California Leafy Greens Research Program
Annual Report April 1, 2023 to March 31, 2024

Title: Evaluation of INSV and Pythium wilt (*Pythium uncinulatum*) on spinach.

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Cooperating personnel: Kelley Richardson (USDA/ARS), Daniel Hasegawa (USDA/ARS), JP Dundore-Arias (CSU-Monterey Bay), Yu-Chen Wang (UC Cooperative Extension), Beiquan Mou (USDA-Salinas), spinach growers and pest control advisors in Monterey, Santa Cruz, San Benito, and Santa Barbara counties, Holaday Seed Company, and Gowan Seed Company.

LIST OF IMMEDIATE OBJECTIVES

1. Screen 70 contemporary varieties on spinach for disease reactions to Impatiens Necrotic Spot Virus (INSV).
   Deliverables: Determine the effects of INSV on commercial spinach varieties based on field observations of different levels of susceptibility.
2. Screen contemporary spinach varieties for susceptibility to *Pythium uncinulatum* at both the seedling stage (damping off) and the mature plant stage (baby leaf and teen).
   Deliverables: Preliminary efforts have shown that *P. uncinulatum* can colonize spinach roots but does not cause significant damping off of spinach under controlled conditions. However, additional information is needed on the levels of variety susceptibility at the seedling and more mature plant stages.
3. Evaluate disease development of *Pythium uncinulatum* on spinach varieties previously infected with INSV to determine if there is a predisposing effect on INSV on root damage.
   Deliverables: Determine if INSV predisposes spinach to root damage caused by *P. uncinulatum.*

Abstract

Progress has been made in both testing field samples for INSV and doing mechanical inoculations to spinach plants under controlled conditions and allowing symptoms to develop on inoculated plants. Symptoms developed on spinach plants within two weeks of inoculation and caused mortality in withing six weeks. In addition, reference strains of *Pythium uncinulatum* (obtained from Dr. JP Dundore Arias) from lettuce were used to develop an assay for the impact of *P. uncinultatum* on pathogenicity to spinach seedlings and roots. Although *P. uncinulatum* was able to infect spinach roots, it was not a very aggressive pathogen relative to other species of Pythium often found on spinach roots. Now that the appropriate protocols have been developed for both INSV and P. uncinulatum, experiments have been initiated to examine the interaction between

the two pathogens. The protocols for the interactions experiments closely follow those that are being used for lettuce developed by Drs. JP Dundore Arias and Daniel Hasegawa to examine the interaction.

Results:

Samples from 12 commercial spinach fields in California were collected that were suspected to possibly be infected with INSV in 2024. However, using INSV immunostrips, none of the samples were positive for INSV (data not shown).

A protocol for mechanically inoculating spinach plants with INSV has been developed and successful inoculations of spinach varieties have developed and the infections were able to cause plant mortality (Figures 1 and 2).

P. uncinulatum was able to colonize spinach roots but did not cause any significant damping off in either a plug inoculum test or a colonized millet soil infestation test (Tables 1 and 2)

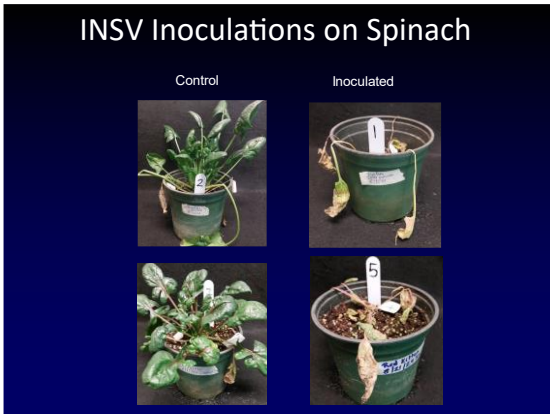


Figure 1. Mortality of mechanically inoculated Viroflay and Red Kitten spinach plants compared to an un-inoculated control plants.

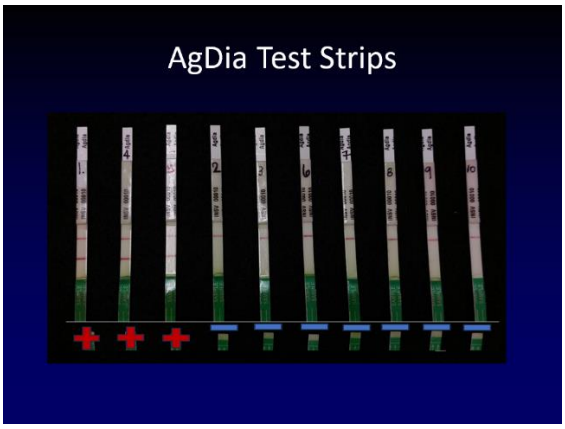


Figure 2. AdDia immunostrips showing positive and negative results for various plants test.

Table 1: Average stand count of Viroflay seeds (20 seeds per plot) for 2 treatments infested with Millet inoculum (Three ratios of amendment) across 5 replications, recorded at 7, 10 days, and 14 post-inoculation (dpi).

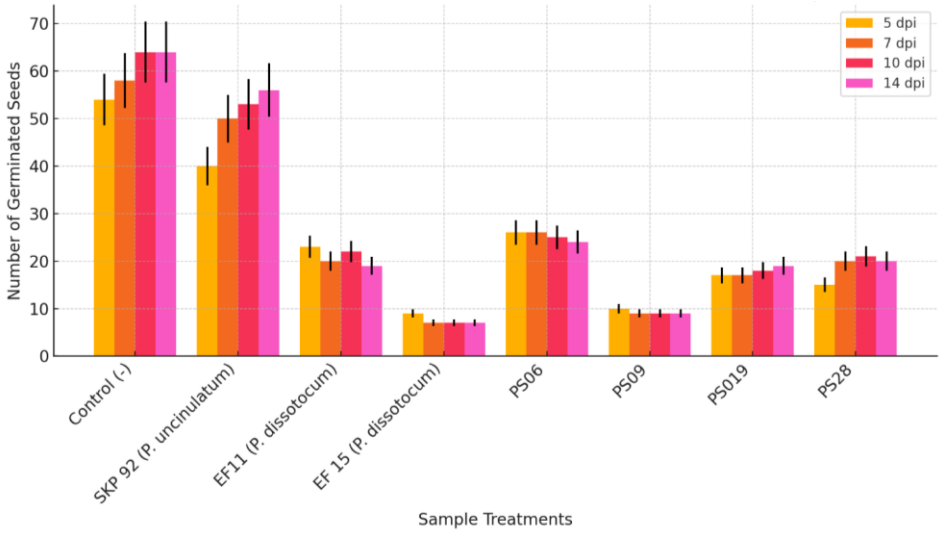
Number	Treatment	Avg. stand count in Three ratios of amendment to soil								
		1:15 (Low level)			1:7 (recommended level)			2:6 (High Level)		
		7 dpi	10 dpi	14 dpi	7 dpi	10 dpi	14 dpi	7 dpi	10 dpi	14 dpi
1	Control (-)	6.0	7.0	7.0	8.8	9.8	9.8	7.6	8.4	8.4
2	SKP 92*	7.0	9.4	9.4	11	11.6	11.6	10.4	11.4	11.4

* *Pythium uncinulatum*

Table 2: Average stand count of viroflay seeds (20 seeds per plot) for 8 treatments infested with 20 colony plugs across 5 replications, recorded at 5, 7, 10, and 14 days post-inoculation (dpi).

Number	Treatment	Tissue Type	Avg. stand count			
			5 dpi*	7 dpi	10 dpi	14 dpi
1	Control (PDA Plugs)		10.8	11.6	12.8	12.8
2	SKP92 (*P. uncinulatum*)	-	8.0	10	10.6	11.2
3	PS06 (*Pythium* sp.)	Symptomatic Psyllium	5.2	5.2	5.0	4.8
4	EF11(*P. dissotocum*)	Spinach	4.6	4.0	4.4	3.8
5	PS28 (*Pythium* sp.)	Psyllium	3.0	4.0	4.2	4.0
6	PS19 (*Pythium* sp.)	Symptomatic Psyllium	3.4	3.4	3.6	3.8
7	PS09 (*Pythium* sp.)	-	1.8	1.8	1.8	1.8
8	EF15 (*P. dissotocum*)	Spinach	1.8	1.4	1.4	1.4

*days post-inoculation



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