3. Frass by Grubbits (active ingredient = frass of the black soldier fly, *Hermetia illucens*), applied as either 2g/kg or 4g/kg to the soil.

4. Water control (no active ingredient), applied as 20mL of water to the soil.

Treatments were all applied as a single dose prior to pathogen challenge. Efficacy was evaluated by quantifying virus titer (ELISA) and plant size at the conclusion of experiments (roughly five weeks post planting), then using generalized linear models to test for statistically significant differences by treatment, and relative to controls (Dunnett's test).

We also evaluated the effects of priming treatments on thrips-plant interactions. Experiments were conducted as choice tests to address attraction and repellency to lettuce plants with the following treatments: 1) Actigard +Regalia (foliar, at 2-leaf stage), 2) Actigard + Regalia (soil, at planting), 3) BSF frass (soil, at planting 2g/kg), 4) BSF frass (soil, at planting 4g/kg), 5. untreated. Sets of three individually potted romaine lettuce plants of each treatment, all at the 3 true leaf stage, were placed in a randomized complete block design fashion inside of a thrips-proof cage (12 plants total per cage). Two-hundred adult thrips were placed inside of the cage and allowed to settle and feed on plants by choice. After 3 weeks, all plants were removed and counted for the number of thrips present on each plant (immatures and adults) and feeding damage rated. A diagram of the assay design is shown below.



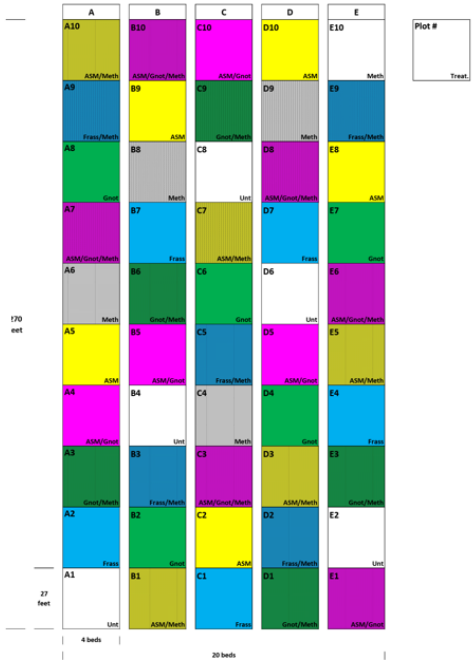
We also attempted to run greenhouse experiments to evaluate the effects of the treatments on Pythium-inoculated and mock-inoculated plants. Efficacy was evaluated by recording the number of symptomatic plants and the severity of infection and comparing plant size and biomass at the conclusion of experiments (roughly five weeks post-planting) against non-inoculated plants, then using generalized linear models to test for statistically significant differences by treatment, and relative to controls (Dunnett's test). However, rodent damage to plants prevented collection of data on this portion of the proposed project. Experiments are continuing under a no-cost extension.

2. Test optimal doses and/or combinations of soil-applied materials for field efficacy.

We performed a field experiment testing different treatments outlined to the right and below, with or without standard treatment with the insecticide methomyl. We included individual treatments with Actigard and Regalia, as we wanted to re-test these treatments following the prior year's field experiment. The design of the field experiment and data collection efforts are outlined below. The experiment was performed in July-September 2023.

1	Untreated
2	Actigard
3	Regalia
4	Actigard + Regalia
5	BSF Frass
6	Methomyl
7	Actigard + Methomyl
8	Regalia + Methomyl
9	Actigard + Regalia + Methomyl
10	BSF Frass + Methomyl

Row spacing = 40 inches
2 seedlines / bed
2 inner seedlines of 2 beds evaluated



Date (2023)	Activity	Days	Application rates	Data collected*
7/19	Frass soil application, plant field	0	BSF frass: 2 g/kg	
7/21	First water	2		
8/1	Flag field	13		
8/3	Elicitor spray (1)	15	Actigard = 1 oz/ac; Regalia = 30 ml/gal; NuFilmP = 1 pint/100 gal; Field rate = 60 GPA	
8/10	Thinning	22		
8/14	Pre-treatment eval (1)	26		1, 2, 3
8/15	Elicitor spray (2)	27	Actigard = 1 oz/ac; Regalia = 30 ml/gal; NuFilmP = 1 pint/100 gal; Field rate = 60 GPA	
8/16	Drip tape installed	28		
8/17	Methomyl spray (1)	29	Methomyl = 10 lbs/ac; NuFilmP = 1 pint/100 gal	
8/21	Pre-treatment eval (2)	33		1, 2, 3
8/28	Elicitor spray (3)	40	Actigard = 1 oz/ac; Regalia = 30 ml/gal; NuFilmP = 1 pint/100 gal; Field rate = 60 GPA	
~~8/31~~	~~Methomyl spray (2)~~	~~43~~	Sprayer broke the morning of the application and replacement parts had to be ordered	
9/5	Pre-treatment eval (3)	48		1, 2, 3
9/11	Elicitor spray (4)	54	Actigard = 1 oz/ac; Regalia = 30 ml/gal; NuFilmP = 1 pint/100 gal; Field rate = 60 GPA	
9/14	Methomyl spray (3)	57	Methomyl = 10 lbs/ac; NuFilmP = 1 pint/100 gal	
9/18	Final eval (4)	61		1, 2, 3, 4
9/22 - 9/27	Crop harvest	65-70		

1 = In-field INSV symptom severity (20 plants per block)

2 = Plant collections for INSV testing using TAS-ELISA (3 plants per block)

3 = Plant collections for thrips counting (3 plants per block)

4 = Above ground wet biomass (3 plants per block)

3. Quantify positive effects of soil-applied materials on pythium levels and pythium-suppressive biological control agents in the laboratory and field.

We attempted to quantify the effects of treatments (Obj. 2) on Pythium wilt will be by evaluating field disease incidence among treatments using previously employed methods (CLGRP and SB170 grant FY 2022). However, Pythium incidence in the field experiment was extremely low and quantitative measurements of disease severity/incidence in relation to the treatments were not possible. We did collect soil samples from each plot immediately prior to planting and at harvest. We used plating methods to assess the densities of indigenous soilborne locally occurring *Streptomyces*. We isolated individual bacterial cultures for future work to assess Pythium suppressive capabilities.

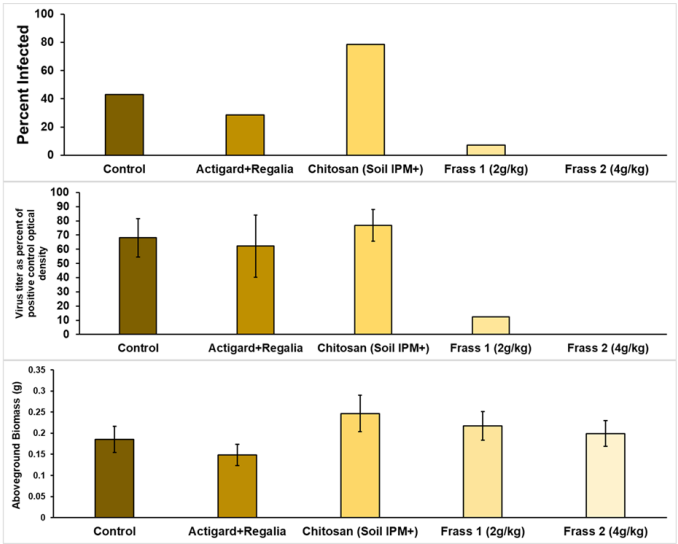


Figure 1. Results of replication 1 of elicitor efficacy assessments performed in the greenhouse with mechanical INSV inoculations. N = 14 plants per treatment.

Results

Objective 1. Evaluate efficacy of soil-applied materials with plant priming and/or biocontrol-enhancing capabilities. We documented encouraging effects of frass and confirmed efficacy of Actigard+Regalia co-applications (Figure 1). Frass prevented infections (0-7% infected) relative to the control (40% infected) (Figure 1 top). Actigard + Regalia also reduced infection frequency (25% infected). Surprisingly, the chitosan-containing product (Soil IPM+) drastically increased infection frequency (80% infection), suggesting that this product actually suppresses immunity

against INSV. In elicitor treatments, if plants did become infected, only frass treatments reduced virus titers (Figure 1 middle graph). There were no significant effects on plant size relative to controls (Figure 1 lower). However, the experiment was only run for 5 weeks and differences may have become apparent if we allowed plants to develop for a longer period.

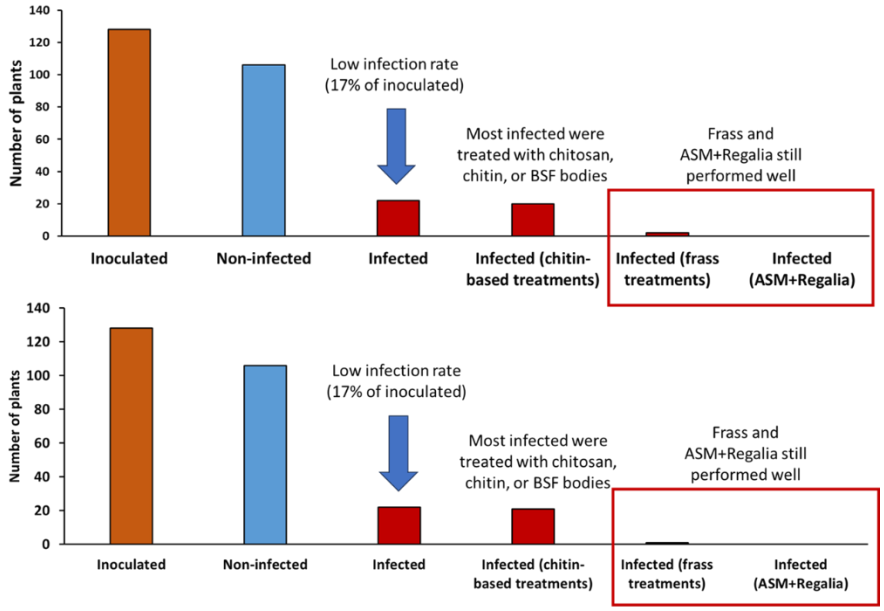


Figure 2. Results of replication 2 (top) and 3 (bottom) of additional elicitor efficacy assessments performed in the greenhouse with mechanical INSV inoculations. N = 14 plants per treatment.

We attempted to repeat these experiments but had limited success in getting good inoculation levels in the controls (Figure 2). This ultimately reduces our ability to draw conclusions from the experiments, as the inoculum was potentially not very infectious. However, we still saw that frass treatments or Actigard (ASM) + Regalia treatments reduced infection levels relative to controls and/or treatments receiving chitin or chitosan active ingredients (e.g., Soil IPM Plus). These experiments are being repeated in Fall 2024 using the no-cost extension funds. We also revised our inoculation protocol to attempt to increase inoculation success overall. This ensures a robust test of the products.

Greenhouse experiments to assess the effects of elicitors on thrips behavior and performance revealed that our selected elicitors do not influence thrips in the greenhouse environment. There were no significant effects of products on thrips preferences among the plants (Figure 3 top) and, correspondingly, no significant effects on the levels of thrips damage (Figure 3 lower). These data suggest that elicitor products are not likely to act by augmenting vector preferences and/or performance (reproduction, growth, or survival) under field scenarios.

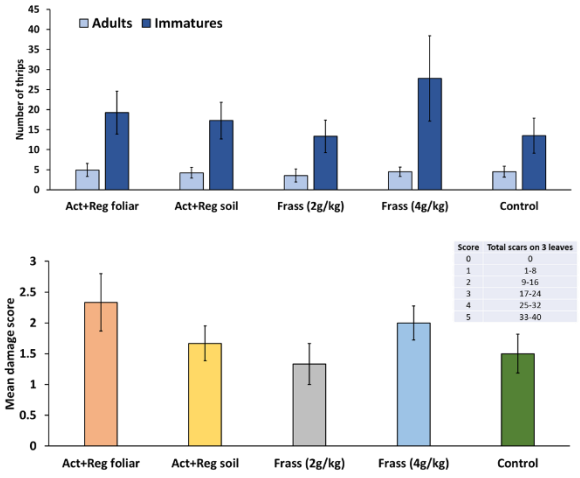


Figure 3. Results of thrips choice test experiments. N = 9 replications total.

Objective 2. Test optimal doses and/or combinations of soil-applied materials for field efficacy. Field evaluations of elicitor efficacy in protecting romaine lettuce from INSV infections were inconclusive due to low disease pressure in 2023. INSV severity scores are rated on a scale from 0-5. However, the average score across all treatments at 61 days post planting was less than one, indicating barely measurable disease pressure (Figure 4). Likewise, ELISA scores for virus titer were very low – most of the plants sampled did not pass the threshold for being considered infected and of those that did, the virus accumulation was minimal (Figure 4).

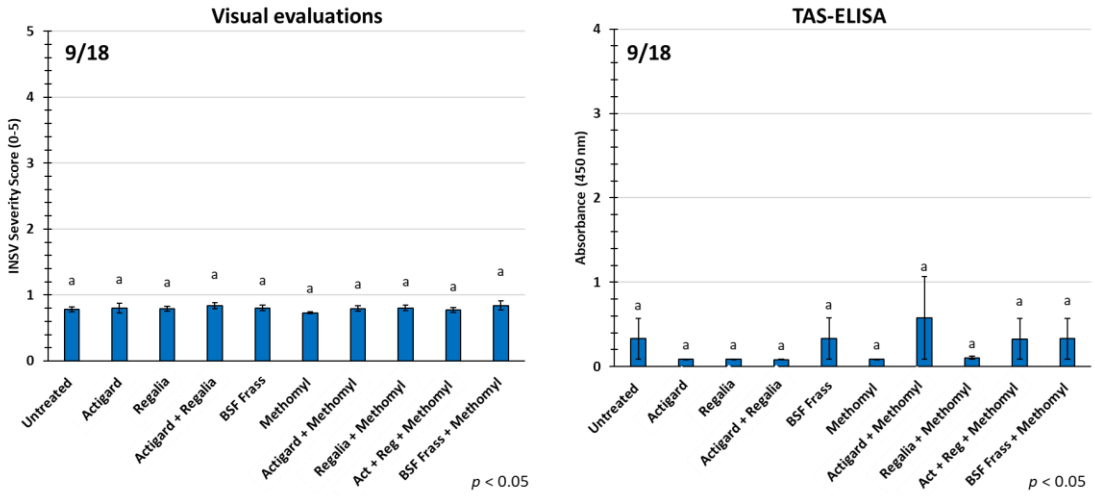


Figure 4. Results of visual and ELISA ratings of INSV infection severity at 61 days post-planting.

Thrips pressure was also low in 2023, with an average of around 2-4 thrips per plant for most of the season, with fewer thrips toward the end of the experiment (Figure 5). This low vector pressure is likely responsible for the reduced disease pressure.

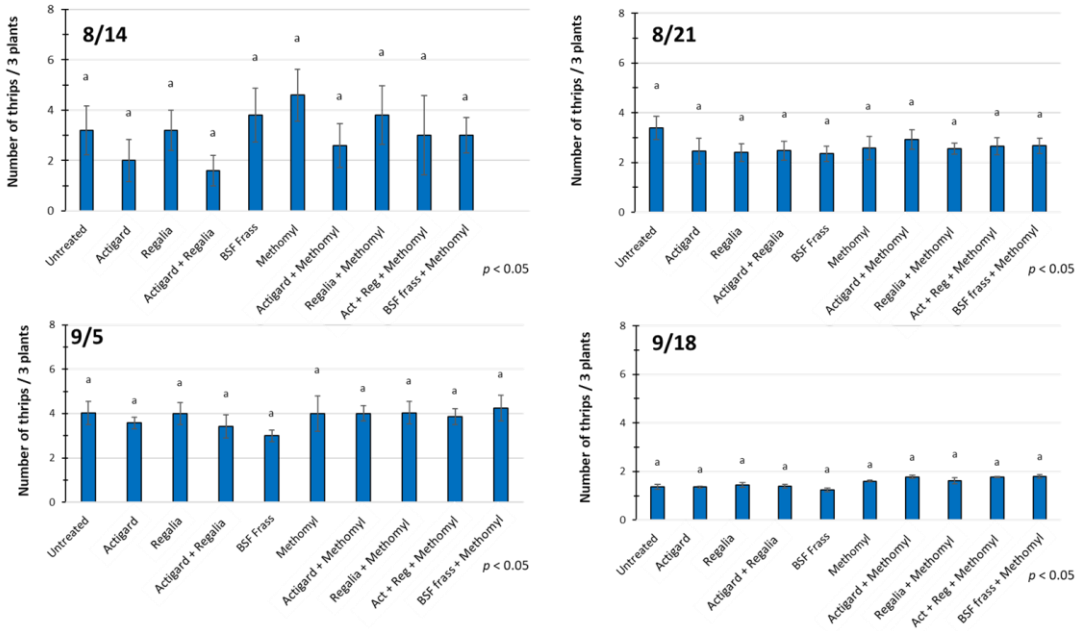


Figure 5. Thrips population counts at four time points during the field experiment (9/18 is 61 days post planting).

We had planned to repeat the experiment in 2024, but disease pressure was tracked throughout the season and was insufficient for a field test of the products we examined in the greenhouse. We are currently proceeding with an alternative test using a semi-field set up of a greenhouse with free roaming thrips colonizing infected plants. Plants treated with the products can be introduced into this space and exposed to foraging thrips for a defined period of time. Following exposure, plants are being monitored for disease symptoms and progression, virus titer, and plant biomass. Although this is not yet a field test, it reintroduces several realistic elements of the virus-host-plant interactions that would be present in the field. These experiments are taking place in Fall 2024 under the no-cost extension.

3. Quantify positive effects of soil-applied materials on pythium levels and pythium-suppressive biological control agents in the laboratory and field.

Elicitors had positive effects on soil bacterial biomass and populations of potentially disease suppressive *Streptomyces* spp. (Figure 6). Testing of disease suppressive capabilities of ~30 *Streptomyces* isolates is ongoing during the no-cost extension period. We also performed a soil mineralization experiment with frass to determine how it influences soil microbial communities over an 84-day period. Samples have been collected and are being processed for microbiome analysis, which will establish how disease suppressive species change with frass addition to the soil over a typical growing season duration.

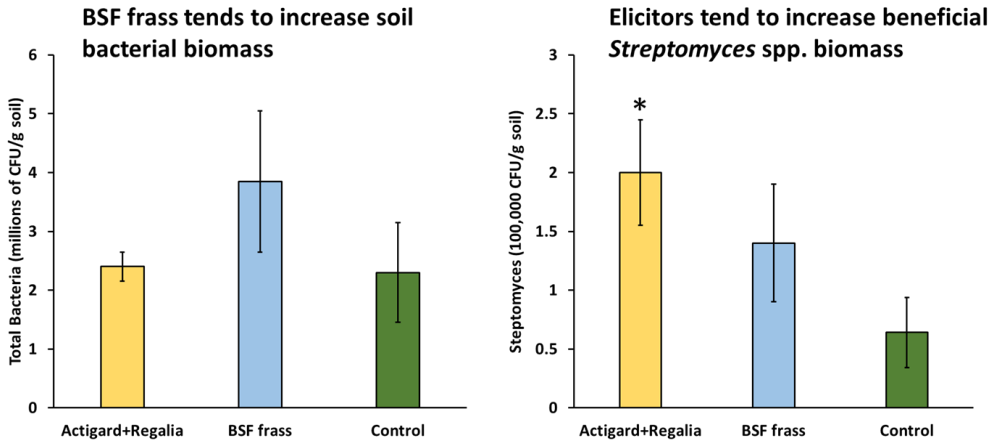


Figure 6. Results of semi-quantitative bacterial biomass assays from field experiment plots.

Summary

We have some promising data that suggest elicitors could be a useful component of INSV management, and possibly of *Pythium* management. Experiments during the no-cost extension period should help clarify these effects. The data generated by this proposal were used to seek additional funding through the USDA NIFA Predoctoral Fellowship program (prepared by the grad student researcher who performed INSV inoculation experiments, grant proposal pending).

Acknowledgements

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