

Characterizing the accumulation of impatiens necrotic spot virus (INSV) in lettuce tissues

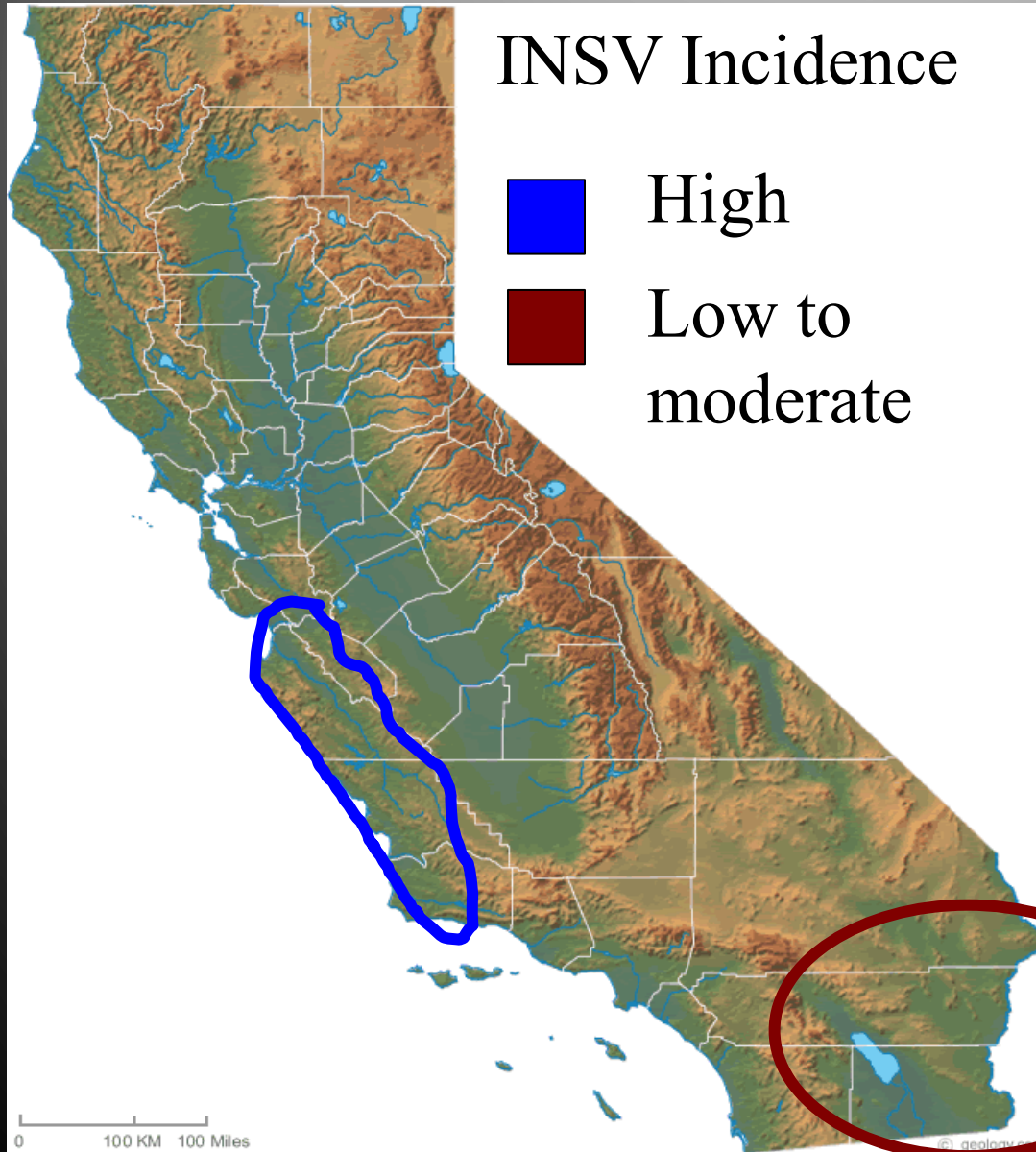
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INSV in Western Lettuce Production



- INSV is most common in the Salinas Valley and coastal production regions in California.
- INSV is less common in desert production regions but can be economically significant for winter production.

INSV can severely impact lettuce yield and marketability in coastal California.



Photos:
Top: W.M. Wintermantel
Bottom: D.K. Hasegawa



Necrotic spots on leaves, expanding into larger areas of necrosis, leaf curling and wilting. Lettuce is not marketable with these symptoms.

INSV can severely impact lettuce yield and marketability in coastal California.

The virus is transmitted in the field by western flower thrips.



Western flower thrips
(*Frankliniella occidentalis*)
Photo by David Cappaert, Bugwood.org



Industry Needs:

- Reduce populations of western flower thrips
- Reduce INSV incidence in lettuce and other virus host plants in the region
- Advance resistance to INSV in lettuce
- Limit the rate of virus spread in lettuce-producing regions

Project Title: Characterizing the accumulation of impatiens necrotic spot virus (INSV) in lettuce tissues and incidence in thrips vectors.

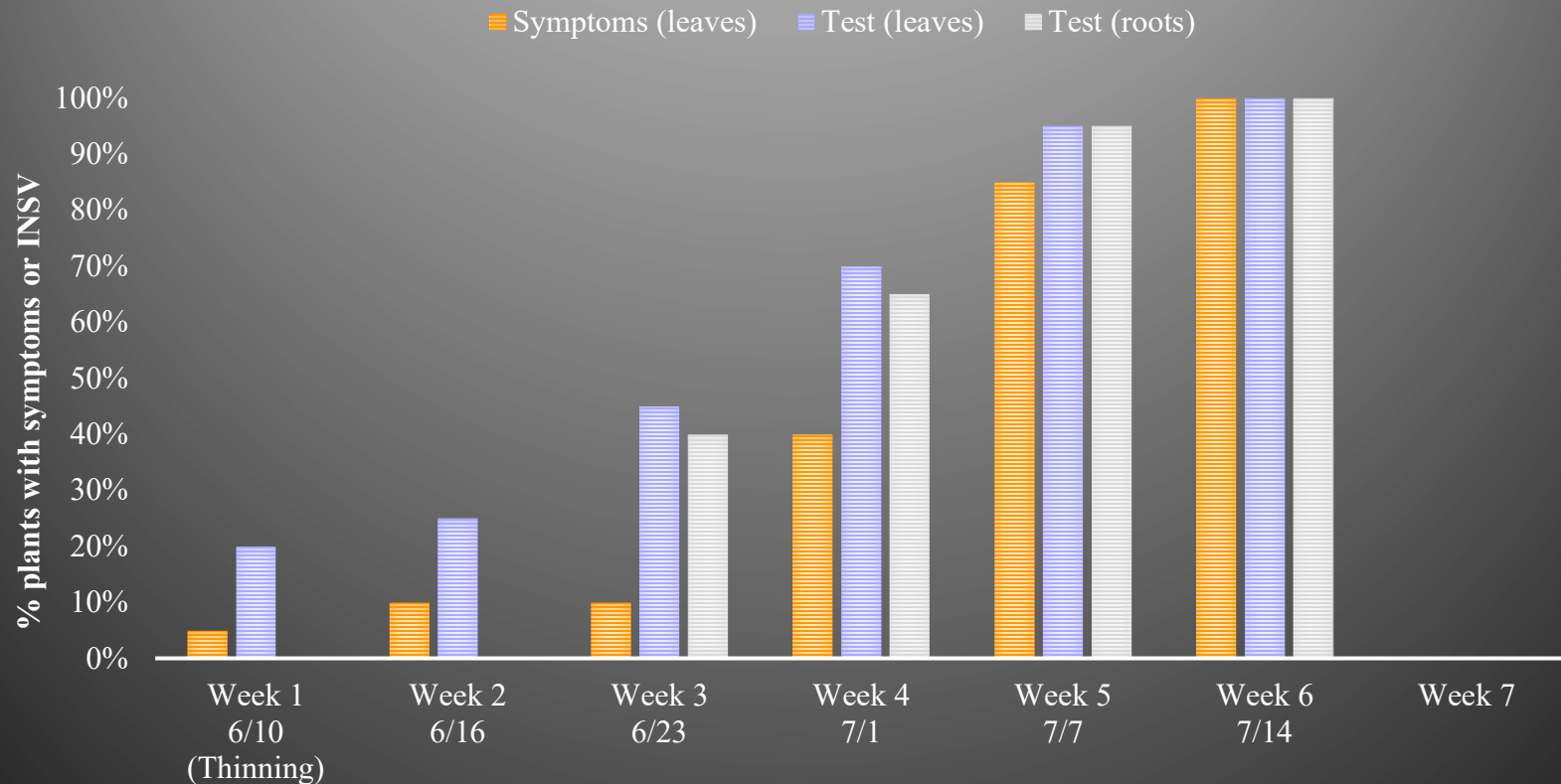
2023 Objectives

1. Determine the distribution and accumulation of INSV in lettuce varieties that are considered resistant or tolerant and assess their impact on plant quality.
2. Assess the potential for INSV to be acquired by thrips from lettuce varieties exhibiting altered virus distribution patterns.

Why are we looking at tissue-specific accumulation of INSV?

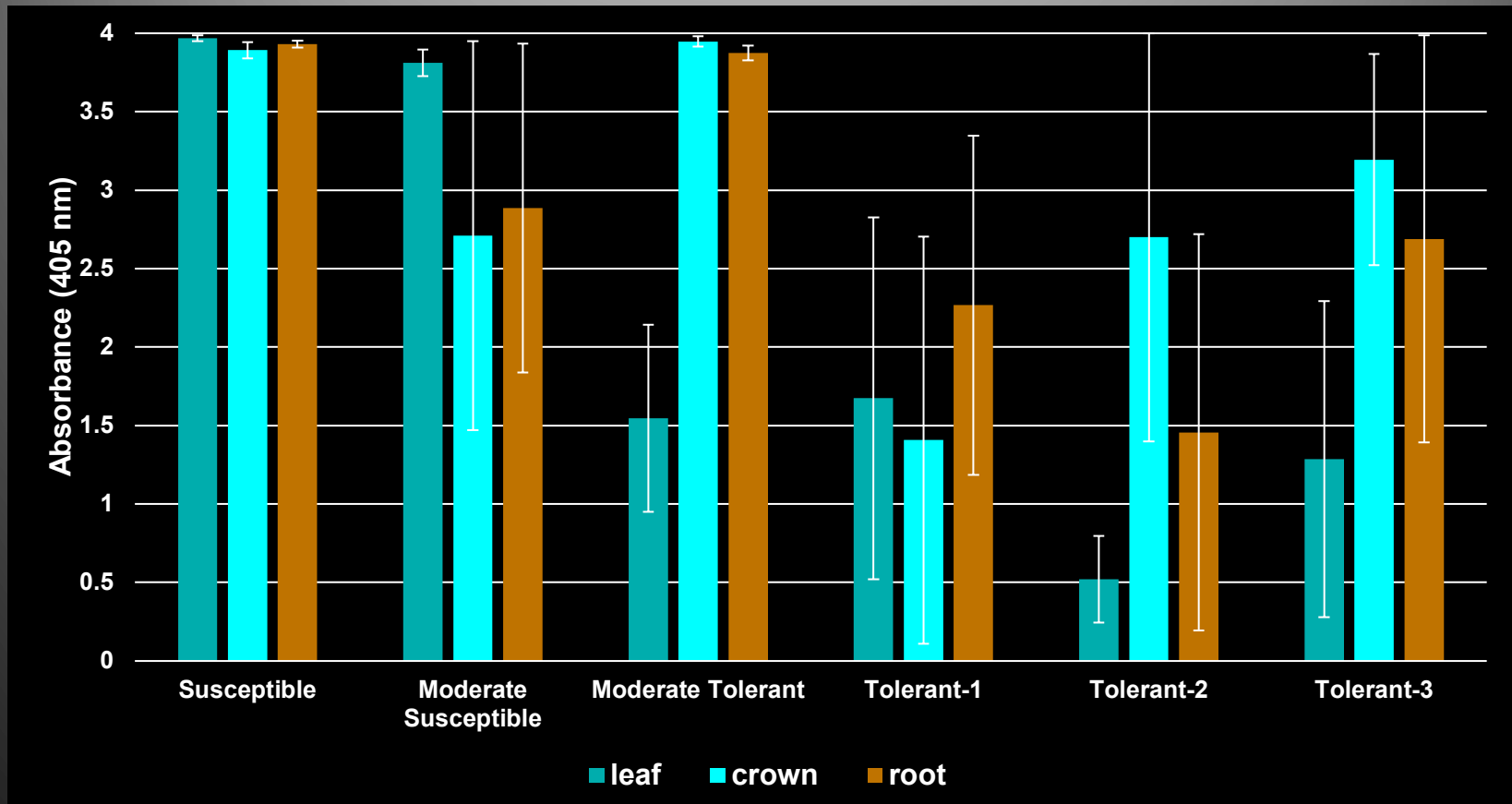
- Studies by the Hasegawa Lab demonstrated that INSV infects both leaf and root tissues in susceptible lettuce varieties.
- Studies conducted in the summer and fall of 2022 in grower fields found differential distribution and accumulation of INSV in leaves and roots of lettuce from six commercial fields using TAS-ELISA (next slide).
 - INSV appeared to be differentially localized in the leaves, crowns, and roots, justifying additional studies to characterize virus distribution in susceptible and tolerant varieties.

Preliminary studies found INSV symptom severity was correlated with progression of weekly virus accumulation.



Representative field: 20 plants were evaluated weekly beginning at the thinning stage and continued until harvest. Root data was not collected from weeks 1 and 2 due to limited amounts of tissue. Data was not collected week 7 due to grower's decision not to harvest the field.

Preliminary studies suggested that INSV may accumulate differentially in leaves, crown, and root tissues in commercial lettuce varieties.



Data represents average absorbance values from TAS-ELISA analysis for three lettuce plants from each of six varieties in a single commercial field trial in Fall 2022. Susceptibility ratings were assigned based on ELISA values and visual field evaluations (data not shown).

Project Design: Objective 1

- INSV was inoculated to sets of lettuce seedlings of each of four lines using viruliferous thrips.
 - Lettuce plants used in experiments included lettuce susceptible to INSV (Parris Island) and commercial varieties and USDA breeding lines with tolerance to INSV.
- At two, four, and six weeks following introduction of viruliferous thrips populations, individual plants of each line were:
 - sampled at leaf, root, and crown.
 - tested for virus infection and titer using ELISA (serological method for virus quantification) with INSV-specific antiserum.

Transmission Methodology

- Western flower thrips at the first instar larval stage were transferred to detached INSV-infected leaves for 24 hr to acquire the virus.
 - Viruliferous larvae were maintained to allow completion of their life cycle to the adult stage.
- Within 48 hr of adult emergence, *specific numbers of* viruliferous adult thrips were collected, placed into a clip cage, and transferred to young lettuce plants at the first to second true leaf stage to allow for virus transmission.
- Plants were maintained in thrips-proof cages in a greenhouse for six weeks.
- Multiple plants of each variety were exposed to viruliferous thrips to assure enough infected plants for each lettuce variety/line being evaluated.

Growth Chamber testing has not yielded consistent infections

ELISA - 6 WEEKS POST INOCULATION

Plant	Mean Leaf	Mean Crown	Mean Root
Tolerant 2	0.092	0.090	0.089
Tolerant 2	0.090	0.087	0.089
Tolerant 2	0.093	0.092	0.090
Tolerant 3	0.090	0.091	0.092
Tolerant 3	0.093	0.090	0.089
Tolerant 3	0.089	0.092	0.087
Parris Island 1	3.744	3.774	1.335
Parris Island 2	0.093	0.101	0.092

A free feeding experiment was initiated to improve infection efficiency, 3/2024



Comparative field trials

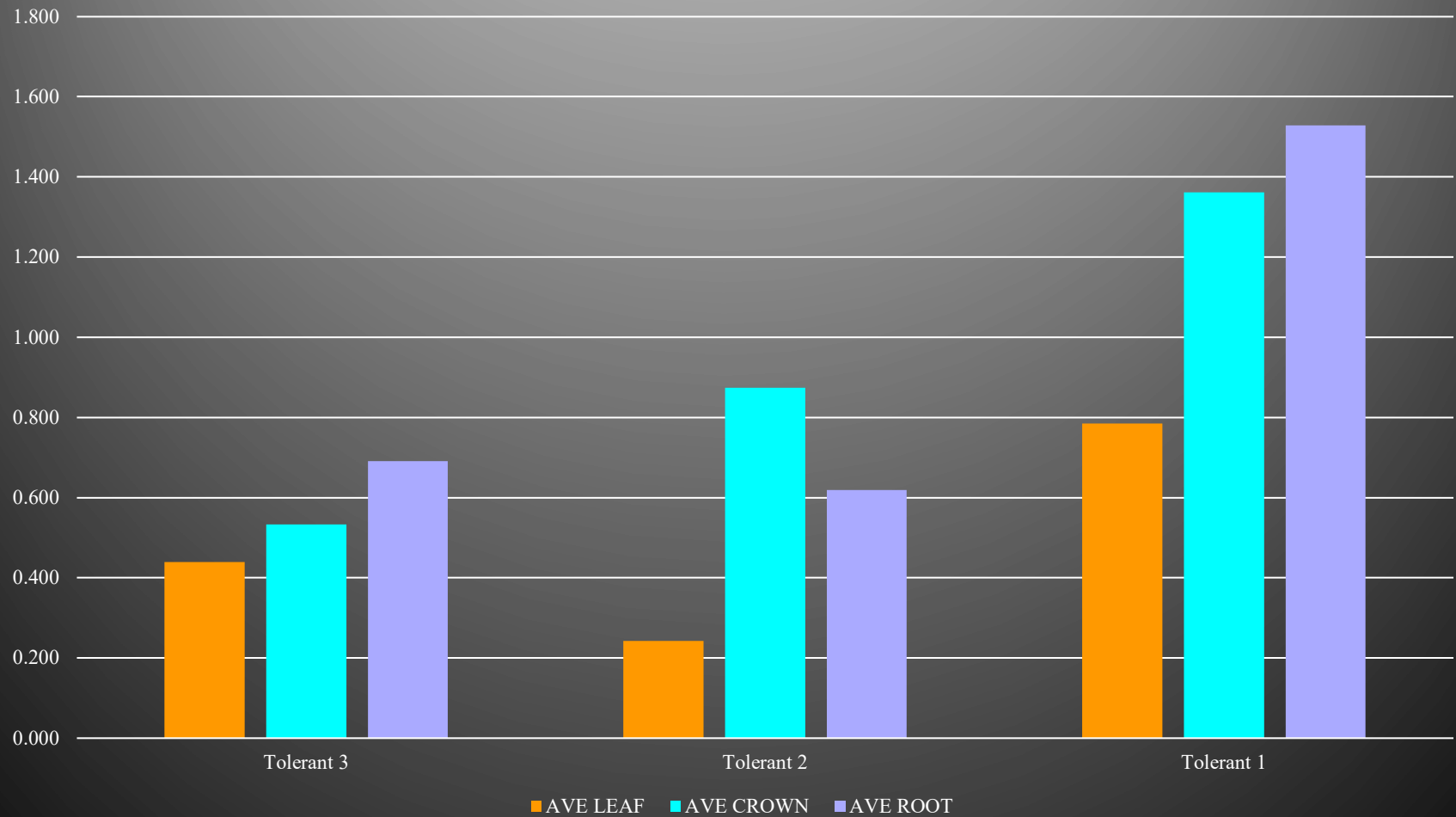


As a follow up to preliminary studies and for comparison to growth chamber, field trials were evaluated for accumulation in root, leaf, and crown.

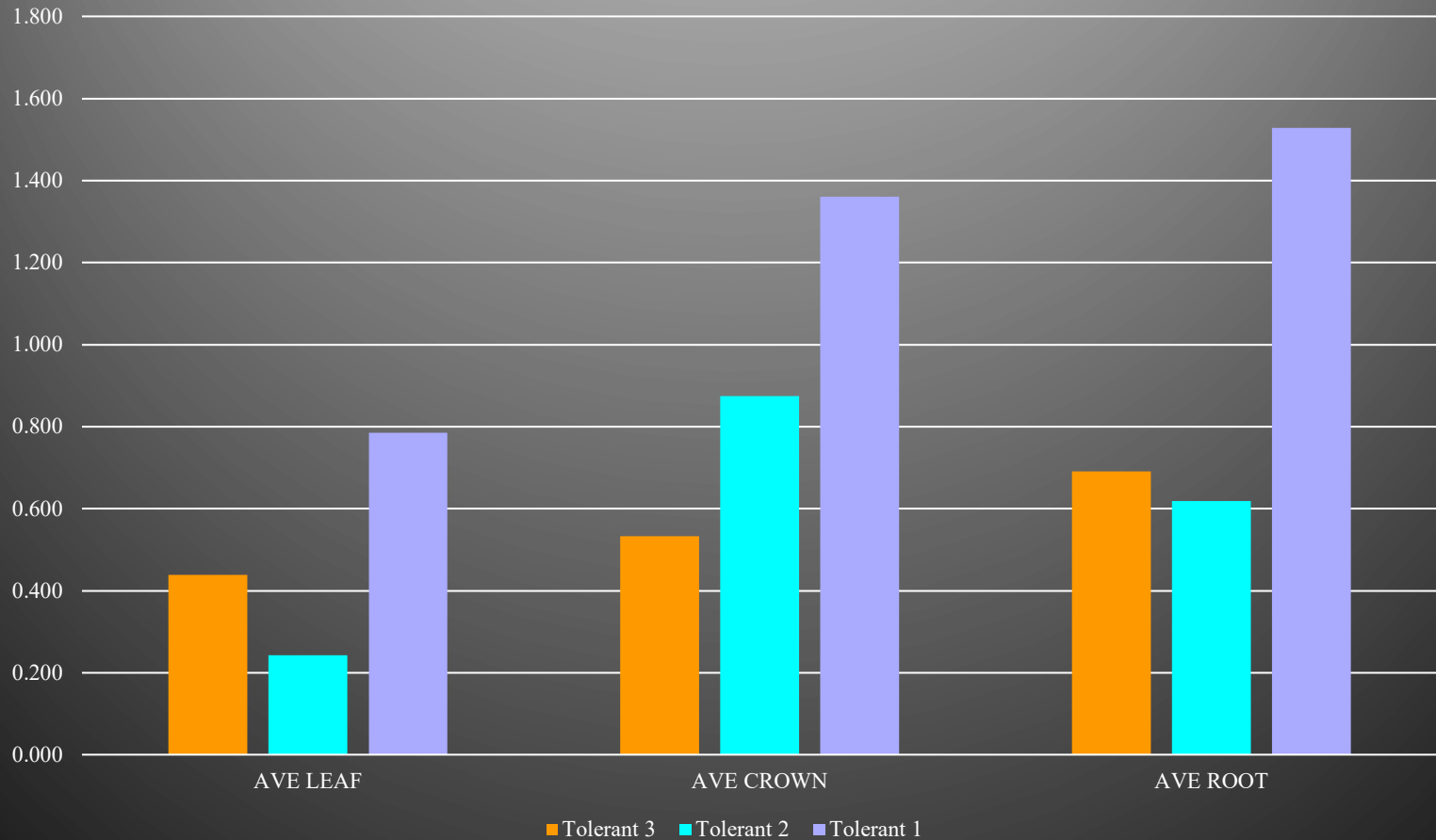
Symptoms were limited among INSV tolerant varieties in a fall 2023 field trial

	Variety/Dis. Severity	1	2	3	4	5	6	7	8	9	10
Rep1	Tolerant 1	0	0	5	4	0	0	5	0	5	0
	Tolerant 2	5	0	0	0	0	0	0	0	5	0
	Tolerant 3	0	0	0	0	0	0	0	0	0	0
Rep2	Tolerant 1	0	0	0	0	0	0	0	0	0	0
	Tolerant 2	0	0	0	0	0	0	0	0	0	0
	Tolerant 3	0	0	5	0	0	0	0	0	0	0
Rep3	Tolerant 1	0	0	0	0	0	0	0	0	0	0
	Tolerant 2	0	0	0	0	0	5	0	0	0	0
	Tolerant 3	0	0	0	0	0	0	0	0	0	5
Rep4	Tolerant 1	0	0	0	0	0	0	0	0	0	0
	Tolerant 2	0	0	0	0	0	0	0	0	0	0
	Tolerant 3	0	0	0	0	0	0	0	0	0	0
INSV severity score: 0-5								Field Trial, October 2023			

Mean INSV ELISA values show differences in accumulation for leaf, root, and crown tissue in tolerant varieties



Mean INSV ELISA values show differences in accumulation for leaf, root, and crown tissue in tolerant varieties



Virus in thrips

- Individual adult thrips are collected from each of the plants tested and evaluated for the presence/titer of INSV.
 - This will provide information on how plants with differences in INSV distribution may influence the ability of INSV to be acquired by thrips and potential for in-field spread.

Project Design: Objective 2

- Based on the outcomes of Objective 1, two varieties that show the greatest differences in virus distribution and accumulation patterns will be evaluated for their potential as hosts for virus acquisition and transmission.
- Following full development of INSV symptoms in the susceptible line, 20 adult thrips will be added to each plant of each variety (3 plants per variety) to allow egg laying for 1 week.
- Adults will be manually removed from the plants and eggs that were laid in the plants will be given 2 weeks to develop to the adult stage.
- Five adult thrips will be collected from each plant and tested for the frequency at which they are infected with INSV.
- Total RNA will be extracted from individual thrips using methods established by Hasegawa and will be tested for INSV using reverse-transcription quantitative PCR (RT-qPCR).
- The frequency of INSV infection will be compared between the susceptible and tolerant varieties to assess their risk as hosts from which thrips can acquire INSV and differences determined by a one-way analysis of variance.

Summary points:

- Preliminary studies indicate INSV accumulates to lower levels in tolerant lettuce varieties than in susceptible varieties.
- Numerically, tolerant root and crown tissue consistently accumulated higher INSV titers than leaf tissue.
- Further standardized experiments are needed to validate this, and the relationship between thrips acquisition and transmission from plants with tolerance compared with susceptible varieties.



Thank you for your
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