ANNUAL RESEARCH REPORT

for

2024

Published as an industry service by the Grower Members of the California Tomato Research Institute, Inc.

Top Research Outcomes

- **Broomrape:** Rimsulfuron chemigation now in grower hands – confirmation of 80% emergence reduction with no yield loss.
- **Sanitation:** Best practices expanded to harvest, hauling and field equipment, with effective quaternary ammonium options identified and other sanitizers ruled out.
- **Genetic Suppression Potential:** Suberin-enhanced root lines demonstrated in vitro broomrape suppression. Trials in 2025 will test hybrid performance and commercial breeding viability.
- **Transplanters:** 70% labor savings achieved with top-performing automated models.
- **Soil Disease Tools:** KPAM trials clarified performance zones; new Fusarium variety guide released.
- **Fusarium Diagnostics:** qPCR tools cut turnaround time from months to weeks.

Membership ROI

- $1.7 million in outside grants secured for grower-priority projects.
- $100,000 in direct CTRI research costs offset by co-funding from CLFP and CDFA.
- Every $1 of CTRI investment more than matched by outside sources or in-kind returns.

Advocacy & Critical Research Infrastructure Wins

- Led successful push to save the **Tomato Genetics Resource Center (TGRC)** at UC Davis. The new Director hire has been confirmed for 2025.
- CTRI named **Research Program Manager** for the new CDFA Broomrape Board, leveraging our platform at no extra cost to the industry.
- Successfully supported UCANR prioritization for the hiring of a Vegetable Weed Specialist and open Farm Advisor roles in key production regions.
- By advocating for USDA IR-4 and partnering with them for product registrations, we are in the pipeline for two novel broomrape mitigation materials earlier than possible otherwise.

What's Next in 2025

- Submitting a **Section 18 to CDPR for sulfosulfuron** to expand broomrape control.
- Deeper dive into yield differences in "new" vs. "old" tomato fields.
- Strengthening breeder pipelines and pre-breeding strategy through national collaboration.
- **Bindweed Suppression via Chemigation:** Testing whether the broomrape Matrix chemigation protocol can also suppress field bindweed, potentially offsetting labor costs and expanding ROI for growers even in broomrape-free fields.

Why It Matters

CTRI membership isn't charity, it's a business investment with a track record of return. Every dollar goes toward solving the problems that hit your bottom line: broomrape control, faster disease diagnostics, evaluating labor-saving tech, and more. These aren't theoretical tools, they're already making a difference in commercial fields. If you're not yet a member, now's the time to join your neighbors in shaping the future of this crop. For questions or to get involved, reach out to Zach Bagley at (530) 405-9469 or zach@tomatonet.org, or visit www.tomatonet.org.

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INTRODUCTION

What do you get when over two-thirds of California's processing tomato acreage unites behind a shared research mission?

You get broomrape control tools in the ground. You get new diagnostics for faster, smarter field decisions. You get millions in outside funding brought back into grower-priority projects. **You get an industry working together – solving today's problems and gearing up for tomorrow's threats.**

This report marks the **56th year of continuous research** sponsored by the voluntary members of the **California Tomato Research Institute (CTRI)** - growers who fund, direct, and benefit from this work.

CTRI exists to identify critical production challenges and to invest in the research solutions that move our industry forward. Projects are selected and funded by a grower-led Board using member assessments ($0.12 per paid ton in 2024). Since 1968, **more than 750 projects** have been supported - focused on pest management (350+), breeding and evaluation (150+), agronomics (150+), market/process quality (75+), and automation (25+).

In 2024, those investments delivered:

- A broomrape chemigation tool with 80% reduction in emergence, with no yield penalty.
- Side-by-side comparisons of new automated transplanters to assess labor savings and ROI.
- Diagnostic tools that cut Fusarium ID time from months to weeks.
- $1.7M in leveraged external funding secured through CTRI's direct support and engagement.
- Industry-wide wins on research policy, regulatory issues, and resource protection.

We're not just funding research - we're building coalitions between growers, processors, allied industry, and researchers to turn results into action. This report shares both our **impact in 2024** and where we're headed in **2025 and beyond.**

If you're already a member, thank you.

If you're not, take a look at what your neighbors are building.

And to every processor field rep, consultant, or industry partner - this is your reference when someone asks: *What is CTRI doing for me?*

Additional resources can be found by joining the CTRI industry email list: https://bit.ly/CTRIemails.

Questions? Contact Zach Bagley at zach@tomatonet.org or 530-405-9469.

California Tomato Research Institute

CTRI GROWER FUNDED RESEARCH: 2024			
2024 TOTAL AFTER FINAL BOARD DECISIONS & COST SHARING: $590,215			
Broomrape Containment, Control and Management		**Research Lead**	**Institution**
2020 Start	Broomrape: Devt. of Long Term Mgmt. Options: CA Commercial Field Conditions & Contained Research Facility Ongoing Work	Brad Hanson	UC Davis
2021 Start	Developing best equipment sanitation practices for broomrape and other high-profile soil borne pathogens; to mitigate field-to-field spread* - CLFP Co-Funding	Cassandra Swett	UC Davis
2022 Start	Determining the population structure of Phelipanche ramosa and Orobanche aegyptiaca field detections in California	Adam Schneider	UW-LaCrosse
2022 Start	Inducible Suberin for Tomato Drought Tolerance (root architecture)	Siobhan Brady	UC Davis
2023 Start	Detection of Broomrape Infestations with Remote Sensing* - CLFP Co-Funding	Alireza Pourezza	UC Davis
2023 Start	Screening of a VOC Sensor to Identify Broomrape Infestations* - CLFP Co-Funding	Cristina Davis	UC Davis
Agronomic/Water/Nutrient Management			
2023 Start	Evaluation of materials to mitigate negative effects of salinity and high temperatures on yields of processing tomatoes	Tom Turini	UC Extension
2023 Start	Climate Smart Mgmt. Innovations for Improved Soil Quality, and Productivity of CA Processing Tomatoes	Amelie Gaudin	UC Davis / UC Extension
2023 Start	KPAM in Soils with RKN AND Fungal Challenges - Impacts on Yield and Disease Severity	Patricia Lazicki	UC Extension
2024 New	Assessment of Novel Transplanter Performance and Economics	Patricia Lazicki	UC Extension
Germplasm and Variety Development			
1991 Start	C. M. Rick Tomato Genetic Resource Center	Roger Chetelat	UC Davis
2024 New	Leveraging germplasm resources for genetic discovery and deployment of salt stress resilience	Greg Vogel	Cornell
Insect & Invertebrate Management			
2024 New	Decoding Resistance-Breaking Root-Knot Nematodes: A Statewide Survey and the Path to Diagnostic Tools in California's Tomato Fields	Shahid Siddique	UC Davis
Pathogen Management			
2017 Start	Disease diagnosis, pathogen movement / emergence monitoring, new pathogen ID and F4 monitoring for the CA processing tomato industry	Cassandra Swett	UC Davis
2018 Start	Developing an integrated mgmt. strategy for F. falciforme vine decline in processing tomato, including co-management with Fusarium wilt	Cassandra Swett & Brenna Aegerter	UC Davis / UC Extension
2024 New	Evaluation of Insecticide Programs in Processing Tomatoes for the MGMT of BCTV and TSWV Vectors and Viruses	Tom Turini	UC Extension
2017 Start	Viral Diagnostics* - CDFA BCTV Control Board Co-Funding	Robert Gilbertson	UC Davis / UC Extension
Weed Control and Management			
2024 New	Controlling in-row weeds with post plant applications of pre-emergent herbicides	Scott Stoddard	UC Extension

Project Title: Field and CRF research towards branched broomrape management

Year of Project Initiation: 2019-ongoing

CTRI Funding in 2024: $54,347

Principal Investigators:
Brad Hanson, Cooperative Extension Weed Specialist
UC Davis Department of Plant Sciences
530-752-8115 bhanson@ucdavis.edu

Co-Investigator(s):
Pershang Hosseini, Postdoctoral scholar
Department of Plant Sciences,
University of California, Davis.
perhosseini@ucdavis.edu

Matthew Fatino, Postdoctoral scholar
UC Davis Horticulture and Agronomy Graduate Group
mfatino@ucdavis.edu

Collaborators and Cooperating Personnel: Eric Schreiner, Schreiner Bros Farming; Gene Miyao, Patricia Lazicki, Cassandra Swett, Coby Goldwasser.

Executive Summary

Branched broomrape is an invasive noxious weed that has been increasingly observed in commercial tomato fields in the Sacramento Valley in recent years. There are limited management strategies for branched broomrape due to its unique physiology, phenology, and development; there are also significant regulatory barriers due to its status as an "A-list" noxious weed in California. Work began in 2019 to evaluate an existing herbicide program developed in Israel based upon two ALS-inhibitor herbicides: sulfosulfuron and imazapic. In 2021, our focus pivoted from imazapic to imazamox due to impassable regulatory barriers for the former chemistry. In 2022, rimsulfuron was evaluated as a chemigated material, and results from that field season were positive; this led directly to a CTRI-held 24(c) Special Local Need Label for the tomato industry in California. In 2023, we continued to evaluate chemigated rimsulfuron alone and paired with preplant incorporated sulfosulfuron along with limited treatments including imazamox and imazapic as standards.

In 2024, several permutations of the annual max rate of rimsulfuron (4 oz/A) were evaluated alone, paired with preplant incorporated sulfosulfuron at different timings, and paired with PPI sulfosulfuron and foliar maleic hydrazide, as well as chemigated sulfosulfuron. Two chemigated rimsulfuron treatments were also evaluated at an additional infested site at a much larger scale that included commercial yield measurements. In addition to the herbicide trials, a grafted variety trial was conducted evaluating several grafted combinations provided by Morning Star Processors. In the small scale and large-scale herbicide trials, all chemigated rimsulfuron treatments significantly reduced broomrape versus the control. More applications of lower doses of rimsulfuron may slightly enhance efficacy, but further research is needed to fully quantify this. Preplant incorporated sulfosulfuron seemed to slightly enhance broomrape suppression when paired with chemigated rimsulfuron, while the stacked treatment had the best numerical results reducing emergence by 96% compared to the control. Results from chemigated sulfosulfuron are very promising and could potentially replace one or more of the chemigated rimsulfuron applications to allow its

use for foliar broadleaf weed control, which could provide much needed nightshade control in fields with high nightshade populations. In the large-scale demonstration study, chemigated rimsulfuron performed very well, reducing broomrape by over 80% while not reducing yields compared to the control.

In concurrent lab and greenhouse work, diverse strategies were explored to manage branched broomrape. Four focus areas for the controlled environment studies included evaluating synthetic strigolactones for "suicidal germination," assessing broomrape seed tolerance to flooding, screening the susceptibility of 34 crops to broomrape parasitism, and examining the effects of nitrogen fertilizers on broomrape seed germination. Synthetic strigolactones effectively stimulated germination in the absence of a host, reducing parasitism in tomato plants and highlighting their potential for integrated pest management (IPM) and we hope to bring this to field evaluation in 2025. Flooding experiments showed reduced germination under high temperatures and longer flood periods, indicating potential viability in certain agricultural contexts. Crop susceptibility screening revealed nearly 50% of tested crops could host broomrape. Lastly, fertilizer studies suggest a potential link between nutrient management and broomrape suppression that should be explored further.

The amended 2024 objectives of this project were to:

A. Field:
   A1. Refine rimsulfuron chemigation treatments to provide better grower support for the 24(c) label approved in 2023 with limited data.
   A2. Continue evaluation of sulfosulfuron PPI treatments in support of future label request once residue packages are submitted to USEPA.
   A3. Field evaluation of a synthetic strigolactone germination stimulant prior to tomato transplanting. (*note: this objective could not be addressed in 2024 and was replaced with a large scale evaluation of rimsulfuron chemigation with commercial yield data*).
   A4. Evaluate a “stacked” treatment program of 1) preplant strigolactone to stimulate germination, 2) PPI sulfosulfuron, 3) chemigated rimsulfuron, and 4) foliar maleic hydrazide to compare the integrated program to single product plots.
   A5. Coordinate with tomato breeding companies to evaluate a limited number of commercial or pre-commercial lines for broomrape parasitism in the infested field site.

B. Contained Research Facility (CRF)
   B1. Continue systematic screening of commercial tomato cultivar sensitivity to broomrape parasitism in the quarantine greenhouse at UC Davis
   B2. Refine small-scale screening assay of seedling plants for broomrape parasitism as a preliminary screening tool.
   B3. Conduct further research on two germination stimulation compounds, starting with controlled Petri dish assays and progressing to pot assays incorporating soil and host plants.
   B4. Design and conduct an experiment to evaluate broomrape seed tolerance to flooding and assess its potential as a viable field management strategy.
   B5. Conduct small-scale evaluations of the susceptibility of various crops and weeds to broomrape parasitism.
   B6. Initiate research on the impact of fertilizers on broomrape seed viability to address unexpected broomrape mortality observed in previous research trials.

Methodology and Results

A1, 2, 4. An efficacy trial refining rimsulfuron-based chemigation treatments was conducted in spring/summer of 2024 in the branched broomrape infested commercial tomato field north of Woodland, CA. The trial evaluated the 24(c) SLN protocol of chemigated rimsulfuron, several permutations of the 4 oz annual max rate split different ways, PPI sulfosulfuron paired with rimsulfuron applied according to different timing regimes, chemigated sulfosulfuron (new treatment), two rates of foliar maleic hydrazide, and a full-stack treatment of PPI sulfosulfuron, chemigated rimsulfuron, and foliar maleic hydrazide. Each plot consisted of a 120-ft 60" bed with a single subsurface drip-line buried 9-10" in the center. The trial was transplanted on April 9, 2024, with 'HM 58841' in a single line with 12" spacing. Treatment applications were made from early May to mid-July (Tables 1, 2). The trial was arranged in a randomized complete block design with four replications. Preplant incorporated and foliar treatments were applied using a CO_2 pressured backpack sprayer and 3-nozzle boom while chemigated treatments were mixed in 3-L bottles and injected directly into each bed's dripline using CO_2. Broomrape emergence data was collected from May until August; yield was not collected to due variability in the crop due to recurring broomrape scouting activity (Table 2).

This season, all treatments except the constant rate maleic hydrazide significantly reduced branched broomrape emergence versus control (Table 2). More numerous chemigated treatments of rimsulfuron at lower rates (4x1 oz, 5 x0.8 oz) tended to have slightly lower emergence than the 24(c) treatment of 3 applications of 1.33 oz, though further research will need to be conducted to confirm this trend. Chemigated sulfosulfuron had one of the lowest numerical number of broomrape clusters, and this treatment will be pursued in the future and could free up some rimsulfuron for foliar use for broadleaf weed control. Maleic hydrazide at both rates had similar results to chemigated rimsulfuron treatments; however, in 2024, these treatments broke about 6 weeks after the last treatment with a late season flush of broomrape emergence. Future research could refine application timings to stretch out control later in the season.

A3. *The original objective was not addressed as planned due to logistical constraints in receiving the germination stimulant products at sufficient quantities for field research from Saudi Arabia. Instead of the planned objective, we were able to acquire an additional grower cooperator to conduct a larger scale demonstration trial for chemigated rimsulfuron efficacy that included commercial yield validation to address industry concerns*.

The demonstration trial was conducted within a commercially planted processing tomato crop and treatments included a three-treatment protocol similar to the 24(c) SLN Matrix label and a four-treatment protocol; both protocols' applications totaled the annual maximum of 70 g/ha (4 oz/A) rimsulfuron. Individual plots were 400 m long and arranged in a randomized complete block design with three replications. 'HM 8237' tomato transplants were mechanically transplanted with 12" in-row and in-line spacing with two lines on each bed. Chemigation treatments were mixed in a 100 L tank and applications were made into individual beds with an electric pump during the last third of an irrigation set. Treatments were applied according to a days after transplant schedule (Table 1). At this location, broomrape emergence was evaluated four times throughout the growing season and tomato yield was collected using a Johnson commercial mechanical harvester (Oxbo, Woodland, CA) and weigh cart equipped with a scale. Tomato yield per 400 m plot was collected at commercial maturity on October 2, 2024.

In the large-scale demonstration study, there was no visual tomato crop injury observed in any of the treated plots (data not shown). Chemigated rimsulfuron treatments had substantially less broomrape emergence versus the nontreated control (Table 3). The control plots had an average of 122 clusters per 400 m plot, while rimsulfuron applied three times at 22.3 g ai/ha (1.33 oz/A x 3) resulted in 21 clusters per plot and rimsulfuron applied four times at 17.4 g ai/ha (1 oz/A x 4) resulted in 15 clusters per plot (Table 3). There was no statistical difference in broomrape emergence between the two chemigated rimsulfuron treatments and

both reduced broomrape by greater than 83% compared to the control (Table 3). Tomato yield ranged from 9,143 kg to 9,306 kg per 400 m plot and there was no significant difference between rimsulfuron-treatments and the control (Table 3). Given the significant reduction in broomrape emergence with both chemigated rimsulfuron treatments and saleable fruit yield that was comparable to the control plots, these results could encourage growers to adopt the 24(c) rimsulfuron protocol as a preventive treatment in fields at-risk of branched broomrape infestation

A5. A trial evaluating several grafted combinations of tomato cultivars was conducted in spring/summer of 2024 in the same Yolo Co infested field. Each plot consisted of a 120-ft 60" bed with a single subsurface drip-line buried 9-10" in the center. The trial was transplanted on April 9, 2024, with a single grafted combination per plot planted in a single-line with 24" spacing. The trial was arranged in a randomized complete block design with 5 replications. The trial was scouted three times throughout the season for branched broomrape, and individual clusters were marked with wire construction flags. Data were analyzed with an analysis of variance.

Every replication of every grafted variety combination in the field trial had broomrape emergence. There were no statistical differences in broomrape emergence among the three grafted variety combinations ($p=0.74$). H1996xCG6094 had an average of 62 clusters per plot, H1996xCG4069 had an average of 60 clusters per plot, and H1996xCG6575 had an average of 49 clusters per plot. There was not an ungrafted control variety included within this embedded trial. However, the trial was within a larger tomato field planted with 'HM 58841' on April 9 and in field trials adjacent to the grafted variety trial, plots planted with 'HM 58841' had an average of around 110 clusters per 120' bed. Due to the lack of a control variety within the replicated trial, statistical inferences cannot be made regarding differences in broomrape emergence between grafted variety combinations and 'HM 58841'. However, it appears that the plots planted with each of the three grafted varieties tended to have less broomrape than plots in adjacent field trials planted with 'HM 58841'. Future studies should be conducted with an ungrafted control to validate these trends as well as screen additional grafted combinations and commercially available rootstocks.

B1. The greenhouse screening trial was initiated in the spring of 2024 and aimed to build upon data from a 2023 cultivar screening (2023 CTRI funded project). This trial was conducted in a newly designated broomrape research greenhouse space outside of the CRF. Tomato varieties from the 2021 top 20 planted cultivars PTAB list and other cultivars of interest from industry partners were seeded into transplant plug trays on May 7, 2024, and inoculated with preconditioned broomrape seed and transplanted into 1 L pots on June 5, 2024. The trial was arranged in a randomized complete block design with three single-plant replications of each variety.

Unfortunately, the new greenhouse space which alleviated a space limitation from the quarantine greenhouse was significantly warmer than the previous space in the CRF; summertime temperatures and our late spring planting resulted in no broomrape emergence. We hypothesize that the higher air and soil temperatures caused the preconditioned broomrape seed to enter secondary dormancy, resulting in no germination or emergence. To address this failure, another run of this trial began in winter 2024/25 and is currently ongoing.

B2. After several iterations, the small-scale seedling assay protocol is working well and is now the primary method being used in our CRF and dedicated broomrape greenhouse space. The system is simply a clear ~32 fl oz plastic beverage cup nested within an opaque cup of the same size; this allows non-destructive observation of a portion of the root zone of each host plant such that broomrape attachments can be observed, measured, and tracked for various data collection purposes. Additionally, in some experiments such as the host studies where plants can be scored as infested as soon as an attachment is observed rather than waiting for the emergence of a shoot several weeks later which expedites the research.

B3: Two synthetic strigolactone products, developed by researchers in Saudi Arabia, were evaluated as potential tools for managing branched broomrape through suicidal germination. These products had previously been tested on striga (witchweed), a root parasite of maize and sorghum, and were applied for the first time to branched broomrape in the United States in 2023. Results from Petri dish germination experiments conducted in a quarantine greenhouse indicated that the experimental products effectively stimulated the germination of branched broomrape seeds, with performance comparable to GR24, the laboratory standard. During 2024, this study continued in vitro and was scaled up to pot experiments with soil and tomato host plants. The previous Petri dish experiments demonstrated that the experimental products effectively stimulated the germination of branched broomrape seeds at levels comparable to GR24, the laboratory standard. The pot experiments confirmed these results, further supporting their potential efficacy (Figure 1). These chemicals were applied either once or twice, at least three weeks prior to planting tomato seedlings, to stimulate branched broomrape seed germination in the absence of a host, thereby causing suicidal germination. Planting tomato seedlings after the chemical application demonstrated a reduction in branched broomrape parasitism on tomatoes across all treatments. We plan to transition this work to field trials in 2025, (and if research approvals can be obtained and sufficient chemicals imported into the US).

B4: A pilot experiment was conducted to evaluate the tolerance of branched broomrape seeds to flooding and assess its potential as a field management strategy. The experiment was conducted under two temperature conditions—cold (10°C) and hot (28°C)—to simulate winter and summer soil temperatures in the Sacramento Valley. Broomrape seeds were sealed in fine mesh nylon bags and placed in one-quart jars filled with sieved soil collected from the UC Davis Vegetable Crops Facility fields. The bags were buried below the soil surface, and the jars were filled with tap water to create a one-inch water layer above the soil. The jars were incubated at 10°C and 28°C for durations of 3, 7, 14 and 28 days. After incubation, the seeds were removed, sterilized, and placed on Petri dishes for pre-conditioning and germination assessment. Germination rates were then evaluated. The results showed that high temperatures had a greater negative impact than cold conditions on broomrape germination. Additionally, the duration of flooding significantly influenced germination rates, with temperature as a secondary factor. The interaction between flooding duration and temperature may also play a critical role in preventing broomrape germination under flooded conditions (Figure 2). A second run of this experiment with more time points is currently underway and should lead to useful information about how soil moisture conditions affect broomrape seed viability.

B5: A small-scale experiment was conducted to assess the susceptibility of 34 crops from 11 plant families to branched broomrape under greenhouse conditions. These families included Solanaceae, Cucurbitaceae, Fabaceae, Brassicaceae, Malvaceae, Asteraceae, and Cannabaceae. The double-pot system was employed to facilitate observation without disturbing the host plants, consisting of a transparent inner pot nested within a black outer pot. Crops were grown in a soil mix containing broomrape seeds, and their susceptibility to parasitism was monitored throughout the study. The results, summarized in Table 4, reveal varying levels of susceptibility among the crops, categorized as high, medium, or low and non-host, based on the number of replicate pots exhibiting broomrape attachment. High indicates that all pots were infested with branched broomrape attachments. Medium signifies that 40–70% of the pots were infested, while low represents an infestation rate of 10–20%. Overall, 16 out of 34 crops demonstrated their potential to serve as hosts for branched broomrape, which accounts for nearly 50% of the crops tested in this experiment.

B6: A series of experiments in a new objective was conducted to evaluate the effects of various nitrogen fertilizers on the germination of branched broomrape seeds and early radicle elongation. The fertilizers were applied at three stages: preconditioning, post-preconditioning, and shortly after germination. The study followed a factorial design with three replications and was conducted using Petri dishes for germination assay. Approximately 30 to 50 sanitized and washed branched broomrape seeds were placed in each dish.

Fertilizers were introduced at various specified stages and included ammonium phosphate, ammonium sulfate, ammonium nitrate, urea, calcium nitrate, potassium nitrate, and potassium chloride. Each fertilizer was tested at multiple concentrations: 0, 1.56, 3.125, 6.25, 12.5, 25, and 50 nM. All Petri dishes underwent a preconditioning stage in a growth chamber at 25°C for at least seven days. Afterward, GR24, a synthetic germination stimulant, was added to induce germination. The Petri dishes were sealed with parafilm during incubation in the growth chamber to maintain optimal conditions. On the 10th or 13th day following the application of GR24, the dishes were unsealed, and germination rates and radicle lengths were recorded. This experiment is ongoing, and the results will be available soon.

Discussion

In the small scale and large-scale herbicide field trials, all chemigated rimsulfuron treatments significantly reduced broomrape versus the control. The “stacked” treatments that included PPI sulfosulfuron, chemigated rimsulfuron, and foliar maleic hydrazide performed very well (96% reduction in broomrape emergence) which suggests that ultimately an integrated management approach may be feasible if/when these treatments are registered. There was some evidence that more applications of lower doses of rimsulfuron may slightly enhance efficacy, but further research is needed to fully quantify this and we are currently analyzing several seasons of field and greenhouse data to model this better. Importantly, the large-scale demonstrations of rimsulfuron efficacy and commercial tomato yield indicated good suppression of branched broomrape and no negative effect of the chemigation rimsulfuron at the max rate. Results from chemigated sulfosulfuron are very promising and could potentially replace one or more of the chemigated rimsulfuron applications to allow its use for foliar broadleaf weed control, which could provide much needed nightshade control in fields with high nightshade populations.

For tomato varieties, again there did not appear to be dramatic differences among current cultivars in terms of broomrape sensitivity. The small-scale test of several grafted tomato varieties was not definitive with regard to broomrape sensitivity but was sufficiently encouraging to continue this line of research in partnership with the industry.

Four distinct controlled environment experiments were conducted, each addressing a different aspect of broomrape management. The findings provide valuable insights into the potential management strategies for controlling this parasitic weed. The products effectively stimulated broomrape seed germination in vitro and in soil-based experiments, with results comparable to the laboratory standard GR24. By inducing germination in the absence of a host, these products cause "suicidal germination," which could significantly reduce the soil seed bank. The reduction in parasitism observed in tomato plants following treatment underscores the potential of these compounds in integrated pest management strategies. While promising, the need for field trials and regulatory approval highlights the next steps in scaling this approach for practical application in the United States.

The pilot experiment assessing broomrape seed tolerance to flooding revealed that prolonged flooding, especially under high temperatures, effectively reduced germination rates. These findings suggest that flooding could be a viable strategy in areas where it is agronomically and environmentally feasible. The interaction between flooding duration and temperature highlights the importance of optimizing conditions for this strategy. While flooding may not be universally applicable, it could be an effective supplemental tactic in certain regions (rice rotation), particularly during the summer months when temperatures are high.

The susceptibility screening of 34 crops provided critical information on the host range of branched broomrape. The categorization of crops into high, medium, low, and non-host groups offers a foundation for selecting resistant or less susceptible crops as part of crop rotation strategies. The finding that nearly 50%

of tested crops could serve as hosts underscores the widespread risk posed by broomrape and the importance of implementing targeted management strategies to minimize its impact.

The ongoing experiment investigating the impact of various nitrogen fertilizers can suggest that different fertilizers and their application timing may influence broomrape seed behavior. This approach could potentially integrate into nutrient management practices, allowing growers to suppress broomrape germination while optimizing crop nutrition. However, further results are needed to validate this strategy and determine its effectiveness under field conditions.

Acknowledgments

We would like to acknowledge and thank those who collaborated on this project. In particular, this project would not have been possible without the generous cooperation of Eric Schreiner and Schreiner Bros. Farming who have hosted the efficacy trials since 2019. We would also like to acknowledge our additional grower cooperator who provided the site for the large-scale herbicide trial in 2024. We would like to thank AgSeeds Unlimited, particularly Ross Lopez, for transplants and transplanting support.

Special thanks, to Gene Miyao and Zach Bagley for being engaged collaborators on the broomrape effort since 2019 and Patricia Lazicki for her recent collaboration on the broomrape effort. Juan Carlos Galaz at the UC Davis Chile Life Sciences Innovation Center and his group for their work on the broomrape effort in the southern hemisphere that has been highly informative to our projects in California. The contributions of the non-broomrape members of the Hanson lab are greatly appreciated for their invaluable help in the field and greenhouse on various aspects of this project.

Lastly, this project is closely coordinated with the CTRI-funded equipment sanitation projects led by Cassandra Swett. The specific broomrape sanitation goals addressed and supported by the weed science team, particularly co-PI Hosseini and lab assistants, are reported under the Swett sanitation project.

Table 1. Application dates from two branched broomrape efficacy trials conducted near Woodland, CA

Treatment		**2024**	**Demo site**
Preplant incorporated	Preplant incorporated	28-March	-
-	Transplant	9-Apr	24-May
Chemigation	400 GDD	9-May	-
Chemigation	600 GDD	16-May	-
Chemigation	800 GDD	30-May	-
Chemigation	1000 GDD	6-June	-
Chemigation	20 DATP	3-May	18-June
Chemigation	30 DATP	9-May	28-June
Chemigation	40 DATP	20-May	8-July
Chemigation	50 DATP	30-May	18-July
Chemigation	70 DATP	6-June	-
Foliar MH, rimsulfuron	100 GDD	22-Apr	-
Foliar MH	200 GDD	27-Apr	-
Foliar MH	400 GDD	9-May	-
Foliar MH	700 GDD	28-May	-
Foliar MH	1000 GDD	6-June	-

GDD: growing degree days, DATP: days after transplant, MH: maleic hydrazide

Table 2. Treatments from a 2024 broomrape efficacy study conducted near Woodland, CA.

	Treatment	Active Ingredient	Rate	Application	Timing	Broomrape Emergence^	
			g ai/ha			Clusters/30 m	
1	Nontreated control					111	ab
2	Rimsulfuron x3	Rimsulfuron	23.3	Chem x3	400, 600, 800 GDD	36	c
3	Rimsulfuron x4	Rimsulfuron	17.4	Foliar, Chem x3	200 (F), 400, 600, 800 GDD	25	c
4	Rimsulfuron x5	Rimsulfuron	13.9	Chem x5	200, 400, 600, 800, 1000 GDD	15	c
5	Sulf+Rim x3 GDD	Sulfosulfuron	37.5	PPI		18	c
5		Rimsulfuron	23.3	Chem x3	400, 600, 800 GDD		
6	Sulf+Rim x3 DATP	Sulfosulfuron	37.5	PPI		34	c
6		Rimsulfuron	23.3	Chem x3	25, 35, 45 DATP		
7	Sulf+Rim Late DATP	Sulfosulfuron	37.5	PPI		32	c
7		Rimsulfuron	23.3	Chem x3	30, 50, 70 DATP		
8	Sulfosulfuron alone	Sulfosulfuron	37.5	PPI		114	a
9	Sulfosulfuron drip	Sulfosulfuron	12.5	Chem x3	400, 600, 800 GDD	16	c
10	MH constant rate	Maleic hydrazide	400 x5	Foliar x5	100, 200, 400, 700, 1000 GDD	44	bc
11	MH split rate	Maleic hydrazide	270 x2, 540 x3	Foliar x5	100, 200, 400, 700, 1000 GDD	27	c
12	Full stack	Sulfosulfuron	37.5	PPI			
12		Rimsulfuron	23.3	Chem x3	400, 600, 800 GDD		
12		Maleic hydrazide	270 x2, 540 x3	Foliar x5	100, 200, 400, 700, 1000 GDD	4	c
13	Rim Chile rate	Rimsulfuron	10	Chem x3	400, 600, 800 GDD	40	c
P-value						<0.0001	

PPI: preplant incorporated, Chem: chemigated, GDD: growing degree days, DATP: days after transplant, sulf: sulfosulfuron, rim: rimsulfuron MH: maleic hydrazide. ^Means that share the same letter are not significantly different from one another.

Table 3. Treatments from a 2024 broomrape management demonstration study conducted near Woodland, CA.

Treatment	Active Ingredient	Rate	Application	Timing	Broomrape Emergence^		Tomato Yield^	
		g ai/ha		DATP	Clusters/400 m		kg/400m	
1	Nontreated control				122	a	9,306	a
2	Rimsulfuron x3	23.3	Chem x3	20, 30, 40	21	b	9,143	a
3	Rimsulfuron x4	17.4	Chem x4	20, 30, 40, 50	15	b	9,158	a
p-value					0.0003		0.44	

Chem: chemigated, DATP: days after transplant. ^Means that share the same letter are not statistically different from one another.

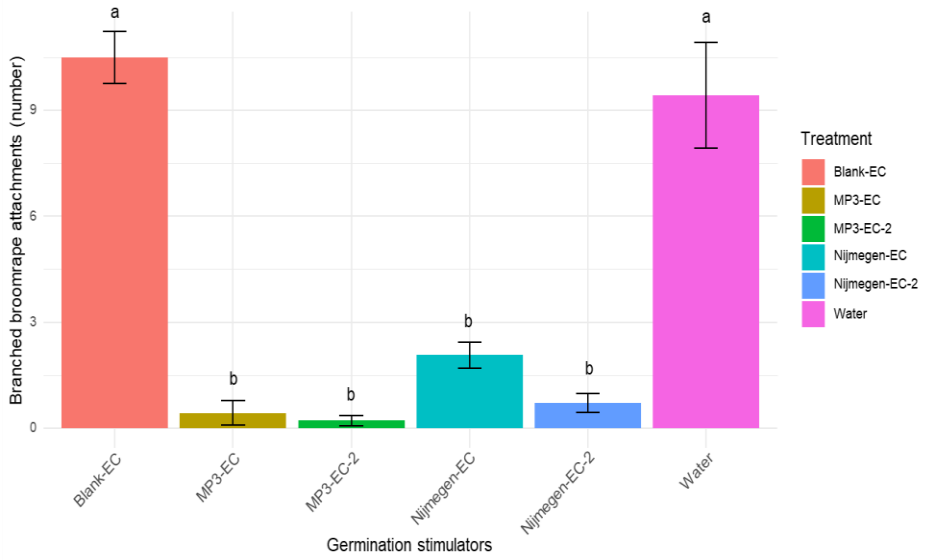

Figure 1: Comparison of germination stimulator treatments, MP3-EC, and Nijmegen-EC for the number of branched broomrape attachments. Data are means ± SE (n = 7), and treatments with various letters differ significantly according to one-way analysis of variance (ANOVA) ($p < 0.05$).

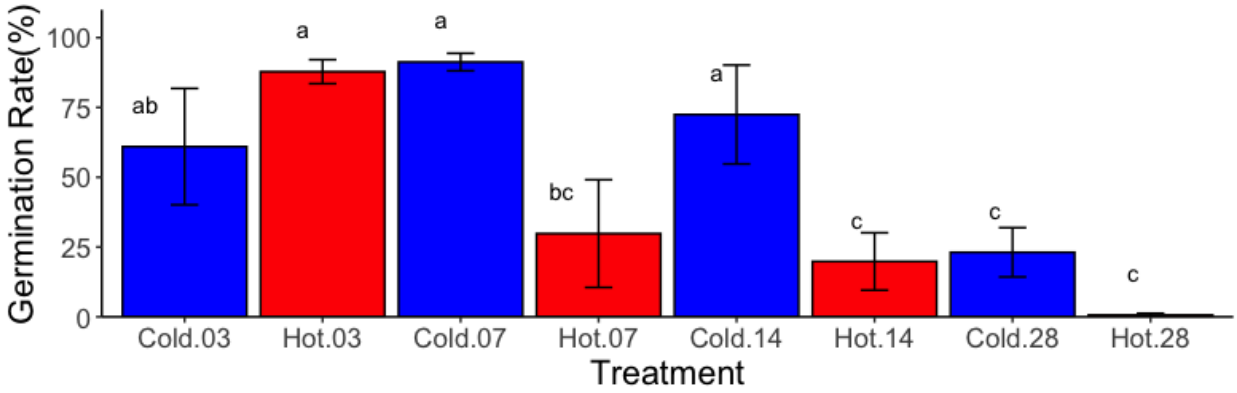

Figure 2: Effect of flooding duration and temperature on the germination of branched broomrape seeds in a preliminary experiment. Hot means 28C incubation and cold means 10C incubation (approximate summer and winter soil temperatures, respectively) and the number indicates the number of days of flooded conditions.

Table 4: Host susceptibility: screening crops for resistance to branched broomrape parasitism

Family	Crop	Broomrape attachment*
Apiaceae	Carrot	High
	Celery	High
	Cilantro	High
	Parsley	High
Asteraceae	Sunflower	Non
	Safflower	Non
	Lettuce	High
Brassicaceae	Radish	Medium
	Cabbage	High
	Broccoli	Non
	Cauliflower	Medium
Cannabaceae	Hemp	High
Cucurbitaceae	Watermelon	Non
	Pumpkin	Non
	Squash	Medium
	Cucumber	Non
	Melon	Medium
Fabaceae	Alfalfa	Non
	Pea	Medium
	Red bean	Non
	Fava bean	High
	Bush bean	Non
	Hairy vetch	Non
	Red clover	Non
	Crimson clover	Low
	Soybean	Non
Malvaceae	Cotton	Non
Solanaceae	Tomato	High
	Eggplant	Non
	Pepper	Non
Pedaliaceae	Sesame	Non
Poaceae	Durum wheat	Non
	Corn	Non
Amaranthaceae	Quinoa	Medium

* High indicates that all replicate plants had branched broomrape attachments, medium signifies that 40–70% of the plants were parasitized, low represents an infestation rate of 10–20%, and non means no attachment observed.

Figure 3: Branched broomrape host screening in dedicated broomrape quarantine greenhouse. Host roots and broomrape attachments visible in the double-pot system (inset).

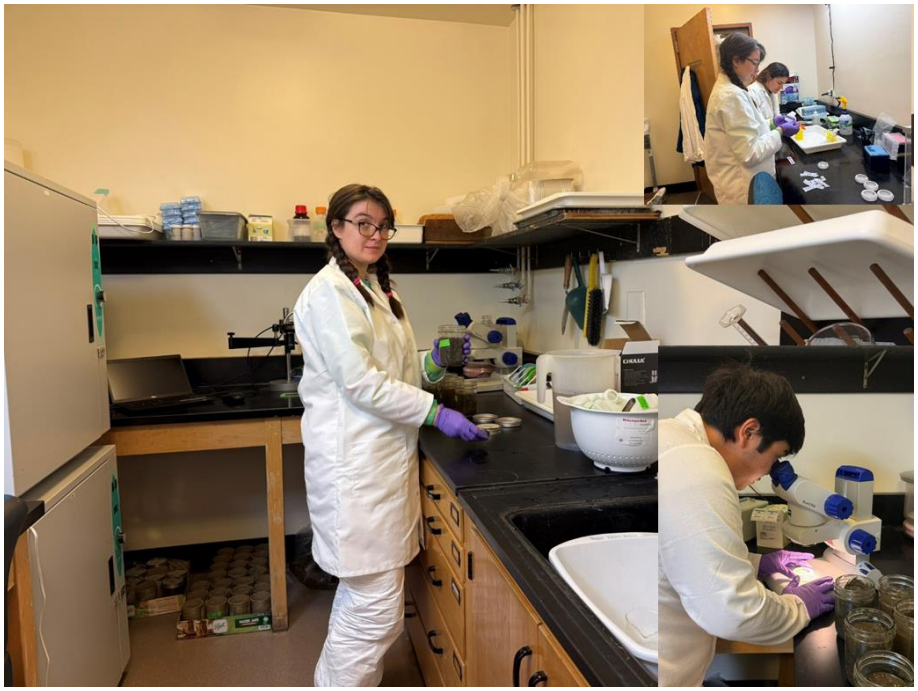

Figure 4: Evaluating branched broomrape germination in broomrape quarantine lab space. Experiments include various seed germination, flooding, and fertilizer screening objectives.

Project Title: Developing best management practices for mitigating spread of branched broomrape and other high-profile soil borne pathogens.

Year of Project Initiation: 2021

CTRI Funding in 2024: $54,158

Project Leader and Co-PIs:
PI: Cassandra Swett, Associate Professor of Cooperative Extension Plant Pathologist, Department of Plant Pathology, UC Davis, 530-752-337, clswett@ucdavis.edu

Co-PI: Brad Hanson, Professor of Cooperative Extension, Weed Specialist, Department of Plant Sciences, UC Davis, (530) 752-8115, bhanson@ucdavis.edu

Co-PI: Patricia Lazicki, Vegetable crops advisor, Yolo, Solano and Sacramento County, Woodland, CA, palazicki@ucanr.edu

Co-PI: Justine Beaulieu, Postdoctoral researcher, UC Davis Department of Plant Pathology, jbeaulieu@ucdavis.edu

Co-PI: Pershang Hosseini, Postdoctoral researcher, Department of Plant Sciences, perhosseini@ucdavis.edu

Co-PI: Zach Bagley, CTRI Director, zach@tomatonet.org

Collaborator(s):

Brenna Aegerter, Vegetable crops advisor, San Joaquin County, bjaegerter@ucanr.edu

Humberto Izquierdo, Agricultural Commissioner/ Sealer of Weights & Measures- Yolo County, Agriculture/Weights & Measures, Woodland, CA, Humberto.Izquierdo@yolocounty.org

David Viguie, Timothy & Viguie Farming

Anthony Terranova and Carlos Zatarain, Anterra Group. anthony@anterragroupinc.com, Carlos@anterragroupinc.com

Executive Summary

Key takeaways:

- Other sanitizers like Star San can maintain efficacy in the presence of debris. Unfortunately, while this sanitizer has dual efficacy against the Fusarium wilt and bacterial canker pathogens, it does not reduce branched broomrape seed or southern blight sclerotia germination.
- Spring field preparation equipment represented a significantly lower risk than harvesters, but this could be related to dry soil conditions, suggesting that soil moisture and/or time of year may influence risk.
- Although cleaning spring field preparation equipment with pressurized air and 1.5% QAC did not always reduce soil debris loads, we did observe reduced microbial risk on all equipment, particularly the Agriplanter and mulcher. This supports our 2023 findings in which microbial loads were reduced when soil debris loads were <1 in.
- As work progresses, the role and importance of outreach and resource creation increases.

Specific outcomes and benefits:

- Growers and canneries have access to recommendations and consultation support for effective in-season and off-season equipment sanitation, including spring field preparation equipment to reduce the risk of spreading broomrape.
- These methods also work to reduce spread of soil borne pathogens including the Fusarium wilt and bacterial canker pathogens. As the former is at high risk of resistance breaking, only equipment sanitation will prevent spread of a new race in the years immediately following introduction.
- BMPs are improved, providing new information on a wider range of sanitizer options, zonation, and information on spring field preparation equipment risk.
- Training slide decks and educational videos are in development.
- Researchers learn about barriers to effective sanitation adoption.

What's next for this project:

- Evaluate risk and cleaning challenges associated with equipment type and time of year the equipment is used.
- Continue to evaluate sanitizer efficacy against broomrape seed and other high impact diseases and update our Sanitizer database with new information.
- Develop and beta test an installed harvester cleaning prototype.
- Develop protocols for a controlled study to examine the efficacy of the volume and pressure of applied QAC sanitizer on reducing microbial and debris loads.
- Continue to create outreach materials for stakeholders to enable method adoption to limit broomrape seed dispersal.

Introduction

A lack of effective strategies to prevent spread of broomrape and other soil borne pests is perhaps the single most important management gap for the California tomato ecosystem. In recent years, branched broomrape, and to a lesser extent Egyptian broomrape, have become a major industry challenge. These parasitic weeds are CDFA "A list" quarantine pests which require field destruction if even a single plant is detected, and field treatment consists of costly methyl bromide applications which are limited in efficacy and economic feasibility. In addition, other soil borne diseases, including Fusarium wilt, *F. falciforme* vine decline, southern blight, bacterial canker, and root knot nematode are major drivers of plant decline and fruit damage every year, in some cases causing producers to abandon whole fields or parts of fields. Genetic resistance is a key tool to manage many soil borne pests, and we lack tools to mitigate spread when resistance breaking strains are detected, making this industry highly vulnerable to rapid loss of resistance-based tools.

With operational consolidation of farming outfits and custom harvesters alike, and the buildup of soil inoculum loads in fields due regulatory loss of true fumigants, there is increasing risk of disease agents and broomrape seeds being spread between fields and into new counties and regions. Movement of infested soil on equipment likely represents the primary means for broomrape spread into new fields, and, together with infested plant material, is the primary means for pathogen spread. Recent studies indicate that quaternary ammonia, while effective on broomrape seed and Fusarium propagules in the lab, rapidly loses efficacy when applied to surfaces containing significant amounts of soil or plant debris. Removal of debris to loads amenable to effective QAC use is time consuming and with tight timetables for many equipment types, is not always operationally feasible. There are several other sanitizers on the market with low corrosivity, that are used to sanitize equipment and one or more of these may be effective against

broomrape, but without sensitivity to debris—if so, this would be far superior to QACs for the purposes of field equipment sanitation, which is near-impossible to get completely debris-free.

Main Goal and Objectives of the funded project: reduce the spread of branched broomrape seed among California processing tomato fields. To enhance project deliverables and industry impact of this work, we aim to adapt seed sanitation protocols to be effective against the suite of soil borne pests impacting California processing tomato production, including pathogens.

- **Obj. 1**. Expand sanitizer recommendations.
- **Obj. 2.** Expand equipment sanitation BMPs to include a wider suite of field equipment.
- **Obj. 3.** Beyond sanitation: Holistic risk management strategies which incorporate other soil movement practices.
- **Obj. 4**. Outreach to enable rapid and effective adoption of broomrape dispersal management methods.

Methodology and Results:
Objective 1: Expand sanitizer recommendations.

1.1 Evaluate efficacy of new sanitizers (2 or more) against branched broomrape.

Methods
The efficacy of two new sanitizers, Star San and Virkon, was tested for preventing the germination of branched broomrape seeds using the general experimental method. Both sanitizers were applied at the label-recommended rates. Virkon at 1% w/v and Star San at 1 ounce per 5 gallons of water v/v. Mg4 at 1% v/v was used as a sanitizer control, along with a control without any sanitizer.

Results
The results showed that neither of the new sanitizers, Star San nor Virkon, was effective in preventing the germination of branched broomrape seeds, with no significant difference observed compared to the control without sanitizer. In contrast, Mg4 nearly completely inhibited seed germination (Figure 1). The findings of this experiment suggest that while the newly tested sanitizers, Star San and Virkon, are effective in other disinfection applications, they are not suitable for preventing the germination of branched broomrape seeds.

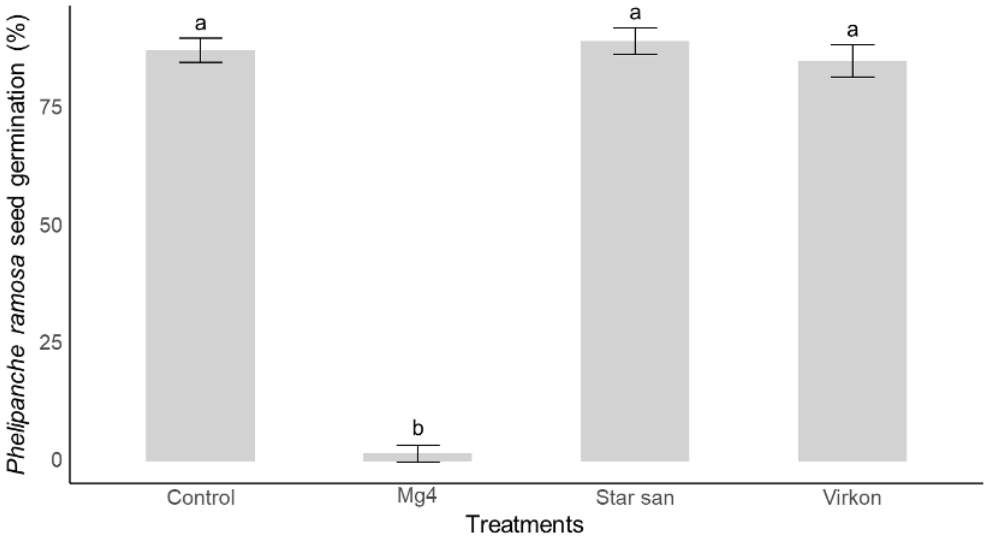

Figure 1: The effect of various sanitizers (Mg4, Star San, and Virkon) on branched broomrape seed germination.

1.2: Evaluate efficacy of new sanitizers against the Fusarium wilt pathogen.

Methods

We conducted two *in vitro* assays evaluating the efficacy of four sanitizers on the Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici* (Fol). A spore suspension (30 uL of approximately $1x10^6$ cells/mL) were fixed on sterile glass slides (3 slide reps/sanitizer) and exposed to hydrogen peroxide (3%), peroxyacetic acid (0.01%), Star San (0.03%), and Virkon (1%) for 1 minute. Because we have observed that MG4 (1%) can completely reduce Fol germination we used this sanitizer as a positive control. Water was used as a "no sanitizer" or negative control treatment. After 1 minute exposure to the respective sanitizers, slides were submerged in a neutralizer solution for 5 min then agitated for 1 min to release bacterial cells from the slides. 250 uL from each rep were spread onto Fusarium selective medium agar (3 plate reps/suspension) for a total of 9 plates per sanitizer. Plates were incubated at 28C and counted after 5 days. Additionally, a dose-response assay was conducted for the Virkon sanitizer (0.001, 0.01, 0.1, and 1%) against Fol. Germination reduction as a result of exposure to the sanitizer was calculated using Equation 1 (see below).

Equation 1:
% Germ Change = -([CFU/mL for water trt] - [CFU/mL for sanitizer trt]) / ([CFU/mL for water trt] * 100)

Results

Fol Sanitizer Assays. Exposing the Fol spore suspension to Star San (0.03%) for one minute completely reduced Fol germination. Virkon (1%) reduced Fol germination by approximately 99%. PAA (5%) and hydrogen peroxide (3%) were less effective, reducing Fol germination by approximately 70% and 78%, respectively (Fig. 2).

Fol – Virkon Dose Response Assays. We observed a complete reduction in Fol loads (CFU/mL) under 1% Virkon (Fig. 3).

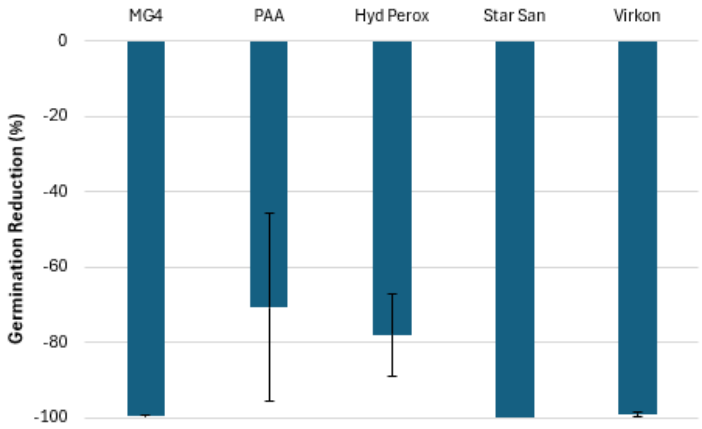

Figure 2. The reduction of *Fusarium oxysporum* germination after treatment with five sanitizers as a percentage of germination after a water (no sanitizer) treatment (assays 1 and 2 combined).

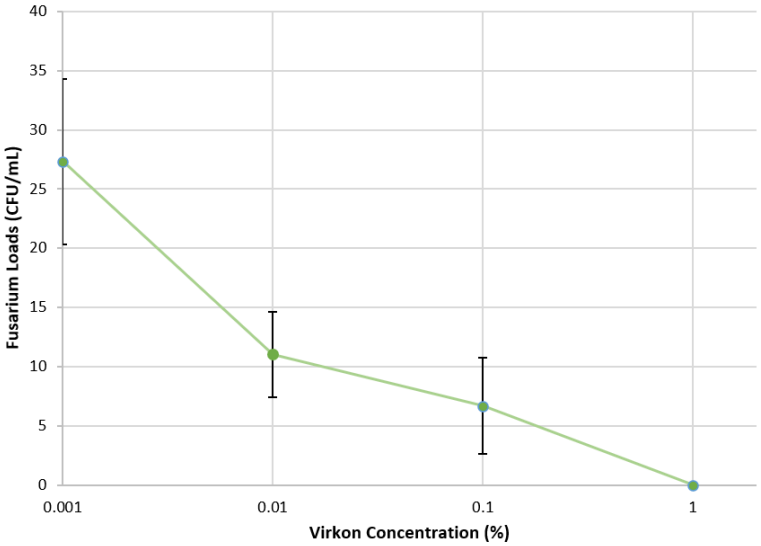

Figure 3. Dose response of *Fusarium oxysporum* loads (CFU/mL) after 1 minute exposure to four increasing concentrations of the Virkon sanitizer.

1.3: Evaluate efficacy of QAC and new sanitizers against the southern blight pathogen, including new protocol development.

Methods: A protocol was developed by technician Kacey Zimmerman and Postdoc Justine Beaulieu to screen sanitizers against the sclerotia. Two *in vitro* assays were conducted testing the efficacy of PAA, hydrogen peroxide, Star San, Virkon, and MG4 on sclerotia germination at 1x and 2x the label rates (hydrogen peroxide was tested only at the 1x label concentration as this was the concentration of the product formulation) at 1 minute exposure. Water was used as a "no sanitizer" or negative control treatment. Sclerotia were exposed to their respective sanitizers for 1 minute then submerged in a neutralizing solution for five minutes. Five replicate groups of five sclerotia were placed in sterile glass Petri dishes and incubated at 28C. After 7 days the number of germinating sclerotia were recorded. Germination reduction as a result of exposure to the sanitizer was calculated using Equation 1.

Results: None of the sanitizers at either 1x or 2x the label rates (Figs. 4 and 5) reduced sclerotia germination by more than 10%.

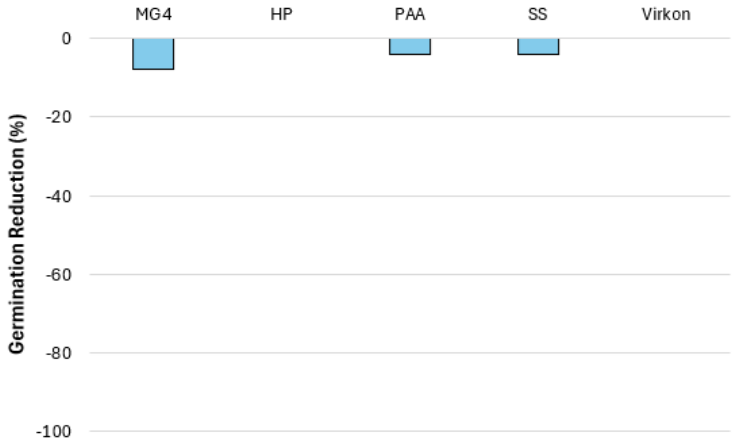

Figure 4. Reduction of Southern blight (*Athelia rolfsii*) sclerotia germination after exposure to five sanitizers at the label rate (MG4 1%, hydrogen peroxide (HP) 3%, peroxyacetic acid (PAA) 0.01%, Star San 0.03%, and Virkon 1%) for 1 minute.

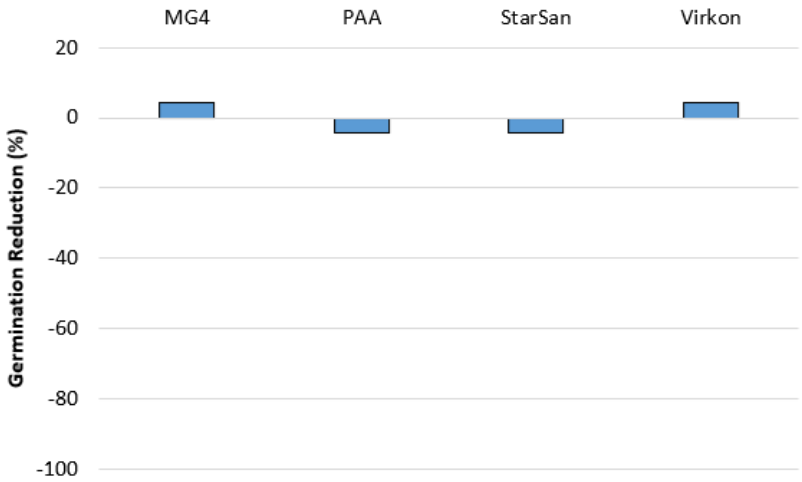

Figure 5. Reduction of Southern blight (*Athelia rolfsii*) sclerotia germination after exposure to four sanitizers at 2x label rate (MG4 2% peroxyacetic acid (PAA) 0.02%, Star San 0.06%, and Virkon 2%) for 1 minute.

1.4: Evaluate efficacy of QAC and new sanitizers against one bacterial pathogen.

Methods: Technician Kacey Zimmerman developed a protocol for evaluating sanitizers against *Clavibacter michiganensis* (Cmm), the cause of bacterial canker. A suspension of Cmm cells (30 uL of approximately $1x10^7$ cells/mL) were fixed on sterile glass slides (3 slide reps/sanitizer) and exposed to MG4 (1%), hydrogen peroxide (3%), peroxyacetic acid (0.01%), Star San (0.03%), and Virkon (1%) for 1 minute. Water was used as a "no sanitizer" or negative control treatment. After 1 minute exposure to the respective sanitizers, slides were submerged in a neutralizer solution for 5 min then agitated for 1 min to release bacterial cells from the slides. 250 uL from each rep were spread onto bacterial growth medium agar (3 plate reps/suspension) for a total of 9 plates per sanitizer. Plates were incubated at 28C and counted after 5 days. Germination reduction as a result of exposure to the sanitizer was calculated using Equation 1.

Results: We conducted this assay twice and have found that all sanitizers effectively reduced Cmm germination in at least one assay, however PAA was inconsistent (Fig. 6).

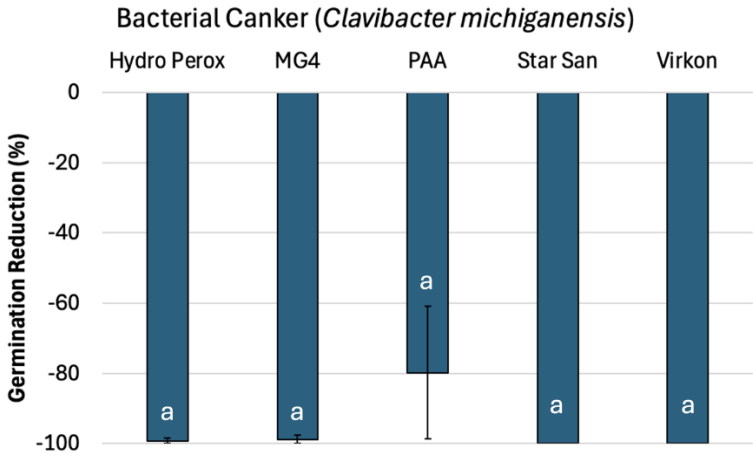

Figure 6. Reduction of *Clavibacter michiganensis* germination after exposure to five sanitizers MG4 (1%), hydrogen peroxide (3%), peroxyacetic acid (0.01%), Star San (0.03%), and Virkon (1%) for 1 minute expressed as a percentage. Assays combined.

1.6: Evaluate sensitivity of all new sanitizers to soil (based on Fol assay).

Methods: We conducted one *in vitro* assay testing the sensitivity of two sanitizers to four concentrations of soil debris (0%, 10%, 30%, and 50%) using Fol as the indicator pathogen. To create the debris treatments, Yolo County soil was sifted through a 63-micron sieve. The resulting powder was mixed with water to create four debris concentrations: 10% (100 mg/mL), 30% (300 mg/mL), and 50% (500 mg/mL) soil. The suspensions were autoclaved twice then plated onto potato dextrose agar to test for sterility. 100 uL of each debris concentration (including 0%) were fixed onto three sterile glass slides for each sanitizer tested including a set of water negative controls by drying for 40 min at 35C. 30 uL of the $1x10^6$ Fol spore suspension was fixed on top of the dry soil debris or blank slides using the same drying method. Exposure of the debris-spore slides to the sanitizers MG4 (1%), Peroxyacetic acid (PAA) (0.01%), Star San (0.03%), and Virkon (1%) and subsequent plating, and calculations are as described in 1.1. Similar experiments with hydrogen peroxide (3%) are currently underway.

Results: As with the Fol sanitizer assays in 1.1, MG4 (1%) was able to completely reduce Fol germination in the absence of soil debris. However, its ability to reduce Fol germination was reduced by the lowest soil debris concentration (10%) and was further reduced as soil debris concentrations increased (Fig. 7). PAA reduced Fol germination by 95% in the absence of debris. Efficacy was reduced by approximately 62% by the lowest soil debris concentration (10%) and further reduced to almost 0% under the highest debris load (50%). Also consistent with the Fol sanitizer assays in 1.1, Star San (0.03%) was able to completely reduce Fol germination in the absence of soil debris. Additionally, Star San was less sensitive to soil debris than MG4 and maintained its ability to completely reduce Fol germination at the 10% soil debris treatment. Virkon (1%) reduced Fol germination by 97.5% in the absence of soil debris. Efficacy was reduced to 83.3% under 10% debris and to 24.4% under 50% debris.

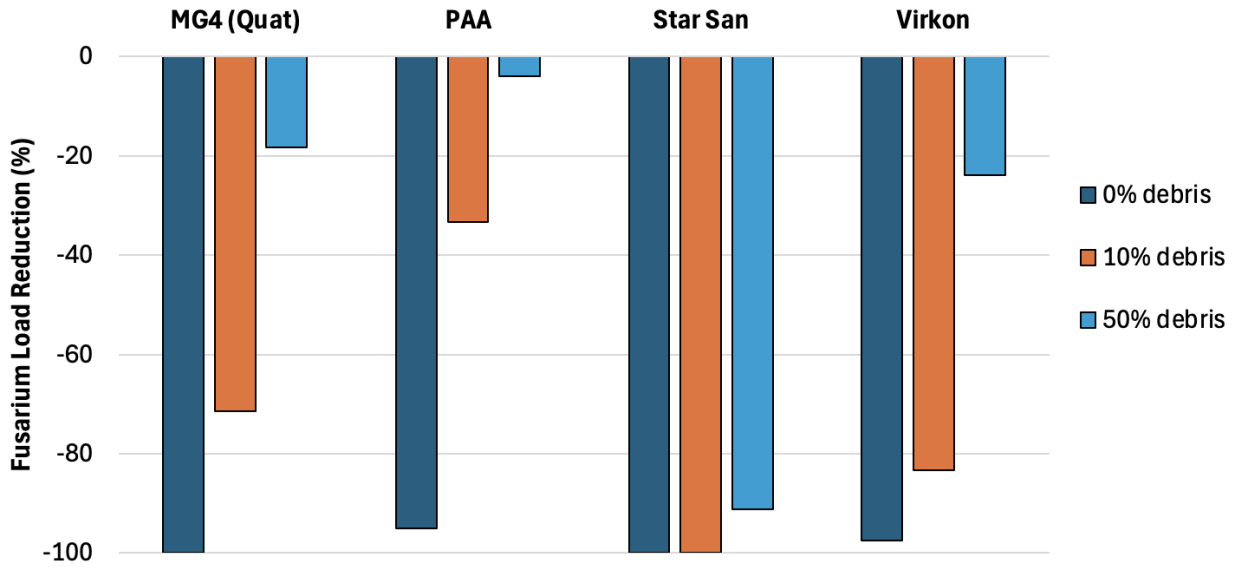

Figure 7. Reduction of Fusarium loads after exposure to MG4 (1%), PAA (0.01%), Star San (0.03%), and Virkon (1%) for 1 minute in the presence of three levels of soil debris (0, 10, and 50%).

1.7: Develop a sanitizer selection quick reference guide (table).

Methods: Product labels and results from 1.1-1.6 were used to create the table below.

Table 1. Sanitizers and their concentrations tested for efficacy against branched broomrape (*Orobanche ramosa*) and the pathogens causing Fusarium wilt, bacterial canker, and Southern blight.

Trade Name	Active Ingredients	Tested Concentration	Sanitizer type	Corrosive on metal	Effective against branched broomrape	Effective against Fusarium wilt	Effective against Southern blight	Effective against bacterial canker	Efficacy in presence of soil debris	Affected by sunlight
Peracetic acid/Peroxyacetic acid (94865-2)	Peracetic acid 4.5-5.4%; Hydrogen Peroxide 25-30%; Acetic Acid 7-13%	0.01% (100 ppm)	Oxidizer	Yes	No	No	No	No	Low	TBD
MG 4-Quat (10324-117-9152)	Octyl Decyl Dimethyl Ammonium Chloride 3%; Didecyl Dimethyl Ammonium Chloride 1.5%; Dioctyl Dimethyl Ammonium Chloride 1.5%; Alkyl dimethyl benzyl ammonium chloride 4%	1% (10,000 ppm)	Quaternary Ammonia	No	Yes	Yes	No	Yes	Low	No
Star San Acid Sanitizer (65001-1)	Dodecylbenzenesulfonic Acid 15%; Phosphoric Acid 50%	0.03% (300 ppm)	Organic Acid	Corrosive on soft metals	No	Yes	No	Yes	Moderate-High	TBD
Virkon S (71654-6)	Potassium peroxymonosulfate 21.41%; Sodium Chloride 1.5%	1% (10,000 ppm)	Oxidizer	Corrosive on soft metals	No	Yes	No	Yes	Moderate	TBD
Jet-Ag	Hydrogen Peroxide 26%; Peroxyacetic Acid 4.9%	0.2-0.3% (2,000-3,000 ppm)	Oxidizer	Yes	TBD	TBD	No	TBD	TBD	TBD
Bleach (67619-32)	Sodium hypochlorite 8.25%	TBD	Oxidizer	Yes	No	Yes	No	TBD	Low	Yes

<u>Objective 2</u>: Expand equipment sanitation BMPs to include a wider suite of field equipment

2.1: Conduct a critical control point assessment to identify target areas for cleaning efforts on commonly used equipment with high risk of broomrape/pest movement.

Methods: We conducted microbial load and debris load assessments on three tractors, five incorporators, a seeder, an Alloway, three bed chisels, and three automated transplanters between April and May of this year across four operations in Yolo and Solano Counties. We also conducted assessments on three discs, two listers, and two triplanes in October (only debris load data could be used). To evaluate microbial risk, two replicate swabs were touched 10 times each per high-risk surface tested on the equipment. Swab washate was spread onto Fusarium selective media (FSM) and incubated for 3-5 days. Fusarium loads, used as a proxy for branched broomrape seed loads, were counted as the number of Fusarium colonies (CFU) per milliliter of washate and averaged across replicates. To evaluate debris load risk, soil debris loads were visually estimated on each surface using a 1-7 scale (Table 2). Microbial and debris load risks per equipment piece was calculated by averaging Fusarium loads and debris load ratings, respectively, across all surfaces on each piece of equipment. These values were also calculated for harvesters and harvester trailers from data collected in previous years (debris load data were not collected).

Table 2. Soil debris load evaluation scale.

Risk level	Soil Debris
1	Clean (no obvious soil)
2	Light dust/soil
3	Uniform dust with more aggregation ≤ 1 mm deep
4	Patchy >1 mm < 1 in. deep
5	Uniform >1 mm < 1 in. deep
6	Patchy aggregation > 1 in. deep
7	Uniform aggregation > 1 in. deep

Results: Fusarium loads on field equipment were significantly lower than loads on harvesters ($P < 0.001$) (Fig. 8). While there were higher Fusarium loads on tractors, mulchers, and bed rollers than on other field prep equipment, differences were not significant. Soil debris load results support these findings as we did not observe significant differences between field prep equipment evaluated in the spring; however, debris loads on equipment evaluated in the fall (disc, lister, and triplane) were higher ($P < 0.001$) (Fig. 9).

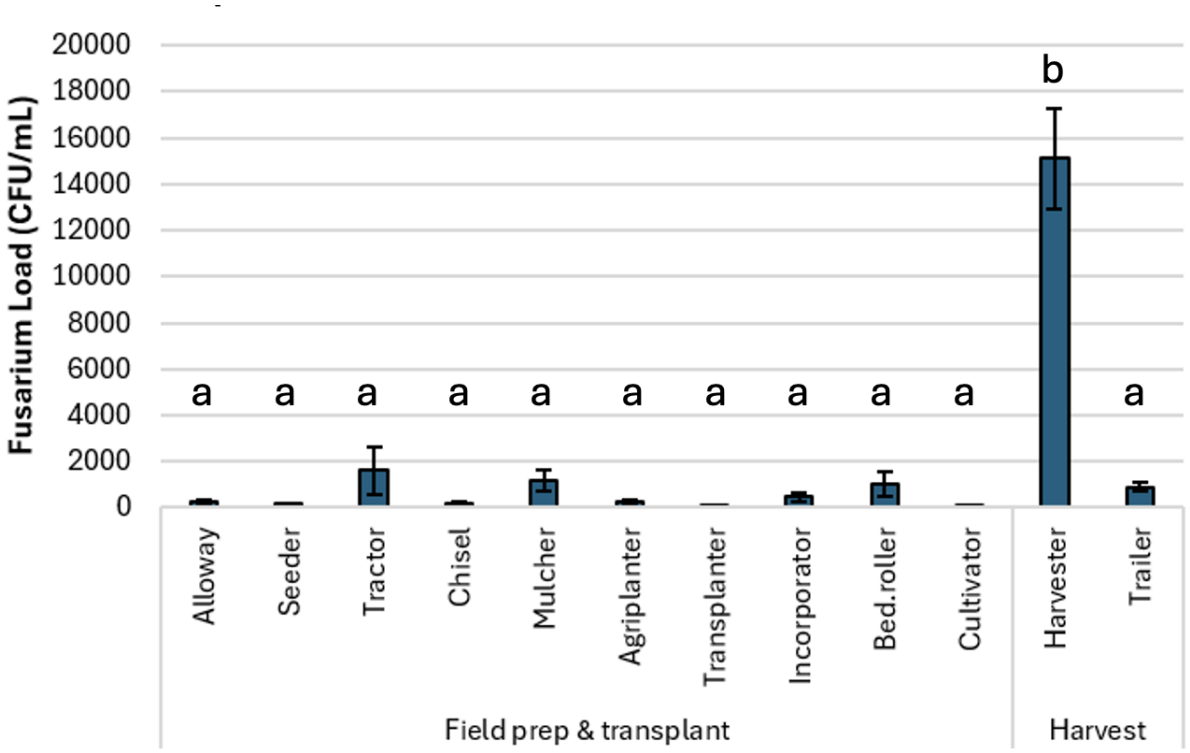

Figure 8. Fusarium loads on commonly used equipment ($P < 0.001$).

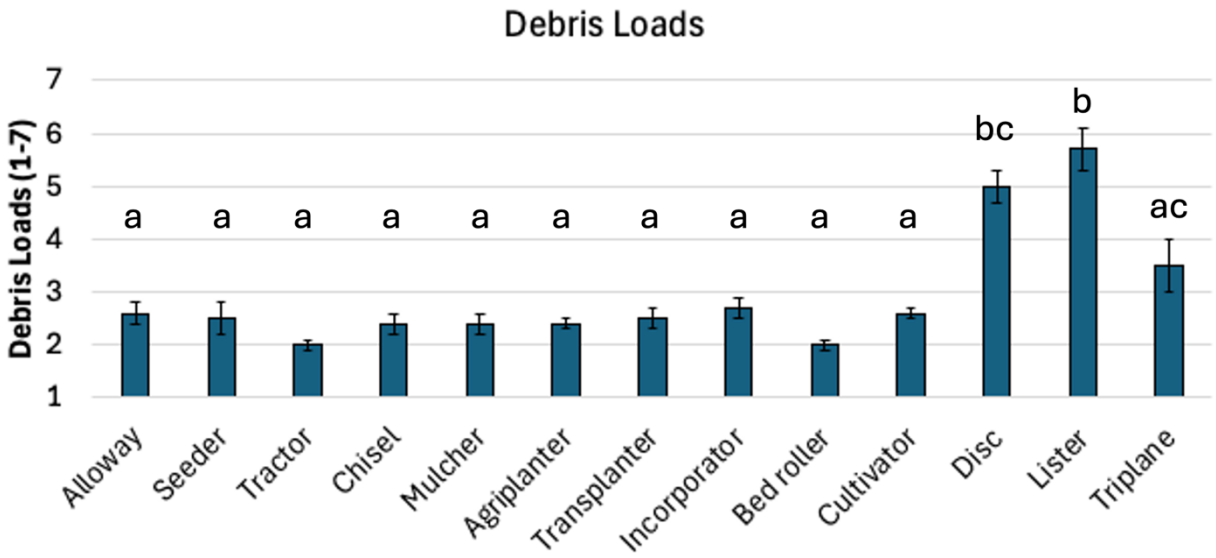

Figure 9. Debris loads (1-7) on commonly used equipment. Debris scale is 1 – Clean (no obvious soil), 2 – Light dust/soil, 3 – Uniform dust with more aggregation ≤ 1 mm deep, 4 - Patchy >1 mm < 1 in. deep, 5 – Uniform >1 mm < 1 in. deep, 6 – Patchy aggregation > 1 in. deep, and 7 – Uniform aggregation > 1 in. deep. $P < 0.001$.

2.2: Within Task 1, conduct critical control point assessments to identify target areas for cleaning efforts on commonly used equipment with high risk of spreading broomrape and other pests.

Methods: We grouped surfaces by function e.g. all surfaces (excepting knives) that are used belowground were grouped into the "Belowground" category. Fusarium loads and soil debris loads from all field prep equipment were averaged for each surface group.

Results: The deck (located on mulchers) had the highest Fusarium loads and loads were significantly higher on the deck than wheels, tires, and knives ($P < 0.001$) (Table 3). The deck also had significantly higher soil debris loads than all other surface groups with an average debris load ($P < 0.001$) (Table 4).

Table 3. Fusarium loads on surface groups averaged across equipment.

Surface Group	Fusarium load (CFU/mL)[a]	No. of Swabs
Deck	11,765.6 ± 5,913.8 a	8
Frame	4,455.4 ± 1,486.8 ab	94
Inner Tire	892.0 ± 736.2 ab	4
Belowground	1,386 ± 1,117.3 ab	59
Axle	396.8 ± 111.1 ab	10
Cabin walls/flaps	178.1 ± 51.0 ab	16
Wheel	119.8 ± 25.0 b	45
Roller	117.4 ± 75.3 ab	18
Tire	115.8 ± 38.9 b	41
Knife	98.4 ± 35.2 b	34
P value Surface group	< 0.001	329

[a] Data are means ± SE, and treatments with the same letters are not significantly different according to one-way analysis of variance (ANOVA) ($P < 0.05$).

Table 4. Soil debris loads on surface groups averaged across equipment.

Surface Group	Debris load[ab]	No. of Surfaces
Deck	5.7 ± 0.6 a	9
Underground parts	3.2 ± 0.1 ab	60
Cabin walls and flaps	2.7 ± 0.2 ab	16
Frame	2.7 ± 0.1 ab	99
Knife	2.4 ± 0.2 ab	36
Inner Tire	2.3 ± 0.2 bc	8
Axle	2.2 ± 0.3 bc	10
Wheel	2.1 ± 0.1 bc	49
Roller	2.0 ± 0.2 bc	18
Tire	1.9 ± 0.1 d	46
P value Surface Group < 0.001		351

[a] Debris loads (1-7) of high-risk surfaces on commonly used equipment. Debris scale is 1 – Clean (no obvious soil), 2 – Light dust/soil, 3 – Uniform dust with more aggregation ≤ 1 mm deep, 4 - Patchy >1 mm < 1 in. deep, 5 – Uniform >1 mm < 1 in. deep, 6 – Patchy aggregation > 1 in. deep, and 7 – Uniform aggregation > 1 in. deep.
[b] Data are means ± SE (n = 3), and treatments with the same letters are not significantly different according to one-way analysis of variance (ANOVA) ($P < 0.05$).

2.3: Assess efficacy of basic cleaning strategy for subset of equipment used by collaborator.

Methods: Working with one collaborator, we evaluated microbial loads and debris loads as described above before and after cleaning (pressurized air then application of 1.5% QAC). Equipment evaluated included a tractor, a cultivator, an Alloway, two bed chisels, a mulcher, and an automated transplanter (Agriplanter). We collected data on cleaning method used and challenges experienced by the workers. Within each equipment type and part, we compared microbial loads and debris loads before and after cleaning to determine cleaning efficacy and identify areas for improvement.

Results: Grower cleaning practices reduced Fusarium loads by almost 100% on the Agriplanter and mulcher. These load changes were significantly greater than load changes on the other pieces of equipment ($P = 0.011$) (Table 5). Cleaning was least effective on the harvester and moderately effective on the chisel, tractor, and Alloway. Broken down by surface group, we did not observe a significant difference in cleaning efficacy ($P = 0.177$) (Table 6), however we did observe a trend of reduced efficacy on the knives. In general, we observed lower efficacy of grower cleaning practices on reducing soil debris loads. However, in support of what we observed with Fusarium load reductions, grower cleaning practices reduced debris loads significantly more on the Agriplanter than on any other piece of equipment ($P < 0.001$) (Table 7). Looking at surface groups, we observed complete soil debris load reduction on the deck ($P < 0.001$) (Table 8). Reductions were below 50% on the other surface groups and there was no reduction on the flaps or inner tires.

Table 5. Fusarium load reduction per equipment type after cleaning by grower (pressurized air then 1.5% QAC).

Equipment	Fusarium Load Change (%)[a]	n[b]
Agriplanter	-99.9 ± 0 a	6
Mulcher	-99.8 ± 0.2 a	5
Chisel	-89.2 ± 6.5 b	10
Tractor	-77.9 ± 19.6 b	5
Alloway	-76.1 ± 10.5 b	14
Harvester	-44 ± 24.3 b	6
P value Equipment = 0.011		46

[a] Data are means ± SE, and treatments with the same letters are not significantly different according to Kruskal Wallace ($P < 0.05$).
[b] n values correspond to the number of pre-cleaned vs. cleaned comparisons per equipment type.

Table 6. Fusarium load reduction per surface type (not including harvesters) after cleaning by grower (pressurized air then 1.5% QAC).

Surface group	Fusarium Load Change (%)[a]	n[b]
Axle	-100.0 ± 0.0 a	2
Flaps	-99.2 ± 0.0 a	1
Wheel	-99.1 ± 0.9 a	6
Frame	-97.1 ± 1.5 a	10
Roller	-91.3 ± 5.8 a	4
Underground	-86.1 ± 10.3 a	6
Tire	-74.7 ± 17.2 a	6
Knife	-50.1 ± 25.3 a	5
P value Surface group = 0.177		40

[a] Data are means ± SE, and treatments with the same letters are not significantly different according to one-way analysis of variance (ANOVA) ($P < 0.05$).
[b] n values correspond to the number of pre-cleaned vs. cleaned comparisons per equipment type.

Table 7. Soil debris load reduction per equipment type (harvesters not evaluated for soil debris loads) after cleaning by grower (pressurized air then 1.5% QAC).

Equipment	Debris Load Change (%)[a]	n[b]
Mulcher	-80 ± 11.5 a	1
Agriplanter	-50 ± 1.7 b	1
Chisel	-25 ± 8.2 c	2
Alloway	-20.3 ± 3.9 c	2
Tractor	12.1 ± 13.7 d	1
P value Equipment < 0.001		7 total

[a] Data are means ± SE, and treatments with the same letters are not significantly different according to one-way analysis of variance (ANOVA) ($P < 0.05$).
[b] n values correspond to the number of pre-cleaned vs. cleaned comparisons per equipment type.

Table 8. Soil debris load reduction per surface group (harvesters not evaluated for soil debris loads) after cleaning by grower (pressurized air then 1.5% QAC).

Surface group	Debris load change[a]	n[b]
Deck	-100 ± 0.0 a	2
Frame	-45.8 ± 6.7 b	16
Knife	-41.7 ± 20.1 b	6
Wheel	-35.0 ± 7.6 b	10
Tire	-31.3 ± 12.0 b	16
Underground	-25.9 ± 6.0 b	16
Roller	-11.1 ± 7.0 c	6
Flaps	0.0 ± 0.0 d	2
Inner tire	0.0 ± 0.0 d	2

P value Surface group = 0.003	76

[a] Data are means ± SE, and treatments with the same letters are not significantly different according to one-way analysis of variance (ANOVA) ($P < 0.05$).
[b] n values correspond to the number of pre-cleaned vs. cleaned comparisons per equipment type.

2.4: Collect footage of all equipment evaluated in Task 1, including pre and post cleaned equipment in Task 3.

Methods and Results: Digital photographs were captured for all equipment evaluated. Footage was collected for a bed roller, bed chisels, an incorporator, an automated transplanter, an Alloway, and a mulcher. Footage of bed chisel and mulcher cleaning were also collected. All photographs and videos are kept in a UC Davis Drive folder that can only be accessed by the Clean Machine Team (Fig. 10).

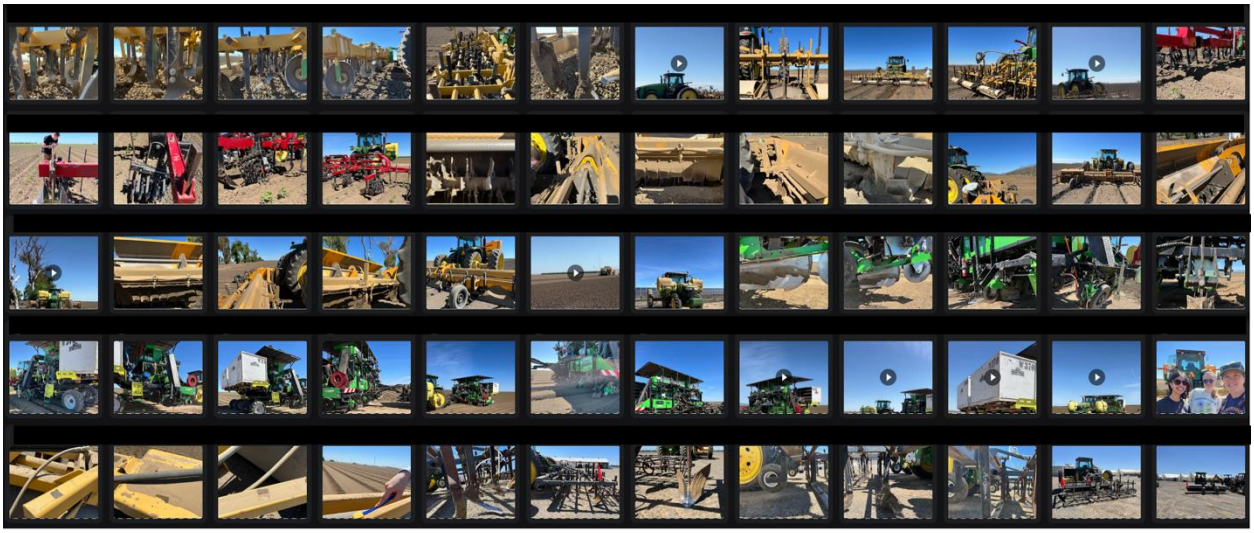

Figure 10. A screenshot of some of the digital photographs and footage collected of field preparation equipment evaluated.

2.5: Add information to "Best management practices for preventing spread of broomrape and other soil borne pests".

Methods and Results: The document was updated in May 2024 and recommendations based on the critical control point assessments of field prep equipment (2.2) will also be added (Fig. 10).

Figure 11. Screen shot of the Field Equipment Sanitation BMP, updated in May 2024.

<u>Objective 3:</u> Beyond sanitation: Holistic risk management strategies which incorporate other soil movement practices

3.1: Risk assessment for common soil movement practices.

Methods and Results: We conducted an initial consult with a collaborator on soil movement practices of top concern in their operation, which they are seeking to manage. We observed their strategies for headland management and discussed challenges.

3.2: Provide a consultation service

Methods and Results: We met with our collaborating grower several times in 2024 to view field zonation practices and discuss challenges.

3.3: Integration into BMPs.

Methods and Results: We drafted a basic zonation practice guideline for integration into our BMPs.

<u>Objective 4:</u> Outreach to rapidly enable the tomato production community to mitigate spread of broomrape and other soil borne pests

4.1: Develop an expanded BMP for "Best management practices for preventing spread of broomrape and other soil borne pests" including a comprehensive quick reference sanitizer table, an equipment sanitation table with relative risk assessment for different types of equipment and critical control points for each type of equipment, cleaning practices for high-risk equipment, field zonation practices to mitigate on-farm spread.

Methods and Results: We created the quick reference sanitizer table (Obj. 1.7), prepared and shared reports with collaborating growers outlining our relative risk assessment for their field prep equipment and drafted a "field zonation practices" guide that will be incorporated into the next version of our BMP.

4.2: Initiate The Clean Machine UC Davis YouTube channel.

Status: We hired new social media support staff in May and are close to completing a video on an introduction to equipment sanitation and others video on branched broomrape and trailer sanitation (Fig. 12). Because the quality of the video is not where we want it to be, we have included a budget for the UC Davis Communication Resources team to polish these videos and develop others including how to scout for branched broomrape and trailer sanitation for FY25.

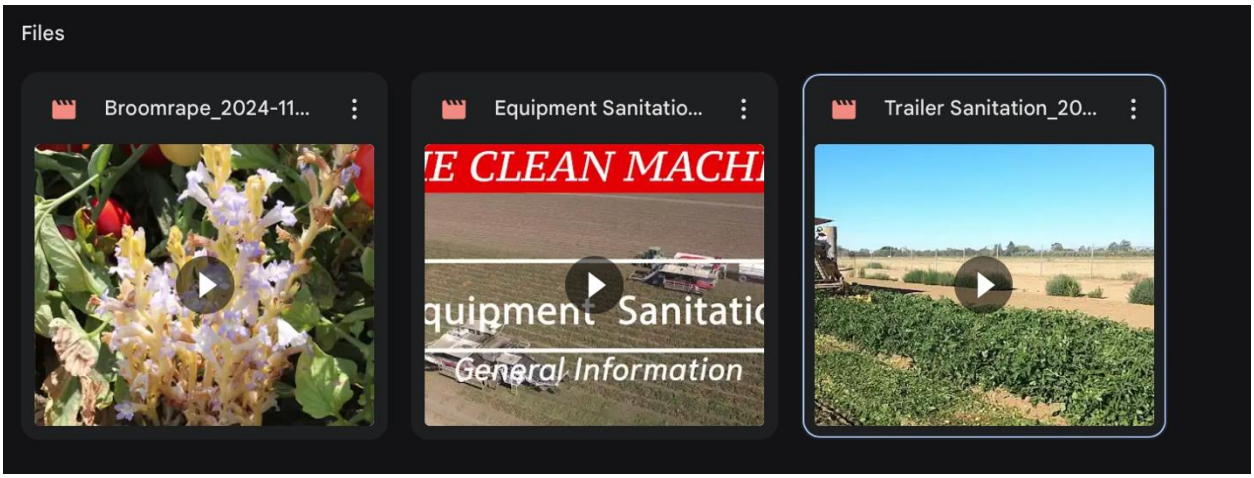

Figure 12. Screenshot of draft videos for the YouTube channel.

4.3: Prepare a sanitation train-the-trainer slide deck and training session.
Status: We have prepared a draft of the slide deck (Fig. 13) and will go through it with a subgroup of farm advisors to provide training and get input. We will make suggested edits and post the slide deck online and email it to all farm advisors.

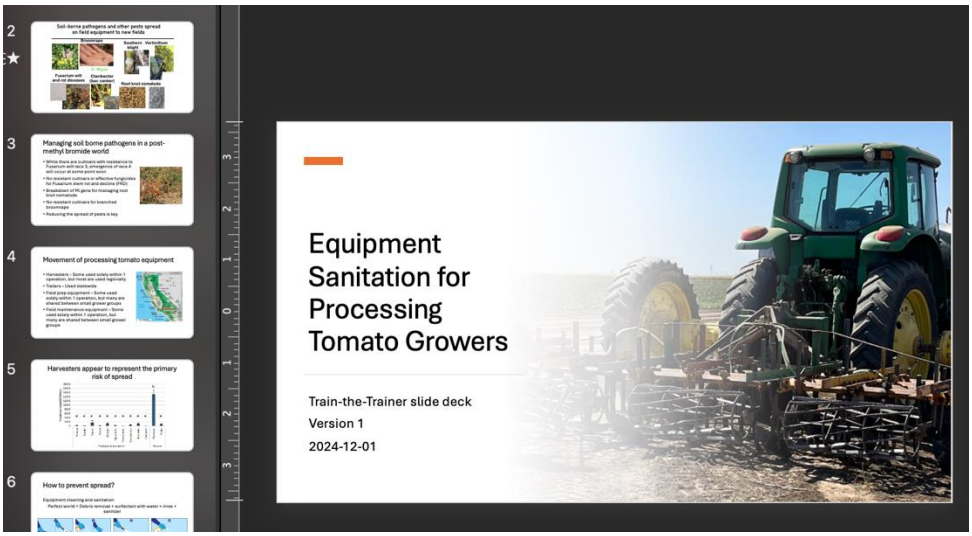

Figure 13. Screenshot of Equipment Sanitation for Processing Tomato Growers train-the-trainer slide deck.

4.4: Disseminate information to enable rapid adoption.

Status: We will post materials to the Swett lab website as they are polished. All materials sent to the CTRI and CE advisors will also be disseminated via newsletters and posted on broomrape targeted websites by the end of the year. We have presented up-to-date information at regional meetings e.g., the Yolo and Fresno County grower meetings. We also hosted a regional field day in June to provide updates on field sanitation.

Acknowledgements: We would like to thank the operations who provided us information and access to their operations for this important work. In addition, thanks to the many growers and industry professionals who provided input on current sanitation practices and concerns.

Project Title: Population structure and invasion history of *Pheliphanche ramosa* field detections in California

Year of Project Initiation: Continuing (2nd year)

CTRI Funding in 2024: $36,876

Principle Investigator: Adam Schneider, Ph.D., Assistant Professor, University of Wisconsin- La Crosse,

Executive Summary: Branched broomrape, *Phelipanche ramosa* is a noxious weed that parasitizes several crops globally, including tomatoes. This species was first reported in California in the 1920s, and subsequently in the 1950s, 1960s-70s. Recent outbreaks, since 2009, have been in San Joaquin, San Benito, and Yolo Counties. The long periods of time between certain outbreaks could be an indication that this species was eradicated from California at least once, and then reintroduced. Alternatively, given the longevity of dormant seeds of this species, and the ability of the seedbank to quickly accumulate due to the fecundity of individual plants, these populations may represent a single introduction with long gaps between detections following intensive treatment efforts. Determining the likelihood that historical outbreaks were caused by imported seed versus existing seeds can help inform whether future containment efforts should be targeted toward screening imported seed stock, or promoting good hygiene so seeds or contaminated soil are not moved between sites on persons or machinery.

This study took a two-prong approach to evaluate the genetic relationships among California branched broomrape. **First**, a "microsatellite" approach used 13 regions of the genome (called "microsatellites") that commonly show variation within and between populations of the same species. A total of 76 samples, representing separate genetic individuals, passed all data quality checks. These came from two sources: (a) multiple individuals from three currently infected fields in Yolo County ("Contemporary Populations"), and (b) single individuals from several dozen herbarium specimens collected from different infected fields throughout California between 1929 and 2024 ("Historical Samples").

Second, a "genome skimming" approach provided a large amount of genome sequence data to confirm the microsatellite results, while also allowing the evaluation of relationships of California branched broomrape to other known populations in North and South America, including cultivated tomatoes in Chile. This genetic data was then analyzed in six ways (Table 1).

Table 1. Summary of Data Analysis.

Method	Purpose	Results	Conclusion
Calculate Summary Statistics	Describe genetic diversity of three populations, and provide snapshot of past time periods	High inbreeding, low genetic diversity; low heterozygosity no clear trend in genetic diversity over time. **Table 2.**	Results are consistent with the reproductive biology of this species, and other studies. A clear trend of decreasing genetic diversity would have supported the "one origin" hypothesis. No clear trend is inconclusive.
Principal coordinate analysis (PCA)	Summarize the genetic diversity and overall similarity of populations through time	Weak variation through time; One contemporary population is unique from others. **Figure 1.**	Gradation between historical and modern samples in genetic make-up, but no clearly distinct groups. Weak support for "one origin" hypothesis.
PCA with European populations on other hosts	Preliminary contextualization of California data; also a quality check	High differentiation between California *P. ramosa* and French populations parasitizing rapeseed, hemp, and tobacco. **Figure 2.**	Samples from California are distinct from European populations on other hosts.
STRUCTURE analysis	Model the best sub-groupings of individual samples; look for correlation between potential biological drivers (time, geography, etc.)	The best genetic sub-groupings distinguish some extant populations, but do not correlate with the timing of different outbreaks. **Figure 3.**	Moderate support for the "one origin" hypothesis, additional support for genetic drift (random change through time)
Cluster analysis (neighbor-joining	Infer a tree showing similarity between individual samples	Older and newer samples tend to cluster together, but with many exceptions. **Figure 4.**	Moderate support for "one origin", with some random change through time.
Phylogenetic Inference (maximum likelihood)	Infer a tree showing similarity among populations in North and South America	Three distinct genetic groups in North America and one in South America. **Figure 5.**	Strong support that branched broomrape populations in California and Chile represent separate introductions from Eurasia; no evidence of gene flow between these populations

In summary, these analyses show clear evidence of separate introductions of branched broomrape to Chile and California. The genetic group that comprises populations in California most likely represents a single introduction from Eurasia, distinct from other populations across the USA and Chile. Repeated outbreaks spaced in time are best explained by a persistent seed bank. Today these populations parasitize tomatoes or weedy nightshades in cultivated fields. Genetically identical plants historically parasitized hemp in Kentucky, and early reports from California indicate spillover into other weed species is possibles well. Fortunately, this appears to be an exception, to the overall good news that containment efforts within California have been successful and not related to the persistence of branched broomrape in other areas.

Practically, the strongest recommendations from this study are: (1) Continue with early detection and containment efforts of infected areas, and (2) Practice good hygiene of equipment and persons to avoid the spread of seed outside of currently infected areas. At the same time, care should continue to be taken to ensure that imported seed are not sourced from infected areas. Potentially useful future work could compare Californian branched broomrape to European populations to leverage the more extensive population genetic research done there. Since tomatoes are not a common host in Europe, it may also provide clues as to what non-tomato crops might be potential reservoir hosts.

Introduction:

Phelipanche ramosa, or Branched Broomrape, is the most widespread species of crop-parasitizing plant in the United States and is rated by the California Department of Food and Agriculture (CDFA) as an "A" grade plant pest, which identifies them as most likely to cause economic harm and are under the strictest regulatory control. This species was first found in Alameda, Sacramento, and San Joaquin Counties in the 1920s and 1950s. In the 1970s, additional outbreaks were reported in Sacramento, Santa Clara, and Ventura Counties.

No reports were made between 1975 and 2009, but the last 15 years have encompassed a new wave of outbreaks: in 2009 *P. ramosa* was observed in tomato fields around Hollister in San Benito County, in 2014 it was again found in fields in San Joaquin County, and from 2017-2023 it has been found at various sites in Yolo County. Most of the recent field detections have occurred in tomatoes. An important question for understanding the invasion dynamics, and ultimately the future threat to crop plants, is if these continued outbreaks are the result of broomrape seed movement between sites, or whether they are the result of repeated introductions to California from contaminated tomato seed stocks.

At the same time, branched broomrape has also been found in central Chile, parasitizing cultivated tomatoes across a variety of sites since the 1980s. It was first collected in 1986 in multiple regions of the country (Matthei 1995). Other populations also exist in the United States but do not parasitize cultivated tomatoes.

My research team performed a population-level genetic analysis of the historic and recent outbreaks in order to compare genotypes within and across populations (contemporary samples) and time-bins (historical samples) to determine if the recent invasions are more likely from a single original invasion, i.e., broomrape seed movement between sites, or whether they are the result of repeated introductions to California, presumably from contaminated tomato seed stock imports.

The main *Goals* and the *Objectives* under that goal:

Goals:

- Compare genetics of existing and historical *P. ramosa* infestations to shed light on likelihood of multiple seed introductions from outside California versus seed spread from a single introduction in the past. (Objectives 1-4)
- Compare genetics of Californian and Chilean branched broomrape populations to determine if seeds are being inadvertently spread internationally. (Objective 5)
- Determine if previously published genetic data from European branched broomrape populations can be analyzed together with New World data to shed light on the origin(s) of Californian and Chilean branched broomrape populations. (Objective 6)

Objectives

1. Survey California herbaria for historical collections of *P. ramosa*
2. Extract DNA from contemporary and herbarium specimens
3. Amplify microsatellite regions of interest
4. Data analysis
5. Compare Californian and Chilean branched broomrape populations using "genome skimming"
6. Compare microsatellite genetics of Old World and New World branched broomrape to determine probable origin(s) of Californian branched broomrape

Methodology and Results:

Sample Collection *(Objectives 1-2)*

To determine available historical genetic material, I consulted the Consortium of California Herbarium online specimen database and made an in-person visit to the UC Davis herbarium. All herbarium samples

came from the California Department of Food and Agriculture and UC Davis Herbaria. While very few individuals were sampled from any one infected field/time combination, the samples could be binned into four time periods allowing for a rough snapshot of genetic diversity and composition across those eras (Table 2).

Fresh material was collected from multiple individuals from three infected properties in Yolo County. These represent true biological populations from a single farm field. These fields can safely be assumed to have been initially infected once, by a single source of seed.

DNA from both contemporary and historical samples were extracted using the same methods.

Table 2. Samples available for microsatellite genetic analysis after DNA extraction and summary statistics

	Population or Group	**Description** *(Years: County)*	**Samples used for genetic analysis**	**Allelic Diversity**	**Heterozygosity** Observed/Expected	**Inbreeding Coefficient** (F_{IS})
Contemporary Populations	Site 01	Seed Farm *(2022: Yolo Co.)*	8	2.53	0.33 / 0.40	0.28
	Site 02	Experimental Farm *(2022: Yolo Co.)*	19	2.86	0.25 / 0.53	0.54
	Site 09	Private Farm *(2023: Yolo Co.)*	9	2.32	0.15/0.37	0.67
	TOTAL		**36**			
Historical Samples (Herbarium specimens)	Pre-1954	*1929, 1952-1953: Various counties*	9	2.31	0.19 / 0.38	0.58
	1968-1974	*1968, 1971-72, 1974: Various counties*	6	2.15	0.19 / 0.41	0.57
	2009-2014	*2009: San Benito Co. 2014: San Joaquin Co.*	9	3.01	0.17 / 0.55	0.71
	2017-2022	*2017, 2019-22: Yolo Co*.	16	2.89	0.19 / 0.49	0.61
	TOTAL		**40**			

Genotyping *(Objectives 3)*

Twenty gene regions (loci) were selected for analysis based on two previously published population-level studies of this species (Le Corre et al 2014; Stojanova 2018). They were validated for the Californian samples by visualizing PCR products with gel electorphoresis. Of the initial list of 20 loci, 14 amplified consistently across all populations (over 67% complete data). One of these 14 showed no variation across Californian samples, leaving 13 loci for downstream analysis. After genotyping, five *P. ramosa* samples (excluded from Table 2) lacked data for >40% of the gene regions, and so were also discarded from analysis.

Microsatellite Analysis and Results *(Objective 4)*

The data were analyzed in five ways to investigate genetic similarity among populations and the likelihood of gene flow:

First, I calculated several statistics related to the genetic diversity of each group (Table 2). In general, the values of lower observed heterozygosity compared to expected and a high inbreeding coefficient conform to expectations for this particular species, which is self-fertile. Through time, no clear trend is apparent, but given the coarseness of historical sampling, conclusions should not be drawn based on any single number.

Second, Figure 1 summarizes the existing genetic diversity among individuals using principal coordinates analysis. This condenses genetic variation across all 13 genes onto a readable plot, such that more genetically similar individuals group together. Only samples from a Yolo Co. seed farm (“Private_Farm1”, dark blue points), are clearly separate from the rest. By contrast, most other groups are partially overlapping, suggesting non-distinct genetic profiles. Nevertheless, more recent samples appear to cluster towards the positive end of axis 1, perhaps reflecting genetic drift (random change over time) due to high levels of inbreeding and population bottlenecks during (near-)eradication events.

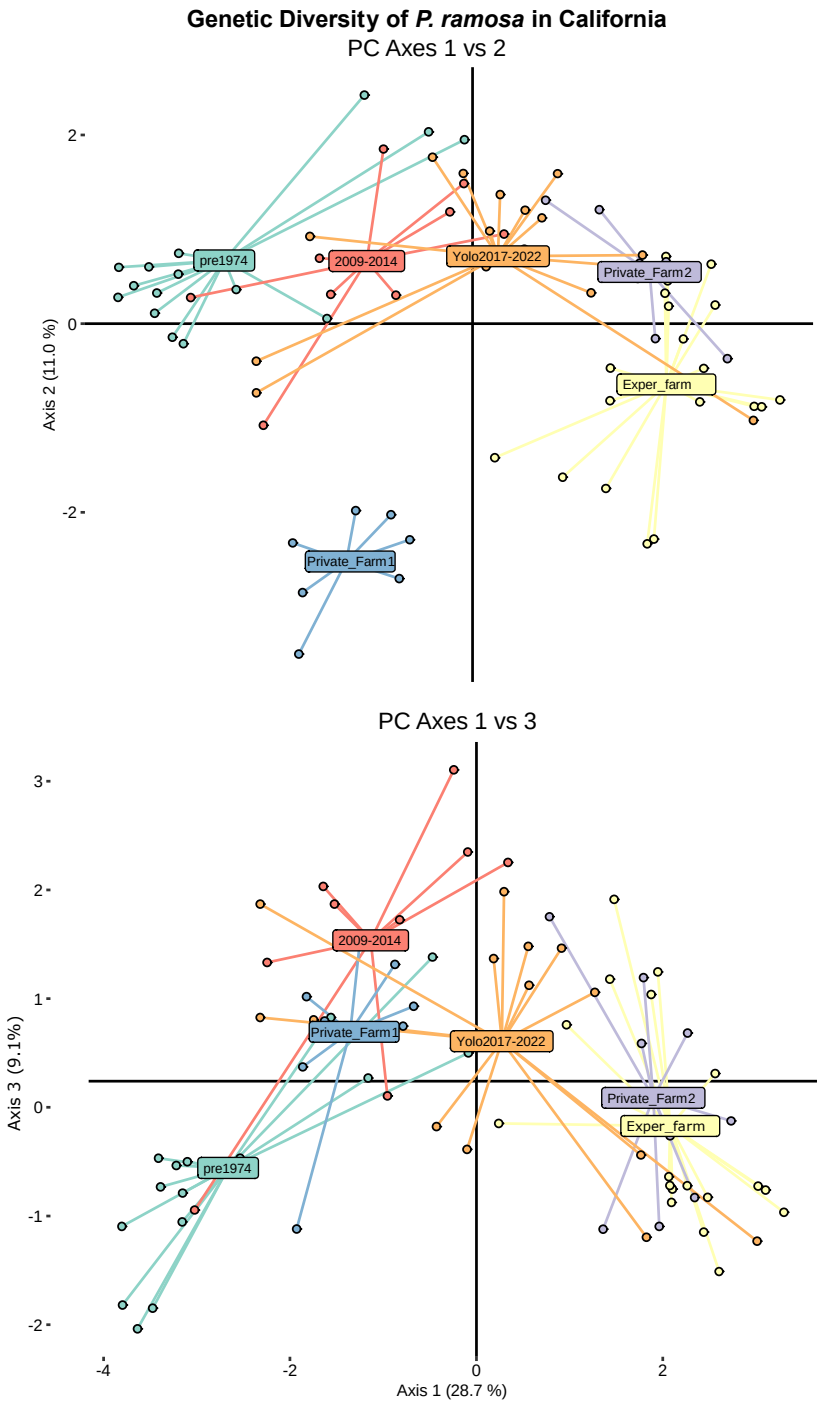

Figure 1. Principal Component Analysis showing genetic variation of Californian samples of branched broomrape, *Phelipanche ramosa*. Plots show the first two ordination axes[1] (top) and first and third axis (bottom), collectively representing nearly 50% of the genetic variation. Points represent individual genotypes. Samples from each pre-determined group are connected by colored lines and labeled at the centroid of each group. For legibility, the pre-1954 and 1968-1974 groups have been shown as a single cluster (teal); samples collected before 1954 generally plotted in the leftmost part of this cluster, whereas samples from 1968-1974 were scattered throughout.

[1] Ordination axes are abstract projections of multivariate data, in this case alleles at each genetic locus, that best display patterns in the data.

Third, to contextualize this genetic variation with respect to the species as a whole, I combined my genetic data with that from six populations of *P. ramosa* parasitizing three other crops in France (originally published by Le Corre et al., 2014). As expected, the branched broomrape from California is distinct from other host races but also falls within the general range of genetic variation seen in this species (Fig. 2).

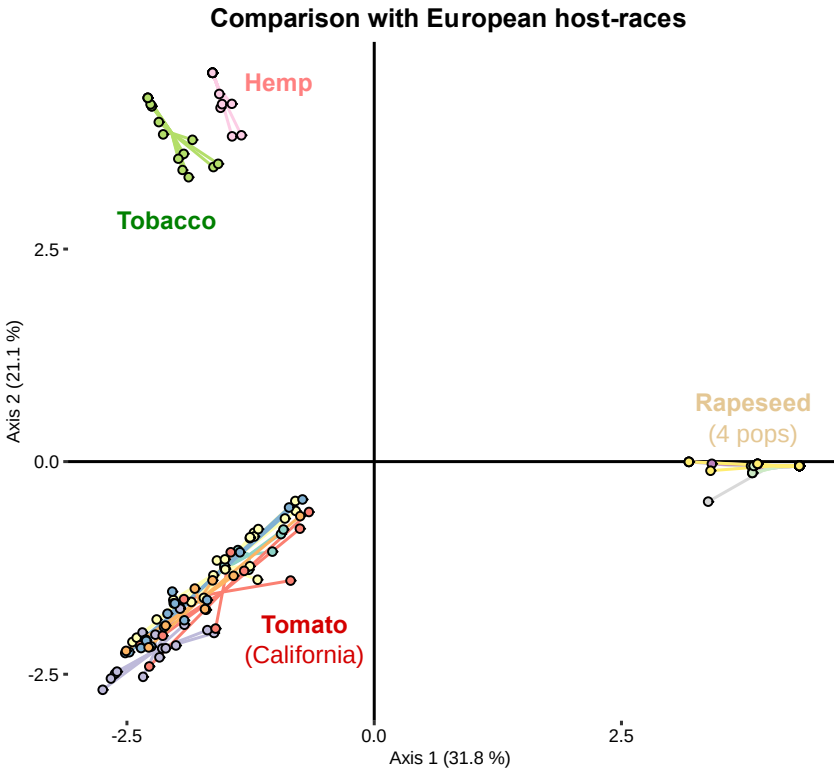

Figure 2. Principal component analysis comparing genetic diversity of tomato-parasitizing branched broomrape in California with six populations parasitizing other hosts. Data from tobacco, hemp, and rapeseed from Le Corre et al. 2014.

While Principal Component Analysis provides a useful summary of variation, a STRUCTURE analysis can assign individuals to genetic subsets of the data independently of predefined labels (Pritchard et al. 2000). Separate introductions of branched broomrape in the early 20th and 21st centuries should show clear genetic differentiation if the introductions came from unique genetic sources.

The STRUCTURE analysis revealed subgroupings that consistently mixed historical and modern samples in manner that does not clearly correlate with any *a priori* hypothesis (Fig. 3). The optimal number of sub-groups that fits the data could not be precisely determined. However, when two subgroups were defined (K=2), the analysis grouped samples from the Private Farm 1 with those from before 1954 and some of the more recent ones. The other group, in yellow, was composed of the Experimental Farm and Private Farm 2 sites, most of the recent Yolo County reports from 2017-2022, and about half of the samples from 1968-2014.

Subdividing the samples further (K=3) splits the yellow group, but not along any recognizable lines, except for maintaining the unity of Experimental Farm samples. Similarly, splitting the red group again (K=4) distinguishes the Private Farm 1 outbreak from all other samples.

Given the reproductive biology of this species (highly inbred, low levels of outcrossing, etc.), the ambivalent subgroup assignments of the Private Farm 1 specimens at K>2 suggests that these divisions are not biologically meaningful.

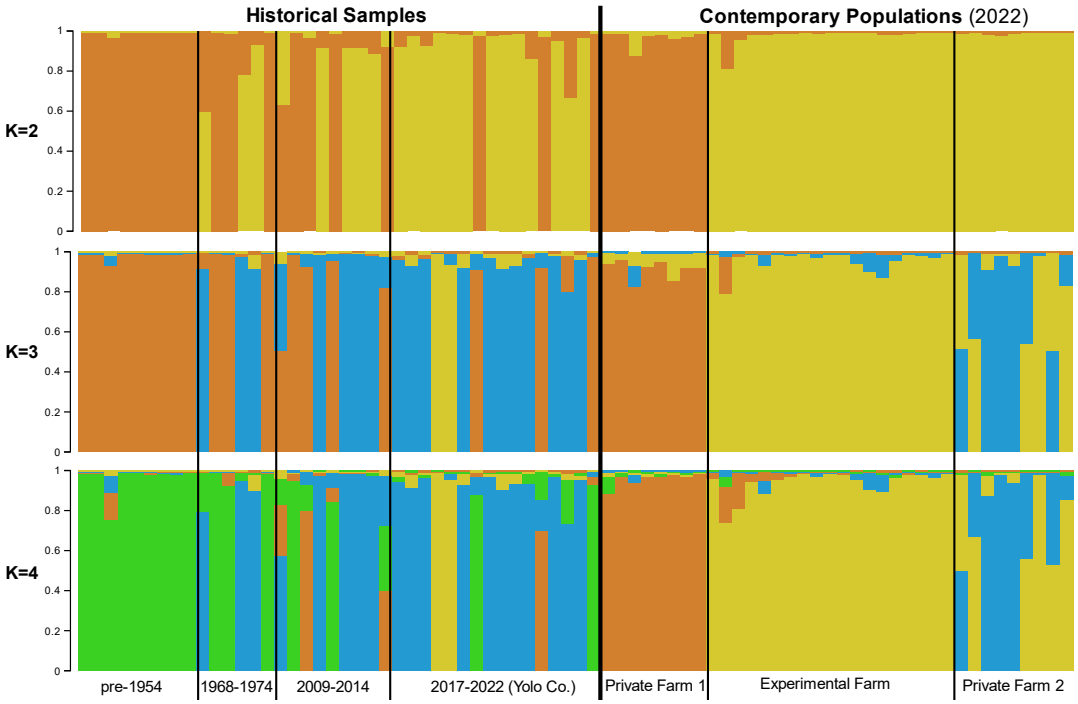

Figure 3. Population structure analysis of Californian branched broomrape for a pre-determined number of subgroupings (K=2, 3, or 4). Each column represents one of 76 genotypes ordered by either time-bin (Historical Samples), or by sampling location (Contemporary Populations), and colored based on the probability of being assigned to each subgrouping. Multiple independent runs at each K gave consistent results.

Fifth, I analyzed genetic similarity between individual samples using two types of cluster analysis: neighbor-joining (Saitu and Nei, 1987) and UPGMA (unweighted pair-group method with arithmetic mean). These analysis generate bifurcating trees based on similarity of each sample to the rest, but differ in the statistical methods applied. Both methods resulted in similar trees, so only the neighbor joining analysis is presented (Fig. 4). In accordance with other analyses, samples show some clustering according to time but with many exceptions. Moreover, no clear difference delineates older (1974 and earlier) from more recent (2009-present) samples, which would be expected if they had unique population histories. As expected, there is strong (but not complete) grouping of samples from each of the three contemporary populations, since all samples of a given population likely started from one or more seeds from the same infection source.

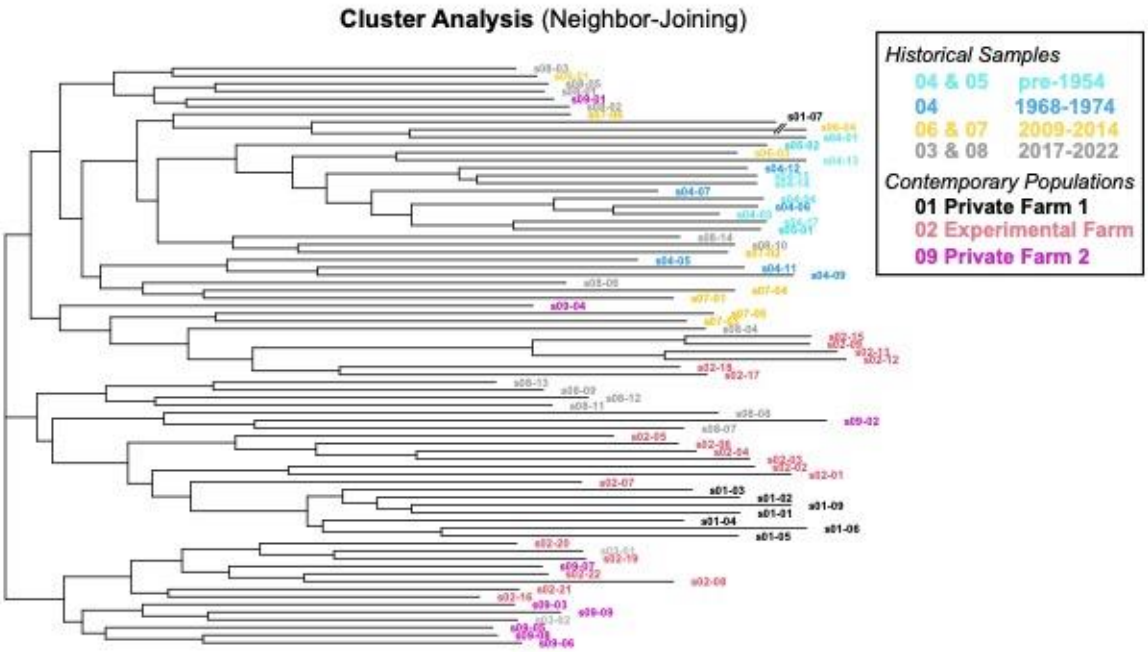

Figure 4. Unrooted neighbor-joining tree showing genetic similarity of 76 branched broomrape samples from California. Each sample is color-coded based on the groupings in Table 2, which mostly corresponds to the sample number. Branch length of sample s06-04 reduced for compactness.

Genetic comparison of American branched broomrape populations using Genome Skimming *(Objective 5)*
To compare the Californian and Chilean populations I used a cost-effective sequencing approach called "genome skimming" to infer relationships among populations (Table 3). A subset of Californian samples used in the microsatellite analysis were used, along with four Chilean samples. Outside funding from the University of Wisconsin supported the addition of 15 additional samples from North America that parasitize hosts other than tomato and two reference populations of European *Phelipanche*.

Table 3. Samples available for genome skimming genetic analysis after DNA.

Sample Group	Group	Description *(Year: Locality)*	Number of samples
California	Pre-1954	*1929 & 1953: Alameda Co.*	2
	1968-1974	*1968: Santa Clara & San Joaquin Co.* *1971: Ventura Co.*	3
	2009-2014	*2014: San Joaquin Co.*	1
	2017-2024	*2017, 2022, 2024: Yolo Co*.	5
			Group Total: 11
Chile		*2024:* Rancagua, Lolol, Quinta de Tilcoco, Talca	**Group Total: 4**
Other North American	Southern USA	*2004-2023: Texas, Louisiana, Alabama* On non-cultivated weeds	12
	Kentucky	*1942: Bourbon Co.* On *Cannabis sativa*	1
	Virginia	*2007, 2016: Norfolk and Hampton Cos.* On non-cultivated weeds	2
	TOTAL		**Group Total: 15**
Europe		*Phelipanche ramosa* on tomato (Germany) *Phelipanche nana* on non-cultivated weeds (Lebanon)	**Group Total: 2**

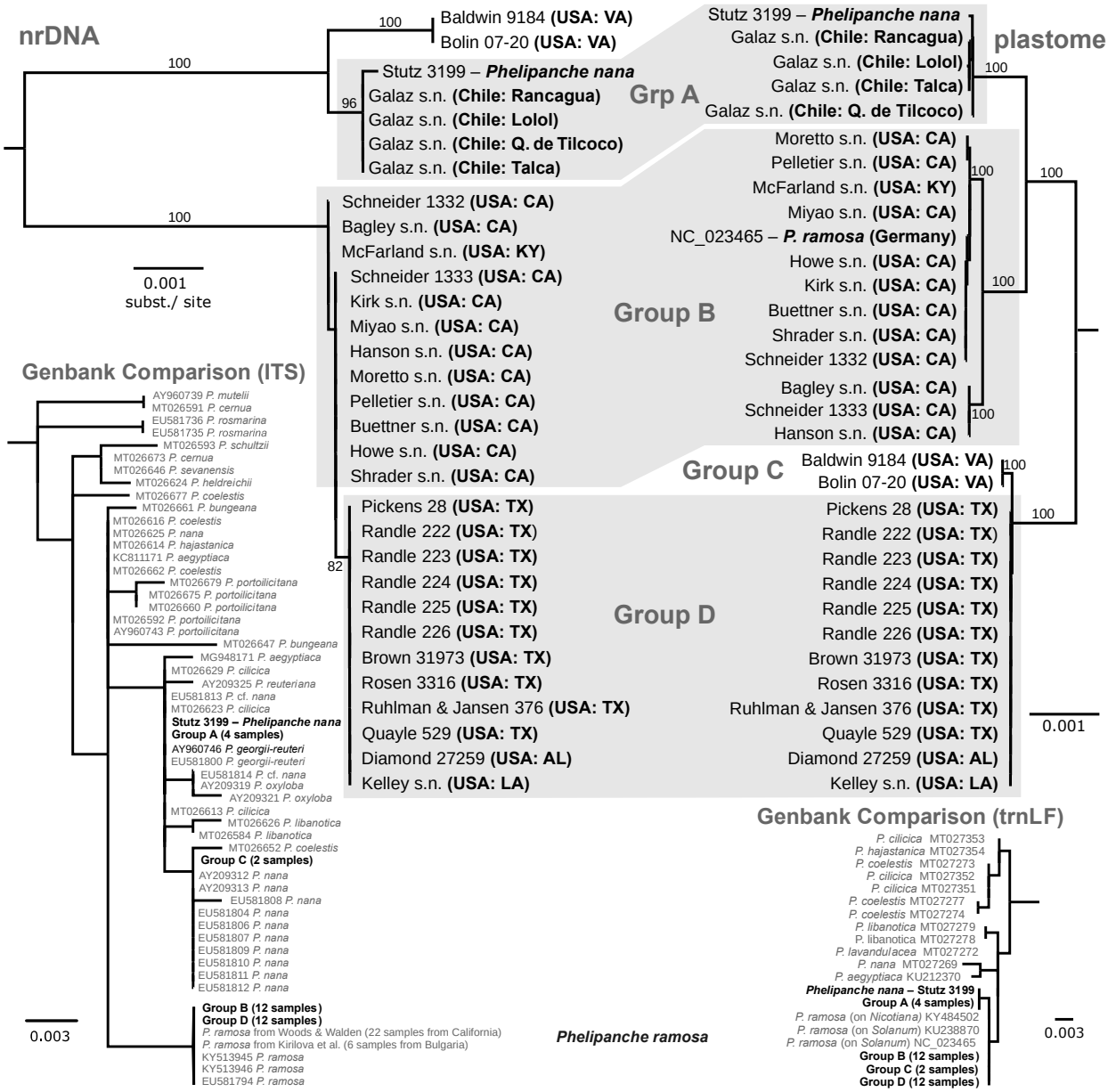

Figure 5. Nuclear ribosomal repeat (nrDNA) and plastome phylogenies of branched broomrape from North and South America showing four distinct genetic groups. Tip labels indicate collector and locality, with voucher information available in Table 2. Bootstrap support of selected nodes shown. **Inset:** Comparison of newly sequenced samples (black tip labels) with other *Phelipanche* spp. (gray labels showing GenBank accession and taxon), showing substantial differences between Chilean (Group A) and Californian (Group B) samples. AL, Alabama; CA, California; LA, Louisiana; TX, Texas; VA, Virginia.

Phylogenetic analysis revealed four genetic groups that were strongly aligned with geography (Fig. 5). Group A is composed of the four Chilean samples from tomato fields. These were nearly identical genetically to the reference sample from Lebanon. Group B is composed of the Californian tomato field samples, and a historic specimen on *Cannabis* from Kentucky. Group C includes the samples from eastern Virginia and Group D includes branched broomrape from the southern USA.

In the last year I also became aware of ITS (internal transcribed spacer) sequence data from 22 other branched broomrape specimens in the CDA herbarium generated by Wood and Walden at the California Department of Food and Agriculture (GenBank Accessions OR690545–OR69056) as part of Plant Protection Act Section 7721 project 3.1272. While this locus is not generally able to provide population-

level resolution, all 22 samples were genetically identical to the Californian broomrape populations samples at this locus, but highly distinct from Chilean or Virginian populations (Fig. 5 inset).

Both findings also confirm the microsatellite results (Figs. 1-4) with respect to the "one origin" hypothesis for Californian branched broomrape by showing the strong genetic similarity of all Californian samples across time and space. At the same time, Californian populations are distinct from branched broomrape in other parts of the United States and Chile, which almost certainly *were* introduced separately. The curious exception is the historical collection on *Cannabis sativa* in Kentucky is in the same genetic group as Californian tomato parasites. This exception may prove the rule, so to speak, that containment efforts within California have been successful. At the same time, *Cannabis* is commonly grown in California and may inadvertently serve as an additional host for branched broomrape to utilize.

Determining European Origins *(Objective 6)*

In the last year I reached out to two research groups who have published microsatellite data of European branched broomrape (V. Le Corre et al. 2023 and B. Stojanova et al. 2019). One of them has responded and shared their raw data with me in preparation for future work on combining my microsatellite dataset with theirs. Given the recent findings confirming a single European origin of Californian populations (Fig. 5), moving forward with this work may be less important.

Discussion/ Conclusions:

Four central questions motivated this research. They are listed below, along with a summary of conclusions.

1. "**Were outbreaks from 1952-1974 from the same original invasion as the outbreaks in 2009, 2014, and 2017-2022?**"

   Available genetic evidence from this study (Figs. 1-5) supports a close relationship among all branched broomrape reported in California. **I found strong support that all California branched broomrape derived from a single introduction.** None of these results show signatures that could be expected of a separate introduction between 1975 and 2009, such as (a) sudden spikes in genetic diversity from the introduction of new genetic material (Table 2) or (b) strong genetic differentiation between two or more subgroups that correlate with particular counties or a particular time of introduction (Fig. 1-4). Genome skimming data (Fig.5) clearly shows the genetic differentiation between California samples (plus one from Kentucky), and other populations in North America. Observed changes in genetic profile of samples over time seem to be more consistent with gradual change, perhaps through genetic drift (Fig. 1).

2. **"Are Chilean branched broomrape populations are closely related genetically to those in California, enough to be from a common origin?"**

   Phylogenetic analysis provides **strong support for separate origins of Californian and Chilean broomrape populations** (Fig. 5). Branched broomrape was introduced to the United States in the late 1800s, with the first Californian populations appearing in the 1920s, long before its introduction to Chile by 1986. If Chilean populations were introduced from California, they would together from a single genetic group, but this is very clearly not the case. It is plausible that branched broomrape seed to persist for over 40 years in California without a field detection? This particular species has extreme fecundity and seed longevity (Joel et al. 2013). Given the large seed bank that can be produced in even just a single generation, it's plausible that enough could remain viable over several decades while kept away from a potential host plant that might stimulate germination.

3. **Are the three branched broomrape populations in 2022 characteristic of most/all current infection sites?**

   With moderate confidence, I conclude that the answer is yes. The genome skimming data (Fig. 5) shows that Californian populations, including recent branched broomrape populations are genetically homogeneous but clearly distinct from other introduced populations of branched broomrape. This supporting the finer-scale microsatellite results (Figs. 1-4), and the expanded sampling across the ITS locus showing identical sequences across California (Fig. 5 inset). Minute genetic differences among some of the samples appear to be negligible and/or reflect genetic drift over time. Analysis of sequenced but unanalyzed 2023 samples from two private fields in Yolo Co. could provide further confirmation.

4. **Can published microsatellite data on several Eurasian branched broomrape populations on different hosts (Le Corre et al. 2023) be analyzed together with the existing California data set (this research).**

   Work towards this objective is in progress. One of the two research groups who have published microsatellite data of European branched broomrape (V. Le Corre and B. Stojanova) shared a large data file with me from their recent study. A preliminary glance at the file indicates some challenges I would face in reconciling these datasets. More detailed analysis is planned for the summer. Given this year's findings confirming a single European origin of Californian populations (Fig. 5), moving forward with this objective may be less important in terms of shaping applied management decisions or informing industry practice.

With these results in mind, future containment of *P. ramosa* is going to be most dependent on early-detection and eradication procedures in infected areas, and care that equipment and personnel are kept clean when moving between sites in areas of recent infestation. At the same time, care should continue to be taken to ensure that imported seed are from high-quality producers and not sourced from infected areas.

Limitations and Continued Work

Herbarium specimens are the only source of genetic data available for historical infections of *P. ramosa*. However, they usually are collected to document one or few representative individuals from a population, not a statistically robust random sampling. While invaluable, the "bins" of historical samples were collected over the span of years from different farms and counties, making these groups not directly comparable to extant populations in terms of the summary statistics in Table 2. At the same time, analyses that compare *individuals* (Fig. 1-4) should be given greater weight. In this study, nearly every herbarium specimen available was used. Only samples that were very old, not in flower, or had been treated in a way that probably had destroyed the DNA, were not used. While more genetic loci could be sampled, no further historical material from additional fields or time periods are available. Looking forward, however, taking care to scientifically document new outbreaks with a physical voucher specimen curated in a herbarium can provide an important data source for future researchers. County Agricultural Commissioners or botanist affiliated with public herbaria (e.g., CDFA, UC Davis, UC Berkeley, Chico State) can assist with this.

Continued work could include further genetic monitoring of branched broomrape in infected fields in California (Question 3 above), and determining the relationship between Californian and European samples of branched broomrape (Question 4). While additional effort toward addressing both of these questions could help better understand the past invasion dynamics of this plant pathogen, it would likely

have diminishing returns in informing industry practice. Nevertheless, because sequencing has already been completed on two populations from 2023, I recommend a no-cost extension to analyze the data in hand. I recommend investing additional resources to sequence samples from 2024 and beyond, a microsatellite analysis of Chilean samples, as I had previously proposed. This is further explained in the Budget Summary.

Acknowledgements: Thanks to the private landowners who allowed sampling from their property for the contemporary populations, and to the following herbaria and curators for specimen access and permission for destructive sampling: BRIT (Tiana Rehman), CDA (Genevieve Walden), DAV (Alison Colwell), ISC (Deb Lewis), TEX (George Yatskyevich), WILL (Beth Chambers). Chris Randle contributed DNA from five of his collections in Texas, and Juan Carlos Galaz, Brad Hanson, and Zach Bagley (CTRI) facilitated the collection of samples from Chile and California. Zach Bagley and Brad Hanson (UCD) assisted with collection of fresh specimens. Dan Potter (UCD) provided access to his research lab for DNA extractions, and Chloe Gale (UWL) assisted with lab work. Transport and use of *P. ramosa* material done in accordance with California Department of Food and Agriculture Permit #3910.

This project as leverage for other dollars: I was awarded a $8646 grant from the University of Wisconsin-La Crosse to compare the results of this project with branched broomrape populations in Alabama, Louisiana, Texas, Virginia, and Kentucky. In the future, this project may be used to seek outside funding to determine the relationship between New World genetic groups (Fig. 5) and Old World host races determined by Stojanova et. al (2019) and Le Corre et al (2023).

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Project Title: Inducible Suberin for Tomato Drought Tolerance

Year of Project Initiation: 2021, completion in 2025

Amount of Funding Requested from CTRI in 2025: 17,000 USD, dependent on obtaining positive results for Objective 2.

Principal Investigator: Siobhan Brady; Dept. of Plant Biology and Genome Center, Howard Hughes Medical Institute, University of California, Davis

PI Contact Information: (530)752-5183; sbrady@ucdavis.edu

Cooperator(s):

- Sukhpreet Sandu, Intellectual Property and Innovation Manager, HM Clause
- Shantel Martinez, Processing Tomato Breeder, HM Clause
- Chad Kramer, Director of Breeding AMPA, HM Clause
- Kebede Muleta, Molecular Genetics Project Manager, HM Clause
- Vincent Asiago, Director, R&D Portfolio, Innovation and IP
- Alessandra Frizzi, Innovation Scout, Bayer
- Brad Hanson (extension specialist, UC Davis)
- Shahid Siddique, UC Davis

Executive Summary:
Water availability is a pressing concern in the State of California and expected to further worsen due to climate change. New strategies and tools are required to maintain the yield of processing tomatoes in California, which accounts for 90% of national tomato production. As part of their long-term strategic planning, over the last three years the California Tomato Research Institute (CTRI) generously awarded our proposal to investigate the effect of enhanced suberin deposition in tomatoes as a strategy **to improve yield in drought conditions and potentially reduce infestation by branched broomrape and nematodes.** Suberin is a biopolymer that accumulates in the tomato root exodermis and whose deposition is correlated with drought tolerance and resistance to several pathogens. We have identified commercially relevant lines with varying suberin content. Using these, we are generating plants within relevant processing lines with the target of increased suberin. Crosses to obtain hybrid plants from these lines will be completed in early 2025. We generated transgene constructs and transgenic plants with targeted enhanced suberin deposition taking advantage of a newly identified transcriptional regulator of suberin deposition within the tomato root exodermis (Canto-Pastor et al. 2024, Nature Plants). The degree to which increased suberization can drive drought tolerance in both the greenhouse and the field, as well as the best mode to accomplish this without growth penalties will be determined. Fruit yield will be directly quantified according to processing industry standards. Branched broomrape and nematode infection will be additionally quantified in these lines. If increased suberin can drive gains in yield under these conditions, then we will work with HM Clause to exploit natural variation in breeding populations or use CRISPR-based gene editing to fix this trait for commercial purposes. We anticipate completion of the project in 2025.

Introduction

Processing tomatoes grown in California are under threat due to increasing water deficit within California. Sub-surface irrigation lessens water demand, (Bagley 2018) but water uptake by plant roots in sub-surface irrigation is still dependent upon root system proximity to drip lines. Root systems are less frequently considered as useful traits for breeding programs due to their presence below ground and decreased experimental tractability. Water enters the root and moves between cells (apoplastically) through passive diffusion. Typically, water molecules face a barrier in the root endodermis *via* cell wall impregnation of a hydrophobic biopolymer called suberin. Endodermal suberin is thus a selective checkpoint for the transmission of water to xylem cells, which will transport this water to the above ground parts of the plant. Water transport from the endodermis to the above parts of the plant is dependent upon xylem hydraulic conductivity, which is in turn dependent on the efficacy of the suberized barrier and leaf evapotranspiration (Schreiber et al. 2005). ***Tomato differs from this classical model*** of water transport as their roots have **an additional cell type with a suberized barrier** (**Fig. 1A-B**) - ***the exodermis***, and the ***tomato endodermis does not contain suberin*** under the conditions we have tested (Kajala et al. 2021, Manzano et al., 2024). Furthermore, the wild tomato species, *Solanum pennellii*, has constitutive suberin production (**Fig. 1C**) within the exodermis which correlates with its drought and salinity tolerance. ***This striking difference in root structure has never been considered to maximize tomato water use efficiency in conditions of water deficit in breeding programs.***

In addition to being required and sufficient for drought tolerance (Baxter et al. 2009, Cantó-Pastor et al., 2023), suberin is also linked to increased pathogen resistance (Holbein et al. 2019), including to root-knot nematodes and to the oomycete *P. sojae* (Thomas et al. 2007), and to parasitic plant infection (Kawa et al., 2022, 2024). Finally, suberin is also linked to higher shelf-life of agriculture products including potato tubers via the periderm (Boher et al. 2013); and in tomato fruits via its related biopolymer cutin which is produced by much of the same biosynthetic pathway (Lara, Heredia, and Domínguez 2019). ***The suberin biosynthetic pathway and its regulation is underexplored in tomato roots.***

Our research has demonstrated that tomato roots produce “developmental” suberin that is deposited as the root ages, as well as “drought-inducible” suberin that is produced in high levels in response to drought (**Fig. 1D**). Furthermore, the wild, drought- and salt-tolerant species, *Solanum pennellii*, produces constitutively high levels of suberin within its root, suggesting that suberin production is linked to its drought and salinity tolerance (**Fig. 1C**). ***This variation in suberin levels can be used in breeding programs*** to introgress regulatory regions from a wild species into domesticated germplasm using available breeding populations by processing tomato seed companies. The Brady lab has identified a transcriptional activator of suberin biosynthesis which is necessary for the drought response and which is used in the research activities described here (Cantó-Pastor et al., 2024).

This proposal focuses on two areas of interest for the California Tomato Research Institute Request for Proposals: (i) *Physiological responses to heat extremes and poor water quality;* and (ii) *Genetic discovery*. We apply our knowledge gained from basic research at UC Davis on genes that regulate suberin production to generate lines with increased suberin production and we will determine the degree to which they can confer resilience to water stress (**Fig. 2A**), resistance to nematodes (Holbein et al. 2019) like *Meloidogyne incognita* and for increased resistance to the parasitic plant *Phelipanche ramosa* (branched broomrape). ***Collectively, the***

generation of these lines should allow for increased exploitation of the soil profile by tomato roots. As a bonus, these lines may facilitate increased carbon deposition within the soil via the suberin biopolymer.

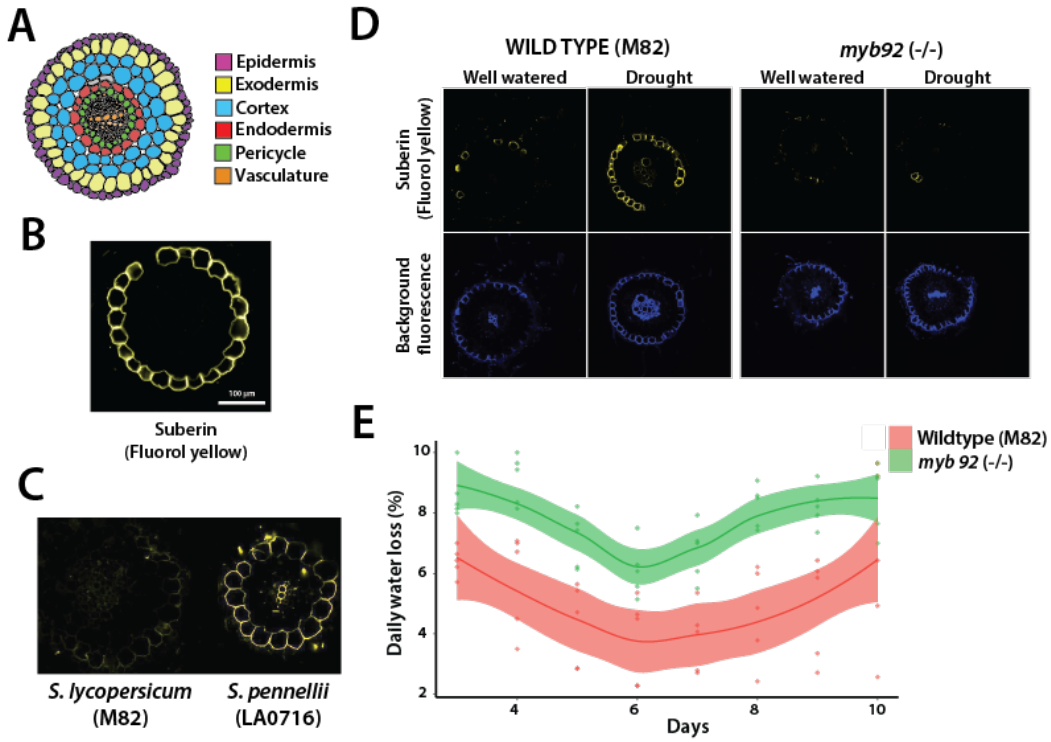

Figure 1: The suberized exodermis in the tomato root and its transcriptional regulation. **(A)** Root cell type organization in tomato roots. **(B)** Cross-section of tomato roots stained with fluorol yellow. Yellow = suberin deposition in the exodermis. **(C)** Comparative deposition of suberin under normal conditions in the M82 processing tomato control vs the wild species *Solanum pennellii*. Suberin intensity is normalized to the maximum observed in *S. pennellii.* **(D)** Cross-sections of M82 and the *myb92* loss-of-function mutant roots (4-week-old plants) under well-watered and drought conditions. The fluorol yellow (suberin) signal is significantly decreased in both conditions. **(E)** Daily water loss, as a percentage of total water content in the pot, over 10 days of imposed drought. Plants were monitored and adjusted daily at 40% water capacity to replicate the effects of water deficit.

Objectives, Methodology and Results
<u>Main Goal:</u> *Increase suberin production in roots to enhance drought tolerance in tomato*

<u>Aim 1.</u> *Generate exodermal and drought-inducible tomato with targeted increased suberin production.*
<u>Objective 1A: (COMPLETED)</u> Test variation in suberin content in commercial germplasm.

<u>Methods/Results</u> We generated data from HM Clause commercial germplasm that quantified variation in exodermal suberin and confirmed that specific varieties are able to deposit robust suberin in control and osmotic stress conditions. Line HM5511 has a moderate level of suberin compared to the other lines and is the most suberin-inducible line under ABA stress (as a proxy for drought) (**Fig. 2B, C**). Upon consultation with HM Clause we decided that this line was the best candidate for increasing suberin deposition. Our rationale was that under control

conditions, we should be able to increase suberin levels; and further maximize the magnitude of suberin under water limitation using our proposed transgene approach.

Deliverables/Measurable Results: We have quantified the levels of suberin within individual root cell types, as well as its drought inducibility in an HM Clause commercial germplasm collection.

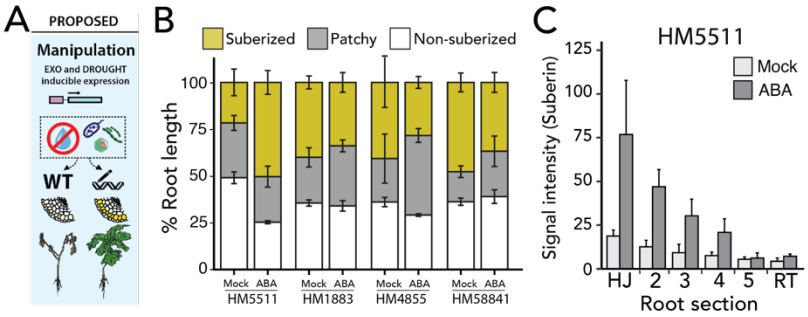

Figure 2. Progress summary of the proposal for Objective 1A. YEAR 1: (A) Graphical overview of the project goals; (B) Developmental stages of suberin deposition of HM Clause lines treated with mock or 1 µM ABA for 48 h. Zones were classified as non-suberized (white), patchy suberized (gray) and continuously suberized (yellow), n ≥ 6, error bars: SD; (C) Quantification of suberin abundance along the root of HM5511 seedlings. HJ = hypocotyl junction, RT = root tip, n ≥ 6, error bars: SD.

Objective 1B: (PARTIALLY COMPLETED IN YEAR 3) Generate tomato lines with increased exodermal suberin upon water deficit.

Methods: We generated (i) *35Spro:MYB92* transgenic lines (*35Spro* is a strong promoter that drives expression in nearly all cells of the plant, MYB92 is a positive transcriptional regulator of suberin biosynthesis); (ii) *35Spro:*ASFT (ASFT is a suberin biosynthetic enzyme); (iii) *ASFTpro*:MYB92 (*ASFTpro* is an exodermal-specific promoter, this line should increase suberin only in the exodermis), and (iv) *RAB18pro*:MYB92 (*RAB18pro* is a drought-inducible promoter, this line should increase suberin only in drought conditions).

Since processing tomato varieties are hybrids, and given the results in Aim 1A, *ASFTpro*:MYB92 and *RAB18pro*:MYB92 lines were generated in the female parent of one of the top-performers of the screen in this aim – HM55111 – in both suberin abundance and inducibility (**Figure 1)**.

Results:
At least 5 independent lines were generated for each construct and seed collected in the T2 generation.

***35Spro*:MYB92**: 5 independent lines in the T2. 1 has increased suberin (Fig. 3A), but with a growth penalty. Therefore, this line will no longer be used.
***35Spro*:ASFT**: 5 independent lines in the T2. Testing for increased suberin is in progress.
***ASFTpro*:MYB92** in HM Clause germplasm (*increased exodermal suberin, in theory, no growth penalty*): 5 independent lines in the T2. **Two lines look promising with increased suberin in a first experimental trial and are being confirmed.** Plants are being crossed to generate the hybrid, 18 crosses were successful (Fig. 3B-C).

***RAB18pro*:MYB92** in HM Clause germplasm (*drought-inducible suberin*): 5 independent lines in the T2. Testing for increased suberin is in progress. Plants are being crossed to generate the hybrid. 5 crosses were successful.

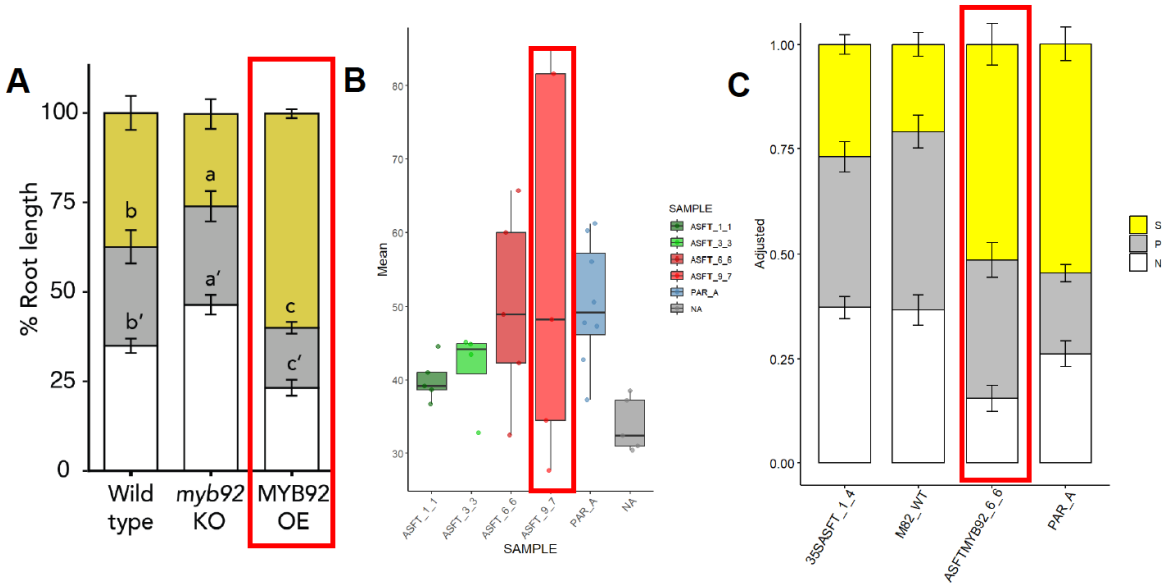

Figure 3. Phenotyping of suberin over-expressor lines. (A) *35Spro*:MYB92 line 1-2 (MYB92OE) has increased suberin compared to wild-type. (B) *ASFTpro:*MYB92 line 9-7 has increased total suberin relative to wild type (PAR_A). (C) *ASFTpro:*MYB92 line 6-6 has increased complete suberin compared to wild type (PAR_A). Yellow = complete suberization, grey = patchy suberization, white = no suberization. In panels A and C, suberin levels are normalized to root length.

Deliverables/Measurable Results: Of the three strategies that have been tested, one is promising for further experimentation in the field and/or greenhouse.

Objective 2B. Determine the influence of increased root suberin on tomato plant growth and yield in control and water deficit conditions.

Methods: In collaboration with Shantel Martinez and Chad Kramer at HM Clause, we experimentally designed a field plot to test water deficit at the UC Davis Plant Sciences Field Station in the *35Spro*:MYB92 plants.

Results: Due to the growth penalty of the *35Spro*:MYB92 plants, and upon discussion with Zach Bagley, we decided to not carry out the field season in 2024. Instead, we will conduct these experiments in 2025 with any lines that have increased suberin.

Objective 2C: Test the influence of increased suberin production on nematode and branched broomrape infection.

Methods: No experiments have yet been conducted for nematode infection. Proof-of-principle experiments have been conducted using the branched broomrape *in vitro* infection system **(Fig. 4).**

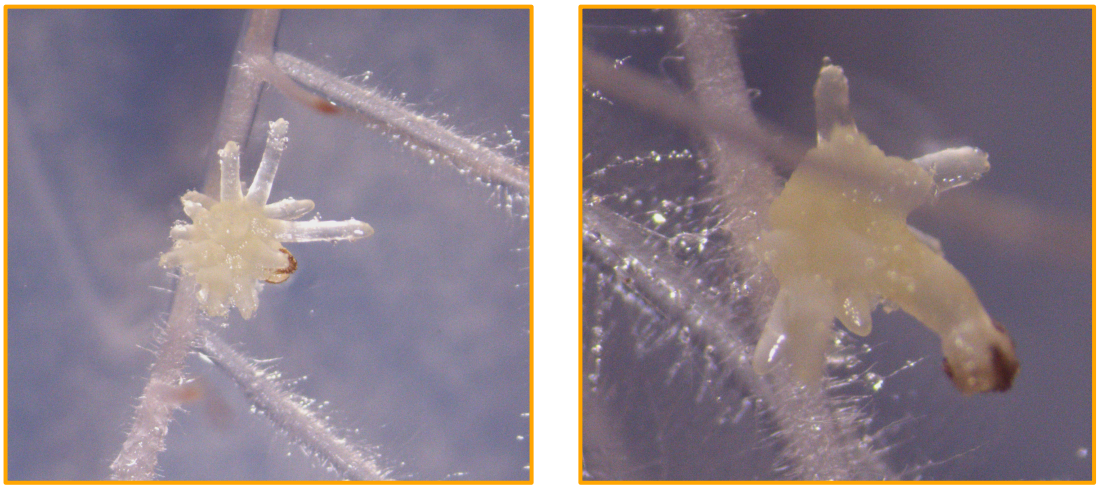

Figure 4. *In vitro* branched broomrape infection system.

Acknowledgements: We thank Shantel Martinez and Chad Kramer for extensive discussions and work to select the best candidate cultivars to generate a hybrid plant, provision of seed for transformation and directions in crossing to generate hybrid plants. Alessandra Frizzi from Bayer was helpful in discussing how to make potential results from these experiments relevant to seed companies.

Other Support: In kind support from HM Clause for the above advice and provision of resources. Bayer provided funds to generate the 35Spro:MYB92 lines. USDA, HHMI and NSF funds were used to pay for graduate student, postdoctoral scholar and faculty salary. USDA and NSF funds were additionally used to purchase supplies for the project and to pay for greenhouse time.

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Budget Summary

CTRI Funds Granted (2022-2024)	CTRI Funds Spent (2022-2024)	Funds from Non-CTRI Sources Spent	Additional Support Sources
$119,254	$32,388	$102,612	NSF, USDA, Bayer, HHMI

The remaining funds from 2024 will be spent in 2025 to complete the phenotyping of the suberin over-expression/induction lines and to conduct greenhouse and field experiments.

Project Title: **Remote Sensing for early detection of branched broomrape in tomato**

Year of Project Initiation: **2023**

Amount of funding requested from CTRI for this year: **$18,173**

Principle Investigator: **Alireza Pourreza (UC Davis, Department of Biological and Agricultural Engineering)**

Co-PI(s) and affiliation(s): **Mohsen B. Mesgaran (UC Davis, Department of Plant Sciences)**

Cooperator(s): **Hanan Eizenberg (Newe Ya'ar Research Center, ARO, Israel)**

Executive Summary:

Branched broomrape (Phelipanche ramosa) is a parasitic weed that poses a significant threat to California's tomato industry, with infestations capable of reducing yields by up to 80%. To address this, we explored the feasibility of using remote sensing to detect broomrape infestations, enabling targeted management interventions. We utilized drone-based multispectral imagery at various growth stages and satellite Time-series imagery over the season. We identified near-infrared (NIR) and shortwave infrared (SWIR) bands as key spectral regions for detecting infestation. The results of this study showed that broomrape infestation impacts the radiative properties of tomato plants that can be differentiated from non-infested plants after 500 GDD. This spectral variability can be detected by monitoring and analyzing temporal satellite imagery of tomato farms.

Introduction:

Branched broomrape (Phelipanche ramosa) is a parasitic weed lacking chlorophyll that attaches to the roots of host plants, notably tomatoes, extracting essential nutrients and water. This parasitism can lead to significant yield reductions, with losses of up to 80% reported in severely infested fields (Osipitan et al., 2021). The weed's subterranean lifecycle renders early detection challenging, as it remains underground until late in the tomato growing season, and a single flower can produce over 100,000 seeds, facilitating rapid spread within and between farms (UCANR, 2020). In California, where over 90% of the United States processing tomatoes are produced, the reemergence of branched broomrape poses a substantial threat to the industry (Osipitan et al., 2021). Traditional management strategies, such as the application of herbicides like *rimsulfuron*, have been employed in regions like Israel; however, these treatments are often applied broadly, affecting both infested and healthy plants, potentially reducing yields and raising environmental concerns (Eizenberg & Goldwasser, 2018). Given California's current limited scale of infestation, there is an opportunity to implement more precise management practices.

Recent studies have demonstrated the potential of remote sensing technologies in detecting broomrape infestations. For instance, drone-based multispectral imaging combined with deep learning algorithms has shown promise in the timely detection of branched broomrape in tomato farms (Narimani et al., 2024). Additionally, research utilizing radiative transfer modeling has indicated that broomrape infection alters the spectral reflectance of host carrot plants and their biochemical and biophysical parameters (Atsmon et al., 2024). Sun-view geometry and radiometric calibration significantly influence spectral reflectance data at the canopy level, necessitating careful consideration to ensure data accuracy (Jafarbiglu & Pourreza, 2023).

This research evaluated the feasibility of using remote sensing as a site-specific, scalable monitoring tool for detecting and mapping branched broomrape at both field and regional scales. Building upon previous findings, we conducted a comprehensive study integrating drone-based multispectral and hyperspectral imagery collected at various growing degree days to monitor broomrape infestations within fields. We also utilized time-series Sentinel-2 satellite imagery to assess infestations at a broader scale. Our results showed that a time-series model can successfully detect broomrape presence at both drone and satellite scales. We identified critical spectral bands, such as near-infrared and shortwave infrared, sensitive to vegetation density, water content, and other biochemicals.

Objectives:

The main goal of this research was to validate a scalable and efficient detection approach for broomrape infestations using advanced remote sensing technologies and AI. Building on the extensive data gathered in the previous year, we aim to examine a multi-tiered monitoring approach that utilizes satellite and drone imagery to address broomrape's threat at varying scales and stages of infestation.
Objectives include:

1. **Satellite Imagery Integration:** To incorporate satellite imagery for broad-scale detection and broomrape mapping in previously unmonitored areas, utilizing historical data to enhance model accuracy.
2. **Drone Imagery Refinement:** To refine the high-resolution drone imaging analytics pipeline, creating a detailed view of known infested fields to inform precision management and containment strategies.
3. **Model Development and Validation:** To develop a range of detection models, from AI-driven to mechanistic, and to validate them using independent datasets, ensuring their efficacy across different conditions and locales.

Through these objectives, we achieved comprehensive knowledge about the potential broomrape detection approaches that can facilitate strategic intervention.

Methodology and Results:

Study 1: Drone-based multispectral image analytics
We employed the MicaSense Altum-PT sensor, which captures synchronized multispectral, thermal, and panchromatic data, providing high-resolution imagery across multiple spectral bands: blue, green, red, red-edge, near-infrared (NIR), thermal, and panchromatic (MicaSense, n.d.). Utilizing a DJI Matrice 210 drone, we conducted aerial imaging over an infected site in Woodland, California. Figure 1 presents the orthomosaics for each spectral band from one of our data collection sessions, applying the Soil Adjusted Vegetation Index (SAVI) mask to isolate vegetation pixels by mitigating soil brightness influences (Huete, 1988).

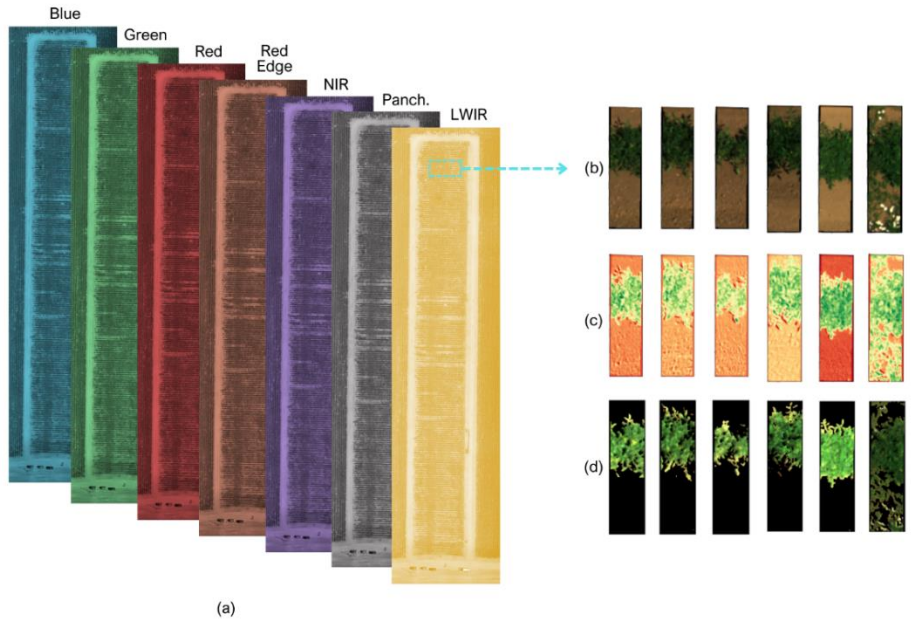

Figure 1. Visualization of data multispectral processing steps: (a) shows the entire spectral range across seven bands, (b) displays cropped RGB visualization of tomato plants, (c) illustrates the application of the SAVI index, and (d) depicts the masking of the canopy from the soil.

For analytical purposes, we extracted images of healthy and broomrape-infected tomato plants. We computed several texture features from these images—mean, standard deviation, smoothness, third moment, uniformity, entropy, and gray-level range—to serve as inputs for our classification model (Pourreza et al., 2012).

To effectively classify infected versus healthy plants and capture temporal dynamics across multiple data collection points, we utilized a Long Short-Term Memory (LSTM) neural network model. LSTM networks are well-suited for sequence prediction tasks because they can learn long-term dependencies in time-series data (Hochreiter & Schmidhuber, 1997).

Our model's performance was evaluated using a separate (unseen) validation dataset. The confusion matrix in Figure 2 illustrates the classification results, where the model accurately identified 41 out of 43 infected plants, demonstrating a high level of precision in distinguishing between healthy and broomrape-infected tomato plants.

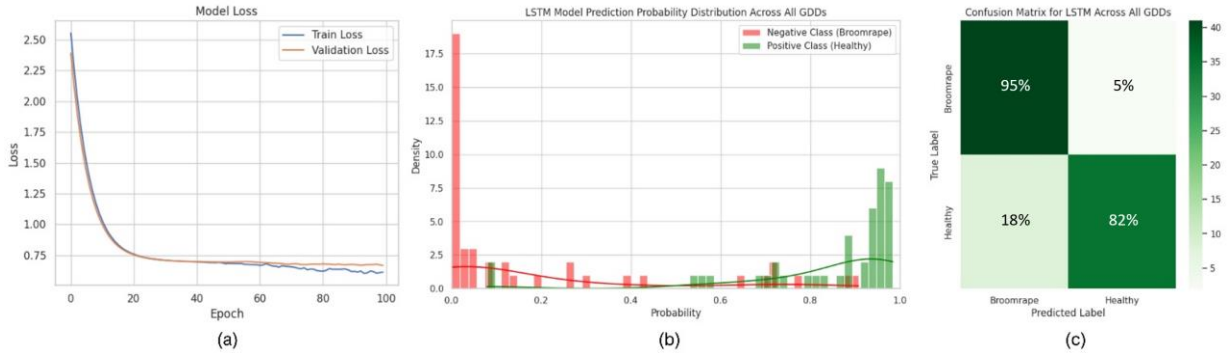

Figure 2. LSTM model with (a) training and validation loss over 100 epochs, (b) KDE versus probability plot of model predictions, and (c) confusion matrix, demonstrating high predictive Accuracy

Study 2: Drone-based hyperspectral image analytics

At approximately 500 Growing Degree Days (GDD), a critical period for early broomrape detection, we conducted aerial hyperspectral imaging over our target farm in Woodland using the Resonon Pika L sensor. This sensor captures 281 spectral bands across the visible to near-infrared (VNIR) range (400–1000 nm). It is well-suited for airborne remote sensing applications due to its lightweight and compact design (Resonon, n.d.). The Pika L was mounted on a DJI Matrice 600 drone for data acquisition.
Post-flight, we performed radiometric calibration on the hyperspectral data to ensure Accuracy. We combined the Normalized Difference Vegetation Index (NDVI) and the Excess Green Index (EGI) to isolate vegetation pixels, effectively masking out soil and non-vegetative elements. Figure 3 illustrates the canopy segmentation process.

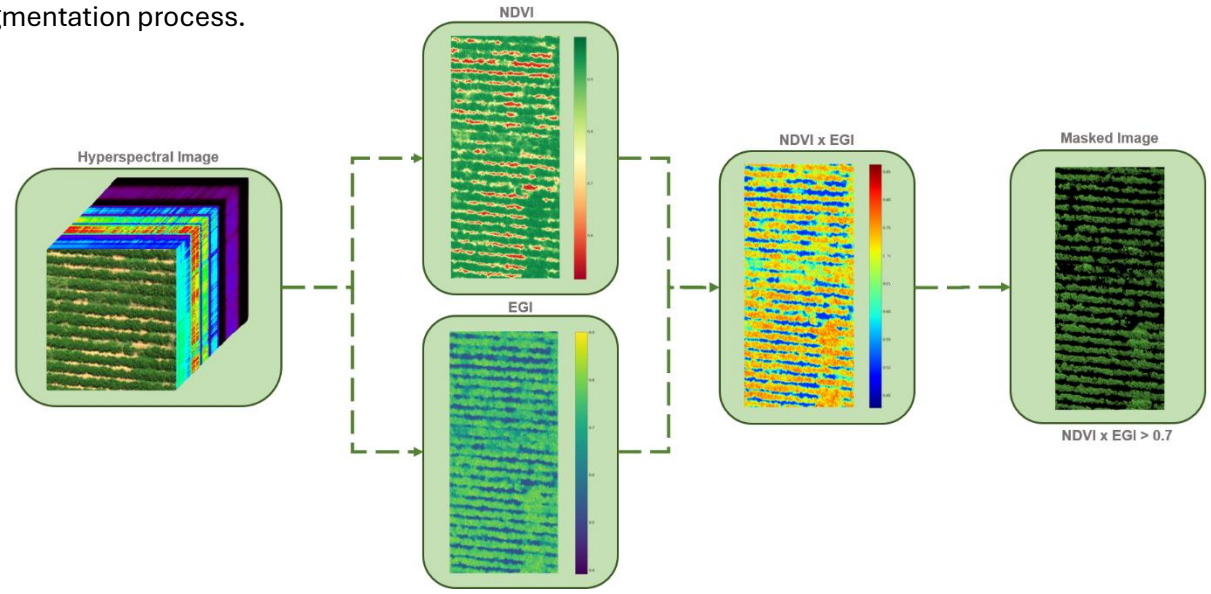

Figure 3. Canopy segmentation process isolating vegetation pixels from hyperspectral imagery.

Analysis of the mean and standard deviation of reflectance spectra revealed significant differences between healthy and broomrape-infected tomato plants, particularly in the red-edge to near-infrared regions (Figure 4). This spectral zone correlates with vegetation density, indicating that broomrape-infected plants exhibit reduced canopy cover over time compared to healthy counterparts.

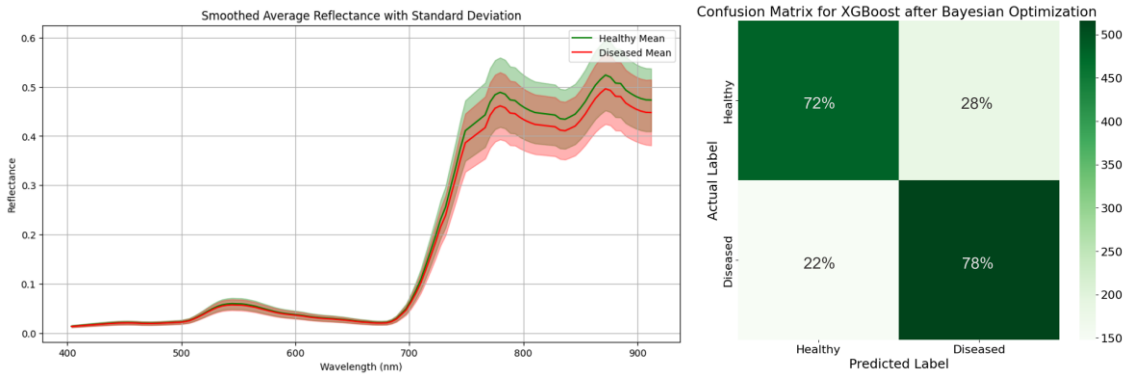

Figure 4. Left: Average reflectance spectra of healthy and broomrape-infected tomato plants, highlighting differences in the red-edge to near-infrared regions; Right: Confusion matrix illustrating the performance of the XGBoost model in classifying healthy and broomrape-infected plant pixels.

To classify infected and healthy plants, we employed an Extreme Gradient Boosting (XGBoost) model, a decision-tree-based ensemble machine learning algorithm known for its high performance in classification tasks (Zhang & Ma, 2022). Our model achieved an accuracy of 75%. Figure 4 presents the confusion matrix and performance metrics on the test dataset, showing that out of 665 pixels corresponding to infected plants, 481 were correctly identified as infected.

These findings underscore the potential of drone-based hyperspectral imaging combined with advanced machine learning algorithms like XGBoost to detect broomrape infestations in tomato crops early.

Study 3: Satellite-based time-series multispectral image analytics

we adopted a comprehensive workflow (Figure 5) for detecting broomrape infestations in tomato farms using Sentinel-2 satellite imagery. Initially, we delineated the exact boundaries of target farms using Google Earth, enabling precise extraction of satellite data corresponding to each field. Subsequently, we retrieved Sentinel-2 imagery and applied vegetation indices, notably the Normalized Difference Vegetation Index (NDVI), to estimate transplanting and harvest dates. This approach aligns with NDVI time series methodologies for monitoring crop phenology stages (Boori et al., 2019). Additionally, we accessed daily temperature data from the nearest weather stations to calculate Growing Degree Days (GDD), facilitating temporal alignment of phenological stages across different farms.

Figure 5. Detecting broomrape infestations in tomato farms using Sentinel-2 satellite imagery.

We created a dataset of five healthy and five broomrape-infested tomato farms in California. Due to the absence of recorded transplanting and harvest dates for some sites, we monitored variation in field NDVI over time to estimate these critical periods, as demonstrated in Figure 6. This method was previously used to estimate crop sowing and harvesting dates using satellite-derived NDVI time series (Rodigheri et al., 2023). To standardize comparisons across farms with varying phenologies, we calculated cumulative GDD using daily maximum and minimum temperatures, as illustrated in Figure 7.

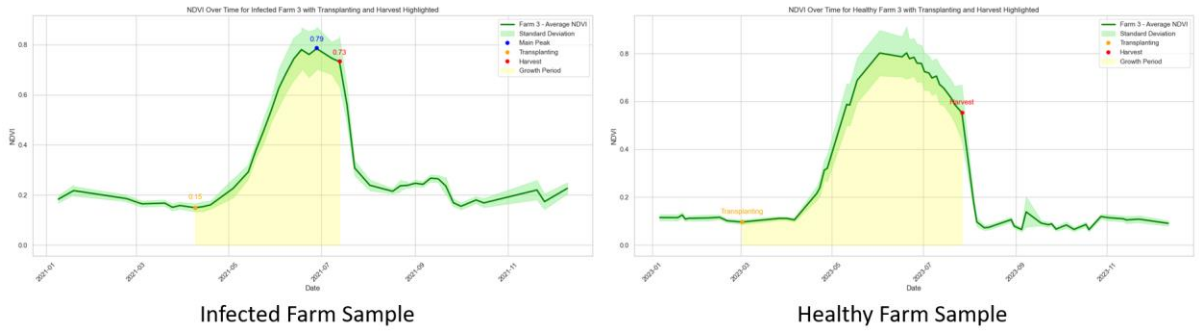

Figure 6. Model estimation of transplanting and harvest dates using historical NDVI data.

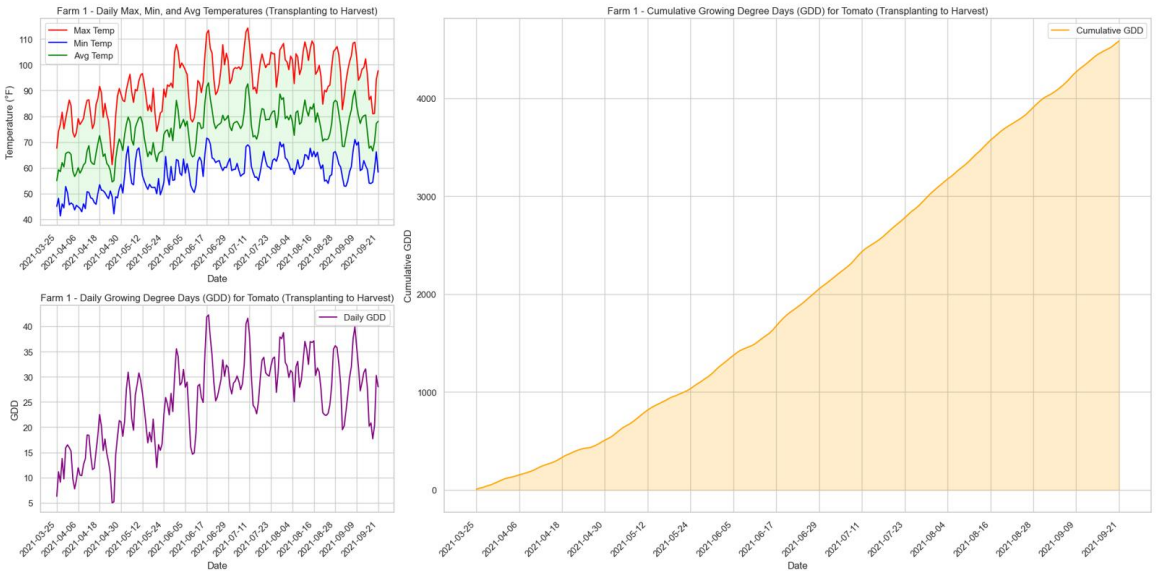

Figure 7. Calculation of cumulative Growing Degree Days (GDD) using daily maximum and minimum temperatures.

We then analyzed Sentinel-2 spectral bands (Bands 1–12, covering visible to shortwave infrared regions) at 100 GDD intervals, selecting images with less than 10% cloud cover. Figures 9 and 10 present the mean and standard deviation of reflectance values for infected and healthy farms, respectively. The more significant variability observed in infected farms reflects differing infection levels and consequent vegetation cover disparities. In contrast, healthy farms exhibited consistent reflectance patterns, indicating uniform vegetation health.

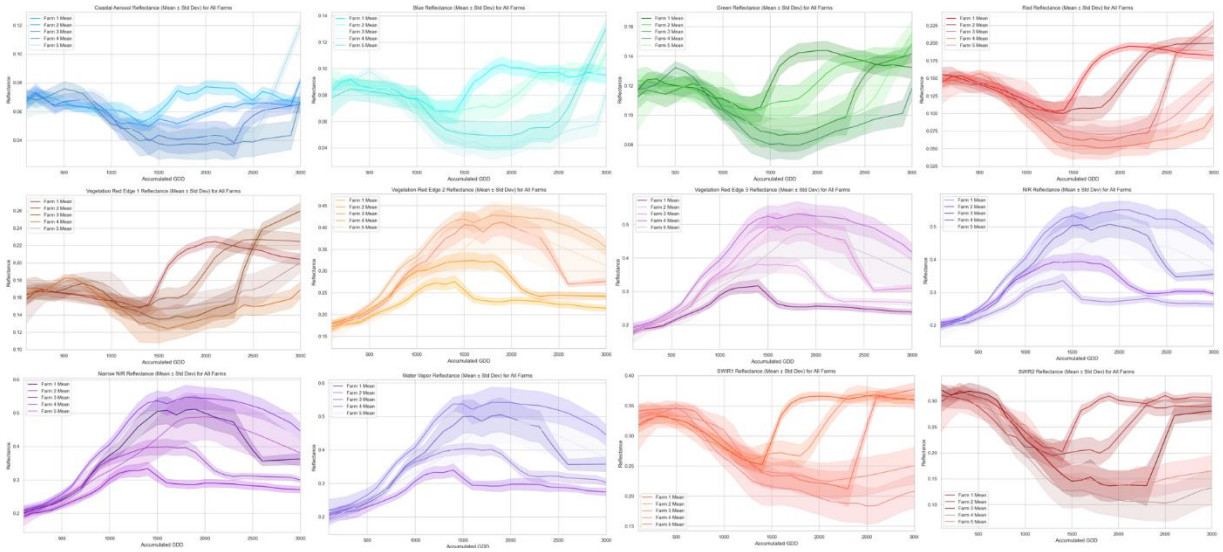

Figure 8. The mean and standard deviation of Sentinel-2 spectral band reflectance values for broomrape-infected tomato farms indicate variability due to differing infection levels and vegetation cover disparities.

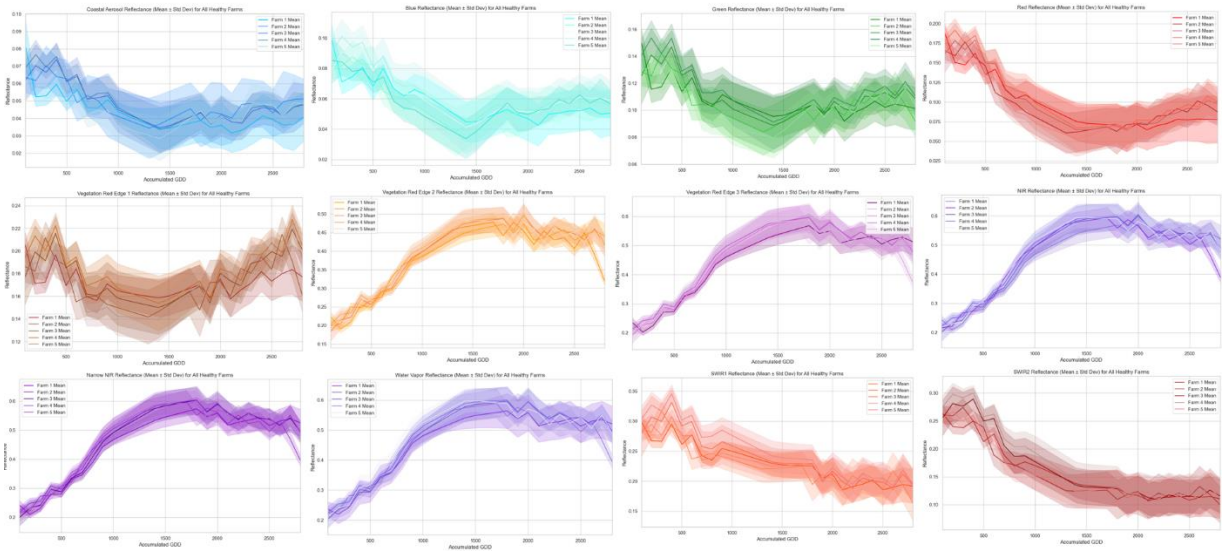

Figure 9. The mean and standard deviation of Sentinel-2 spectral band reflectance values for healthy tomato farms demonstrate consistent reflectance patterns indicative of uniform vegetation health.

Figure 10 compares the average reflectance spectra of infected and healthy farms at 500, 1000, 1500, and 2000 GDD. Notably, healthy farms displayed higher reflectance in the Near-Infrared (NIR) region over time, corresponding to greater vegetation density. Conversely, healthy farms exhibited lower reflectance in the Shortwave Infrared (SWIR) region, suggesting higher water content in plant canopies. These findings are consistent with our previous leaf-level analyses.

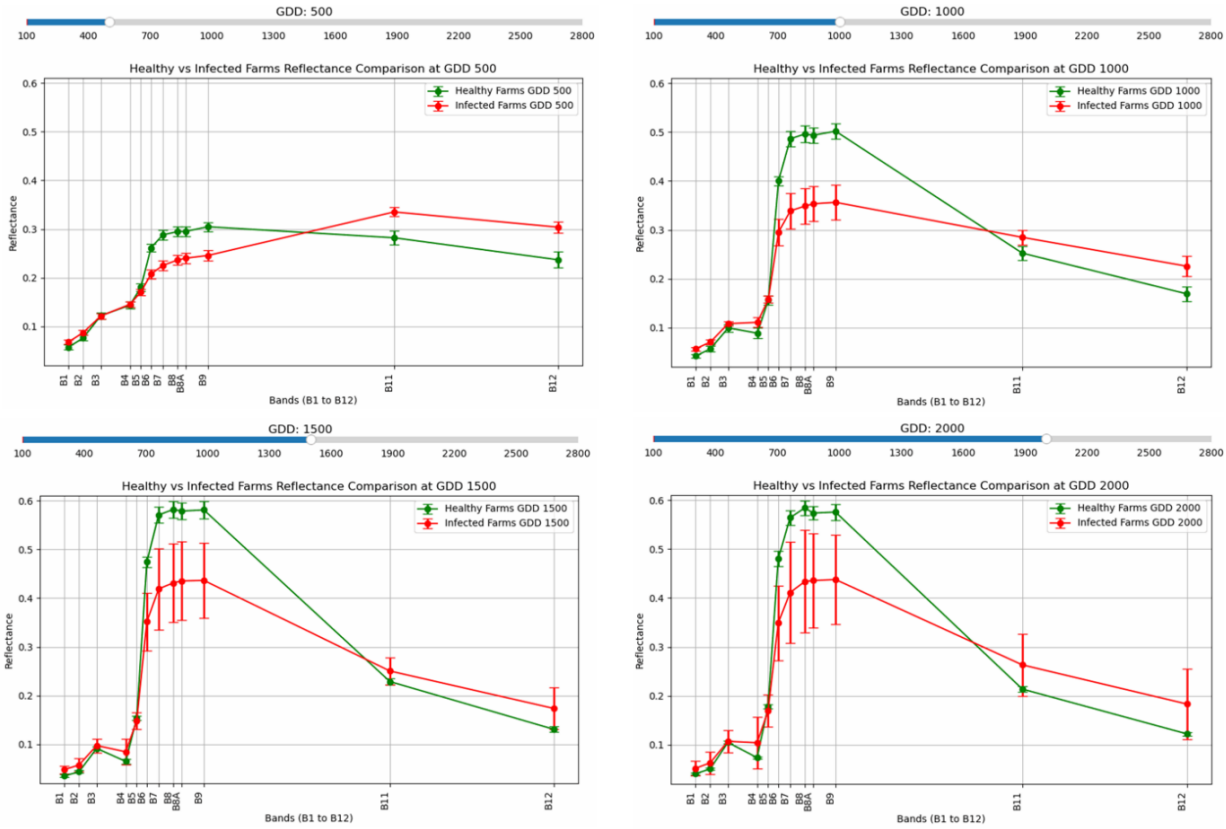

Figure 11. Comparison of average reflectance spectra between infected and healthy tomato farms at 500, 1000, 1500, and 2000 GDD, highlighting differences in NIR and SWIR regions indicative of vegetation density and water content.

Beyond farm-level classification, we developed a clustering-based machine-learning approach to generate infestation maps within infected fields. Figure 12 depicts the progression of the infestation region from 100 to 1400 GDD at 100 GDD intervals, revealing the spatial spread of broomrape from initial localized areas to broader regions within the field.

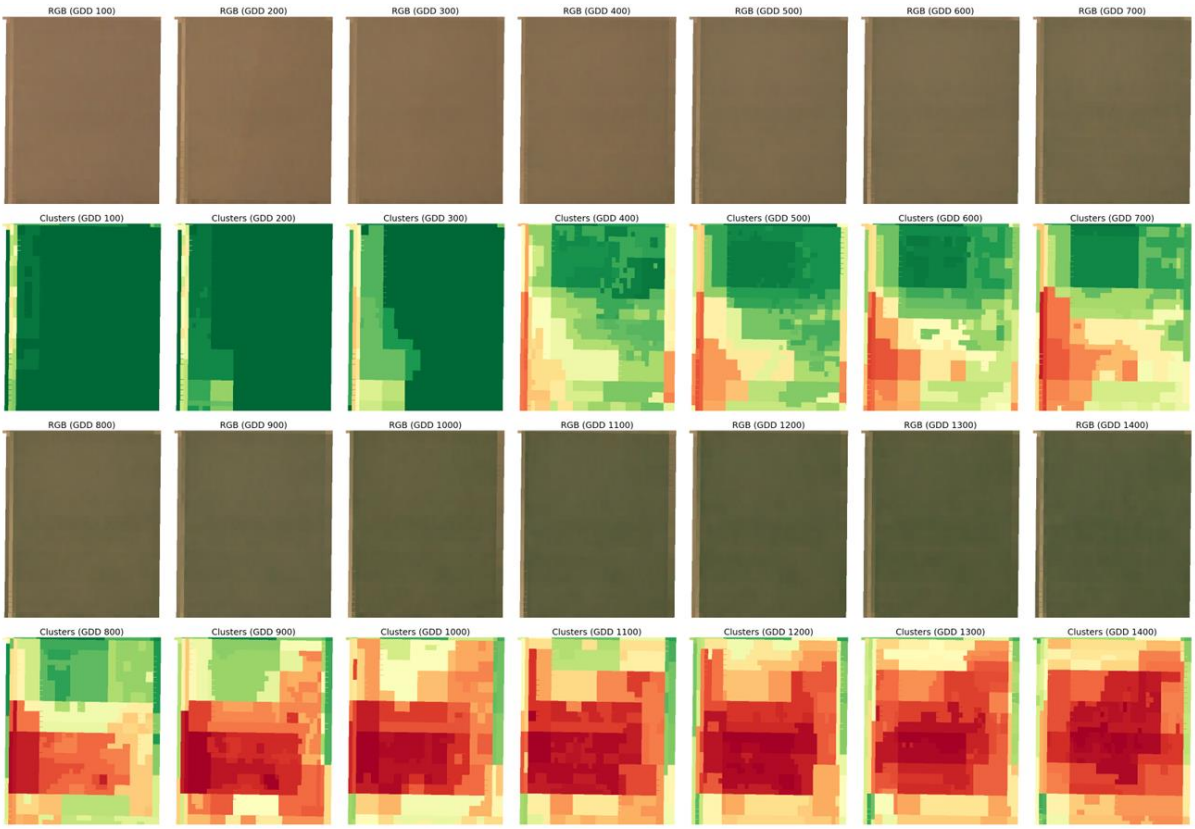

Figure 12. The temporal progression of broomrape infestation within a tomato field illustrates spatial spread from localized areas to broader regions between 100 and 1400 GDD.

These results show the capability of a hybrid technique that integrates satellite imagery, temperature-based phenological modeling, and advanced machine learning techniques to detect and map broomrape infestations at both farm and sub-field scales. This scalable approach offers a valuable tool for early detection and precise management of broomrape in tomato cultivation.

Discussion:

Branched broomrape (Phelipanche ramosa) poses a significant threat to California's tomato industry, with infestations capable of reducing yields by up to 80% (Osipitan et al., 2021). Traditional management strategies, such as the application of herbicides like rimsulfuron, have been employed in regions like Israel; however, these treatments are often applied broadly, affecting both infested and healthy plants, potentially reducing yields and raising environmental concerns (Eizenberg & Goldwasser, 2018).
Our research aimed to develop a validated, scalable, and efficient early detection system for broomrape infestations using advanced remote sensing technologies. We constructed a multi-tiered monitoring approach that utilizes satellite and drone imagery to address broomrape's threat at varying scales and stages of infestation.

We achieved early detection of broomrape infestations at the field level through drone-based multispectral and hyperspectral imaging combined with machine learning models.
At a broader scale, we integrated Sentinel-2 satellite imagery with phenological modeling and machine learning techniques to detect infestations across multiple farms. Satellite-based spectral image analytics identified critical spectral bands, such as near-infrared and shortwave infrared, sensitive to vegetation density and water content, enabling differentiation between healthy and infected plants. We identified

optimal performance between 500 and 1000 Growing Degree Days (GDD) to distinguish healthy from infested fields effectively. Additionally, unsupervised learning approaches provided insights into the spatial progression of infestations within fields that can provide valuable information about targeted management interventions.

Implementing this remote sensing framework offers several advantages:

- **Timely Interventions:** Detecting infestation severity in each county and within the crop's growing season permits prompt action to intervene.
- **Precision Management:** Early detection allows for targeted application of control measures, reducing the need for broad-spectrum herbicide use and minimizing environmental impact.
- **Scalability:** The integration of satellite imagery enables monitoring at regional scales, supporting large-scale management strategies.

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Project Title: Screening of a VOC sensor to identify broomrape infestations

Year of Project Initiation: 2023 **CTRI Funding in 2024**: $34,414

Project Leader and any Co-PIs: Cristina E Davis, PhD
Professor, Department of Mechanical and Aerospace Engineering
Associate Vice Chancellor of Interdisciplinary Research and Strategic Initiatives
University of California Davis
cedavis@ucdavis.edu | 530-754-9004

Cooperating Personnel: Brad Hanson, PhD
Professor, Department of Plant Sciences
University of California Davis

Executive Summary: The long-term goal of this project is to develop a portable device that can screen tomato fields for broomrape infestations. The device would be field deployable, either attached to a tractor or a drone, providing real-time results in the field without destructive sampling nor sending samples to a laboratory for analysis. The concept is based on the phenomenon that plants release volatile organic compounds (VOCs) into the air, which are end products of metabolism. Changes in plant health alter these chemical emissions, providing an opportunity to "sniff" tomato plant odor and identify plants that are infected by broomrape.

In the first project year, a proof-of-concept greenhouse study was used to adapted existing protocols to detect a broomrape infestation in tomato plants through odor compounds, called volatile organic compounds (VOCs). Based on Year 1 findings, direct "sniffing" of broomrape through its own odor seemed unlikely, because of how little plant mass of broomrape there is relative to typical tomato vines. Instead, we determined broomrape detection through altered tomato odor profiles was promising. A machine learning algorithm correctly identified broomrape-infected tomato plants from non-infected controls with an accuracy of nearly 80% (79.2%) from an odor sensor. Our models identified 9 specific tomato metabolites that are altered by broomrape and "sniffed" by our models for screening. These metabolites may help understand biochemical processes impacted by broomrape so that future treatments or cures can be developed using this knowledge.

During this Year 2 of the project, we continued our greenhouse study and have made steady progress in bettering our adaptation of the VOC/odor collection process for tomato plants. We expanded the number of infected and control plants to better train our machine learning models to differentiate broomrape infected plants versus non-infected plants. Our results indicate that using our technology, we can identify broomrape-infected plants prior to the emergency of broomrape in the field (as early as 3 weeks after underground broomrape exposure). In addition to conducting a greenhouse control study using gold standard laboratory instruments, in Year 2 we also intended to develop and deploy a sensor into a broomrape-infested tomato field in Yolo County. While the sensor is constructed and operational, development delays did not allow for field deployment within this funded period. Instead, our team is seeking funding to continue sensor development and commercialization.

Introduction: Long term, this project will develop a mobile sensor to screen for broomrape infestations in tomato fields. Our objective is for a device that is field-deployable either by attaching the sensor to a

tractor or by attaching the sensor on a drone. Results will be provided at the point-of-sampling (ie, in the field), rather than sending samples to a laboratory for analysis.

Our sensor relies on the fact that every living organism releases odors, also called volatile organic compounds (VOCs). Some of these chemicals are responsible for what humans perceive to be odors or scents (in other words, the unique smell of a tomato plant is caused by its VOC emissions).

VOCs serve many different roles in plants, however they are often the end products of plant metabolism. Thus, the odor profiles of a plants can indirectly reflect disease state, pathogens infection, the presence of parasites, and more [1] since these stressors alter the plants metabolism. While the human nose is unable to distinguish these differences, chemical sensors with sensitivities beyond the human capability can.

The main *Goal* and the *Objectives* under that goal: The main goal of this program is to help tomato growers identify broomrape in their tomato fields through development of a new screening technology. We would develop a sensor that could "sniff" tomato fields to detect broomrape, which could be used via drone or attached to a tractor, reporting results back to a central base station.

Specifically for this Year 2, we had two major objectives:

- Objective 1: Continue our proof-of-concept greenhouse study from Year 1 to assess whether broomrape or infected tomato plants emit a detectable chemical signature.
- Objective 2: Test a custom, mobile chemical sensor platform in a broomrape-infested tomato field in nearby Woodland, CA.

Methodology and Results:

Objective 1: Continue our proof-of-concept greenhouse study to assess whether broomrape or infected tomato plants emit a detectable chemical signature. (**Completed**)

We successfully continued our method development to measure tomato and broomrape plant VOCs in the Contained Research Facility. Below is a more detailed look into each step of the process.

O1.1: Plant materials

During Year 2, a total of 36 plants **(Figure 1)**, double Year 1's total, were maintained in a greenhouse at the Contained Research Facility (CRF) at the University of California, Davis (Davis, CA). Control and experimental tomato plants were germinated simultaneously without exposure to Branched Broomrape. At 14 days post germination, 18 of 36 tomato plants, known as the experimental group, were randomly selected to be exposed to broomrape seeds, which were placed on the outer perimeter of the tomato plant container. By using a clear container, we monitored when broomrape attached to the tomato plant root system **(Figure 2).** During this experiment, broomrape-infected tomato plants showed visual symptoms of broomrape parasitization. All control plants were confirmed not to have been infected with broomrape.

O1.2: Branch enclosures

Note: Branch enclosures are a unique necessity of the Contained Research Field. They are not needed in tomato fields for sampling. The CRF contains a unique air recirculation system to prevent broomrape from

escaping the facility accidentally. Consequently, the greenhouse background is saturated with tomato plant emissions. To measure individual tomato plants, an enclosure is placed around the plant to isolate its chemical emissions.

A branch enclosure manifold for tomatoes and broomrape (**Figure 3**) was adapted from previously used in citrus VOC studies, which are common in atmospheric biogenic organic compound emissions studies. Due to limited space in the CRF, our manifold could accommodate four plants at one time.

All tubing in the system was composed of ⅛ in. (3.175mm) outer diameter PTFE. Connections were made with stainless steel fittings. The air flow was generated by an oil-free electric diaphragm pump (Part 9341310, USAThomas). We improved the pump type from Year 1 to Year 2 was changed to ensure constant air flow. Air flow was passed through a hydrocarbon trap (Part 21991, Restek Corp., Bellefonte, PA) to scrub the air of background greenhouse VOCs. Flowmeter was used to adjust total volume of air supplied to the manifold (Part VFA-21-SSV, Dwyer). Precision-adjust air flow control valves and controlled the flow to eight individual purge lines, and the lines were monitored and recalibrated to each to maintain a flow of 40 sccm.

A single tomato plant was placed inside PTFE bags to isolate the VOC emissions from the plant. Although translucent, the bags allowed photosynthetically active radiation to pass through to allow for normal plant photosynthesis. The purge line was placed along the branch and the bag was closed around the branch using a zip tie. During Year 2, entire plant VOC signal was collected by enclosing the PTFE bags over the entire plant and sealing it at the base of the stem above the soil **(Figure 3).** Whereas in Year 1 samples, depending on the size of the plant, may have been collected from a single branch. This modified collection method ensured a higher signal strength during Year 2.

***Figure 1**: With expanded but still limited space in the CRF, we added additional plants to the increase the population size of the experimental (right) and control (left) groups. (Not all 36 plants pictured)*

***Figure 2**: Close up of clear container with evident broomrape parasitization. The container is normally covered in a black covering to block any solar radiation to affect root systems and parasitization.*

Figure 3: *Close up of VOC collection. Tomato plants are placed into branch enclosures (Teflon bags). The material allows for photosynthetically active. radiation to pass through, and tubing provides fresh air. Twisters are suspended to the sides of the bags, slightly above the plants, through magnet*

O1.3 Determine the exact timepoint tomato plants exhibit a shift in volatile profile upon infection by broomrape

To collect volatile emissions from tomato plants, we used an analytical chemistry approach as used in Year 1 of the project using a VOC sponge, commercially called "Twisters", a small tool that plant VOCs stick to. Twisters are placed inside the branch enclosure (**Figure 4**) for 24 hours, saturating with the plant VOC profile. Prior to removing samples from the CRF, Twisters are placed into a freezer for 4 weeks (to ensure deactivation of any broomrape seeds), then returned to our laboratory for chemical analysis.

Along with every plant measurement, blank samples were collected using a bag that was never exposed to plant matter. This bag shared the same air supply as the experimental and control groups. These samples were then run in an identical manner as tomato samples to determine the background VOC profile **(Figure 5).** Background was subtracted from the samples in some models in order to identify actual differences between experimental and control groups.

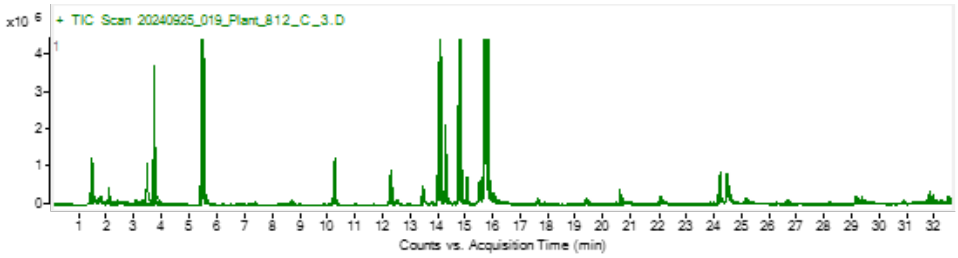

Figure 4*: Example of a typical VOC profile of a single tomato plants as measured by GC-MS. Shown is the complete profile. Each peak represents a chemical emitted by the plant, with the peak height related to the concentration of that chemical.*

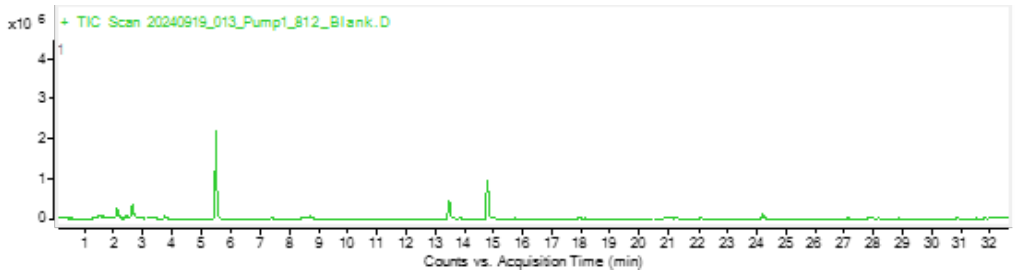

Figure 5: Example of a typical VOC profile of a blank as by GC-MS. Shown is the complete profile.

Year 2 results continue to suggest broomrape attachment alters the tomato plant metabolism, and these changes are reflected in the tomato plant VOC emissions. During the second year of the project, the primary focus of data analysis was to determine the timepoint where the shift of VOC profile could be used in a machine learning algorithm to identify broomrape-infested plants. Using Partial Least-Squares Discriminant Analysis models over 4 different weeks (**Figure 6)**, we were able to see a suggested separation from the two groups as early as three weeks after tomato plants were exposed to broomrape seeds. At this timepoint, the Receiver Operating Characteristic (ROC) curve showed an area of the curve (AUC) of 0.75 with a high specificity, but low sensitivity. This demonstrates the model's highly effective ability to classify a plant group. By Week 5, the model's ability to discriminate between the control and experimental groups highly increased. With an AUC of 0.82, sensitivity of 0.80, and specific of 0.78, by the model has a promising ability to successfully discriminate between control and experimental groups. These models demonstrate the potential for early detection of broomrape infestation since emergence from the soil did not beginning until after Week 5.

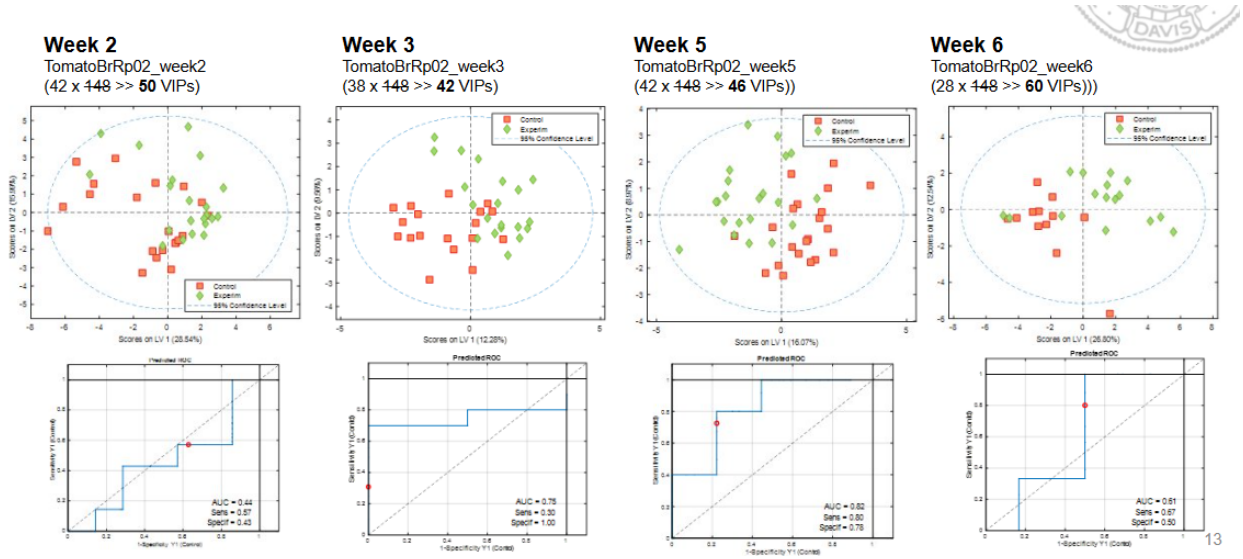

Figure 6: *PLS-DA models and ROC curves for weeks 2, 3, 5, and 6 after broomrape exposure to the experimental group. Separation between the control (red) and experimental/infested (green) improves from Week 2 to Week 5. Best discrimination by Week 5. Results are suggesting VOC changes can be used to identify infestation before broomrape emergance.*

Objective 2: Test a custom, mobile chemical sensor platform in a broomrape-infested tomato field in nearby Woodland, CA. (**Incomplete – seeking further funding (eg USDA) to complete**)

For Objective 2, we had intended to test a custom, mobile chemical sensor platform in a broomrape-infested tomato field. We were to deploy a prototype sensor in a commercial tomato field infested with broomrape working with collaborator Prof Brad Hanson to determine the real-world feasibility of detecting broomrape. However, we were leveraging funds from an outside project to support the development of the custom mobile chemical sensor, but because of unforeseen challenges with the senor, we had delays in its completion. As a result, we missed the optimal window of opportunity to test the sensor in the infested field, which limited our ability to test the sensor in real world conditions.

At the time of this report, the sensor is fully developed (**Figure 7**) and is operational (**Figure 8**). The system consists of several components to collect and measure the odor compounds coming off tomato plants. First, it contains a specialized sample chip that helps enhance odor compounds collected from tomato plants, so that they are easier to detect. Then, a gas chromatography column separates the 150+ odor compounds from tomato plants. Finally, a specialized detector called a differential mobility spectrometer is used to measure the amount of each odor compound in the same.

Once the chemical profile is measured, this data is fed to AI models who determine whether that sample came from a broomrape-infested tomato plant.

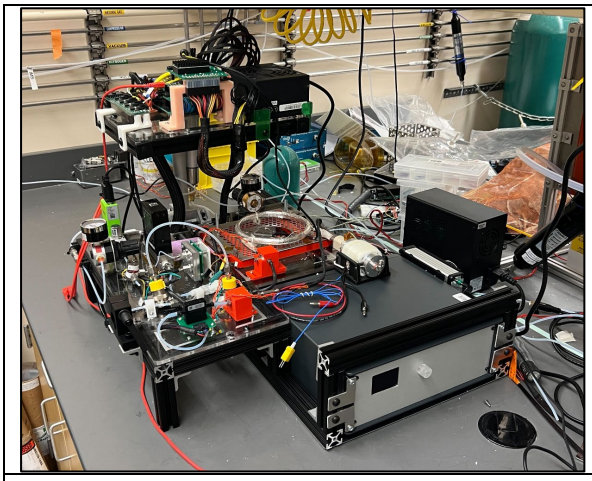

Figure 7: The sensor developed in part by CTRI funds to measure plant VOCs.

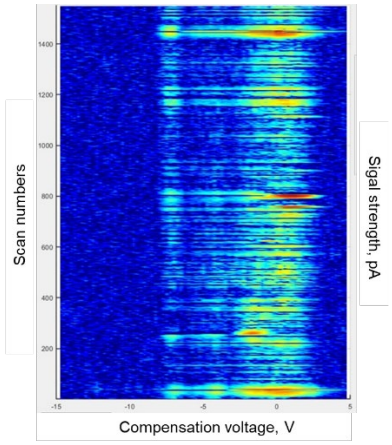

Figure 8: Example of chemical data coming from the sensor.

Discussion: The objective of Year 2 was to build on our initial proof-of-concept study by refining our methods to use volatile organic compound (VOC) profiles in detecting broomrape infestation in tomatoes and testing the feasibility of a mobile sensor in a real-world field setting in Woodland, CA.

We had success in expanding our greenhouse study by doubling the size of the control and sample group populations by x2 and bettering our air recirculation system in hopes to refine our VOC collection methods. Because of the expanded population, we were able to build a more developed machine learning model, which showed that the ability to detect broomrape infestations can increases over time and narrowed down the detection window. The findings demonstrated that there are detectable changes in the VOC profile of the tomato plants as early as three weeks after broomrape exposure. The model that

achieved the best performance was 5 weeks after broomrape exposure. With an area under the curve of 82%, the model preforms with a high level of distinction between control and infected groups. These results have demonstrated that there is a potential for early detection of broomrape and that broomrape detection betters over time. Although these results are from a greenhouse study, we believe that it has the possibility to be applied to real world conditions with modified time points.

Objective 2 to test a custom sensor in broomrape infested tomato fields was delayed. We were unable to make the optimal window for field testing due to foreseen challenges with sensor development. The groundwork has been laid for this objective and our team has the continued desire to test the feasibility of real-world conditions.

This projects long term goal was to help provide tomato farmers with an inexpensive, screening tool for broomrape infestations. This would enable target inspections of fields and help to minimizing any spread of broomrape. Additionally in Year 1, the identification of any tomato metabolites which are altered by broomrape can help in future research. We hope to partner with CTRI in the future to continue this study.

Acknowledgements: We are thankful for Prof Brad Hanson, who is a collaborator on this project. Brad Hanson and his team provided and maintained all plants used in this project.

This project as leverage for other dollars: Results from this Year 2 of funding helped to obtain a substantial (>$10m) award from the National Science Foundation (NSF) for the UC Davis Campus. Led by Prof Christine Johnson, the new Center for Pandemic Insights will help our team develop VOC sensors like the one developed with CTRI funds. Additionally, we applied for the USDA PPDMDPP to develop sensors for detection of another tomato pest, the South American Leafminer, though it was not selected for funding.

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Project Title: Evaluation of materials to mitigate negative effects of salinity and high temperatures on yields of processing tomatoes

Year of Project Initiation: 2023

CTRI Funding in 2024: $21,312

Principle Investigator:
Thomas A. Turini
University of California Ag and Natural Resources
Fresno County Cooperative Extension
550 E. Shaw Avenue, Suite 210-B
Fresno, CA 93611
taturini@ucanr.edu
559-375-3147

Co-Principle Investigators:
Isaya Kisekka
UC Davis
Associate Professor of Hydrology and Irrigation
ikisekka@ucdavis.edu

Felix Ogunmokun
UC Davis
Agricultural Water Center Project Manager
fogunmokun@ucdavis.edu

Executive Summary: Processing tomato yields can be reduced by stress due to drought, high temperatures and/or high salinity levels. Rigorous evaluation of approaches to reduce or eliminate the negative impacts of plant stress on yield can aid decision makers in the absence of consistent and definitive results regarding the impacts of these products. The objective of this study is to evaluate biostimulant and soil surfactant approaches in mitigating losses in California processing tomatoes.

Biostimulants applied to either foliage or roots may trigger physiological processes resulting in tolerance to stress conditions to alleviate yield reductions or negative quality impacts due to environmental stresses. Skeepon, an acetic acid-based plant activator, showed promise in greenhouse and laboratory studies in reducing drought stress symptoms, and it is currently being tested in production settings. The studies at UC West Side Research and Extension Center was the first evaluation of this material on processing tomatoes in this area. The material was evaluated in late-season processing tomatoes under three levels of stress on two varieties. During both years, temperatures were above average and challenges with the irrigation system resulted in greater levels of stress than was intended.

Under the high stress conditions of this study, the few treatment differences present were attributable to the varieties and there was no interaction between stress levels and the varieties/Skeepon treatment. With combinations of substantial heat and drought stress, Skeepon did not significantly improve production nor quality. This outcome was similar to that of 2023. Based on these studies, Skeepon is not effective under extraordinary levels of drought and heat stress. However, under stress conditions more typical, it is not possible to determine based on this work.

Soil surfactants injected through sub-surface drip irrigation may alter water distribution in the soil profile and provide benefits that include improved water use efficiency and decreased nitrogen leaching. Sub-surface drip irrigation reduces evaporative losses, improves irrigation uniformity and increases production per unit of irrigation water as compared with furrow irrigation. However, in this system, there remains an opportunity to learn more about water and fertilizer distribution in soil profile.

The study to evaluate Proliferate, a penetrating humectant and soil surfactant, was conducted in 2023, but was abandoned in 2024 due to poor plant growth attributable to insufficient water delivery to the trial site at the UC West Side Research and Extension Center. In the 2023 study, the Proliferate injections began two-months post-plant and was repeated at three-week intervals. No treatment effect was observed under the conditions of that study.

Challenges presented by abiotic stress should be addressed and the potential of these materials should be re-evaluated under milder stress conditions than what the crop was subjected to in these trials. University of California ANR has been making considerable investments in the West Side Research Extension Center, and I would be willing to engage in irrigation-work at the site once the irrigation infrastructure issues are completely resolved.

Introduction:
Drought events and regulatory actions limit availability of water for agricultural purposes in processing tomato production areas, so increased water use efficiency is critical to ensuring agricultural sustainability (Ghorbanpour et al., 2022; Foley et al., 2011). In addition, above optimum temperatures for tomato production, which is 70° – 75°F depending upon developmental stage and cultivar genetics, yield can be substantially reduced due to reductions in pollen release and germination (Sato et al., 1998). With the additional consideration of potential yield reductions due to salinity stress, multiple approaches may be needed to mitigate impacts of abiotic stress on tomato production.

Plant activators are used in many crops to trigger physiological processes within a plant in order to mitigate losses due to biotic or abiotic stresses. Skeepon is a plant activator that stimulates the acetate synthesis, which influences the priming of the jasmonic acid pathway: Skeepon-treated potted tomato plants exhibited tolerance to water stress as compared to the untreated controls in replicated greenhouse experiments (Kim et al., 2017). This material is currently being field tested internationally. These trials are the first evaluation under Central Valley field conditions in California processing tomato production.

In the 2023 study conducted at UC West Side Research and Extension Center, transplant trays with two processing tomato varieties (H5608 and H1293) soaked in Skeepon for 24 hours were transplanted on 6 Jun. The two treated varieties and the same two varieties without the treatment made up the sub-plot treatments in a four-replication split plot experimental design. The four subplot treatments were randomized within three main plot treatments, which included a late-season deficit irrigation treatment, a saline water treatment and a standard irrigation treatment. Stresses imposed were greater than intended. Due to irrigation infrastructure challenges at the station, it was not possible to maintain consistent pressure, which led to under-irrigation early in the season. There were other challenges with the equipment for delivery of the saline irrigation water, so that treatment was consistently under irrigated as well. Under the conditions of substantial season-long stress, Skeepon did not improve yields nor quality of either of the varieties included in the study.

Soil surfactants applied through sub-surface drip irrigation (SDI) could provide an opportunity to improve water distribution in the soil profile. In California, the majority of processing tomatoes are irrigated by sub-surface drip irrigation SDI, which helps to reduce evaporative losses, improve irrigation uniformity and

increase production per unit of irrigation water as compared with furrow irrigation. However, in this system, there remains an opportunity to learn more about water and fertilizer distribution in soil profile. Applied water and fertilizers that are not taken up by the plant constitute a waste of resources and could be a source of pollution to the environment (Zhang et al., 2021). Because most roots involved in the active uptake of water and nutrients are located within the upper 8 inches of the soil profile (Jackson et al., 1996; Schenk & Jackson, 2002), SDI-applied water may be poorly utilized by the roots, especially at early stages of plant development when the roots are shallow. Furthermore, where the capillarity of the soil is reduced, irrigation would favor downward rather than upward water distribution, which would be beyond the root zone under the influence of gravity. These scenarios could result in increased leaching and reduced yields of processing tomatoes, ultimately decreasing water use efficiency.

University of California West Side Research and Extension Center provides the controlled conditions needed to test the hypothesis of this study in an area and under the conditions of a critically important tomato production area that is challenged by water availability. The 2023 study was planted on 8 Jun and harvested on 24 Oct.

The main *Goal* and *Objectives*:
Generate information leading to mitigation of yield losses due to plant stress:

- Evaluate impacts of a plant activator at planting on tomato yield and quality.
- Assess influence of a soil surfactant on yield and quality.

Methodology and Results:
General: Each objective was addressed in separate field studies conducted at the University of California West Side Research and Extension Center in Five Points, CA. Similar production techniques were used at both sites. Irrigation tape (Jain Turbo Cascade 0.26 gph emitters at 12 in spacing) was injected at a depth of 10-in at the center of the 60 in bed center-to-center. Soil Phosphorus-P was deficient at both sites (17 at the plant activator trial site and 9 ppm at the surfactant study location) and 11-52-00 was applied in a 10-inch-wide band at the center of the bed and incorporated on 25 May. On 22 May, the plant activator study was transplanted and the soil surfactant study was planted on 23 May. A Checchi Magli carousel planter was used and the plants were set 14 inches apart. In both studies, Admire Pro 5 fl oz/acre was injected on 29 May to reduce risk of beet curly top virus. Treflan 1.2 pt and Dual Magnum 1.5 pt were applied before transplanting and incorperated. From five to thirteen weeks after planting, 160 lbs N/acre as UN-32 injected into the drip weekly. On 19 Jul, Agri-Mek SC 3.5 fl oz, Radiant SC 6 fl oz, Warior II 1.9 fl oz and Quadris Top 8 fl oz were applied in a 30 gal/acre mixture for russet mites, worms, stink bugs and powdery mildew. At both experimental sites, an irrigation manifold was constructed that included dedicated lines for each irrigation treatment (three in each study), a water meter on each line (the reading was recorded weekly) and a ½ inch Mazzei® Venturi for injecting fertilizer, pest control materials and surfactant into the sub-surface drip. In addition, each line was equipped with a ball valve, back flow valve, pressure regulator, pressure gauge and air valve. The manifold connected each line to lay-flat that was plumbed into sub-surface drip in each of the main-plot treatments.

On 2 October, the plant activator study was harvested. However, the soil surfactant study was abandoned prior to the injection of the first treatment because the field lacked sufficient uniformity. Due to insufficient pressure, areas within the trial area failed to develop and root intrusion into the drip irrigation system had occurred. In the plant activator study, 6 row ft of each plot were hand harvested and total weight was recorded. A sub-sample in a 5-gallon bucket was collected and weighed. The sub-sample fruit were hand sorted into categories that include red, green, sunburn and rot. The percentages of fruit in each category appear in the tables below. There was no sunburn, so that column is excluded from the tables. Forty red

fruit were collected and color, solids and pH analysis were determined by Processing Tomato Advisory Board (PTAB) laboratory located at the Los Gatos cannery in Huron, CA.

Plant Activator Evaluation

The performance Skeepon under three irrigation/salinity conditions, on two varieties was compared in a split plot design with main-plot treatments being irrigation/water quality and sub-plot being Skeepon or no Skeepon on two processing tomato varieties.

Main-plot treatments included the following:

1. Standard irrigation
2. Deficit irrigation from 80 to 130 days post-transplant
3. Severe deficit irrigation from 80 to 130 days post-transplant.

Sub plot treatments were variety and plant activator (Skeepon):

a) H5608 with Skeepon*
b) H5608 without Skeepon
c) H1293 with Skeepon*
d) H1293 without Skeepon

Transplant trays (192 cells/tray) were soaked in a solution of 0.4% Skeepon for 24 hours prior to planting.

Actual applied water relative to calculated crop evapotranspiration rates over the season is charted (Fig. 1). The UC West Side Research and Extension Center irrigation system had inconsistent pressure levels throughout the season, was not operational in periods in mid-July and intermittently unavailable in August.

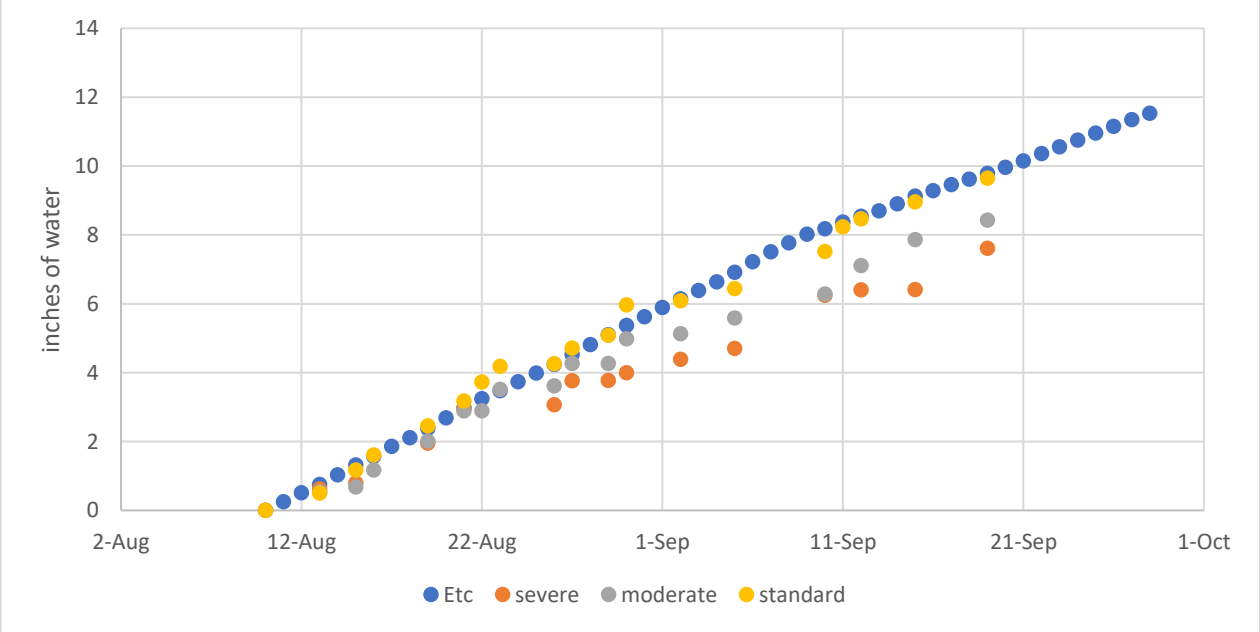

Figure 1. Cumulative calculated water use (evapotranspiration rates CIMIS station #2 Five Points x crop coefficient based on canopy coverage) and water applied in each main plot irrigation treatment in the plant activator evaluation.

The treatments were arranged in a four-replication split plot design with main-plots being irrigation/salinity treatments and sub-plots being the products evaluated over two processing tomato varieties. Main plots were a single 60-inch bed x 150 ft and sub-plots will be one bed by 35 ft. Each sub-plot treatment was

randomized within each main plot. A planted untreated buffer was positioned between each treatment row and on the outside of the trial, which was irrigated by a separate irrigation line. Yield and fruit quality data was subjected to Full Factorial Analysis of Variance and, where appropriate, means were separated by Tukey HSD P=0.05.

Overall, yields were low due to drought and temperature stress at critical stages of development (Table 1). In 2023, H1293 variety plants treated with Skeepon had fruit with significantly lower color ratings than fruit of the same variety without the treatment (data not shown). However, similar differences were not seen in 2024, and the differences that were present in yield, color and pH were associated with the varietal differences rather than the treatment (Table 2). No interactions between irrigation schedule and variety/Skeepon were observed (Table 2).

Table 1. Influence of Skeepon plant activator on yield, maturity and rot in Fresno County, 2023.

main plot [z]	sub plot [y]	total fruit	Hand sort of 15 to 25 lbs fruit [x]		
		(lbs/7 row ft)	red (%)	grn (%)	rot (%)
Standard irrigation		41.25	61.47	25.31	12.29 a [w]
Moderate deficit		45.19	56.20	38.50	8.96 b
Severe deficit		37.69	47.94	36.69	8.65 b
Main plot P [v]		0.2968	0.1902	0.1614	0.0417
	H5608 - Skeepon [u]	51.59 a	58.11	36.36	9.01
	H5608 - No Skeepon	47.29 a	54.72	31.40	9.11
	H1293 - Skeepon	37.12 b	54.96	34.72	9.99
	H1293 - No Skeepon	29.50 b	53.02	31.51	11.76
Sub plot P		0.0013	0.4975	0.9987	0.6904
Main x sub plot P		0.6366	0.5977	0.5588	0.6206

[z] Main plot treatments (irrigation schedules/water quality) were arranged in a randomized complete block within 4 replications.

[y] Four sub plot treatments (variety and Skeepon treatment) are arranged randomly and each appears once in every main plot treatment.

[x] A sub-sample of each harvested area is collected in a 5-gallon bucket and hand-sorted into categories that include reds, greens and rot. Percentage of the total sample collected is presented.

[w] Means within a row that are followed by a different letter are statistically different at P=0.05 according to Tukey's HSD.

[v] Probability (P) values 0.05 or smaller indicate that there are significant differences between the means appearing directly above the P value within the column.

[u] Transplant trays (192 cells/tray) were soaked in a solution of 0.4% Skeepon on for 24 hours prior to planting, which was on 23 May.

Table 2. Influence of Skeepon plant activator on fruit quality character in Fresno County, 2024.

main plot [z]	sub plot [y]	wt/ 50 fruit (lbs)	PTAB [x]		
			color	solids	pH
Standard irrigation		3.86	20.156	6.150	4.4488
Moderate deficit		3.51	20.531	6.106	4.4175
Severe deficit		3.34	19.844	6.038	4.4338
Main plot P [w]		0.4276	0.3508	0.9521	0.3830
	H5608 - Skeepon [v]	3.94	19.250 b [u]	5.875	4.4075 b
	H5608 - No Skeepon	3.87	18.792 b	5.800	4.3908 b
	H1293 - Skeepon	3.23	21.208 a	6.092	4.4600 a
	H1293 - No Skeepon	3.22	21.458 a	6.625	4.4750 a
Sub plot P		**0.4406**	0.0001	0.0652	0.0083
Main x sub plot P		0.5749	0.6091	0.1133	0.1741

[z] Main plot treatments (irrigation schedules/water quality) were arranged in a randomized complete block within 4 replications.

[y] Four sub plot treatments (variety and Skeepon treatment) are arranged randomly and each appears once in every main plot treatment.

[x] Color, solids and pH analysis were performed on a sample of 40 red fruit from each sub plot at the Processing Tomato Advisory Board (PTAB) laboratory at Los Gatos cannery in Huron, CA

[w] Probability (P) values 0.05 or smaller indicate that there are significant differences between the means appearing directly above the P value within the column.

[v] Transplant trays (192 cells/tray) were soaked in a solution of 0.4% Skeepon on for 24 hours prior to planting, which was on 6 Jun.

[u] Means within a row that are followed by a different letter are statistically different at P=0.05 according to Tukey's HSD.

Discussion:

Production of late season tomatoes is consistently challenging in Central California. However, the conditions present in late season-planted tomatoes at the West Side REC under conditions of inconsistent water availability were particularly unfavorable. Very high temperatures were present at critical stages of flowering and early fruit development: High temperatures from the 97° to 105°F occurred from 13 Jul to 1 Aug in 2023 and exceeded 110°F during July 2024 (Fig. 2). While the plant activator tested has provided tolerance of heat and drought stress in tomato in greenhouse studies, under the extreme field conditions of the 2023-24 studies, we did not see an effect.

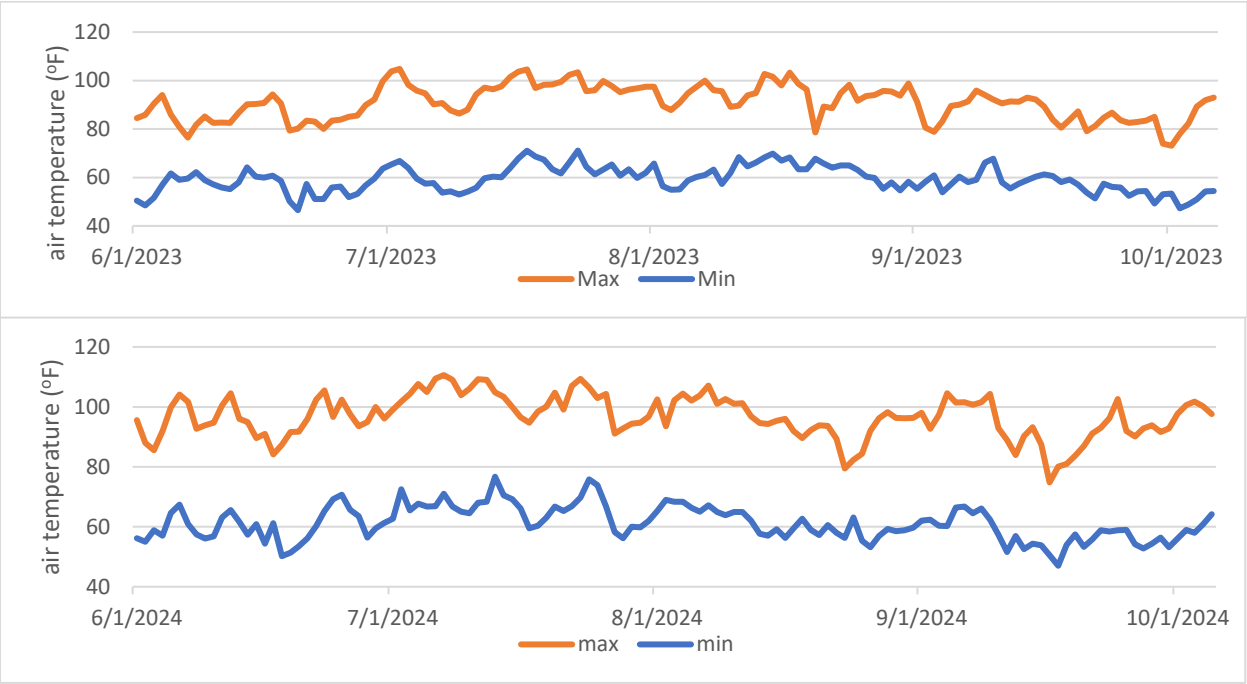

Figure 2. Daily high and low temperatures at Five Points from June to early October, 2023 and 2024.

In the studies conducted during 2023 and 2024, performance under extreme conditions were evaluated and there were no significant differences attributable to the product tested. However, a slightly lower stress season and less challenging plant date in mid-May rather than in early-June, would be more representative of the local commercial conditions and may provide an environment in which we can see treatment differences.

There remains a need for this work.
Challenges presented by abiotic stress should be addressed and the potential of these materials should be re-evaluated under milder stress conditions than what the crop was subjected to in these trials. University of California ANR has been making considerable investments in the West Side Research Extension Center, and I would be willing to engage in irrigation-work at the site once the irrigation infrastructure issues are completely resolved.

Acknowledgements:
University of California West Side Research and Extension Center staff.

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Project Title:

Climate Smart Management Innovations for Improved Soil Quality, and Productivity of California Processing Tomatoes

Year of Project Initiation: 2023 **CTRI Funding in 2024:** $18,997

Project Leader and any Co-PIs:

Principal Investigator:
Dr. Amélie Gaudin, Associate Professor, Endowed Chair of Agroecology, Department of Plant Sciences, University of California Davis. Email: agaudin@ucdavis.edu, 530-650-5101, http://gaudin.ucdavis.edu

Cooperating Personnel:
Ryan Vroegindewey, Senior Manager, Sustainable Agriculture, Campbell Soup Company, Email: ryan_vroegindewey@campbells.com, 856-676-8147.
Peter Geoghan, M.S., PhD Graduate Student Researcher, Graduate Group in Ecology, Department of Plant Sciences, University of California Davis.
Mariana Munoz-Araya, M.S., PhD Graduate Student Researcher, Graduate Group in Horticulture and Agronomy, Department of Plant Sciences, University of California Davis.
Sarah Light, UCCE Farm Advisor, Sutter-Yuba and Colusa counties. Email: selight@ucanr.edu, 530-822-7515
Brenna Aegerter, UCCE Farm Advisor, San Joaquin County. Email: bjaegerter@ucanr.edu, 209-953-6114

Executive Summary:

As industry leaders, producing 30% of the world's processing tomato supply, growers have a unique opportunity to leverage their vast array of knowledge and experiences with Climate Smart (CS) agricultural management practices to innovate towards improving soil health to buffer against these pressures, create resilience to environmental shocks, and new growth opportunities. There is however a critical gap and need to capture and categorize the diversity of Climate Smart practices utilized at the farm system level and evaluate the benefits and tradeoffs to enhance sustainability and resilience. This project categorizes the diversity of CS practices utilized in processing tomato systems and quantifies their impacts on soil health, yields, and input management. The unique partnership we have created between private industry and academic researchers allowed us to quantify the effect of these practices on soil health indicators and yields across 18 fields on a gradient from none/few to stacked CS practices implemented. We have started to leverage the UC Davis Century Experiment (UCDCE) long-term dataset and review of the literature to establish relevant background information on best practices and impacts over decades at the UCDCE. The outcomes of these efforts will culminate in soil health and yield analyses for each farm included in the survey with a comparison against study averages; and scientific publications. These will provide growers and the industry with guidance to adopt CS practices and help capture opportunities for new revenue streams through incentive programs and labeling schemes which are growing in prominence to support such transitions.

Introduction:

There is growing interest in supporting markets and incentives for Climate Smart (CS) system adoption and regenerative practices, as indicated by the partnership we have fostered with Campbell Soup Company. However, significant knowledge gaps remain in understanding cost effective implementation strategies, impacts on yields, input use/efficiency, and soil health on commercial farms. Climate Smart solutions such as subsurface drip irrigation (SDI) have shown tomato production improvements, field scale water savings, reduced weed pressure, and nitrous oxide (N_2O) emissions reductions[1,2]. There are however tradeoffs with single CS solutions, such as potential negative impacts of SDI on soil salinity, microbial activity in non-wet zones in the field, carbon storage related to microbial activity, and groundwater recharge rates[3]. Systems level approaches, stacking multiple CS practices, are likely more effective at overcoming the multitude of climate stressors and sustainability challenges growers face.

Past research and grower experience shows stacked CS practices can improve water and nutrient use efficiencies, and support soil function necessary to catalyze growth, while minimizing or eliminating yield gaps associated with transitions to different management frameworks[4]. In California, CS practices implemented on organic and conventional farms include but are not limited to: reduced tillage, cover crops, crop residue retention, mulching, crop rotations, compost and/or manure applications and other organic amendments, and the use of livestock to graze cover crops and/or crop residues. After 20 years of continuous conventional, hybrid, and organic management of corn-tomato rotations at the UC Davis Century Experiment (UCDCE), soil organic matter (SOM) and soil carbon at depth (0-200cm) increased significantly in the organic systems utilizing cover crops, composted manure, and compost[5]. Yields in the organic treatment were similar to conventional after 24 years, with increased resistance to losses in unfavorable years, an important climate consideration[6]. Similar soil carbon impacts have been realized across long-term experiments across North America with the addition of cover crops[7]. Soil health indicators have also been shown to significantly increase in treatments with cover crops and mostly so in treatments with no-tillage after 20 years of cotton-tomato rotations at the UC WSREC experiment station[8]. Tomato yields across the UC WSREC experiment have increased by 9.5% in the no-till treatment compared to standard tillage. Some studies also suggest fruit nutritional content can be higher in organically managed systems, related to flavonoids at the UCDCE[9] and sugars and vitamin C in studies from other parts of the world[10–12], a potential additional yield outcome consideration. Although long-term trials provide important insights, there are limitations with highly controlled field station experiments. Furthermore, narrow comparisons of organic vs conventional limits knowledge-generation for potential incremental benefits of different CS practices combinations in non-organic systems.

Increased field studies on commercial farms, representing a range of strategies and soils, are therefore needed to provide growers with critical real-world information regarding outcomes management legacies. A looming question for growers is what are the effects of none or a limited number of CS practices, towards multiple stacked CS practices? While soil health and yield considerations above suggest that making this transition could be beneficial in the long-term, the transitional phase can be variable when it comes to yield outcomes and benefits to soils may occur in the longer term. We acknowledge that our study doesn't address this phase, rather the outcomes of legacy management (5+ year), however our multi-stakeholder partnership has provided opportunities for open dialogue on what is needed to create pathways for these transitions. Some soil processes can also respond quickly to shifts towards organic inputs and disturbances (i.e. N availability, microbial pools). Furthermore, our study includes the implementation of these practices from short (5 years) to longer term (decades) and could help elucidate some earlier outcomes as well as long term management legacies.

Previous on-farm field trials at 13 organically managed farms in Yolo county that produce processing tomatoes provide insights on mechanisms of importance to soil health and yield benefits,

namely high carbon inputs (manure, compost, cover crops) which are optimized for use efficiency in the presence of high available inorganic N (i.e. tighter coupling of C:N cycling)[13]. These benefits have also been realized in cropping systems with increased crop diversity, stimulating higher rates of N mineralization from organic compounds[14]. Other research also shows how complex these outcomes can be in the context of which CS practices to incorporate. For instance, while compost is key to storing carbon, the use of cover crops alone in conventional systems can reduce soil carbon at depth over time in the semi-arid Western U.S.[5] These surprisingly different effects of two popular practices for building soil carbon and health further indicate the importance of stacking practices and avoiding a one-size-fits-all approach.

Climate Smart strategies that build soil health must be flexible and adapted to context, goals of the grower, and reflect several core principles: minimize soil disturbance to better maintain habitat for the soil ecosystem, increase organic inputs as carbon source for soil, reduce agrichemical use, eliminate spatiotemporal bare soil events to conserve soil and produce resources for soil organisms, and maximize plant diversity with robust crop rotations[15,16]. In California, the most popular CS practices implemented on large-scale farms include crop rotations, compost and/or manure applications, other organic amendments, a growing interest in cover crops, and grazing of cover crops and crop residues. These versatile practices can be implemented in different ways to fit the management goals and constraints of each farm. Adoption potentially increases management complexity, requiring new knowledge, increased effort and energy, and cost, but could also lead to input cost savings over time due to increases in water and nutrient use efficiency. Additionally, there is potential for growers to capture new revenue streams for verified CS practices, evidenced by the growth of certified organic tomato sales,[17] increasing diversity of alternative labeling schemes (e.g. the new Regenerative Agriculture label), and the emergence of ecosystem services markets. These opportunities position growers for economic return on investment with the adoption of stacked CS practices.

Main goals and objectives

The first main goal of this project is to identify the potential of sets of practices, on a gradient from none/few to multiple stacked CS practices, for building soil health , improving input use and tomato yield and quality outcomes. This three-year project was set up to combine a literature review, data mining from the UCDCE long-term dataset, and a survey of commercial fields to identify whether and where CS practices produce tangible benefits.

1. **Objective 1 (year 1&2):** Analyze the literature and the UCDCE dataset to determine long term soil health, sustainability, and yield outcomes of CS practices and identify pertinent soil indicators
2. **Objective 2 (year 1&3):** Measure soil and productivity outcomes in commercial fields along a gradient of CS adoption
3. **Objective 3 (year 2&3):** Synthesize knowledge to develop a best management practice guide to assist growers in adopting CS practices

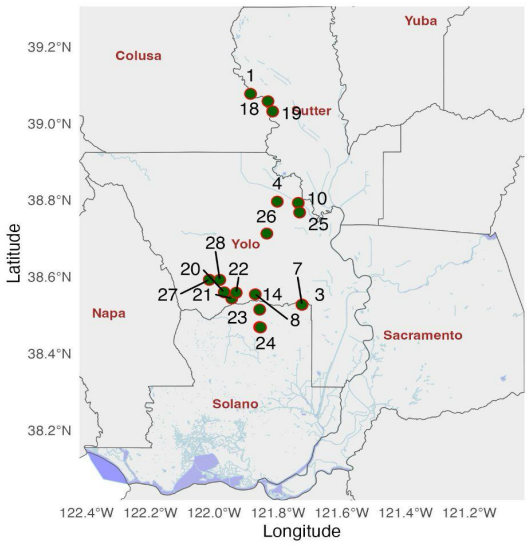
Methods and results:

Objective 1 (year 1&2): Analyze the literature and the UCDCE dataset to determine long term soil health, sustainability, and yield outcomes of CS practices and identify pertinent soil indicators

We have organized the UCD CE dataset and conducted a preliminary analysis leveraging a one-year data consolidation effort by PhD student Peter Geoghan and the building of a yield, input, and soil indicator database. Efforts have been dedicated to finalizing the long-term input data (C, N, fuel, labor,

water) now that the output data on soil health and yields is available and coarsely analyzed. The tomato-corn rotations are being assessed for their legacy effects on biological, chemical, and physical soil health properties based on a comprehensive soil survey conducted by the Soil Health Institute in 2019 following standard protocols. These rotations include conventional (synthetic inputs only), mixed (synthetic inputs + cover crops), and an organic treatment (no synthetic inputs, compost + cover crops). Preliminary results, capturing soil health status 26 years after adoption of CS practices confirmed the sensitivity to management of some of the soil health indicators used in our survey of commercial farms, especially the one related to C pools (ie. Active Carbon, soil organic matter). The combination of compost and cover crop use in the organic system showed benefits to soil health with significant increases in soil organic matter (+0.8% compared to the conventional system) and soil proteins, an indicator of microbial activity (+~70% increase compared to conventional). The amount of arbuscular mycorrhizae and fungi in general was also significantly higher in the organic system compared to conventional. Use of compost largely increased soil potassium and P content (P: 22 mg kg Conv to 250 mg kg in organic). Water infiltration rates were also double in the organic system compared to the conventional plots, despite no detected changes in aggregate stability. The use of cover crops without compost (in the Mixed system) provided more modest soil health benefits and intermediate levels of C increases and no significant improvements in infiltration rates. Work on both the literature review and data analyses was halted in year 2 (2024) to process the samples and data for Objective 2. Progress on the literature review and data analyses will resume in 2025 and be included in the PhD dissertation of Peter Geoghan to be published in 2026.

Objective 2 (year 2 progress): Measure soil and productivity outcomes in commercial fields along a gradient of CS adoption



We finalized soil health analysis of samples from 18 processing tomato fields in Northern Sac (**Figure 1**). This included soil extracellular enzymes, total soil nitrogen, total and organic soil carbon, aggregate stability, ammonium (NH_4^+) and nitrate (NO_3^-), soil respiration (CO_2 24hr burst test), and potentially mineralizable carbon (PMC) (**Table 1**). Results were analyzed and soil health reports for individual fields were shared with participating growers (see an example in **Appendix 1**). The soil health report includes all biophysical data from a specific field with comparisons to study wide averages.

Figure 1. Location of the 18 tomato fields sampled in this project.

Table 1. Indicators that were measured during the project

Soil Ecosystem Function	Indicators	Insights for Growers
Soil fertility	-Cation Exchange Capacity -pH -Macro/micronutrient availability -Soil soluble salts -Organic/Inorganic N pools	Ability of soils to store/retain nutrients, crop available nutrient pools, salinity.
Soil water/water use efficiency	-Water holding capacity -Infiltration	Water use efficiency, storage, and availability to the crop.
Soil structure	-Texture -Wet aggregate stability -Compaction (Bulk density)	Soil potential and ability to create structure/pathways for water movement and root and crop growth.
Soil organic matter and carbon	-Total Soil organic matter -Soil Organic Carbon -Carbon mineralization (24hr burst test) -Potentially Mineralizable Carbon (PMC, 72 hr)	Energy and nutrient flow potential for crop productivity and carbon storage potential.
Soil microbial and ecosystem communities	-Phospholipid Fatty Acids (PLFAs) -Microbial biomass carbon and nitrogen -Carbon cycling enzymes: ● β-Glucosidase (BG) ● Cellobiohydrolase (CBH) - Nitrogen cycling enzymes: ● N-acetyl-β-glucosaminidase (NAG) ● Leucine Aminopeptidase (LAP) - Phosphorus cycling enzyme: ● Phosphatases (PHOS)	Ability to build soil quality, optimize and ensure function related to crop productivity is supported.

We followed up with the growers in our study cohort in 2024 to collect more nuanced management data to categorize fields based on a **Management Scoring Index**. The Management Scoring Index was constructed to capture the implementation of core agroecological principles: increased biodiversity, continuous living roots, soil cover and strategic disturbances. Fields were scored based on several metrics reflecting the application of these agroecological principles:

1. **A crop diversity index (CDI)** which considers the number of summer and winter cash crop species in rotation, the number of cover crop species grown, and the length of the rotation. To account for perennialism, the length in years of the perennial crop was added to the total duration of the rotation. The index was calculated using the rotation diversity index from Bowles et al., (2020) [18] by taking the square root of the length of rotation multiplied by the number of species. Scoring

was awarded from: *0 = fields in the lower third index scoring; 1 = fields in the middle third index scoring; and 2 = fields in the upper third index scoring.*

2. **Cover crop frequency** was included separately from the CDI and winter cover as cover crops are intended to provide some benefit to the soil and do not export water and nutrients compared to cash crops. Additionally, cover crop species diversity was captured in the CDI. Scoring was awarded from: *0 = never plants cover crops, 1 = sometimes plants cover crops, 2 = cover crops every year.*
3. **Winter cover** was determined by how frequently a field is planted during the winter, which both provides a cover for the soil, and the duration for living roots in a field across a calendar year. Scoring was awarded from: *0 = never plants a cover crop or winter cash crop; 1 = sometimes plants cover crops and/or a winter cash crop; 2 = plants a cover crop or winter crop every year.*
4. **Fertilizer class** scoring was awarded from: *0 = only uses synthetic fertilizers, 1 = uses synthetic fertilizers and compost at least sometimes; 2 = only uses organic inputs.*
5. **Tillage intensity** was calculated using the RUSLE 2 program (NRCS) to derive a soil tillage intensity rating (STIR) score. Higher scores indicate higher tillage intensity and are calculated based on the type of implement used and the number of times an implement is used during a season. Typically STIR scores are compiled for entire crop rotations. We only included tillage intensity for the tomato year in the rotation as gathering tillage data across entire rotations was not possible given the quantity of other data we asked growers to provide. Scoring was awarded from: *0 = fields in the highest third quantile STIR score; 1 = fields in the middle third quantile STIR score; 0 = fields in the lower third quantile STIR score.*
6. **Livestock grazing** of cover crops or crop residues was included to capture potential benefits from reduced mowing, tillage, and improved decomposition observed in crop-livestock systems. Scoring was award from: *0 = never grazes; 1 = sometimes grazes cover crops and/or crop residues; 2 = grazes cover crops and/or crop residues annually*

Results

CS practice implemented and Management Scoring Indexes

Below are the compiled scores for each of the categories and the total combined scores for each of the fields in the study. The scoring system provided for a possible 12 total points. Fields were categorized from few adopted climate smart practices (0-4 points), moderate adoption of climate smart practices (5-6), and stacked adoption of climate smart practices (7-12). There are limitations associated with arbitrary cut-offs using ⅓ quantiles and scores were also considered as a continuous variable (i.e. scale from 0-12) to compare differences in each model. Fields ranged from no adoption of any climate smart practices to fields that had full adoption of each practice, except for grazing which had only occurred sometimes for any field that included this practice.

Table 2. Grower Management Scoring Indexes

Fields are listed with their field ID number to maintain confidentiality of results. Scores for cover crop frequency (CC Freq), winter cover, and grazing are scored 0 = never implemented, 1 = sometimes implemented, 2 = always implemented. Scores for the crop diversity index (Crop Index) are scored 0 = low diversity, 1 = moderate diversity, and 2 = high diversity. Scores for fertilizer class (Fert Class) are 0 = all synthetic fertilizers, 1 = synthetic and compost, 2 = all organic. Scores for tillage are scored 0 = higher tillage intensity, 1 = moderate tillage intensity, 2 = lower tillage intensity.

Field ID	Crop Index	CC Freq	Winter Cover	Fert Class	Till Class	Grazing	Total Score	Category	Notes from farmer-assessed management characterization
23	0	0	0	0	0	0	0	few	Clay texture, drip irrigation, 4-year rotation with two years of tomato and min-till, synthetic fertilization only.
24	0	0	0	0	0	0	0	few	Clay texture, drip irrigation, 4-year rotation with two years of tomato and min-till, synthetic fertilization only.
3	0	0	1	0	0	0	1	few	Clay loam texture, drip irrigation, 3-year rotation with conventional till, synthetic fertilization only.
14	1	0	1	0	0	0	2	few	Clay texture, furrow irrigation, 4-year rotation with conventional till, synthetic fertilization only.
4	0	1	1	0	1	0	3	few	Clay loam texture, drip irrigation, 3-year rotation with conventional till, 2-species cover crop, synthetic fertilization only.
10	1	0	1	0	2	0	4	few	Clay loam texture, drip irrigation, 4-year rotation with conventional till, synthetic fertilization only.
25	0	0	1	1	2	0	4	few	Clay loam texture, drip irrigation, 3-year rotation with conventional till, synthetic fertilization and compost application.
20	1	1	1	1	1	0	5	moderate	Clay texture, drip irrigation, 3-year rotation with conventional till, single species cover crop, synthetic fertilization and compost application.
21	1	1	1	1	1	0	5	moderate	Clay texture, drip irrigation, 3-year rotation with conventional till, single species cover crop, synthetic fertilization and compost application.
22	1	1	1	1	1	0	5	moderate	Clay texture, drip irrigation, 3-year rotation with conventional till, single species cover crop, synthetic fertilization and compost application.
26	1	0	1	1	2	0	5	moderate	Silty clay texture, drip irrigation, 4-year rotation with conventional till, synthetic fertilization and compost application.
27	2	1	1	0	1	0	5	moderate	Clay texture, drip irrigation, 4-year rotation with conventional till, single species cover crop, synthetic fertilization and compost application.

Field ID	Crop Index	CC Freq	Winter Cover	Fert Class	Till Class	Grazing	Total Score	Category	Notes from farmer-assessed management characterization
28	2	1	1	0	1	0	5	moderate	Clay texture, drip irrigation, 4-year rotation with conventional till, single species cover crop, synthetic fertilization and compost application.
7	0	2	2	1	1	1	7	stacked	Clay texture, drip irrigation, 2-year rotation with conventional till, 3-species cover crop, sometimes grazed, synthetic fertilization and compost application.
8	1	2	2	1	1	0	7	stacked	Clay texture, drip irrigation, 3-year rotation with conventional till, 3-species cover crop, synthetic fertilization and compost application.
1	2	2	2	2	2	1	11	stacked	Clay texture, furrow irrigation, 4-year rotation including alfalfa with mini-till, 2 species cover crop, sometimes grazed by sheep, chicken manure compost, organic certified.
18	2	2	2	2	2	1	11	stacked	Clay loam texture, furrow irrigation, 4-year rotation with min-till, 3-species cover crop, sometimes grazed by sheep, compost application, organic certified.
19	2	2	2	2	2	1	11	stacked	Clay loam texture, furrow irrigation, 4-year rotation with min-till, 3-species cover crop, sometimes grazed by sheep, compost application, organic certified.

Soil health indicators: topsoil layer (0-6 inch)

Biological Indicators

When controlling for soil texture in our models (%sand), soil organic matter (SOM) and soil organic carbon (SOC) were not different across the climate smart practices score spectrum or categories. Fields increasing in practice adoption scores had moderately higher (Figure 2, *p* value 0.08) microbial biomass carbon (MBC), and significantly higher microbial biomass nitrogen (MBN, Figure 2). We did not observe a difference in microbial (PLFA, bacterial + fungi) biomass or in the microbial diversity across the climate smart score spectrum. Fields with stacked practices had a moderately higher percentage *(p* value 0.08) of SOC stored in MBC, as measured by the microbial quotient (microbial biomass carbon (ug/g soil) / soil organic carbon ug/g s).

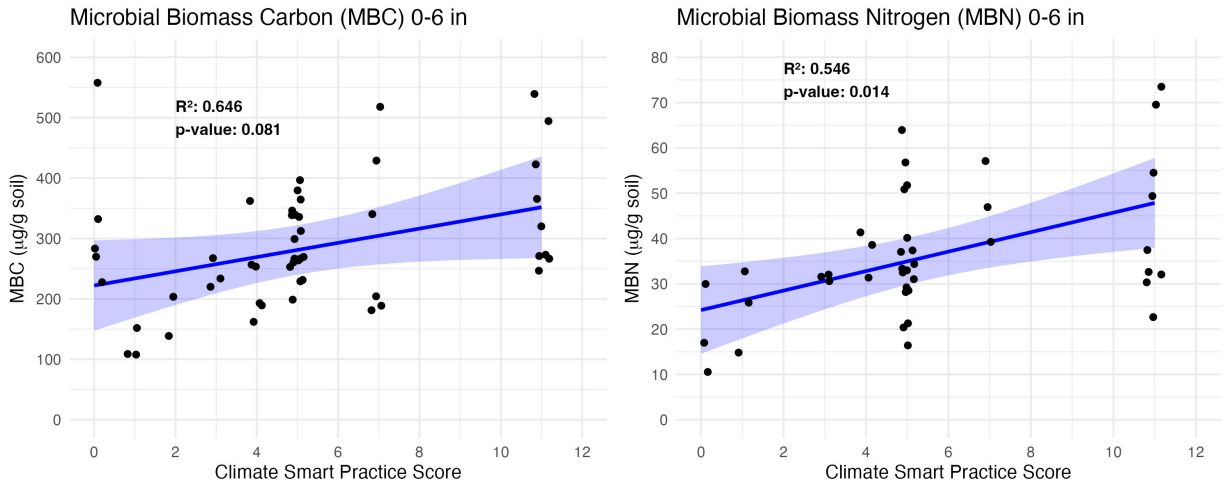


Figure 2. Microbial biomass carbon and nitrogen in the topsoil layer
Microbial biomass refers to the content of carbon or nitrogen stored in living soil microorganisms. Both reflect the level of nutrient cycling activity and abundance of microbial communities and are related to soil health.

Respiration, as measured by carbon dioxide (CO_2) respired by microbes (24hr CO_2 burst test), was higher (Figure 3, *p* value 0.03) with increasing adoption of practices. Potentially mineralizable carbon (PMC) (72 hr CO_2 test), a measure of available energy over time in soils, was moderately higher (Figure 3, *p* value 0.07) with increasing score adoption. Across the category spectrum there was no difference in these two indicators. As a measure of carbon use efficiency (CUE) the microbial metabolic quotient, i.e the amount of carbon respired by microbes relative to the amount of MBC, was not different across either the score spectrum or categories.

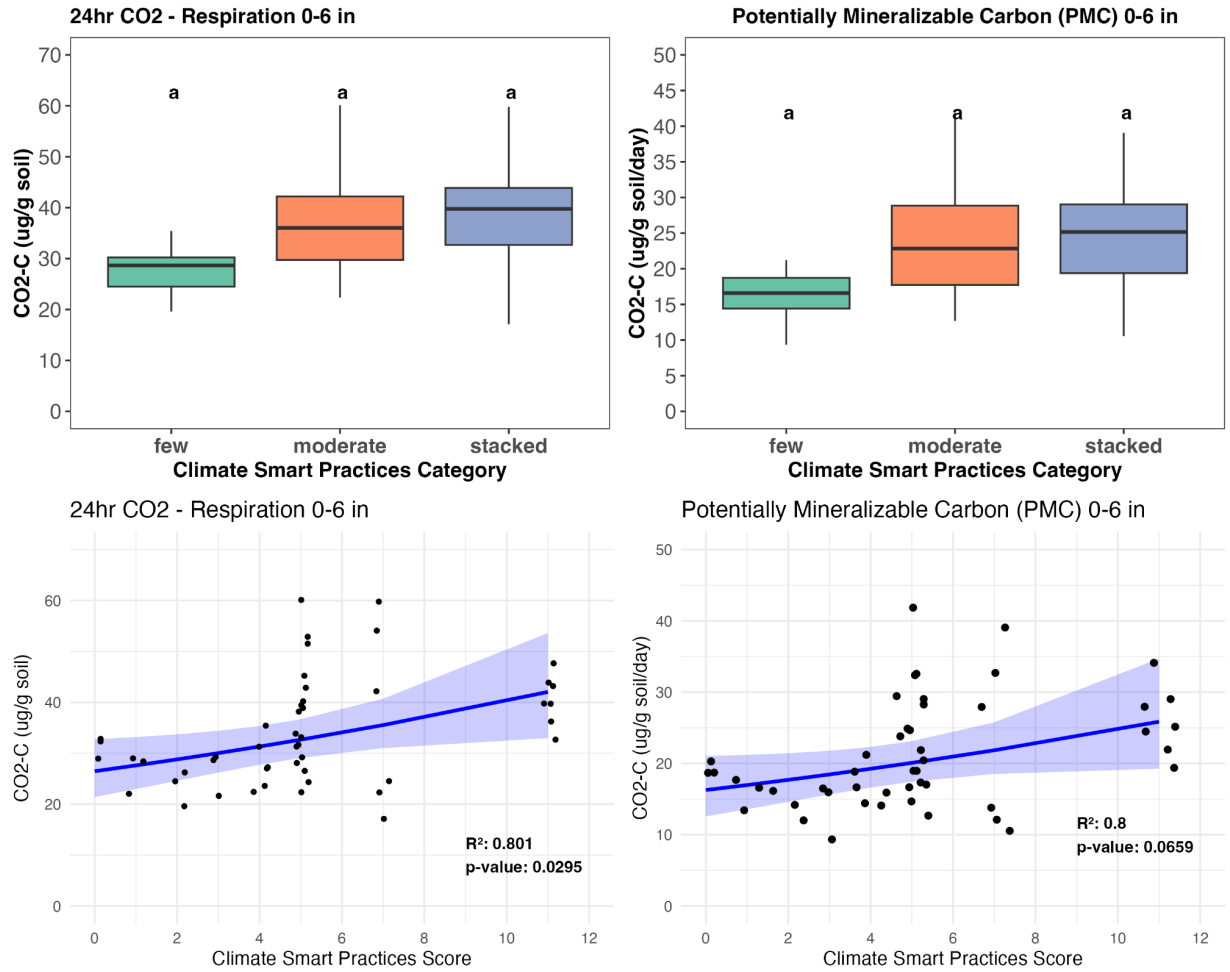


Figure 3. Carbon mineralization in the topsoil layer
Microbial respiration is captured by measuring the accumulation of CO_2 respired by microbes in rewetted soils after 24hrs. Potentially mineralizable carbon follows the same procedure but is measured after 72 hrs and provides an indication of potential C mineralization potential over time.

Potential microbial enzymatic activity related to the breakdown of organic compounds to mineralize carbon, Beta-glucosidase (BG) and Cellobiohydrolase (CBH), were moderately higher (Figure 4, *p values* 0.06 and 0.09) in fields with increasing practice adoption. Enzymes related to nitrogen mineralization and cycling, Leucine Aminopeptidase (LAP) and β-1, 4-N-acetylglucosaminidase (NAG), were significantly higher with increasing practice adoption across the score spectrum (Figure 5).

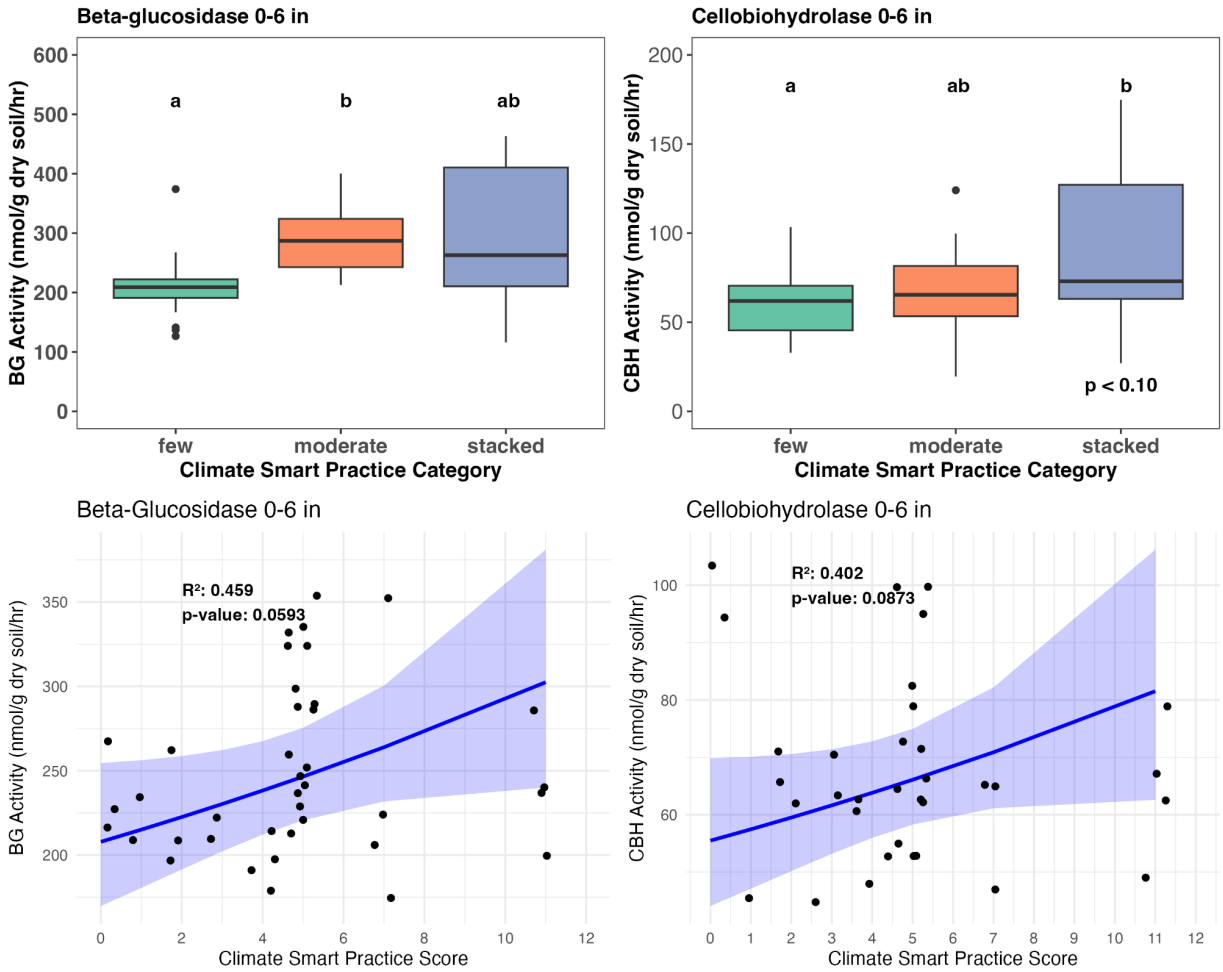


Figure 4. Carbon Cycling Extracellular Enzymes in the topsoil layer

Beta-Glucosidase (BG) is associated with the breakdown of glucose, and is a key indicator of decomposition rates. Cellobiohydrolase (CBH) is associated with the breakdown of cellulose, and is important in the process to free glucose for further decomposition. These two enzymes together are key indicators of soil decomposition and carbon cycling.

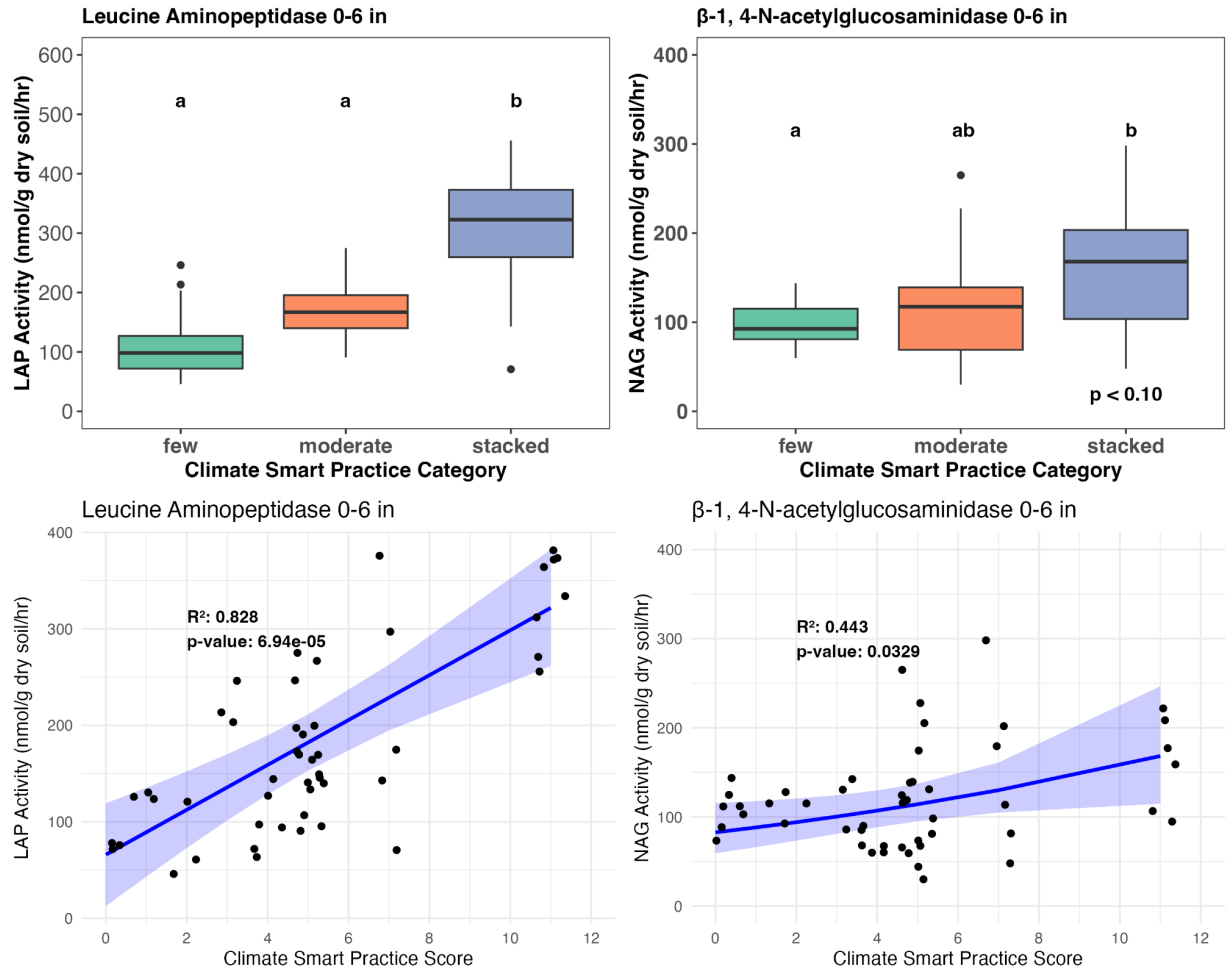


Figure 5. Nitrogen and Carbon Cycling Enzymes in the topsoil layer
Leucine Aminopeptidase (LAP) is associated with the breakdown of the amino acid, Leucine, which helps mineralize nitrogen. β-1, 4-N-acetylglucosaminidase (NAG) is associated with the breakdown of chitin, the material that makes up insect exoskeletons and fungi cell walls, and is important for mineralizing carbon and nitrogen. The two enzymes together are important indicators for N mineralization potential.

Chemical Indicators

Chemical indicators associated with climate smart management scores show fields with low adoption trended towards higher nitrate-N, potassium, soluble salts, and have higher cation exchange capacities (Figure 6.). None of the fields had any concerning levels of soluble salts. Fields with higher adoption scores trended towards more ammonium-N and phosphorus. pH was variable but trended higher with lower adoption scores (Table 3).

Table 3. Chemical indicators in the topsoil layer
Macronutrients, cation exchange capacity (CEC), and soluble salts organized in increasing order on the climate smart practices adoption scale.

Field ID	Management score index	CEC meq/100 g	pH	NO_3-N (lb/ac)	NH_4-N (lb/ac)	P (ppm)	K (ppm)	Soluble Salts mmhos/cm
23	0	29.00	6.93	118.70	2.95	72.47	312.87	0.55
24	0	30.83	6.97	94.17	2.15	76.60	279.17	0.76
3	1	21.73	8.17	16.10	0.71	49.43	136.53	0.35
14	2	24.23	6.27	143.61	21.16	85.40	273.03	0.68
4	3	19.90	7.33	27.18	0.03	54.97	180.07	0.22
10	4	19.40	6.87	16.23	0.10	179.67	146.20	0.14
25	4	21.07	7.43	26.87	0.00	47.50	133.40	0.31
20	5	20.17	7.17	18.23	0.00	45.77	207.23	0.25
21	5	26.27	7.33	3.34	0.26	70.00	366.80	0.19
22	5	25.23	7.50	34.88	1.68	20.23	248.67	0.32
26	5	21.67	7.30	70.76	0.02	413.50	841.63	0.44
27	5	18.23	6.83	29.48	3.36	133.73	218.00	0.30
28	5	21.60	6.73	65.55	13.65	49.50	281.10	0.32
7	7	22.50	7.60	25.05	2.57	45.10	192.97	0.26
8	7	26.73	7.40	11.83	0.41	149.37	176.97	0.13
1	11	22.30	6.87	32.62	0.05	183.00	258.73	0.43
18	11	14.37	6.70	8.13	0.00	110.23	216.53	0.23
19	11	18.20	7.43	44.36	3.37	173.50	194.27	0.44

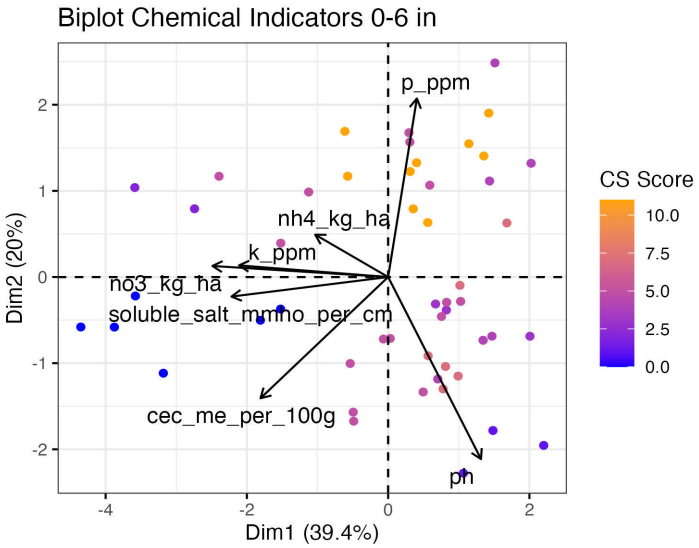


Figure 6. Principal Component Analysis (PCA), showing how much variation in the data is associated with fields of varying practice adoption. Arrows indicate an association of an indicator (e.g. p_ppm) with surrounding colored points. The colored points are designated along the scoring spectrum in the legend on the right hand side of the plot.

Soil Health Indicators: rootzone soil layer (6-12 inch)

Physical Indicators

Climate-smart practices did not affect most of the physical indicators measured in our study, except for aggregate stability and water-holding capacity in the root zone (Figure 7). Moderate adoption of practices increased aggregate stability compared to few practices (*p*-value 0.01). Accordingly, water holding capacity is higher with more climate-smart practices adopted (*p*-value 0.03, R^2 0.706). This effect could be explained by a lower permanent wilting point and higher field capacity as the practices are stacked, but these trends are not significant.

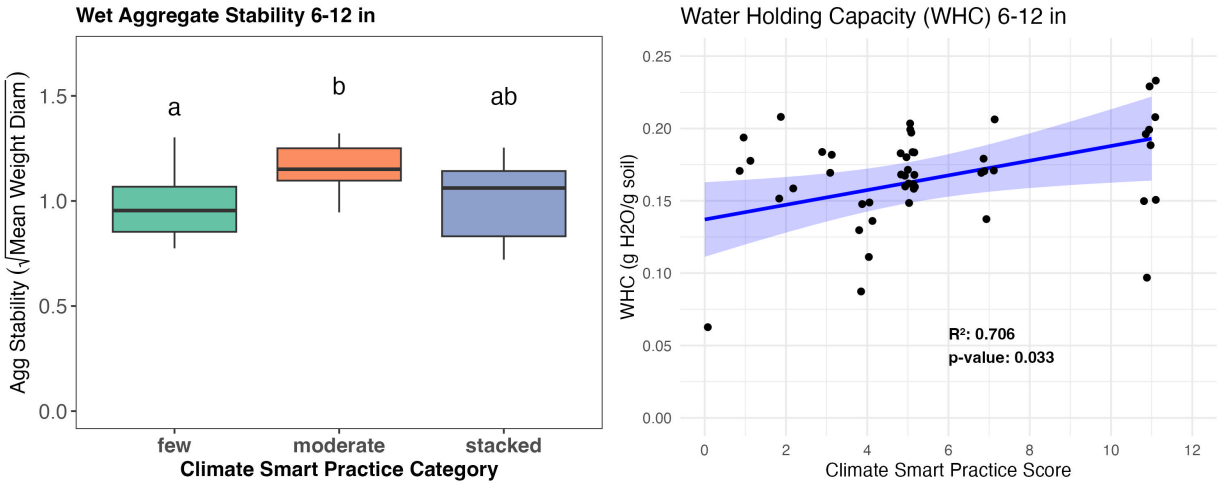


Figure 7. Physical indicators in the 6-12 inches soil layer

Wet Aggregate Stability is a soil structure indicator that influences the resistance of soil to compaction and erosion and determines water movement and storage. Water Holding Capacity is an indicator of the water available to plants, and it depends mainly on soil texture, soil structure, and soil organic matter content.

Biological Indicators

Soil organic matter and SOC followed a similar trend than topsoil and were not different across the practice score spectrum or categories. Microbial biomass carbon and MBN followed a similar response to climate-smart practices in the 6-12 in soils. Microbial biomass carbon was higher in the moderate adoption category, and MBN increased with the practice adoption score (Figure 8.). This is consistent with PLFA biomass which was higher in the moderate adoption category. Similar to the topsoil layer, microbial diversity was not different. The microbial quotient was significantly higher with increasing climate smart practice adoption and across practice categories (Figure 9). We did not measure soil enzymes in the 6-12 in soils as it is expected that more biological activity occurs in the topsoil layer.

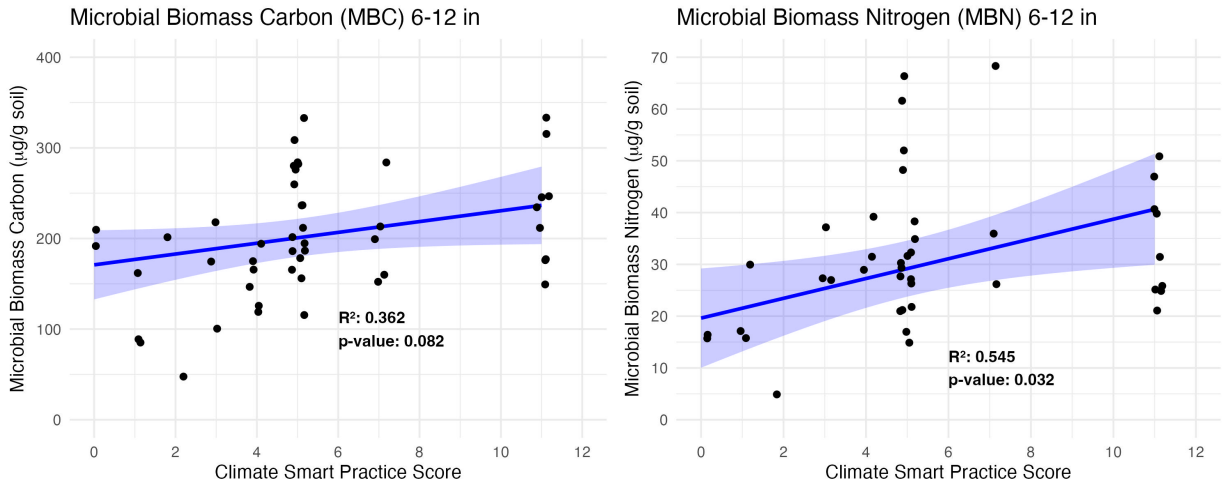


Figure 8. Microbial biomass carbon and nitrogen in the bottom soil layer.

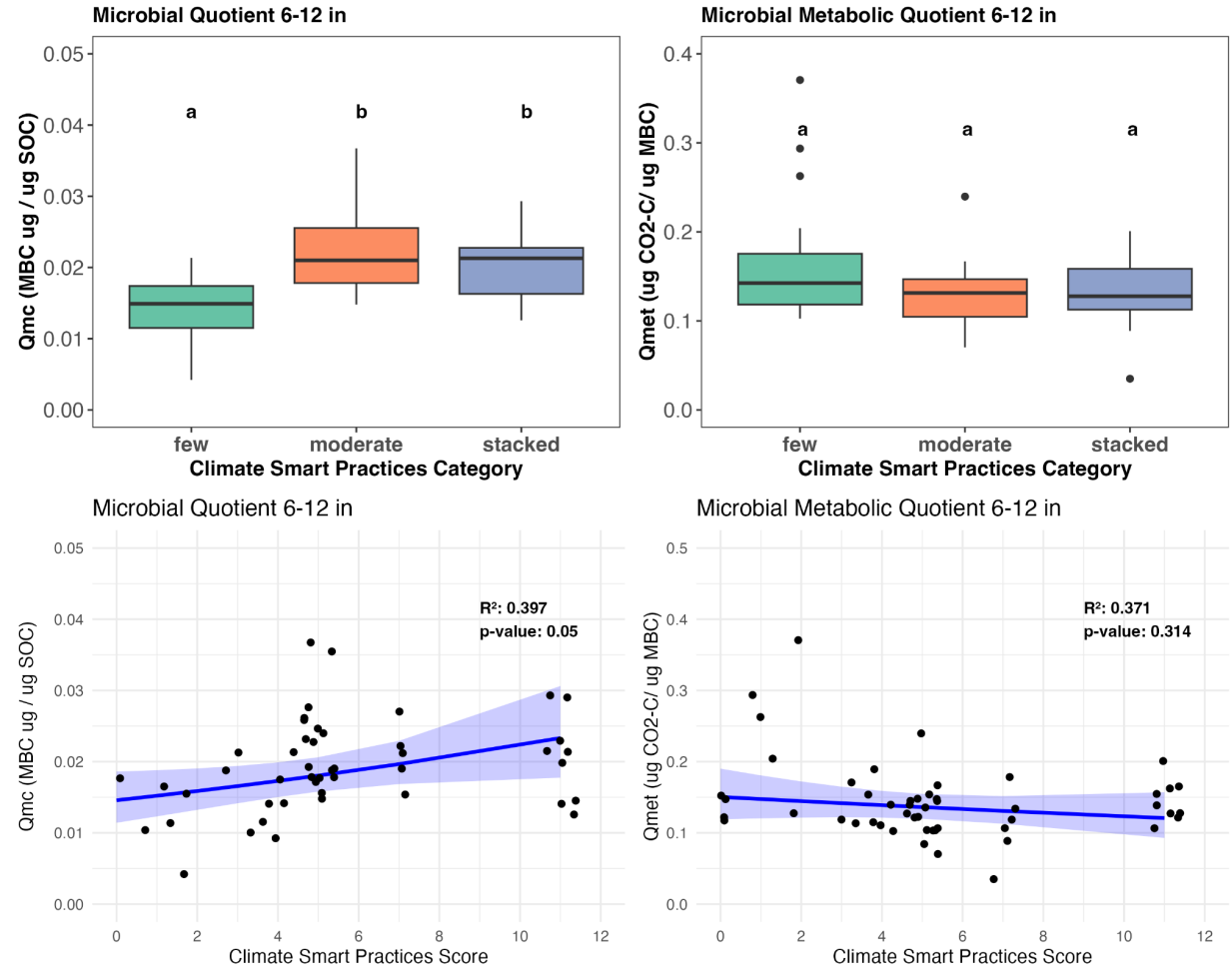


Figure 9. Microbial Quotient and Metabolic Quotient in the bottom soil layer.

The microbial quotient or the amount of carbon stored in microbes (MBC), relative to the amount of soil organic carbon in soil (SOC). The microbial metabolic quotient is a measure of carbon use efficiency with the amount of respiration (CO_2) relative to the size of the amount of carbon stored in microbes (MBC).

Chemical Indicators

Fields showed similar patterns in chemical indicators associating with scores along the practice adoption spectrum in this lower soil layer. Phosphorus was again most strongly associated with fields with high adoption practices

Table 4. Chemical indicators in the 6-12 inches soil layer

Field ID	Total Score	CEC meq/100 g	pH	NO_3-N (lb/ac)	NH_4-N (lb/ac)	P (ppm)	K (ppm)	Soluble Salts mmhos/cm
23	0	25.87	7.13	53.51	1.92	53.60	211.93	0.34
24	0	27.63	7.13	38.94	2.33	73.53	186.43	0.42
3	1	22.83	8.07	43.11	3.27	36.87	145.03	0.35
14	2	22.00	6.73	47.66	1.95	61.87	217.37	0.42
4	3	19.33	7.43	7.23	0.00	38.33	132.20	0.14
10	4	20.27	7.60	6.88	0.00	104.47	119.10	0.14
25	4	21.90	7.87	18.41	0.51	31.07	103.17	0.23
20	5	19.93	7.47	18.96	0.00	29.17	174.87	0.20
21	5	25.17	7.67	7.09	0.00	30.07	198.27	0.16
22	5	25.70	7.43	32.65	2.90	9.63	197.97	0.26
26	5	20.57	7.40	36.06	0.02	347.73	562.77	0.36
27	5	18.80	7.10	23.47	3.25	89.13	153.00	0.22
28	5	20.33	7.00	73.51	20.85	28.50	190.30	0.25
7	7	21.37	7.60	11.08	0.92	59.07	180.90	0.22
8	7	25.67	8.00	4.73	1.35	93.53	145.33	0.11
1	11	22.33	7.13	12.68	0.00	152.47	171.57	0.27
18	11	17.07	7.30	8.42	0.87	79.90	155.60	0.21
19	11	17.97	7.90	13.26	0.37	136.57	126.80	0.29

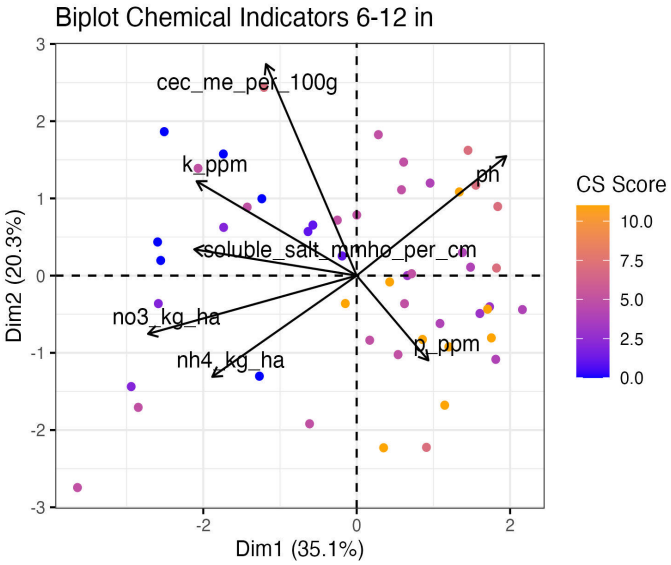


Figure 10. Principal Component Analysis (PCA), showing how much variation in the data is associated with fields of varying practice adoption.

Discussion

Our results were variables and highlight the complexities of building up and measuring soil health in our climate and processing tomato systems. Climate-smart practices positively impacted aggregate stability and water holding capacity, which is consistent with the results at the 20 year UC WSREC experiment[8]. Shifts in other physical soil properties were not significant in our study, probably due to greater variability in on-farm surveys and a shorter time of practice adoption. However, these results demonstrate the benefits of climate-smart practices in two key physical indicators after only five years of adoption. The lack of difference in soil organic matter (SOM) and soil organic carbon (SOC), may be related to the amount of tillage occurring in tomato fields, and/or the shorter time of practice adoption relative to long term experiments[5,8]. A common theme in discussions with growers was to create a soft soil bed to transplant into, to ensure tomato transplants establish their roots. Despite sampling two depths (0-6 in and 6-12 in) tillage operations tend to go 8-12 in in most cases and thus the layers we sampled experience high rates of disturbance.

Fields with increased carbon and nitrogen stored in microbes (MBC and MBN), with higher respiration rates, and potentially mineralizable carbon (PMC) are indicators of larger and more active microbial communities. Respiration provides us insights on how much active carbon is available for use by microbes, which represent the relative amount of energy microbes have at their disposal for growth and to carry out processes related to supporting crops. The four fields that cover crop annually had some of the highest respiration and PMC rates, which is consistent with increased rates at the UC WSREC experiment[8]. Increasing potential soil enzyme activity in the topsoil layer with increasing practice adoption indicates increased potential carbon (C) (i.e. energy) and nitrogen (N) mineralization. This trend is consistent with what has been observed in other studies showing increased N cycling in fields with increased crop complexity and organic inputs[18,19]. In soils with C and/or N limitations, high rates of these enzymes may signal tradeoffs in growth by microbes[20] and should be considered in the context with ensuring ample supply of carbon and nutrient rich organic inputs to meet soil ecosystem and crop needs. Enzyme data should also be cautioned as intracellular enzymes (i.e. inside live microbial cells), those in the near cellular environment (i.e. recently released by microbes), and those adhered to clay particles released previously that may not reflect current microbial activity[21]. Despite these limitations the BG and CBH enzymes, and LAP and NAG enzymes, capture multiple enzymes related to carbon and nitrogen mineralization and cycling to provide a broader picture of potential mineralization rates. Fields in the moderate to stacked end of practice adoption (Table 2.) have increasingly diverse organic inputs via crop diversity, cover crops, and organic inputs. In organic tomato fields in Yolo county, shifting abundance of C and N related enzymes were shown with varying rates of C and N availability[22]. Generally lower mineral N in the stacked fields (Tables 3-4.) may have led to higher amounts of LAP and NAG.

The next steps of analysis will 1/ clarify soil health potential in our region and 2/ some of the benefits growers might harness to inform next steps of outreach and research.

Objective 3 (year 2&3): Synthesize knowledge to develop a best management practice guide to assist growers in adopting CS practices

More time to further summarize management and analyze results is needed before outreaching more broadly. Funds from CTRI have been key to catalyze this new research and partnerships and funding from CDFA (2023-2026) will allow some continuity beyond the duration of this grant. As part of this effort, we will outreach projects learnings from Obj 1 and 2, including the development of a best management practice guide being spearheaded by UC-ANR colleagues (Obj 3).

Acknowledgements:

We would like to thank the California Tomato Research Board (CTRI) for their support in funding this project, who along with the Campbell's Soup Company's support made this research possible. Our incredible cohort of growers: Tom Barrios (Barrios Farms), Tim Beeman (Bullseye Farms), Tommy Bottoms (Tremont Farms), Seth Cooley (Cooley Enterprises), Chris Gnos (E&H Farms), Michael Ledesma (Muller Ag), Scott and Brian Park (Park Farming Organics), Bruce Rominger (Rominger Brothers) and Tony Turkovich (Button & Turkovich); were instrumental in making this project a reality. We are grateful for their willingness to work with them, for the time they have taken out of their busy schedules to work with us, and for the permission they granted us to sample their fields.

This project as leverage for other dollars:

The CTRI award, with the match commitment from Campbell soup ($160K) allowed for the leveraging of awards to the CDFA specialty crop block grant program. An additional gift of $50,000 was added to this project by Campbell Soup to support fruit nutrient density analyses in collaboration with Selina Wang, which expands upon the indicators this project will produce to create a broader systems level assessment of processing tomato systems in the Sacramento Valley.

The CDFA award from their Specialty Crop Block grant totals $396,000 for a two and a half-year period from November 2023 through May of 2026. This award is intended to provide support for the nutrient density analyses in 2023, and for a two-year deficit irrigation experiment. This experiment will expand upon the work conducted through the CTRI award, by collecting multi-year data and through the testing of an environmental stress on soil health/quality and yield outcomes for processing tomatoes. This continued effort will also allow us to sustain salary to finish key deliverables of this project, including the full analysis and publishing of a literature review and results collected from UCD CE experiment (Obj 1) and informing best management practice guides being developed (Obj 3).

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Project Title: Quantifying the effects of K-Pam on soil-borne disease and yields in lower Sacramento Valley processing tomato fields

Year of Project Initiation: 2023 (continuing)

CTRI Funding in 2024: $4,725

Project Leader and any Co-PIs: Patricia Lazicki, UCCE Vegetable Crops Advisor for Yolo, Solano, and Sacramento Counties

Cooperating Personnel:
Blake Harlan and Chris McAllister, Harlan Family Ranch
Cassandra Swett, Prof. of Cooperative Extension in Plant Pathology, UC Davis
Spencer Bei and Aaron Black, Robben Ranch (2023)

Executive Summary

Goal

Soilborne pathogens are a growing threat to processing tomato production in California's Central Valley. In response to these increasing pressures, some growers are expanding their use of preplant soil fumigants. The goal of this study is to quantify the effect of a pre-plant application of the fumigant K-Pam at 30 gal/acre through subsurface drip on: a) incidence and severity of soil-borne pathogens, especially the *Fusarium falciforme* complex of pathogens responsible for causing the disease fusarium stem rot and vine decline (FRD) and b) fruit yield and quality.

Experimental Design and Monitoring

The 2024 trials replicated the design used in 2023. In 2024, two field trials (were established in Yolo County. The grower chose these fields as both having a history of vine decline, which he believed to be due to FRD pressure. At each site, twenty adjacent beds were chosen in areas of the field where FRD was historically high. One site also had a sandy streak which had known issues with root knot nematode; to avoid this additional variation the plot was established well outside this area. Adjacent 200-ft sections within each bed were designated as the trial study area. Both fields were fumigated with K-Pam at 30 gal/acre via chemigation in spring as a preplant. Prior to fumigation, a shutoff valve was installed in the dripline of alternating rows at each site, to create ten paired plots, each containing one fumigated and one non-fumigated row.

At each site, once symptoms started to manifest, disease monitoring was performed every three weeks within the 200-ft observation plots. At each monitoring I assessed the proportion of diseased and dead plants within the observation plots along with a tentative classification of the disease, assigned a rating of soilborne disease severity, and took an average NDVI value using a Trimble Greenseeker. I collected samples of whole plants with representative symptoms from each field outside the experimental area and submitted them to Dr. Cassandra Swett's plant pathology lab at UC Davis to verify disease presence. Tomato spotted wilt virus (TSWV), which can have similar symptoms to FRD and other soil pathogens but is not soilborne, was also monitored using immunostrips in the field. At harvest, fruit biomass yields were quantified by mechanical harvest into a GT weigh cart. Marketable yield was calculated proportionally by hand-sorting culls from 5-gallon bucket samples collected from the harvester after the color sorters. Samples of red fruit were submitted to the Processing Tomato Advisory Board for quality analysis.

Results

At the B65 site, diseases present included FRD caused by both *F. noneumartii* and *F. martii*, as well as Verticillium wilt (caused by *Verticillium ahlia*). Disease pressure was patchy, and none of the plant health metrics differed significantly between fumigated and nonfumigated plots at any time during the season. The proportion of severely declined or dead plants averaged 18%, ranging from 4% in the lowest pressure plot to 45% in the highest. Average yields were high, ranging from 64 – 78 t/a, and were similar between fumigated and non-fumigated plots (70.3 t/a in the K-Pam plots, 70.8 t/a in the control plots). There were no differences in culls or fruit quality.

At the D77 site, the only confirmed disease was FRD caused by *F. noneumartii*. The soil at this site was lighter, and preplant soil samples indicated potential K deficiency; however, leaf samples taken in-season samples were within sufficiency range and did not differ between treatments. Root knot nematodes were also present in the field, though their presence was not confirmed in the trial area. The proportion of severely declined and dead plants ranged from 11-52%, with an average of 25%. Interestingly, the proportion of dead and declined plants tended to be lower in the control than in the K-Pam plots ($p=0.05$). The NDVI readings just before harvest were also significantly higher in the control than K-Pam plots ($p=0.002$). However, K-Pam plot yields exceeded those of the control by an average of 7.5 t/a ($p<0.001$), at 69.3 t/a vs 61.8 t/a. K-Pam plots also had a lower proportion of green fruits (5% vs 9% in the controls, $p<0.001$). No significant differences were observed in fruit pH, solids, or color.

Discussion and Recommendations

Fumigation with K-Pam at 30 gal/acre costs an estimated $300/acre. Thus, at the 2024 price of $112.50/ton the mean yield boost from fumigation was more than sufficient to cover the cost at Field D77, but there was no evidence it increased yields at B65. Extrapolating yield averages across the field, fumigation with K-Pam resulted in a profit of $543.75 at site D77, but a loss of $243.8/acre at site B65. Combined with the narrow profit margins measured from similar field trials in 2023, these results are in line with previous work done in Yolo and San Joaquin counties, which suggests that while K-Pam can lead to considerable yield increases (up to 26 t/acre in one trial) under some conditions, its efficacy varies strongly by site and year.

Additionally, it is uncertain why K-Pam caused such a large, significant yield increase in Field D77. FRD is a slow disease, and is thought to lower yields by causing a premature vine decline which exposes ripening fruits to sunburn injury and increases the risk of black mold. However, vines in the control plots were healthier and greener at harvest than were those in the K-Pam plots. This suggests that K-Pam fumigation increased yields by some other mechanism than by lowering FRD pathogen loads. There results, while difficult to explain, are not uncommon. Out of the six field trials performed since 2019 in which K-Pam has significantly increased yields, in three this yield increase has not been accompanied by any significant reduction in disease symptoms. Given K-Pam's expense, uncertain efficacy, and the risks it poses to the human and environmental health, it is an interesting question where this increase is coming from, and whether it can be achieved by other means. It is worth noting that

Introduction:

Fusarium stem rot and decline (FRD, caused by multiple pathogens in *Fusarium solani* complex and formerly known as *F. falciforme*) is a widespread and damaging disease of processing tomatoes across California. Symptoms include rot of the roots, crown, and stem as well as deformation and chlorosis of the foliage and rapid canopy decline starting mid-season. In certain cultivars, FRD can reduce yields by up to 60% and kill up to 100% of plants by harvest. Until a team led by UCCE extension specialist Cassandra

Swett began management trials in 2019, there were no known methods to mitigate losses. Experiences in similar pathosystems, such as soybean sudden death syndrome, caused by the closely related *F. virguliforme*, indicate that an IPM program for this type of pathogen is not straightforward and relies on a combination of quantitative cultivar resistance (e.g., tolerance), chemical control, cropping system management, and soil moisture management (Leandro et al., 2018, Weems et al. 2015).

A body of research over the past several years has established some of the basic parameters comprising an effective IPM program for FRD in processing tomato; these include use of chemical control complimented with selection of tolerant cultivars, and rotations with non-host crops immediately following and preceding tomato plantings. Chemical controls vary in their effectiveness. One method which has shown positive effect in trials conducted in Yolo and San Joaquin counties since 2019 is metam buried-drip fumigation (often with K-Pam) prior to planting (Aegerter et al., 2023). However, the effect is variable, with trials showing average yield effects ranging from a slight decrease to an increase of 26 tons/acre (Table. 1).

Table 1. *Summary of chemical trials in Yolo and San Joaquin counties. Data partly obtained from Aegerter et al. (2023), used by permission*

	Site	UC Davis	Yolo Co.	San Joaquin Co.	San Joaquin Co.	San Joaquin Co.	Yolo Co.	Solano Co.
	Year	2019	2019	2019	2020	2021	2023	2023
	Disease	FRD	FRD	FRD	Fol & FRD	Fol & FRD	Fol, FRD, Forl, s. blight	FRD, Forl
Product	Vine decline in non-treated control	47%	73%	20%	31%	30%	55%	16%
K-Pam ~30 gal	*Disease*				+	++	*NS*	++
	Yield			7.2 t/a	*NS*	26 t/a	4.7 t/a	3.5 t/a
K-Pam ~15 gal	*Disease*		*NS*		+	++		
	Yield		11.9 t/a		*NS*	13.6 t/a		
Miravis	*Disease*	+			+	++		
	Yield	*NS*			*NS*	9.2 t/a		
Rhyme	*Disease*				+	++		
	Yield				*NS*	10 t/a		
Velum	*Disease*	+			-			
	Yield	*NS*			*NS*			
	Disease P-value	NS	NS	Not tested	p=0.06	p=0.0004	NS	p=0.008
	Yield P-value	NS	P=0.01	p=0.016	NS	p=0.015	p=0.05	p=0.01

Yield benefits have not always been statistically significant, and significant differences in vine decline between fumigated and nonfumigated treatments have not always meant higher yields. The trials were conducted with different varieties under different conditions, under a range of disease pressures and with different pathogens present (Ff=Fusarium falciforme, now called FRD, associated with the pathogens *F. martii, F. noneumartii, F. falciforme*, and Fol=fusarium wilt (*F. oxysporum fsp lycopersici*). However, no consistent patterns have emerged which would help predict when chemical treatments are most likely to be efficacious.

Goal

The goal of this study was to quantify the effect of a pre-plant application of the preplant fumigant K-Pam through subsurface drip on: a) incidence and severity of soil-borne pathogens and b) fruit yield and quality.

Objectives

- *Objective 1: Quantify effects of fumigation with K-Pam on incidence and severity of soilborne disease*
- *Objective 2: Quantify effects of fumigation with K-Pam on yields and fruit quality*
- *Objective 3: Share results*

Methodology and Results:

Objective 1: Quantify effects of fumigation with K-Pam on incidence and severity of soilborne disease

I performed a replicated trial on two processing tomato fields (B65 and D77) with a history of decline due to soilborne disease. I used a similar methodology in 2023, and so with the 2024 trials will have four site-years of data. On each field, in consultation with the grower I selected a location with high past disease pressure. The grower chemigated K-Pam at around 30 gal/acre at least two weeks prior to planting through the buried drip. On one of every two adjacent rows, tape was sealed off during fumigation as a non-fumigated control. Treatments were thus applied to the entire row length. Twenty adjacent 200-ft observation plots were established 300 ft from the tail end of each field, creating ten K-Pam/control paired plots with replication across rows. Drip tape was a single line, 10 to 12" deep centered on a 5-ft centered bed. 30 gpa of K-Pam is the lower rate of the 30 to 60 gpa labelled chemigation rate of Amvacs HL at 5.8 lbs of active ingredient per gallon. Tomatoes were transplanted on 4/3 and 4/11 at the B65 and D77, respectively. The variety HM 5235 was used at B65, and HM 8237 was used D77.

Prior to fumigation I took soil samples to determine initial nutrient status. Field B65 had a silty clay loam texture and high fertility, while Field D77 had a very fine sandy loam texture and lower fertility. In particular, potassium (K) at Field D77 was about 150 ppm and was at 2.4% on the cation exchange capacity. For comparison, former Yolo County UCCE farm advisor Gene Miyao found that a yield response to K and manure was most likely at levels below 200 ppm or below 2% on the CEC. However, leaf samples taken at early flowering showed that K levels were sufficient, and did not differ between K-Pam and non K-Pam plots, and neither vines nor fruit showed any symptoms of K deficiency. Initial soil samples were also taken for nematode analysis, as Field D77 had a history of root knot nematode (RKN) pressure, to provide baseline values for comparison should nematode damage start to manifest during the season. No RKN were detected at Field B65. Levels were low at D77 (24 per 200 ml soil), and no galling was observed within the trial area during the season. It was therefore assumed that the effects of fumigation were due to K-Pam's effect on soil-borne disease, rather than the addition of K or reduction in nematode populations.

Throughout the season, I assessed disease pressures by counting affected plants in 200-ft observation plots (Table 2). Early in the season I counted all symptomatic plants, for each making a provisional diagnosis of disease and a severity rating (Table 3). I gave each a qualitative rating to each row based on overall appearance, and took a measurement of average NDVI using a Trimble Greenseeker. At both sites, there was some early infection with tomato spotted wilt virus (TSWV), which can have very similar symptoms to FRD. At both sites, I verified suspected TSWV infections using AgDia immunostrips, and did not include these plants in subsequent counts. I submitted whole plant samples with representative fusarium-like symptoms, taken from areas near but outside of the observation plots, to UCCE specialist Dr. Cassandra Swett's fungal pathology lab for disease confirmation. At both trials, two different sets of disease symptoms presented. One was a

NDVI and disease incidence data were analyzed as a one-way ANOVA in a randomized complete block design with 10 replications, using the *lmerTest* package in R (Kuznetsova et al., 2017). This approach was taken to help account for the localized nature of the disease patches. Fumigation treatment was designated as a fixed and replicate as a random factor.

Table 2. Disease and NDVI assessment dates at B65 and D77 field sites. WAT=Weeks after transplanting. Field B65 was planted on 4/3 and harvested on 18/15, and the Field D77 was planted on 4/11 and harvested on 8/31.

B65		D77	
Stage	**WAT**	**Stage**	**WAT**
Early green fruit	8	Early green fruit	8
Early red fruit	14	Early red fruit	14
~80% ripe fruit	17	~80% ripe fruit	17
Just before harvest	19	Just before harvest	20

Table 3. Semi-quantitative rating scale used to give an average score for 200-ft observation plots. In all cases, "symptoms" refer to symptoms attributable to soilborne disease. ***Mildly symptomatic***=symptoms show on a few leaves, can be seen standing next to the plant; ***Evidently symptomatic***=symptoms affect up to 50% of the plant, can be seen from further away; ***Severely symptomatic***=Symptoms affect almost all of the plant; ***Dead***= No green tissue, or no turgor in any leaves

Scale	Appearance	Approximate criteria
1	Perfect	No visible symptoms
2	Excellent	Few (<2%) plants mildly symptomatic
3	Generally healthy	Several (2-10%) plants mildly symptomatic OR few plants evidently or severely symptomatic
4	Slightly unhealthy	Many (10-50%) plants mildly symptomatic AND/OR several (~2-10%) plants evidently symptomatic
5	Not too healthy	Most (>50%) of plants are symptomatic, several (2-10%) severely symptomatic
6	Unhealthy	Most (>50%) of plants are symptomatic, of these, almost all are evidently/ severely affected, few (<2%) dead
7	Declining	Most plants are evidently to severely symptomatic, many (10-50%) are dead
8	Dead	Almost all plants dead

The NDVI readings, recorded using a Trimble GreenSeeker at each monitoring date, did not significantly differ between treatments at any date at Field B65 (Fig. 1a). Average visual ratings also did not differ between treatments (Fig. 1b). However, at Field B65, at the final assessment date just before harvest the K-Pam plots had significantly more dead and declining plants and lower NDVI readings than the control plots (Fig. 1). Disease pressure was relatively low in both. While vines senesced during fruit ripening at Field B65, at Field D77 they remained green and vigorous up until harvest (Fig. 2). The variety planted in our plot area at Field D77, HM 8237, has a large, vigorous vine. Additionally, there was relatively little HM 8237 planted in the field, and the other field varieties were declining rapidly due to combined nematode and disease pressure, so the trial was harvested at an earlier stage than was optimal.

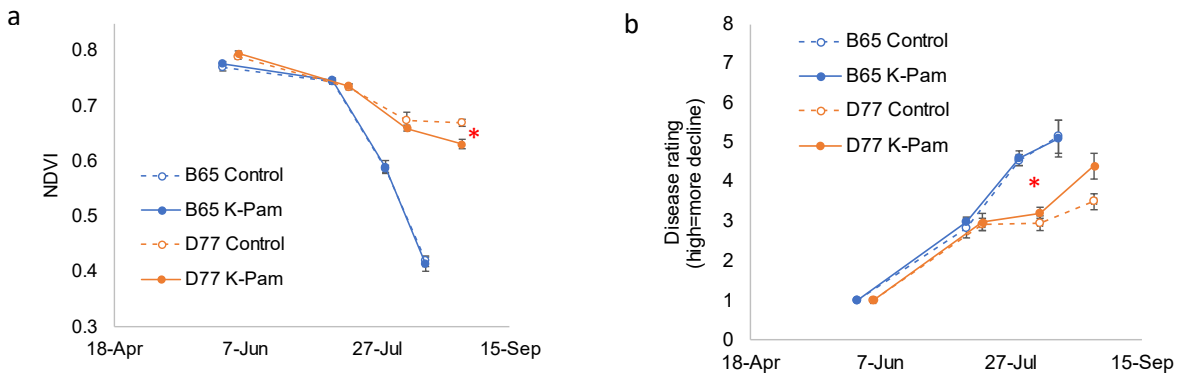

Figure 1. *Average a) NDVI readings and b) soilborne disease visual rating for fumigated (K-Pam) and non-fumigated (Control) plots. Error bars represent standard error (n=10). Red asterisks= treatments are statistically significant ($p<0.05$).*

Figure 2. *Field D77 trial just before harvest. Vines were large and green, there were relatively few dead or declined plants.*

According to samples submitted to the fungal pathology lab from plants with characteristic symptoms, diseases present at Field B65 included FRD (*F. martii*, *F. noneumartii*),and verticillium wilt (*Verticillium dahliae*). Only FRD (*F. noneumartii*) was identified at Field D77. Root knot nematode was present outside the trial area but

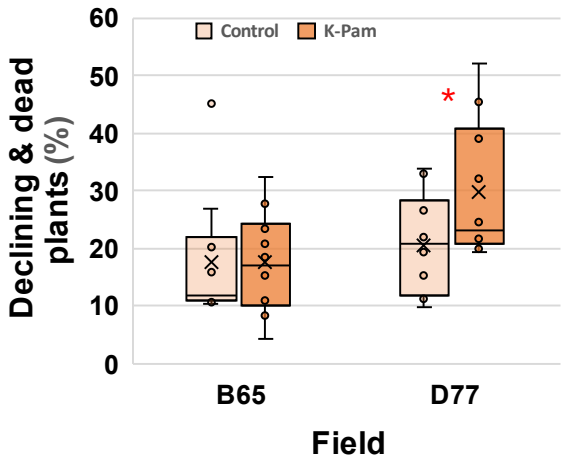

Figure 3. Percentage of dead and severely declined plants just before harvest (n=10)

The proportion of declined and dead plants were counted just before harvest. At Field B65, the proportion of dead and severely declined plants ranged between 4% and 45% and did not differ between treatments (Fig. 3). At the Solano site, the proportion of dead and severely declined plants ranged between 10-452%, and was marginally (p=0.05) higher in the K-Pam than control plots.

Disease pressure was generally low at both sites. In neither of the sites was there evidence that declined plants were significantly contributing to the number of sunburned or moldy fruit

Objective 2: Quantify effects of fumigation with K-Pam on yields and fruit quality

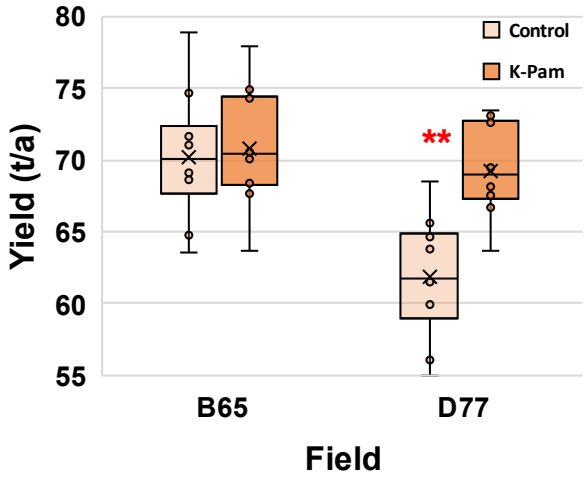

Figure 4. *Total yields at B65 and D77 sites. **Indicates significant difference between treatments within a site at p<0.001 (n=10).*

Yields were quantified by mechanical harvest of the 200-ft observation plots into a GT cart scale. At both sites, the color- and dirt-sorters were turned on. One five-gallon bucket sample was collected per row from the harvester belt after the color sorter. This sample was sorted by hand to determine the proportion of unmarketable fruit in the following categories: green, pink, sunburn, blossom-end rot, and mold. A portion of the marketable fruit from the 5-gallon bucket subsample was submitted to the Processing Tomato Advisory Board for analysis of processing characteristics.

Yields were very high at Field B65, and did not differ between treatments. The average yield for the treated rows was 70.8 t/acre and untreated rows was 70.3 t/acre), a difference of 0.5 t/acre (Fig. 4). The average yield for the treated plots at the Solano site was 69.3 t/acre while the untreated plot yield was 61.8 t/acre, with a difference of 7.5 t/acre (p<0.001).

The proportion of green fruit was lower in the K-Pam treated rows than in the control rows at Field D77 (5% vs 9%; p<0.001, Fig. 5). Other than this, K-Pam did not affect any category of unmarketable fruit at either of the two sites. The relatively high proportion of greens at Field D77, combined with low proportion of pinks and the lack of vine senescence, suggest that split set was an important issue in this field.

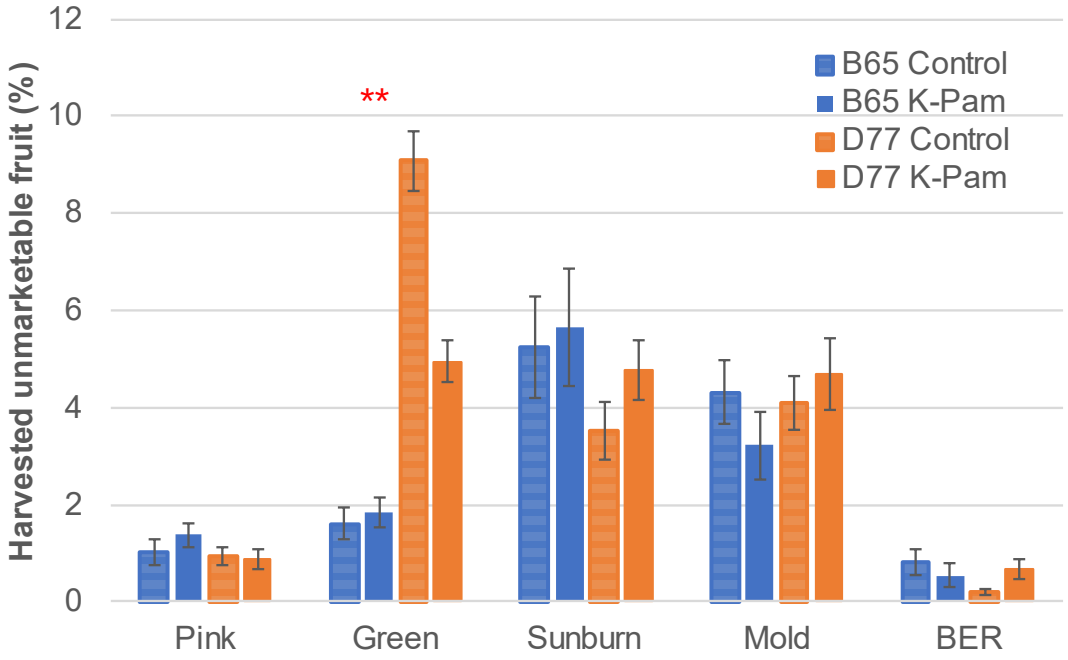

Figure 5. Unmarketable fruit at field sites B65 and D77. Bars represent the standard error. Red asterisks indicate statistical significance at p<0.001.

Objective 3: Share results

Results have been or will be shared with the CTRI, other researchers, and the processing tomato community through meetings including the 2025 CTRI research meeting and regional processing tomato production meetings for the southern Sacramento Valley and the northern San Joaquin Valley. These results will also be added to results from previous chemical trials testing efficacy of different products against FRD in a new IPM guideline which is being developed by UCD fungal pathologist Cassandra Swett and Vegetable Crops Advisor Brenna Aegerter. I will also share summaries of the results through my email newsletter, and directly with the grower.

Discussion:

Despite a sustained, severe heat wave which hit in late June and early July, as the fruit were beginning to ripen, yields at both sites were well above the statewide average (~50 t/a). FRD is generally a later-season disease, which is thought to decrease yields by causing a premature vine decline and exposing fruit to sunburn and mold. The low proportion of sunburn and mold suggest that while FRD was present at both sites, it may not have been present at yield-reducing levels. The low levels of sunburn were unexpected, as the June heat wave caused considerable early necrosis at both sites and there was some evidence of vine decline relatively early in the season. However, particularly in Field D77, many vines appeared to recover. It is possible that some of the fruit which was damaged in the early heat wave may have rotted by harvest time.

If FRD was not a major contributor to yield loss, it is an interesting question why K-Pam increased yields in field D77. In this field, in which the first fruits were ripening during the record heatwave in early July, there was an issue with split set. When the field was harvested, vines were generally large and green and there were a high proportion of small green fruit. A plausible explanation for the observed results could be that the K-Pam contributed to increased early fruit set by increasing the availability of some resource on this

coarse-textured, low-fertility soil. Fruit can be strong sinks for nutrients such as K, and a relatively high proportion of fruit can limit vine growth in determinate tomato cultivars (Widders and Lorenz, 1979). Thus, if K-Pam promoted a heavier earlier fruit set by improving availability of some limiting resource, it could explain the paradoxical observation of higher yields but great vine decline. K-Pam could improve nutrient availability through a variety of mechanisms: e.g. the direct contribution of K, by decreasing microbial competition for nutrients, releasing nutrients contained in microbial bodies as they are lysed during the fumigation process, or improving root growth and vigor by killing pathogens and parasites.

The lack of association between yields and vine decline, while paradoxical, is fairly common. Including the two 2024 trials, effect of K-Pam at different rates on disease pressure and yields has been measured by UC researchers in 7 FRD-infested fields since 2019. In only 2 of these trials did K-Pam both increase yields and decrease vine decline (Table 3). In 3 trials K-Pam increased yields without decreasing vine decline, while in one trial K-Pam decreased vine decline while having no effect on yields, and in one trial it did not affect either yields or decline.

Table 3. Summary of trials on FRD-infested fields conducted by UC researchers since 2019. "Vine decline" represents the average decline observed in the untreated control. + indicates a statistically slight positive effect of K-Pam on disease pressure, ++ indicates a statistically strong effect. Fol= fusarium wilt. Forl= F. crown and root rot; vert=Verticillium wilt.

	Site	UC Davis	Yolo Co.	San Joaquin Co.	San Joaquin Co.	San Joaquin Co.	Yolo Co.	Solano Co.	Yolo Co.	Yolo Co.
	Year	2019	2019	2019	2020	2021	2023	2023	2024	2024
	Disease	FRD	FRD	FRD	Fol & FRD	Fol & FRD	Fol, FRD, Forl, s. blight	FRD, Forl	FRD, vert	FRD
Product	**Vine decline in non-treated control**	47%	73%	20%	31%	30%	55%	16%	18%	21%
K-Pam ~30 gal	*Disease*				+	++	*NS*	++	*NS*	-
	Yield			7.2 t/a	*NS*	26 t/a	4.7 t/a	3.5 t/a	*NS*	7.5 t/a
K-Pam ~15 gal	*Disease*		*NS*		+	++				
	Yield		11.9 t/a		*NS*	13.6 t/a				
Miravis	*Disease*	+			+	++				
	Yield	*NS*			*NS*	9.2 t/a				
Rhyme	*Disease*				+	++				
	Yield				*NS*	10 t/a				
Velum	*Disease*	+			-					
	Yield	*NS*			*NS*					
	Disease P-value	NS	NS	Not tested	p=0.06	p=0.0004	NS	p=0.008	NS	p=0.04
	Yield P-value	NS	P=0.01	p=0.016	NS	p=0.015	p=0.05	p=0.01	NS	p=0.0006

Fumigation with K-Pam at 30 gal/acre costs an estimated $300/acre. Thus, at the 2024 price of $112.50/ton the mean yield boost from fumigation was more than sufficient to cover the cost at Field D77, but there was no evidence it increased yields at B65. Extrapolating yield averages across the field, fumigation with K-Pam resulted in a profit of $543.75 at site D77, but a loss of $243.8/acre at site B65. Combined with the narrow profit margins measured from similar field trials in 2023, these results are in line with previous work done in Yolo and San Joaquin counties, which suggests that while K-Pam can lead to considerable yield increases (up to 26 t/acre in one trial) under some conditions, its efficacy varies strongly by site and year. Given that K-Pam is a restricted use material, as well as being expensive and risky, it is a worthwhile question what mechanisms are at work and whether similar yield bumps could be obtained with some safer material.

More research is needed to better understand the environmental and management factors under which fumigation is likely to be most effective. Interesting areas for future research include further examination

of the additional benefits of K-Pam when a tolerant variety is used, testing K-Pam against other products whose mode of action is to improve nutrient availability or improve root competitive ability, the relative effectiveness of different rates, and how environmental factors (e.g. moisture, nutrition, planting date, nematode pressures) interact with disease severity and K-Pam effectiveness.

Acknowledgements: This work was conducted in collaboration with Harlan Family Ranch (Blake Harlan, Chris McAllister) and the previous trials in 2023 with Robben Ranch (Spencer Bei and Aaron Black). UCCE Vegetable Crops Advisor Brenna Aegerter and Emeritus advisor Gene Miyao assisted greatly in helping with planning, training me in field identification of diseases, and harvest logistics. Dr. Cassandra Swett and Dr. Brad Hanson at UC Davis also provided me with students when I needed harvest help. Dr. Amanda Hodson provided pre-fumigation nematode analysis. The California Tomato Research Institute supported this work both through direct funding and through funding diagnostics through Dr. Cassandra Swett's fungal pathology lab at UC Davis

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Project Title: Assessment of novel transplanter performance and economics

Year of Project Initiation: Pilot study initiated in 2023; funded by CTRI in 2024

Amount of funding requested: $9,305

Principle investigator: Dr. Patricia Lazicki (UCCE Vegetable Crops Advisor for Yolo, Solano, and Sacramento Counties; palazicki@ucanr.edu)

Co-PI: Dr. Ahmed Kayad (Agricultural Engineering Advisor at the UCANR Intermountain Research and Extension Center)

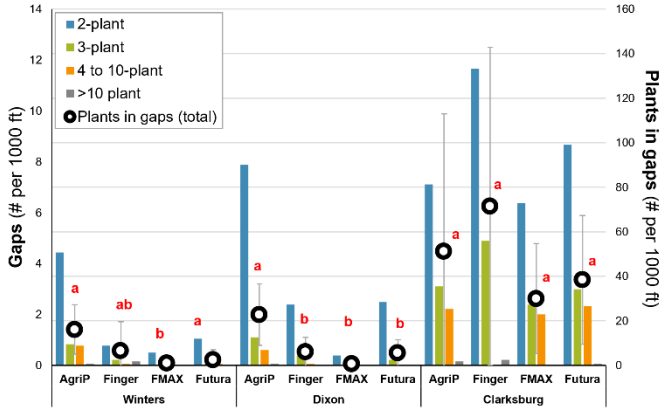
Executive Summary:

Background

According to a recent cost study of processing tomato production in California, transplanting can represent the single largest cash expense in a grower's operation, around 20% of the total seasonal cash expenditure (Aegerter et al., 2023). The increasing expense and uncertain availability of labor make the new technology of automated transplanting an attractive option. However, questions remain as to automated transplanters' planting uniformity and stand establishment, which given today's high tomato prices and the high cost of seed and transplants are also important considerations. The purpose of this study was to conduct replicated multisite side-by-side on-farm trials of five planter types: the finger planter, which is the most labor-intensive and the current area standard, the Ferrari FMAX carousel planter, which uses somewhat less labor and is popular in the southern tomato-growing counties, the Agriplanter and Ferarri Futura, two automated transplanters which were recently introduced to the California market, and the PlantTape system, an automated planter design currently used in other vegetables and recently adapted for processing tomato. Because of scheduling conflicts, however, PlantTape was unable to participate in the study. All planters were tested in a 3-bed, single-row configuration.

Methods

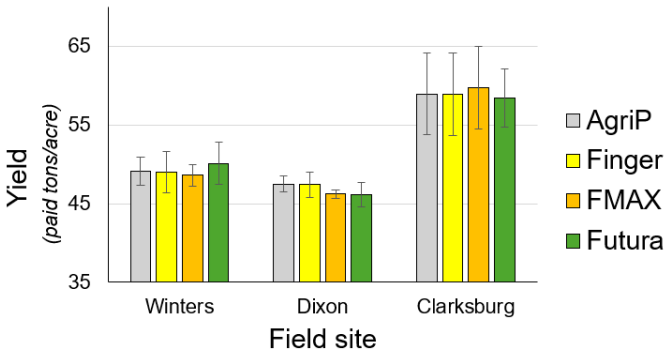
Through three large-scale, replicated on-farm trials, we collected agronomic data including planting depth, planting skips, stand establishment, and yields. Within each field we also documented planting speed and the size of the planting crew. Through interviews with growers and distributors I collected economic data on the planters, including machine purchase and maintenance costs, equipment requirements, labor requirements, and factors influencing machine speed and performance. I also performed a pilot study in 2023, in which I conducted a replicated, randomized side-by-side trial of the Agriplanter, FMAX, and finger planter.



Agronomic performance

Four field trials over two years demonstrated that the automated planters are more prone to planting skips than traditional planters. In particular, the

Agriplanter tended to have the most skips and the FMAX carousel planter the fewest. The FMAX also tended to have the fewest stand gaps 3 weeks after planting. However, the large majority of these skips and gaps were 2 plants long or less, and were relatively rare in all planters. In the 2023 pilot field trial, the Agriplanter had a shallower and more variable planting depth than the finger planter. However, there were no depth differences among planters in any of the 2024 field trials.



There were no differences among planters in either yield or quality in any of the four field trials. In-field differences among replicates tended to be larger than differences among planters.

Cost comparisons

Measurements from the field trial were used along with interviews with growers and manufacturers to compare purchase and operation costs among planters. Results are summarized in Table 1

Table 1. Example purchase and operational costs

		AgriP 3-row	Futura 3-row	FMAX 3-row	Finger 3-row
Measured from trial	Speed (mph)	1.4 - 2.8	1.0 - 1.3	0.79 - 1.1	0.8 - 1.4
	Crew size*	2 - 3	2	5 - 6	8 - 10
	Acres/ man-hr (active time)**	1.3 - 2.5	0.9 - 1.3	0.3 - 0.4	0.2
From grower & distributor interviews	Acres per shift (seasonal avg)***	20 - 30	15 - 20	10 - 11	11 - 12
	Shift length (hr)	10 - 12	8	8 - 8.5	8 - 8.5
	Acres/ man-hr (seasonal avg)$	0.6 - 0.9	0.6 - 0.8	0.2 - 0.3	0.1 - 0.2
	Avg crew wage($/hr)$$	$80	$80	$123	$191
	Avg labor cost ($/acre)	$32 - 43	$29 - 44	$90 - 105	$127 - 135
	Estimated diesel cost ($/acre)$$$	$5.44 - $7.25	$7.16	$4.63	$3.86
	Estimated maintenance cost ($/acre)†	$3.00	$5.10	$4.50	$11.00
	Total average running costs ($/acre)	**$46.85**	**$48.76**	**$106.63**	**$145.86**
Cost per acre (5-year depreciation schedule)	Example purchase price	$352,000	$198,000	$63,000	$1500 (used)
	1000 acre/yr	$117.25	$88.36	$119.23	$146.16
	1500 acre/yr	$93.78	$75.16	$115.03	$146.06
	2000 acre/yr	$82.05	$68.56	$112.93	$146.01

* As observed at field trials. Not including water truck and forklift operators

** Calculated using observed crew size, pass length, and measured speed over 2 passes and one turn (n=9).

*** Grower and distributor-reported seasonal estimate (integrates breaks, cleaning, maintenance)

$ Calculated using grower estimates of daily acreage, crew size, and shift length; not including water truck/forklift
Assumes 3 crew on automated planters

$$ Calculated using averages of grower-reported wages for farm and contract labor
(Contract wage: base: $16; supervisor: $18; contract fee: 42%. Farm wage: base: $19, machine-operator: $22; benefits: 35%)

$$$ Calculated using grower reported diesel usage (per hour or per acre), California 5-yr average diesel cost of $4.63/gal

† As reported by Ray Yeung (AgriPlanter, FMAX, Finger) and Todd Diederich and Brad Strock (Futura)

Trial results suggest that under a range of representative growing conditions for high-yielding processing tomato production in the southern Sacramento Valley, planter type is unlikely to influence fruit yield or quality. While the automated planters (especially the Agriplanter) have more

frequent skips, they were small and rare enough that they didn't influence yields. Planting rate, labor and maintenance costs depend on several internal and external factors, such as field size, plant density, tray and plant quality, bed preparation, and soil type. Example costs and a list of these factors are provided, with the goal that they will be useful to individual operators in assessing the economics of different planter types on their own farms.

Results provide strong evidence that there are no consistent, intrinsic yield differences among tested planter types under the conditions represented by the trials (e.g. experienced planting crews, good bed preparation, good plant quality, daytime planting). However, they do not exclude the possibility that yield differences may exist under other conditions, or provide information about other automated planter types that were not tested.

Introduction

Starting in the 1990s, the processing tomato industry has moved away from direct seeding and now nearly all fields are established with transplants (Hartz et al., 2008). Commonly, growers purchase hybrid seed which is grown out by commercial greenhouses. Transplants are planted either by growers using their own equipment, or by custom operations. According to a 2023 cost study on processing tomato production the seeds, transplants, and planting together can constitute 20% of the yearly cash costs and are the greatest single operational expense apart from harvest (Aegerter et al., 2023).

A large and increasing contributor to this cost is labor, due to new California legislation increasing wages and mandating overtime pay for farm workers. Additionally, tomato transplanting occurs over a period in the agricultural calendar when there is high demand for seasonal labor from other sectors. As timely planting is key both to good agronomic performance and contract fulfillment, the risk of the needed labor being unavailable is another incentive towards shifting to planting methods with reduced labor requirements.

In the Sacramento Valley the finger planter is the most commonly used type. This is a semi-mechanized system in which a planting crew drops plants from front-mounted trays into a series of cups mounted on vertically rotating wheels, which deposit plants into trenches opened by a foot that are closed as the tractor passes. Transplant water is dispensed under the transplant row. A typical crew includes two workers per row, someone to change out trays, someone walking behind to fill in any skips, and the driver. Carousel-style planters have also been used in California for a few decades and are more popular in the southern tomato growing counties. These use a somewhat similar mechanism, but the plants are dropped into cups in a flat rotating carousel. This can allow for one worker per row, with additional workers to change out trays, fill in gaps, and drive the tractor. A popular model in the Central Valley, the Ferrari FMAX, uses a finer foot and more directed water delivery system than the finger planters, which the company claims allows for better plug-soil contact and more uniform moisture.

Automated transplanters have only been used in California processing tomato production for a few years. These systems automate the removal of plants from trays, so the only labor needed is the driver and workers to change out trays. The AgriPlanter is manufactured by a Belgian company, and has been adopted by a few California processing tomato growers in the last two years. Ferrari, the Italian manufacturer of the FMAX carousel planter, has recently introduced an automated model

(the Futura) for California processing tomatoes. PlantTape, based in Salinas, was originally developed by the vegetable industry. In this system, transplants are grown in soil pockets between two layers of a biodegradable tape, which the planter unspools to feed them into the planting trench. According to the company this method allows for both better spacing precision and a reduction of transplant shock compared with other methods. Automated planters are able to plant at a greater speed with fewer workers than other types.

However, the speed and automation of the automated planters come at a cost. It's not practical to fill in skips behind the planter, leading to a higher potential for gaps in the stand. While expert workers can reject poor quality transplants within a tray, this isn't possible in automated systems. Slight variations or faults in the trays or misalignment with the automated system can lead to long unplanted stretches, especially when planting is done at night and the problem isn't immediately evident (personal observation). Such misalignments can also cause long delays in the field while workers diagnose the problem and make the necessary adjustments. Automated systems can be less accommodating of variations in transplant size within a tray, which may also lead to issues with stand establishment. Similarly, they may be less likely to work well with transplants that are too large or small, making availability a potential issue. And like all machinery, these planters need to be optimized for individual growers' operations. Large stand gaps can mean both yield losses and increased weed pressure. Stands that need to be replanted can represent both a substantial additional cost and a delay from optimal timing. Thus, it's an important question whether the lower labor costs offset the risks of a poor stand establishment.

In a preliminary study in 2023, three planter types (finger, the FMAX, and the Agriplanter) were compared in a randomized, replicated side-by-side trial on a grower field in Sacramento County. Additionally, planting skips and stand establishment were measured in randomly chosen AgriPlanter fields around Yolo and Solano Counties. The field trial found significant differences among the planters in the number and length of planting skips, average planting depths, and stand establishment (Fig. 1).

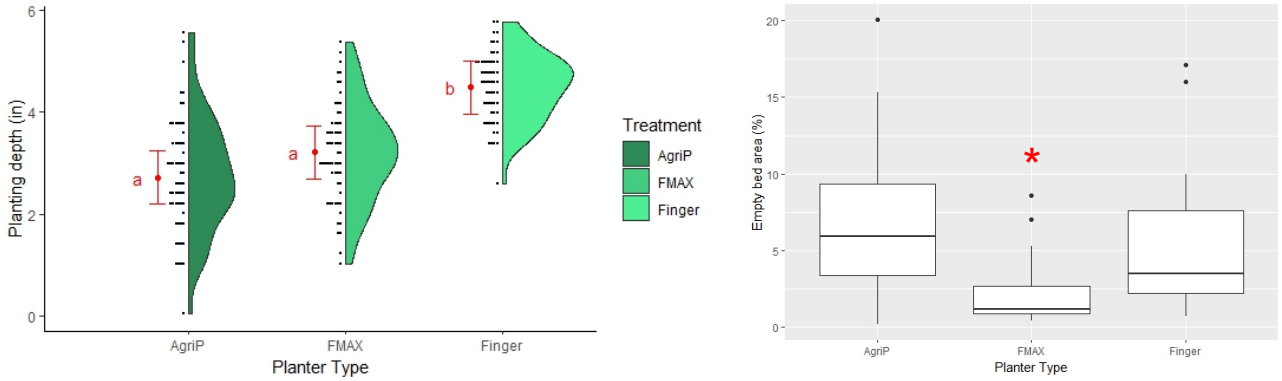


Figure 1. *Side-by-side field trial data on a) average planting depths and b) empty bed area measured just prior to harvest, calculated as a percentage of total area for the Agriplanter, FMAX, and finger transplanters (n=3)*

Automated planters were most liable to long skips and had a greater variability in planting depth (Fig. 1a). Additionally, while the finger planter had the lowest probability of long planting skips, a heat wave just after planting caused high mortality in some of the Agriplanter and finger planter

beds while the FMAX beds were relatively unaffected, leading to a lower percentage of empty bed space in the FMAX-planted beds just prior to harvest (Fig. 1b). The number of single- and double-plant skips were uncorrelated with empty bed space prior to harvest, suggesting tomatoes were largely able to compensate for missing neighbors. This was borne out by the yields, which did not differ among the three planters tested

Table 1. *Yields from 2023 pilot study (n=3).*

	Total	**Paid**
	Tons/acre	
Finger	56-75	50-68
FMAX	57-73	47-64
Agriplanter	56-67	50-62

However, the survey of grower fields suggest that Agriplanter performance depended heavily on other factors including soil conditions, weather, and transplant quality. Additional replicated randomized trials on multiple grower fields are needed in order to draw conclusions about relative performance of the different planters.

Objectives

1. **Measure parameters of transplanter performance under different conditions** Quantify planting skips, average planting depth, stand establishment, and yields for each transplanter type from randomized side-by-side trials on three grower fields (single-row configuration on 60-inch beds). All will be 3-bed models, with the exception of PlantTape which is single-bed.
2. **Demo new planters to the grower community** At the planting of one field trial, host a field day at which interested growers can observe and compare the different planter types in action
3. **Compile relevant economic information** Gather information from growers and transplanter company representatives about relevant parameters such as:
    a. The range and average of planter costs and weights,
    b. Range and average of labor crew size, shift length, and wage
    c. Average daily acreage planted
    d. Fuel requirements
    e. Water requirements during planting and establishment
    f. Other parameters identified as being of interest, as needed
4. **Assess relative costs, benefits, and risks associated with different planters** Use field and interview data to perform a strengths, weaknesses, opportunities and threats (SWOT) assessment for different planter types, as well as an informal comparative cost-benefit analysis for each field trial. (The analysis is understood to be informal, as it represents data from a single year and a few sites, and cannot accurately account for costs like maintenance over time or risks like labor or transplant shortages or need for replanting).

Methodology and Results:

Objectives 1 & 2: Field Trial

Large replicated side-by-side trials were planted in three fields, each with different growers, locations, varieties, and planting dates and conditions (Table 2). All planters were tested in the

three-row configuration. Transplants for each planter were randomly chosen from the lot supplied to the grower for planting the whole field. Plants came from a different transplant house for each field but were generally a good size, healthy, and fairly uniform within the trays. Trial planting in all fields started around 7:00 am. Agriplanter rows were planted by each grower, using their own machine and crew. The other planters were operated by custom transplant businesses accustomed to their use, using their own machines and crew. As proposed in Objective 2, the public was invited to the Clarksburg planting day to observe the planters in action. PlantTape was not able to participate as part of the formal trial, due to a scheduling conflict; however, a machine was available to plant 18 rows as a demonstration at the Clarksburg site. The PlantTape transplants did not reach moisture, and due to their rapid mortality the grower elected to replant those rows. Thus, only four of the five proposed planters were represented.

Table 2. 2024 field trial parameters

Field site	Winters	Dixon	Clarksburg
Variety	SVTM 9034	H 2016	SVTM 9016
Planting date	March 27, 2024	May 8, 2024	May 17, 2024
Temp at planting (Low/High °F)	47° / 60°	58° / 82°	52° / 80°
Avg transplant height & variability*	6” (CV=9.8%)	4.6” (CV=10.2%)	5” (CV=16.2%)
Harvest date	July 24-25	Sept 11-12	Sept 29-30
Trial size	19.8 acres	13.8 acres	18.1 acres
Main soil type	Silt loam, silty clay loam	Silty clay loam	Clay
Site-specific challenges	Heavy bindweed and vine decline in one replicate	Strong north wind whole of planting day	Weed pressure, early-season irrigation challenges

** “Height”= plant height in the tray from the soil line to the growing tip. Variability measured as coefficient of variation (CV=standard deviation/average*100)*

Three replicates were planted per field, using a layout that allowed for harvest in a carousel pattern (Fig. 2). Each replicate consisted of two passes of each three-row planter (6 rows * 3 replicates = 18 rows per planter field). Planter order was randomized within each replicate separately, so that no planter would always be at the center or outside position. Replicates were located at least 12 rows from a field edge and at least 12 rows apart from each other.

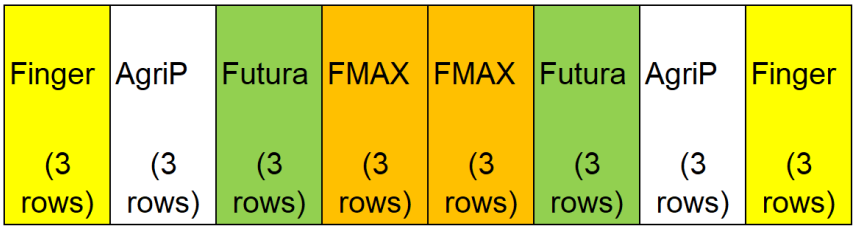


Figure 2. Example design of one replicate in one field. There were three replicates in each field.

Data collected:

- Planting depth--calculated by subtracting an average plant height (obtained by measuring the distance between soil line and growing tip of five random plants in three random trays from each transplant box that was used to supply each planter) from the height of each of ten random plants measured along one row of each pass
- Planting skips (counted just after planting on one row per pass)
- Stand establishment (measured by drone 3 weeks after planting)
- Yields and quality (weights and PTAB grades from processor for each unmixed load)

Planting depth

In each of the three 2024 field trials, all planters had a similar planting depth ($p>0.05$). They also all had similar variability at all sites ($p>0.05$ for Levene's test of homogeneity of variance). Figure 3 shows the combined data for all three sites in 2024.

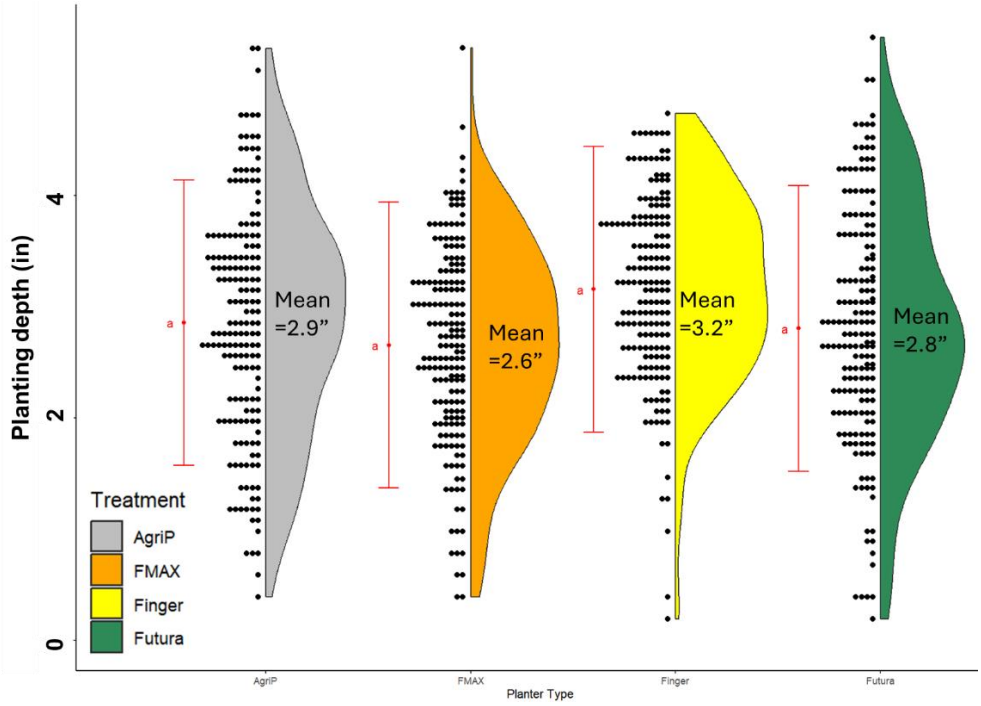


Figure 3. Range, mean, and variability of planting depth in the 2024 field trials. Each black dot represents one plant measurement.

This is in contrast to the results from the 2023 trial, in which the finger planter planted at a greater depth and with less variability than the Agriplanter or FMAX (the Futura was not included in the 2023 trial). These results show that while there can be differences depending on site-specific operating conditions, there very likely aren't any intrinsic issues with any of these machines that would make planting depth different or more variable than the others.

Planting skips

Normal / grower practice for replanting was followed for all planters-- for the finger and FMAX planters at all sites, workers followed behind filling in skips by hand. At the Winters site only, the grower filled in any long skips behind the Agriplanter using a single-row planter.

For the skips counted immediately after planting, the Agriplanter generally had more 2-plant or greater skips than the other planters. At the Clarksburg site, the Futura had the greatest number of single-plant skips (not shown). However, all skips greater than 2 plants were relatively infrequent, on average less than one per thousand feet (Fig. 4). Only one skip greater than ten plants was measured; a 40-ft skip in one of the Agriplanter rows at the Winters site.

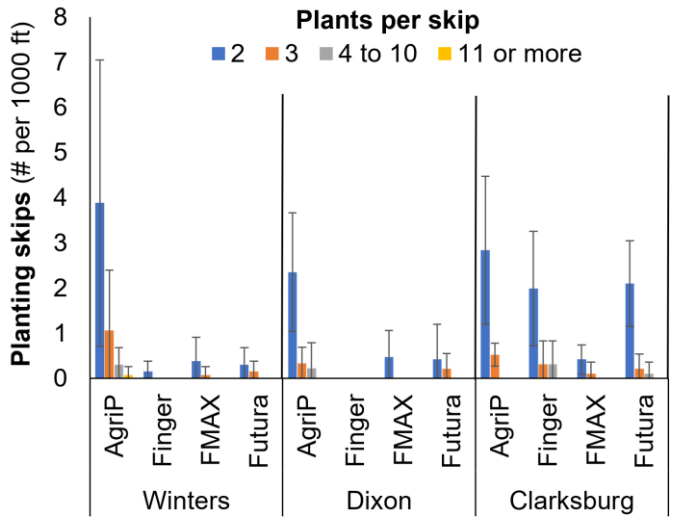


Figure 4. Skips approximately the length of 2 plants or greater, measured immediately after planting

Drone imagery taken approximately three weeks after planting was used to assess stand establishment (Fig. 5). Mortality was relatively low at the Winters and Dixon sites. At the Winters site, the AgriPlanter and Futura both had significantly more plants in gaps than the FMAX, while at the Dixon site the AgriPlanter had more plants in gaps than all other planter types. However, as with planting skips, there were few gaps greater than 2 plants wide (less than 1 per 1000 feet). At the Clarksburg site, an irrigation issue led to a patchier stand in some rows, increasing the number of skips in all planter types. There were no differences among planters types at this site.

Yield and quality

Neither total nor paid yield differed among the four planter types at any of the three sites (Table 3). At the Winters site, there was a slight tendency for the Futura rows to have fewer greens (p=0.08); otherwise, there were no significant fruit quality differences among the planter

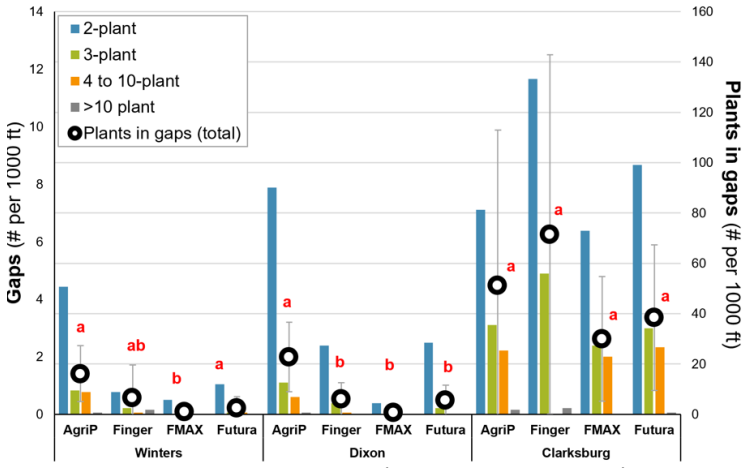


Figure 5. Gaps measured from 1000-ft segments of drone imagery

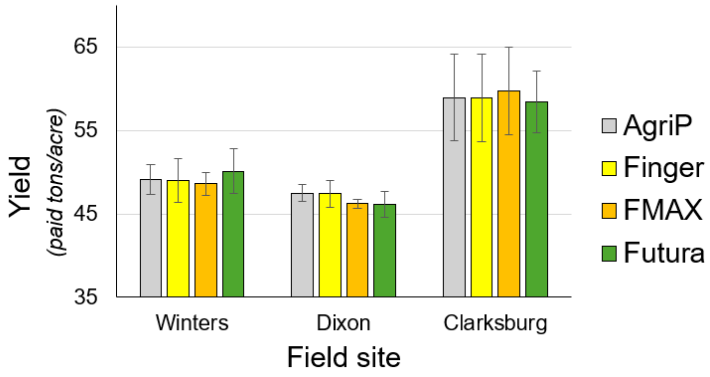


Figure 6. Average yields at the three sites. Bars represent the standard deviation of the mean (n=3)

Table 3. Means and standard deviations for yield and quality parameters (n=3). There were no significant differences among planters for any parameter

		Means								Standard deviation							
Field	**Planter**	*Net yield* t/a	*Paid yield* t/a	*Unpaid* %	*Greens* %	*Lim. use* %	*Solids*	*pH*	*Hue*	*Net yield*	*Paid yield*	*Unpaid*	*Greens*	*Lim. use*	*Solids*	*pH*	*Hue*
Winters	AgriP	49	49	0.7	0.50	0.83	5.42	4.43	21.67	1.72	1.77	0.38	0.25	0.52	0.03	0.06	0.38
	FMAX	49	49	1.9	1.11	0.61	5.46	4.44	22.22	1.53	1.34	1.28	0.67	0.35	0.13	0.05	0.25
	Finger	50	49	2.0	0.58	2.17	5.35	4.44	21.67	2.43	2.67	1.15	0.14	2.02	0.09	0.06	0.14
	Futura	50	50	0.6	0.17	1.58	5.52	4.45	20.83	2.49	2.66	0.52	0.14	1.66	0.13	0.08	1.04
Dixon	AgriP	50	48	5.0	3.25	0.58	5.63	4.29	19.08	1.16	1.00	1.12	0.82	0.38	0.05	0.06	0.38
	FMAX	49	46	5.5	3.50	1.00	5.73	4.29	19.58	0.71	0.55	0.51	0.63	1.14	0.29	0.06	0.38
	Finger	50	47	4.5	3.08	0.75	5.65	4.31	19.58	1.66	1.63	0.67	0.38	0.82	0.14	0.06	0.38
	Futura	48	46	5.0	3.58	0.25	5.75	4.29	19.58	1.17	1.57	0.98	1.16	0.61	0.08	0.02	0.38
Clarksburg	AgriP	63	60	5.3	3.28	0.06	4.59	4.28	22.28	4.49	5.20	2.16	2.00	0.17	0.14	0.04	0.87
	FMAX	65	60	7.9	5.17	0.20	4.53	4.28	21.87	4.64	5.26	2.73	1.35	0.35	0.15	0.05	0.47
	Finger	63	59	6.8	4.60	0.05	4.54	4.27	22.05	4.92	5.26	2.45	1.79	0.16	0.08	0.05	1.07
	Futura	63	58	7.8	5.00	0.10	4.62	4.25	22.05	3.55	3.64	1.63	0.88	0.32	0.10	0.03	0.50

Overall, the variation between fields (combined effect of variety, planting date, and other management and site-specific factors) and the variation between the replicates within a field were much greater than the differences between planters. For example, in the Winters field, the first replicate (red dots) had issues with bindweed and decline which had a much stronger yield affect than planter type (Fig. 6).

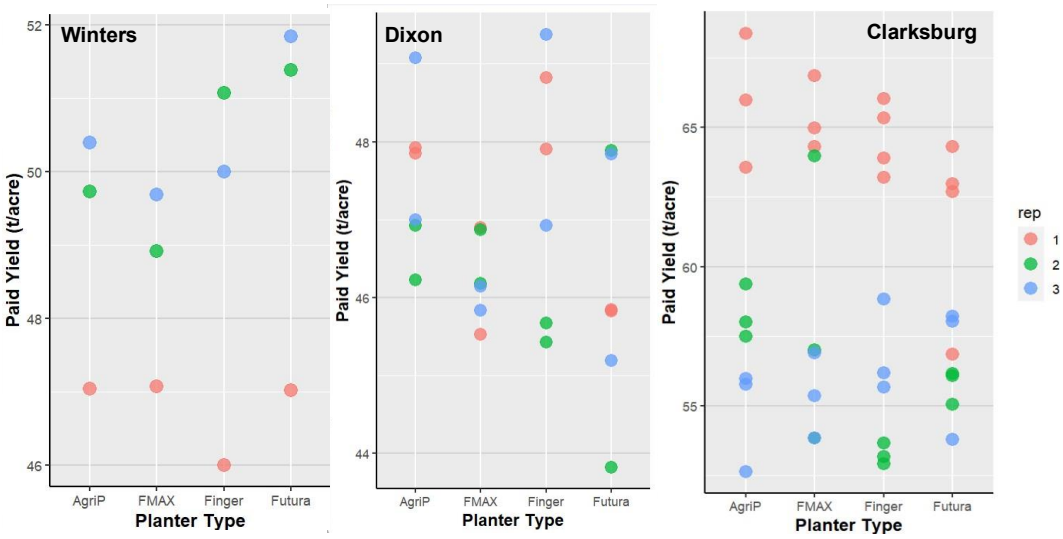


Figure 6. Yields for each planter, separated by replicate. Each dot represents a load (or the average of 2 or more loads, for the Winters site)

Objectives 3 & 4: Compile relevant economic information into a preliminary cost-benefit analysis

In consultation with Dr. Brittney Goodrich, an agricultural economist formerly working with the UC Davis Cost & Return Study team (now working for the University of Illinois), two surveys were developed. They are appended at the end of this report. One contained questions for the manufacturers and distributors, and the other for growers or custom planters with experience using the machine in question. In the case of the Futura, the custom planting business is also the US distributor for Ferrari, so in this case the two questionnaires were administered to the same team (MTD Transplanting). Other interviewees were Eric Puehler (of Puehler Ag, the US distributor for AgriPlanter), and Ray Yeung, who uses the AgriPlanter, FMAX, and finger planter in his custom transplant business and on his own farm. I also interviewed a grower who is using the 5-row configuration of the AgriPlanter, although this was not a part of the field study, given local interest.

Surveys contained questions concerning costs such as purchase price, labor needs, maintenance costs, and resale value (Table 4), as well as more general questions about special challenges associated with the machine, or conditions under which it performed especially well or poorly. Since the automated planters are relatively new and are evolving rapidly some information that would be part of a formal cost study, such as lifespan and end-of-life resale value, are not available. Also, each machine is currently assessed using information from one source. Therefore, the results should be regarded more as case studies from individual operations rather than as a comprehensive and representative cost study.

Table 1. Planter speed, crew size, and calculated acres per man-hour as observed in the field trials

	AgriP 3-row	Futura 3-row	FMAX 3-row	Finger 3-row
Speed (mph; measured from 2 passes, 1 turn)	1.4 - 2.8	1.0 - 1.3	0.79 - 1.1	0.8 - 1.4
Crew size*	2 - 3	2	5 - 6	8 – 10
Acres/ man-hr (active time)**	1.3 - 2.5	0.9 - 1.3	0.3 - 0.4	0.2

* As observed at field trials. Not including water truck and forklift operators

** Calculated using observed crew size, pass length, and measured speed over 2 passes and one turn (3 replicates in 3 fields, n=9).

Table 2. Estimated costs, from grower & distributor interviews. ***Costs reflect only those directly associated with the machine itself, not the full cost of the planting operation.*** *Calculations exclude forklift/water truck operator.*

		AgriP 3-row	Futura 3-row	FMAX 3-row	Finger 3-row
	Acres per shift (seasonal avg)*	16 – 30	10 – 20	10 - 11	11 - 12
	Shift length (hr)	10 – 12	8	8 - 8.5	8 - 8.5
	Acres/ man-hr (seasonal avg)**	0.5 - 0.9	0.4 - 0.8	0.2 - 0.3	0.1 - 0.2
	Avg crew wage($/hr)***	$80	$80	$137	$205
	Avg labor cost ($/acre)	$29 – 44	$32 – 43	$100 - 117	$137 - 145
	Estimated diesel cost ($/acre)[$]	$5.44 - $7.25	$7.16	$4.63	$3.86
	Estimated maintenance cost ($/acre)[$$]	$3.00	$5.10	$4.50	$7.00
	Total average running costs ($/acre)	**$45.85**	**$49.76**	**$117.63**	**$151.86**
Cost per acre (5-year depreciation schedule)	Example purchase price	$352,000	$198,000	$63,000	$7500 (used)
	1000 acre/yr	$116.25	$89.36	$130.23	$153.36
	1500 acre/yr	$92.78	$76.16	$126.03	$152.86
	2000 acre/yr	$81.05	$69.56	$123.93	$152.61

*Grower and distributor-reported seasonal estimate (integrates breaks, cleaning, maintenance)

** Calculated using grower estimates of daily acreage, crew size, and shift length; not including water truck/forklift

Assumes 3 crew on automated planters

*** Calculated using averages of grower-reported wages for farm and contract labor

(Contract wage: base: $16; supervisor: $18; contract fee: 42%. Farm wage: base: $19, machine-operator: $22; benefits: 35%)

[$] Calculated using grower reported diesel usage (per hour or per acre), California 5-yr average diesel cost of $4.63/gal

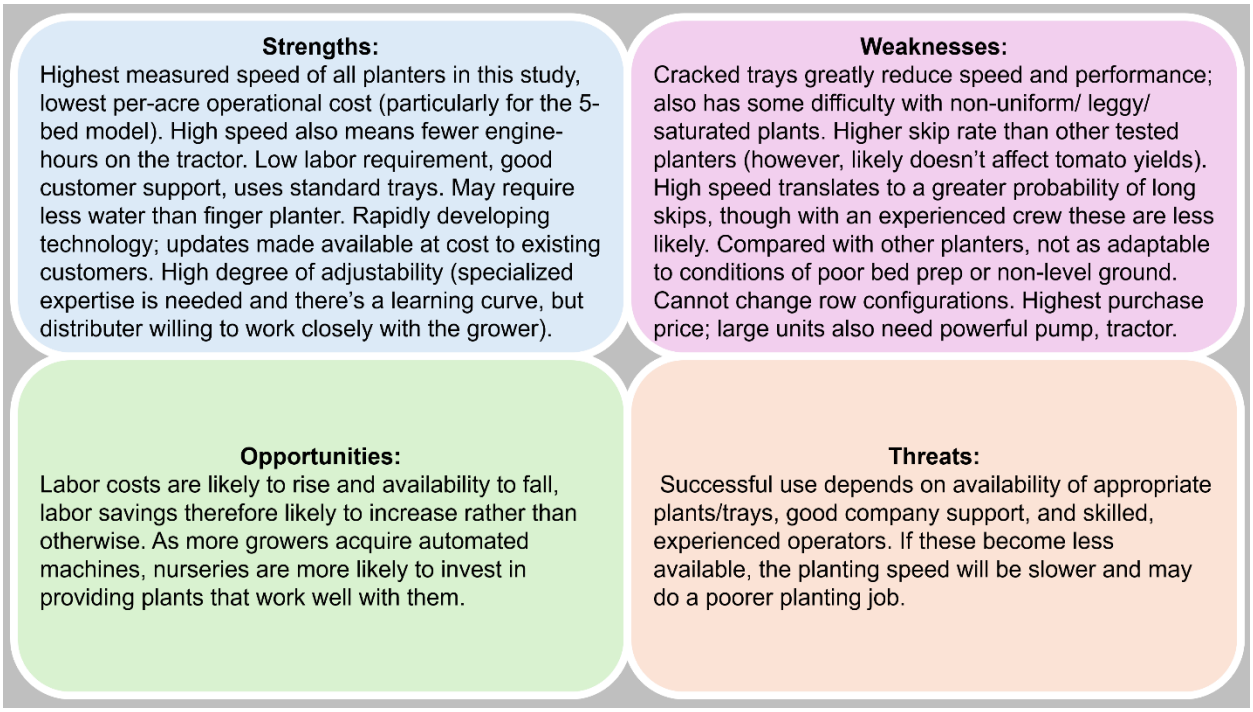
[$$] As reported by Ray Yeung (AgriPlanter, FMAX, Finger) and Todd Diederich and Brad Strock (Futura)

Table 3: Operational case studies from two custom planting operations (MTD Transplanting & Kubo Yeung Farms)

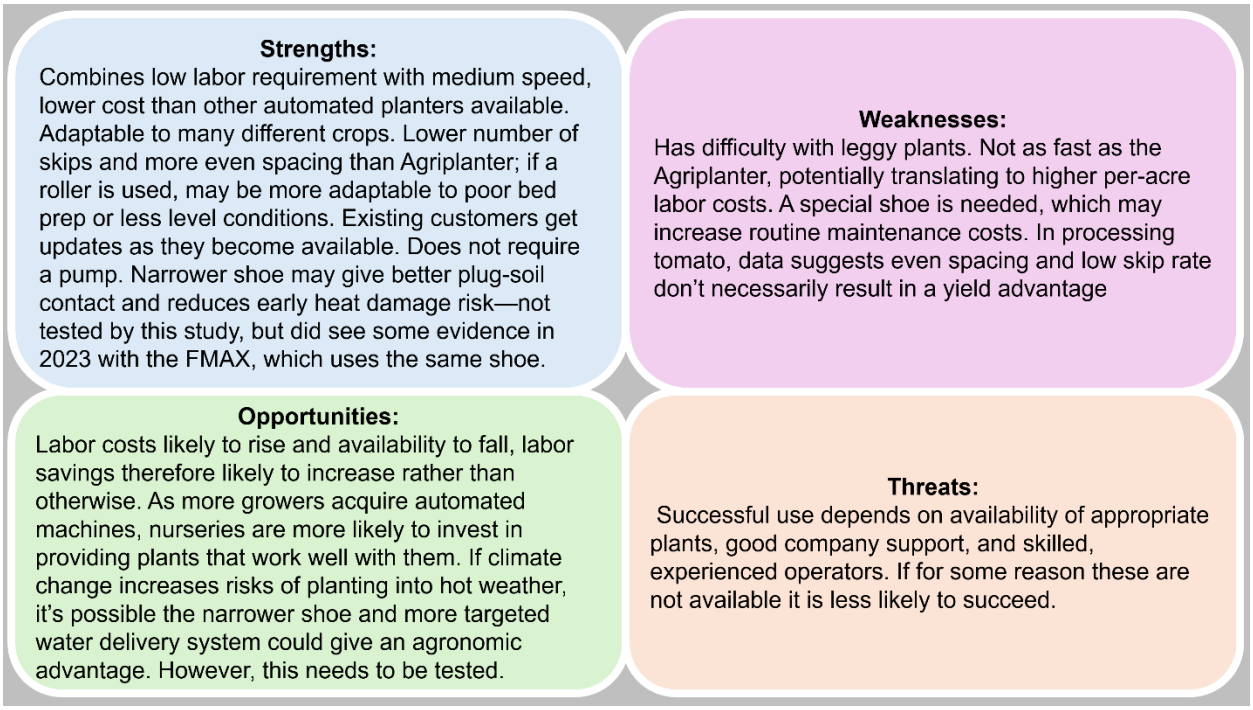
		Futura (3 row)	AgriPlanter (3-row)	FMAX (3-row)	Finger planter (3-row)
Purchase & equipment	Purchase costs	$198,000 (includes training)	$335,811 - $368,195 (lower costs for purchases Jan-May; includes training)	$63,000	~$7500 at auction.
	Weight	4500-4600 lb	~7500 lb	~3000 lb	~3000 lb single-line, 4500 lb double-line
	Tractor needs	70-90 hp, 540 PTO and 3-point hitch	160-175 hp; PTO depends on what grower wants to carry for water (200 for 1000 gal)	125 hp	125 hp
	Additional equipment	Common add-ons include rubber rollers to promote good soil-plug contact, custom built bin rack	Requires a variable-rate GPS, pressure washer for regular cleaning, appropriate pump	Quick hitch	
	Updates	Most updates are to the software, and the cost to the consumer is minimal	Currently supplied at-cost through distributer		
Speed & labor	Crew size	2-4 (1 driver, 1 crew on own farm; more if using for custom planting)	3 (driver, 2 on machine)	5.5 (1 driver, 3 contract labor planting, 1 supervisor. Mechanic, half-time)	8.5 (1 driving, six contract labor planting, 1 supervisor. Mechanic, half-time)
	Acres / day	10-20 acres per 8-hr day	16-20 acres per 10-hr day	10-11 acres per 8-hr day	11-12 acres/8-hr day
	Acres/ man-hr	0.4 - 0.8 (assumes 3-man crew)	0.5 - 0.7 (assumes 3-man crew)	0.2 - 0.3	0.18 - 0.19
	Avg labor cost/acre	$44/acre	$41/acre	$109/acre	$142/acre
	Reasons for a slower day	Higher planting density, short field length	Cracked/broken trays. When they first started the average was closer to 13-15 acres per day, but speed has increased as they learn how to run it better, make adjustments	Tall, tangled plants; poor plant quality	The machine rarely has problems; the more important issue is managing the people and cars
Inputs & maintenance	Diesel (estimated)	Diesel use estimated about 1.5 gal/acre (~4-4.5 gal/hr). Hauled by 90 hp tractor.	Diesel use estimated 22-25 gal/day; 1.2-1.5 gal/acre at 16-20 acre/day. Hauled by 160 hp tractor.	Estimated diesel use is about 0.95 gal/acre; hauled by 125 hp tractor.	Estimated diesel use is about 0.8 gal/acre; hauled by 125 hp tractor.
	Seasonal maintenance	~$5 per acre. Replacement shoes are the only regular wear item, at $320 per row, replaced about every 200 acres	~ $3.00/acre . Bearings, belts are regular replacement items. Shoes are about $400/row, replaced about every 2500 acres	~ $4-5/acre; shoes are regular replacement items	~$7/acre (rubber plant holders, chains, fingers, shoes, guides, wheels)
	Service visits	Service visits are $130-$180 per hour, but they have not had any issues yet that can't be fixed with a phone call	The distributer comes to do the winterazation, at a charge of $200/hr. The bill for a major servicing, after around 5000 acres of use, was $5000-$6000	Service visits haven't been needed	Service visits haven't been needed

I used a SWOT (Strengths, Weaknesses, Opportunities, Threats) analysis capture issues which are not quantifiable in a cost analysis. This analysis summarizes internal (strengths, weaknesses) and external (opportunities, threats) factors which may positively and negatively affect machine's success

Agriplanter:



Futura:



FMAX

Strengths:
Fewest skips of all planters tested. In the 2023 pilot trial FMAX rows had less heat damage than the other tested planters (finger and AgriPlanter), possibly due to narrow shoe which is designed to increase plug-soil contact and plug contact with transplant water. Adaptable to many crops, bed configurations, planting conditions. Can change plant spacing more quickly than the finger planter. Accommodates cracked trays. More output per person than finger planter, less training necessary, may need less water.

Weaknesses:
Slowest of the tested planters. Higher labor requirement than automated planters, requires contract labor. Does not perform well on hard clay soils. Less adaptable than the finger planter, requires better ground prep. Special shoes wear faster and increase maintenance costs.

Opportunities:
If incidence of hot weather during planting increases, it's possible the narrower shoe and more targeted water delivery system could give an agronomic advantage. However, this needs to be tested

Threats:
Increasing cost and decreasing availability of contract labor. Also, if more customers move to automated planters, which can't deal with non-uniform or leggy plants or cracked trays, it's possible nurseries will start assigning poorer quality plants to customers using non-automated planters

Finger

Strengths:
Most adaptable of all the planter types (e.g. if in-field adjustments are needed for planting depth). Least sensitive to hard or rocky soils, or poor bed prep or plant quality. Wide, robust shoe, wears less quickly than that of the FMAX. Used for many crop types, bed configurations, planting conditions. The machine is the least expensive to purchase.

Weaknesses:
Highest labor requirement of the tested planters; contract labor necessary. Labor has to be trained and skilled. Many people at planting leads to a lot of cars, traffic, and confusion, can also be a safety risk to the workers. Wide shoe may lead to poorer plug soil contact and pooling of transplant water at the bottom of the transplant furrow; meaning more water may be required. Relatively high maintenance cost, as cited in case study.

Opportunities:
As processing tomato production expands to areas where it's less typical (e.g. into Northern California, Washington), adaptability to non-ideal plant and soil conditions and utility for many vegetable transplants may be an advantage

Threats:
Increasing cost and decreasing availability of contract labor. Also, if more customers move to automated planters, which can't deal with non-uniform or leggy plants or cracked trays, it's possible nurseries will start assigning poorer quality plants to customers using non-automated planters

Discussion: Transplanter performance, like any agricultural activity, is highly contextual. Important factors affecting how a planter design performs include weather, operator experience, plant and tray quality, soil type, and others. The goal of this study was to test planter performance across a range of conditions representative of high-yielding processing tomato production in the southern Sacramento Valley. A secondary goal was to provide examples of the associated purchase and operational costs.

We did not see any differences among tested planters in either yield or quality in any of the three field trials. These were conducted on fields managed by different growers, planted at different times of the year, and with different site-specific challenges. However, in all cases the planting weather was not too hot and plant quality were good, and all the machines were run by experienced operators. As expected, the AgriPlanter was the fastest, and also had the least even spacing and the greatest number of both short and long skips. Our results suggest that these short skips are currently inherent in the AgriPlanter's performance, but that tomato is able to compensate and under normal conditions they do not affect yields. It's also worth noting that the manufacturer states that that have recently made a change to the design that will help reduce skip issues.

At the Dixon, a strong north wind blew through the whole planting day; however, we did not see wind damage in any of the treatments. At the Clarksburg site, irrigation issues post-planting caused considerable mortality in some rows. In the most affected part of the field (Replicate 2), the rows with the patchiest stand and lowest yields tended to be those planted by the finger planter. However due to the very high variability there was no statistical difference between planters, and this may have been by chance. In the 2023 pilot study, which was planted in June, there was considerable death from heat damage following planting. There was significantly better stand establishment in the FMAX rows than in the AgriPlanter or finger planter rows. The manufacturers attribute this to the better soil-water contact facilitated by the water delivery mechanism and narrower planter shoe compared to the other planters. This result was not replicated in 2024; however, none of the trials was planted in hot weather. More data is needed to determine whether the Ferrari design does in fact give better survivability for planting in heat; but the results so far suggest it is plausible. However, in the 2023 study the better stand establishment did not result in higher yields.

While it was not a part of the final study, it is worth remarking that the PlantTape machine planted three full replicates (approximately 3.7 acres total) at the Clarksburg site. Plants did not reach moisture, and mortality was near 100 percent in these rows. A representative from PlantTape noted that the machine was being run for maximum speed at this trial. Additionally, since their participation was confirmed at the last minute, they may have been using leftover plants that would not normally have been used on a customer field. Nevertheless, this result suggests that the PlantTape technology may not yet be a reliable option for processing tomatoes.

Costs and savings

Our cost example for the AgriPlanter is likely conservative, as the data comes from a business which mostly uses it in custom planting. This business normally runs AgriPlanter with a somewhat larger crew and more slowly than is typical (based on conversations with other AgriPlanter growers). On average for this business, the 3-row AgriPlanter saves around $106/acre in operational costs compared with the 3-row finger planter. Over 1500 acres/yr, at this rate the AgriPlanter would pay

itself off in about 2.2 yr. (For comparison, another local grower who uses it calculated a savings of $230/acre in labor compared to his 2022 labor costs with the finger planter). Another local grower, who replaced two 5-row finger planters with a single 5-row AgriPlanter, reports spending $22,000 in parts and labor on planting in 2024. Compared with the calculated labor cost alone with the finger planters, this represents a savings of $237/acre. Assuming the same wages and crew size as that reported for the 3-row AgriPlanter, the savings of a Futura over a 3-row finger planter are calculated to be around $102/acre, which over 1500 acres would pay itself off in around 1.3 yr. This also may be conservative, as it is also based on data from a custom planting operation which uses larger crews for a custom planting job than is recommended for someone planting on their own farm.

Lifespan & resale value

There is insufficient data on the lifespan and resale value of the automated planters. The oldest Agriplanters in use in California were purchased in 2021. The AgriPlanter US distributor, Puehler Ag, reports that they are aware of a machine in Italy which has been running for 22 years. They estimate that an Agriplanter could be sold for about 25% of the original cost after 20 years of use with reasonable maintenance. Shoes, belts, and bearings all need regular replacement, and the hydraulic pump also will need replacement at some point. The US distributor for the Futura, MTD Transplanting, said it was difficult to give an estimate for the lifespan since the machines are rebuildable and repairable. They estimate that a major overhaul may be needed every 10 to 12 years on a planter doing 1000 acres per year, and that a used Futura would probably sell for about 60-70% of its original purchase price.

Other concerns: plant quality, training needs, machine weight, available configurations

For both automated planters, the distributors emphasize that success depends on good communication with the nursery. For both planters, tall and leggy plants and poor uniformity can lead to problems, and for the Agriplanter cracked and broken trays are also a major issue. These can lead to slower days and long skips. Both also noted that it's very important to have someone on the machine who is well-trained and motivated to learn. Both Puehler Ag for AgriPlanter and MTD Transplanting for Futura offer staff trainings as part of the purchase price, as well as continuing support over the phone.

Automated planters are large, heavy machines, and the potential for delayed field entry or soil compaction was one of the initial concerns for their use. Weights are reported in Table 3. However, the users I spoke with report that the weight seems well-distributed and they have not had issues so far.

All planters were tested in the 3-row, single-line configuration, as all planters needed to be in the same configuration and this is the most common locally for the Agriplanter. However, for both Agriplanter and Futura other configurations (e.g. 5- or 6-row, double-line) can be requested.

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C. M. Rick

T G R C

Tomato Genetics Resource Center

Roger Chetelat (Director/Curator)
Xiaoqiong Qin (Postdoctoral Researcher)
Matt Valle (Assistant Curator)
Plant Sciences Department
University of California, Davis
trchetelat@ucdavis.edu
https://tgrc.ucdavis.edu

ANNUAL PROGRESS REPORT

2024

Leaves of a *S. sitiens*-derived introgression line (center) are more highly subdivided than the background genotype, cv. NC 84173 (L), a trait also seen in the wild parent *S. sitiens* (R). Crossing tests at the TGRC indicate that this increased leaf subdivision trait is conferred by an allele of *bip* (*bipinnata*), a gene known from early tomato mutagenesis experiments (Stubbe 1959 *Kulturpflanze* 7:84). The *bip* mutation in *S. sitiens* is part of this species' distinctive, deeply cut leaves which fold along the midvein, features which likely reduce water loss under drought conditions in this desert-adapted nightshade. (See below for more information on our work with the introgression lines).

SUMMARY

Acquisitions: We accessioned the cultivar 'Healani', an improved 'Anahu' type with additional disease resistance and determinate habit, from Glenn Teves at the University of Hawaii. 'Healani' contains the genes *sp, Mi* (root knot nematode resistance), *Sw*^{a} (partial resistance to tomato spotted wilt virus), *I* (Fusarium wilt resistance), *Tm-2*^{2} (ToMV resistance), and *Sm* (grey mold resistance). We also recovered an 'extinct' TGRC accession of *S. corneliomulleri* from the IPK genebank in Germany. The total of number of accessions maintained by the TGRC is now 4,536.

Maintenance and Evaluation: Over 490 cultures were grown for various purposes, of which 278 were for seed increase, including 63 wild species accessions. Germination tests were run on 690 seed lots. Progeny tests were performed on 31 stocks of male-steriles and other segregating mutants, or to check accessions with unexpected phenotypes. GMO tests were performed on 46 recently acquired stocks, all of which were negative. All plants were monitored throughout development for evidence of disease. Newly regenerated seed lots were split, with one sample stored at 4° C for filling seed requests, the other stored in foil pouches at -20° C for long term preservation. 258 samples were sent to the USDA's National Laborator for Genetic Resources Preservation in Ft. Collins, Colorado for off-site security backup.

Distribution and Utilization: A total of 4,179 seed samples representing 1,625 different accessions were distributed in response to 212 requests from 163 researchers and breeders in 15 countries. The overall utilization rate (# samples distributed / # active accessions) was 92%. Many purely informational requests were also answered. Information provided by requestors indicates our germplasm continues to be used for a wide variety of research and breeding projects. Our annual literature search found 139 publications that mentioned use of TGRC stocks.

Documentation: After our website was redesigned last year using the SmartSite platform, with virtually all pages and database queries being rewritten, the changes made this year emphasized new content rather than major structural changes. We added pages describing groups of related accessions, as well as biographical articles about researchers and plant collectors who played important roles in establishing the TGRC collections. On the database side, we added new queries and filters pertaining to seed requests and accessions, and reformatted our seed packet labels to include more condensed and relevant descriptors for each accession. As in the past, seed request records and passport information on seed samples submitted for off-site back up were provided to the USDA for uploading to the GRIN-Global database.

Research: We further characterized several QTLs/genes contributing to seed vigor/dormancy and response to heat stress in a set of *S. sitiens* introgression lines, each of which contains a defined chromosomal segment from the wild nightshade in the genetic background of cultivated tomato. We tested whether several ABA-related genes that map near the QTLs could be responsible using CRISPR-induced mutants. This work was funded by a grant from the Foundation for Food and Agriculture Research. We also continue to study the molecular mechanisms of pollen rejection in tomato interspecific crosses.

ACQUISITIONS

The TGRC added the cultivar 'Healani', donated by Glenn Teves from the University of Hawaii, College of Tropical Agriculture, Cooperative Extension Service. 'Healani' was bred by Dr. James Gilbert, also with the University of Hawaii, as an improved 'Anahu', one of the first nematode resistant varieties to be released, by crossing Anahu x STEP305, followed by crosses to STEP559 (Teves 2017 *Molokai Native Hawaiian Beginning Farmer Quarterly*, 1-17). Like 'Anahu', 'Healani' contains the *Mi* gene for resistance to root knot nematodes, but also contains the *Tm-2* gene for resistance to Tomato Mosaic Virus, as well as the genes *I, Sm,* and Sw^a for resistance to Fusarium wilt, gray mold, and Tomato Spotted Wilt Virus, respectively. 'Healani' also has determinate habit and is less prone to fruit cracking than 'Anahu'.

Leaves, flowers and fruit of *S. corneliomulleri* LA0118.

We also 'rescued' a very old, inactive accession of *S. corneliomulleri,* LA0118, thanks to seed obtained from the IPK genebank in Gatersleben, Germany. Originally collected by Charley Rick near Canta, Lima, Peru, in 1949, it was one of his earliest collections of what was then named *Lycopersicon glandulosum*, and later *L. peruvianum* f. *glandulosum*. His seed collection had never been successfully regenerated at Davis and the accession was considered 'extinct'. However our records indicated that samples of original (wild collected) seed had been sent out to several researchers in the 1950's, including a scientist in Japan. In 1991 the National Institute of Agrobiological Resources in Tsukuba, Japan deposited a sample of LA0118 with the IPK genebank in Germany (accession LYC 3515). The IPK kindly provided a sample to the TGRC where it is once again available for distribution after being 'lost' for nearly 75 years. We are not aware of any unique traits in this accession, and we have other accessions of *S. corneliomulleri* collected in later years from near Canta, Peru. Nonetheless, these older, extinct populations such as LA0118 are still worth retaining because a) having multiple accessions from regions of high diversity provides a more complete sampling of genetic diversity, b) there is evidence that many historical populations are either threatened or have already been extirpated due to human factors, and c) it has become impractical to make new collections in the current regulatory environment.

The current total of number of accessions maintained by the TGRC is 4,536.

Table 1. Number of accessions of each species maintained by the TGRC. The figures include accessions that are temporarily unavailable for distribution.

***Solanum* spp.**	**# Accessions**	***Solanum* spp.**	**# Accessions**
S. lycopersicum	3,113	*S. corneliomulleri*	59
S. lycopersicum var. *ceras*.	421	*S. chilense*	114
S. pimpinellifolium	331	*S. habrochaites*	122
S. cheesmaniae	42	*S. pennellii*	47
S. galapagense	28	*S. lycopersicoides*	23
S. chmielewskii	16	*S. sitiens*	13
S. neorickii	47	*S. juglandifolium*	6
S. arcanum	44	*S. ochranthum*	7
S. peruvianum	71	Other	5
S. huaylasense	17	**Total**	**4,536**

MAINTENANCE AND EVALUATION

The TGRC grew over 490 families for various purposes. 278 plantings were for seed increases, of which 215 were cultivated tomato and 63 were wild species accessions. 31 cultures were for progeny tests to verify the presence of segregating genes (e.g. male-sterility loci) or to confirm phenotypes. 16 families were grown for introgression and analysis of the *S. sitiens* genome, while 70 were planted to study interspecific reproductive barriers. Testing for the presence of GMOs was performed on 46 recently acquired accessions of *S. lycopersicum* – all were negative for three standard transgene markers.

The *wrinkled fruit* mutant.

Identifying accessions in need of regeneration begins with seed germination testing. We start testing seed lots after 10 years in storage. Seed samples that do not meet our minimum of 80% germination response after two weeks are normally regenerated in the current year. Seed lots that exceed this threshold are retested again every two to three years. Other factors, such as available greenhouse space, age of seed and supply on hand, are also considered. Newly acquired accessions are typically regenerated in the first year or so after acquisition because seed supplies are limited and of uncertain viability. This year, 690 germ tests were run on seed lots produced in 2014 or earlier. Average germination rates were satisfactory overall, except for *S. galapagense* for which a large share of seed lots did not meet our 80% minimum viability (Table 2). Continued improvements in our seed testing procedures (see guidelines on our website) have allowed us to obtain higher rates of germination overall, which reduces the number of accessions that need to be regenerated.

Table 2. Results of seed germination tests. Values are based on samples of 25-100 seeds per accession, and represent the % germination after 10-14 days at 25°C. Seed lots with a low germination rate are defined as those with less than 80% germination.

Solanum Species	Tested Seed Lots From	# Lots Tested	Avg % Germ	# Low Germ
S. arcanum	2005 - 2011	5	78.8	2
S. cheesmaniae	2008 - 2014	6	82.3	1
S. chilense	1994 - 2014	34	91.4	1
S. chmielewskii	1997 - 2013	16	89.7	1
S. corneliomulleri	1992 - 2014	15	95.1	0
S. galapagense	2004 - 2014	6	40.7	4
S. habrochaites	1991 - 2014	29	90.3	0
S. huaylasense	1992 - 2002	3	82.0	1
S. lycopersicum	2003 - 2014	480	93.0	43
S. neorickii	1996 - 2014	11	90.5	1
S. pennellii	1997 - 2012	21	93.8	0
S. peruvianum	1996 - 2014	13	91.2	0
S. pimpinellifolium	2004 - 2014	51	96.4	1
Total		**690**		**55**

Most stocks of *S. lycopersicum* and the predominantly selfing accessions of *S. pimpinellifolium* are grown for seed multiplication in the field unless they require greenhouse culture. Each family is typically represented by 8 or 9 plants, except for segregating families (e.g. male-steriles), which are grown from larger plantings. Our field plot this year occupied one acre. As usual, sequential plantings were made to spread the workload, with the first transplanting on April 16, the last on May 10. Conditions were generally favorable throughout the growing season, despite the usual summer hot spells, and plants were mostly healthy, although as usual we lost some plants to TSWV. Growth under drip irrigation – initially surface, then subsurface – was again quite good and we shut off the water early to keep plants to manageable size. As in the last two year, our plants went into ground that had not seen tomatoes in many years. As a result, there were virtually no volunteer tomatoes sprouting within the beds, which avoided the need to pull out the young plants to prevent seed admixture. However, our field plot was heavily infested with bindweed (morning glory), which required repeated hoeing. We also lost a number of groups to ground squirrels, which eventually was solved by fencing and poison baits.

Fruit of *S. chilense* LA4119.

Most of the wild species, many mutants and certain other genetic stocks require greenhouse culture, either for isolation purposes or because they do not grow or flower well under field conditions. Our schedule of greenhouse plantings of the wild species is based on photoperiod responses: those with the least sensitivity are planted first, in the early spring; those with intermediate reaction are planted in early summer; the most sensitive (i.e. flower best under short days) are planted in mid-summer for fall blooming. Wild accessions are grown from large population sizes (50-75 plants) to maintain diversity, maximize heterozygosity, and avoid inbreeding across successive rounds of seed increase. This year we had difficulty getting fruit set on some accessions of *S. habrochaites*, despite multiple rounds of pollinations. The problem was most pronounced in mid-winter, probably due to the short days and low light intensity. These accessions were replanted in the summer/fall in order to produce sufficient seed from most plants in each population to avoid inbreeding.

Preventing the spread of seed borne pathogens is an important aspect of our seed regeneration program. We inspect all our plantings throughout the growing cycle for disease symptoms. Plants displaying signs of disease are tested with Agdia ImmunoStrips. Our most persistent disease challenge is TSWV, vectored by the difficult to control Western flower thrips.

All stocks grown for seed increase or other purposes were systematically checked to verify that they expressed the expected phenotypes. New accessions were evaluated in greater detail, with the descriptors depending upon the type of accession (wild species, cultivar, mutant, chromosomal stocks, etc.). Plantings were reviewed at different growth stages to observe foliage, habit, flower morphology, fruit set, and fruit morphology. Images of selected accessions were uploaded to our website.

Cut stem of the *red vascular tissue* mutant.

Many genetic stocks, including various sterilities, nutritional, and weak mutants, cannot be maintained as true-breeding lines and must be transmitted from heterozygotes. Progeny tests are therefore made after each generation of seed increase to verify that individual seed lots segregate for the gene in question. Other accessions may show unexpected segregation or off-types due to outcrossing or mix-ups and need to be progeny tested to establish true breeding lines with the desired traits. This year we progeny tested 31 seed lots of male-steriles, other segregating mutants, and

stocks with questionable phenotypes, including the mutants *ms-12, ms-32, tl, tl*^{2}*, gh, gh-2, xan-2, nc,* and a *d* mimic. Other stocks were grown for observation and checking.

Samples of newly regenerated seed lots were catalogued, with most of the seed stored at -20°C for long term storage, and smaller aliquots stored at 4°C for filling seed requests. Following our standard practice, samples of seed were treated with mild acid and bleach to prevent transmission of seed borne pathogens and to meet export requirements for certain countries. As in the past, up to 1000 seed of newly regenerated seed lots were sent to the USDA National Laboratory for Genetic Resources Preservation in Ft. Collins, Colorado for long-term backup storage. A very high percentage of our accessions have been backed up to Ft. Collins, however some of the samples have been stored there for several decades; for accessions that have been stored for over 30 years we are submitting samples of more recently regenerated seed lots as well. This year 258 seed samples were backed up to NLGRP

DISTRIBUTION AND UTILIZATION

A total of 4,179 seed packets of 1,625 different accessions were distributed in response to 212 seed requests from 163 scientists, breeders, and educators in 15 countries. Relative to the size of the TGRC collection, the number of seed samples distributed represents a utilization rate of 92% (4179 samples/4536 accessions). Approx. 36% of our accessions were requested at least once in 2024. We also answered many informational requests regarding our stocks, growing recommendations, and related questions.

We continue to receive many requests for prebred lines such as introgression lines (ILs), recombinant inbred lines (RILs), and backcross inbred lines (BILs). A total of 362 seed samples of the *S. pennellii* ILs were distributed, 111 samples of the *S. habrochaites* ILs, 95 samples for the *S. lycopersicoides* ILs, and 288 samples of the *S. sitiens* ILs. We also sent out 5 samples of *S. lycopersicum* x *S. pimpinellifolium* RILs and BC-RILs, and 45 samples of *S. pennellii* BILs. The large number of *S. sitiens* ILs requested this year is notable, reflecting substantial interest in this germplasm resource which was recently released by the TGRC. Exotic germplasm libraries such as these require considerable time and expense to develop, but the investment is clearly justified because they are permanent resources that are widely used for breeding and research.

The various steps involved in filling seed requests – selecting accessions, treating, and packaging seeds, entering the information into our database, providing cultural recommendations, obtaining phytosanitary certificates, etc. – involve a large time commitment. The TGRC crew worked diligently to fill seed requests in a timely manner. Overseas shipments involve ever changing and increasingly stringent phytosanitary requirements. Shipment of seed to the European Union and many other countries continues to be challenging due to requirements for Tomato Brown Rugose Fruit Virus (ToBRFV) testing, however researchers can obtain a Letter of Authority or import permits granting exception to this rule. Fortunately, the ToBRFV restrictions so far apply only to seed of cultivated tomato, and not to its wild relatives.

Information provided by recipients regarding intended uses of our stocks are summarized in Table 3. As in previous years, there was a notable emphasis on biotic stresses, especially viral, bacterial, and fungal diseases, both for breeding purposes and for research. There continues to be great interest in screening for responses to ToBRFV, a tobamovirus which not only impacts crop production in many areas but also affects the seed industry because the virus is a quarantine pathogen in many countries. There seems to be increasing interest in research on human bacterial pathogens transmitted by plant products and implications for food safety. We received a large number of requests for research on plant parasitic nematodes, including *Meloidogyne incognita*. There continues to be strong interest in abiotic stress responses, especially drought, extreme temperatures (high and low), and salinity. Many other requests mentioned fruit traits (quality, carotenoids, etc), or breeding-related uses, especially marker development. Our stocks continue to be used for much genetics research, with emphasis this year on functional genomics, including association genetics, gene cloning, gene editing and transformation experiments. Finally, a wide variety of physiological/developmental studies were initiated this year, with many requests mentioning ethylene responses or root/rhizosphere biology.

Table 3. Intended uses of TGRC stocks as reported by requestors. Values represent the total number of requests mentioning each area of investigation. Requests addressing multiple topics may be counted more than once.

Biotic Stresses	
Viruses:	
ToBRFV	7
ToMV	2
TSWV	2
TYLCV	2
Unspecified viruses	1
Bacteria:	
Bacterial canker	1
Bacterial speck	1
Bacterial spot	1
Bacterial wilt	2
Endophytes	1
Human pathogenic bact.	3
Unspecified bacteria	3
Fungi:	
Alternaria alternata	1
Anthracnose black dot	1
Botrytis cinerea	1
Fusarium wilt	3
Late blight	1
Pythium	1
Phytopthora root rot	1
Unspecified fungi	1
Nematodes	5
Unspecified diseases	10
Insect pests:	
Spider mites	1
Tuta absoluta	1
Unspecified insects	3
Unspec. biotic stresses	8
Abiotic Stresses	
Boron toxicity	4
Drought	10
Flooding	2
High temperatures	9
Low temperatures	9
Salinity	7
Unspec. abiotic stresses	17
Fruit Traits	
Carotenoids, color	4
Flavor, volatiles	1
Fruit quality	3
Fruit shape	1
Fruit sugars	1
Other fruit metabolites	2
Parthenocarpy	1
Postharvest traits	1
Other fruit traits	1
Other Breeding	
Grafting, rootstocks	3
Germplasm diversity	1
Bee pollination	1
Horticultural traits	1
Linkage drag	1
Marker development	7
Male sterility	3
Molecular breeding	2
Ornamental traits	1
Prebreeding, wide cross	3
Reproductive traits	2
Seed testing	1
Somatic hybrids	1
Tissue culture	2
Unspecified breeding	13
Genetic Studies	
Allele mining	1
Association studies	6
Cytogenetics	1
Epigenetics	1
Gene cloning/function	6
Gene editing	4
Gene expression/RNAseq	2
Genome sequencing	4
Introgression lines	1
Quantitative traits, QTLs	3
Transformation	3
Physiology / Develop.	
Apical dominance	1
Autophagy	1
Ethylene responses	5
GeranylGeranyl Diphos.	1
Jasmonic acid responses	1
Light response/flowering	3
Metabolomics	1
Mineral nutrition	4
Mycorrhizae, rhizosphere	3
Phenylpropanoids	1
Photosynthetic capacity	1
Plant growth/develop.	2
Plant microbe interact.s	1
Reproductive barriers	1
Roots	6
Seed biology	1
Starch synthesis	1
Trichomes	2
Wounding, herbivory	1
Miscellaneous	
Backup seed storage	2
Instructional uses	2
Unspecified research	19

Our survey of the 2024 literature and unreviewed papers of previous years uncovered **139** publications, including journal articles, preprints, abstracts, theses, and patents (most reviews were excluded), that mention use of TGRC stocks. This impressive list of papers (see Bibliography below), many in high impact journals, demonstrates the positive impact of TGRC germplasm on research and breeding involving tomato. Many additional papers were undoubtedly missed, and utilization of our germplasm by seed companies and other commercial interests are generally not publicized.

DOCUMENTATION

Xiaoqiong Qin sampling plants in the greenhouse. Qin was featured in an article by Trina Kleist on the *S sitiens* introgression lines.

Our website was thoroughly redesigned and rewritten last year as part of a migration to the SiteFarm platform. This year's changes were more modest and focused on adding content or improving query functionalities. We added a biographical page recognizing the contributions of the late Dr. Carlos Ochoa, an expert on potato taxonomy and genetic resources with the International Potato Center in Peru who also donated many important wild tomato collections to the TGRC. We also added a page on our development of a set of introgression lines representing the genome of the wild nightshade *S. sitiens* via individual chromosomal segments in the genetic background of a large fruited fresh market variety. We improved our database with new queries and filters to facilitate information retrieval related to accessions and seed requests. We also improved labels on the seed packets we send to researchers by including only the most relevant, key descriptive information pertaining to each accession. As in the past, we continued to update our records on accessions with the latest information from our own observations or from data in the literature, including traits reported in specific accessions. We provided the USDA National Plant Germplasm System with basic passport data on accessions backed up to Ft. Collins for uploading into the GRIN-Global database, as well as our seed distribution statistics for reporting purposes.

RESEARCH

One of our current research projects focuses on identifying the genetic factors (QTLs) controlling seed vigor/dormancy, seed size, and seed and fruit set under heat stress. We are using the set of *S. sitiens* introgression lines (ILs) in cultivated tomato, developed at the TGRC, to map QTLs for these traits. We carried out greenhouse experiments to validate potential fruit set and seed set QTLs under heat stress. For seed trait QTLs, increased dormancy (i.e. reduced germination vigor) was mapped to several chromosomal regions, several of which harbor ABA-related genes that are known affect seed germination. We generated loss of function CRISPR mutants in three ABA-related genes in the corresponding IL background (e.g. *abi4* mutants were generated in the background of the IL containing the *ABI4* gene). Transgenic plants and their respective wild type IL lines were grown in the greenhouse to produce seeds for germination tests. We evaluated germination vigor under control, high and low temperatures, and saline conditions. This project was funded by a grant from the Foundation for Food and Agriculture Research.

Leaves of F_1 *S. lycopersicum* x *S. pennellii* LA0716 direct (L) and reciprocal (R) hybrids.

Our other research project focuses on the mechanisms of pollen rejection in wide crosses. The wild, green-fruited tomato species are cross-compatible with cultivated tomato, but typically only if the cultivar is used as the female parent. In the reciprocal crosses (wild species pollinated by cultivar), pollen tubes are arrested in the wild species' pistils, a barrier known as unilateral incompatibility. We previously showed that pollen rejection in crosses onto *S. pennellii* pistils results in part from high level expression of an ornithine decarboxylase (*ODC2*) gene in the pistils. We generated *S. pennellii* lines with loss of function mutations in both *ODC2* and a second pistil barrier gene, and found the double knockout lines accept cultivated tomato pollen, yielding viable F_1 hybrid plants. These hybrids are expected to have the same genetic makeup in their nuclear genomes, but not in their chloroplast and mitochondrial genomes, which are inherited only from the female parent. These alloplasmic lines (i.e. either *S. pennellii* or *S. lycopersicum* cytoplasms in a constant genetic background) provide tools to study the effects of the cytoplasmic genomes on plant growth and reproduction.

SERVICE AND OUTREACH

Roger Chetelat gave presentations on the TGRC, research projects, and related topics to the San Joaquin County Master Gardeners, Syngenta Seeds, the Foundation for Food and Agriculture Research, the Seed Biotechnology Center's Plant Breeding Academy (two cohorts), the USDA's Plant Germplasm Operations Committee meeting, and PLS 222 (Advanced Plant Breeding). Matt Valle and Xiaoqiong Qin led tours of our greenhouse facility for the PGOC meeting, and the PLS 222 and HRT 200B (Horticultural and Agronomy Practices) courses. Roger, Matt and Qin consulted with visiting scientists from East West Seeds, the Crop Trust, Meiogenix, and the Institute of Plant Genetic Resources in Sadovo, Bulgaria, and provided interviews to Bloomberg News and the Plant Sciences Department communications specialist.

PEOPLE

Roger Chetelat retired as TGRC Director/Curator, and is now working on a part-time, recall basis. The Dept of Plant Sciences is recruiting an Assistant Professor in Plant Genetics to become the new TGRC Director. Assistant Curator Matt Valle supervised undergraduate students Mercury Komjak, Kallan Arimura, Elizabeth Paul and Lola Bran. Elizabeth also did a research internship in the lab. Former student Jessica Garver graduated and was hired by Norfolk Healthy Produce to work in their purple tomato breeding program. Dr. Xiaoqiong Qin

continues to lead our research on seed vigor traits using *S. sitiens* introgression lines and the mechanisms of pollen-pistil incompatibility. Qin and her students also provided DNA marker services to the TGRC.

Sadly, our former graduate student Dr. Elaine Graham passed away unexpectedly this year. Elaine did her M.S. and Ph.D. research at the TGRC. Her Ph.D. project focused on genetic diversity and relationships in populations of *S. chilense,* and described the strong population structure and distinctive geographic races within this diverse species. She also found evidence for incipient crossing barriers (seed sterility) between the northernmost populations and the central or southern populations. She also participated in field work in Chile that expanded our collections of several wild tomato species from that country. And she mentored several undergraduate students in the lab and assisted other UCD researchers with projects on tomatoes. She went on to do a post-doc at the World Vegetable Center in Taiwan, then later took a position with Seminis/Monsanto (later Bayer) in Woodland. She continued to make contributions to tomato breeding and genetics throughout her career.

Elaine Graham, Roger Chetelat and Ricardo Pertuze above Putre, Chile, 2001.

TESTIMONIALS

"Thank you very much for your important service." – Sebastien Debande

"I would like to express my gratitude for your hard work and goodwill." – Yoonjung Lee

"If I had not met you 32 years ago, there would not have been any wild tomatoes in my greenhouse. Thank you for your continued support." – Toshihito Tabuchi

"Thank you very much for providing the seeds and it is indeed a great service. If anything I Can contribute to TGRC as a researcher, please let me know, I will definitely do my best to serve the TGRC if I can." – Channappa Gireesh

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Project title: Leveraging germplasm resources for genetic discovery and deployment of salt stress resilience

Year of Project Initiation: 2024

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Principle Investigator: Greg Vogel, Cornell University. Email: gmv23@cornell.edu. Phone: 607-255-9233

Co-PI(s) and affiliation(s): NA (Collaborator: Neil Mattson, Cornell University).

Executive Summary: Tomato is a moderately salt-sensitive crop, suffering yield losses in soils with an electrical conductivity (EC) higher than 2.5 dS/m, a value exceeded by many soils in the western San Joaquin valley. Salinization is expected to become more severe in the future as climate change affects precipitation patterns, water availability, and temperature. One potential solution is to develop varieties with increased tolerance to salt stress.

Last year, we began a project to discover novel salt stress resilience genes derived from a wild tomato species, *Solanum sitiens*, which is adapted to the driest non-polar desert on earth, the Atacama desert of northern Chile, and populations of which have been found growing in soils with an EC approaching 100 dS/m. Recently, Roger Chetelat at UC-Davis and collaborators released a population of introgression lines (ILs) of *S. sitiens* in the background of fresh-market *S. lycopersicum*. This population contains 56 lines which each contain a single fragment of *S. sitiens* DNA, representing an excellent genetic resource for the discovery of abiotic stress tolerance genes. Our objectives for Year 1 of this project were to validate the salt stress resilience of *S. sitiens,* increase seed of the IL population, and screen the *S. sitiens* ILs for their salinity resistance.

In a first experiment, we evaluated the response of *S. sitiens* accession LA4133, *S. pennellii* accession LA0716, and two tomato varieties – processing tomato hybrid SVTM9033 and the fresh-market parent of the IL population, LA4354 – to five doses of salinity ranging from a fertilizer-only control with an EC of 2.5 dS/cm to a 200 mM NaCl treatment with an EC of 22.5 dS/cm. Plants were grown to maturity in the greenhouse and treated with salt through drip irrigation with every irrigation event. The two domesticated tomato varieties showed similarly severe declines in yield with increasing salt stress, with an average 72.25% decline in yield from the control to the 17.5 dS/cm treatment. This yield decline was realized in different ways, with LA4354 predominantly demonstrating a decline in fruit size and SVTM9033 predominantly demonstrating a decline in fruit number. We could only evaluate vegetative biomass, and not fruit yield, for the two wild species accessions, since a negligible amount of their biomass is allocated to fruit. *Solanum sitiens* LA4133 was slow-growing in general, and in absolute terms, its decline of 0.27 lb in vegetative dry weight from the control to the 17.5 dS/cm treatment was the slightest of the four accessions. However, as a percentage of the control, its decline was actually the highest, showing a 73.39% decline compared to 47.87% for *S. pennelli* LA0716, 41.44% for SVTM9033, and 20.05% for LA4354.

At the start of this project, 39 of the 56 ILs were available for dissemination. We procured these lines and successfully increased seed of 35 of them, comprising 27 lines with introgressions in the homozygous state and 8 with introgressions in the heterozygous state. Due to limited greenhouse space, we proceeded to screen for salinity resistance only the 27 homozygous introgression lines, given the additional complexity in maintaining and using the heterozygous lines in downstream breeding.

The ILs and their domesticated tomato parent LA4354 were grown to maturity under a fertilizer-only control treatment and a salt treatment. Based on the results of the first experiment, we decided to treat the plants in the salt treatment with a 75 mM NaCl solution, targeting a total EC of 10.0. All 28 experimental entries (LA4354 and the 27 ILs) showed a decline in yield under the salt treatment compared to the control, with LA4354 showing a 77.73% decline and the ILs ranging in their decline from 45.35% to 81.69%. LA4354 outyielded 23 of the ILs under the control treatment but only 10 of the ILs under the salt treatment, and its relative decline in yield was more severe than that of all but 2 of the ILs. In addition, all but one of the ILs showed a greater increase in soluble solids under the salt treatment as under the control compared to LA4354, with the largest percent change for any IL corresponding to a 52.44% increase in Brix from 4.1 under the control to 6.25 under the salt treatment for LA5297.

Despite the sensitivity to salt demonstrated by its decline in vegetative biomass, the performance of the ILs indicate that *S. sitiens* LA4133 possesses genes that confer resilience to salinity stress. The fact that almost of the ILs were superior in salt resistance compared to their domesticated tomato parent, as indicated by their lower percent difference in yield between control and salt conditions, suggests that multiple genes are involved in this trait and that they are distributed across the *S. sitiens* genome. Our next steps in the short term are to fine-map these genes, further characterize their effects, and determine the extent of additional salt resistance that is achieved by pyramiding multiple genes together. The ultimate goal of this project is to develop breeder-ready donor lines and molecular markers to disseminate salt stress resilience genes for use by the tomato seed industry.

Introduction: Salinization is the result of water-soluble salts accumulating in soil. It occurs most frequently in arid and semi-arid regions of the world and is often the result of human activity, largely due to the usage of saline irrigation water. In California, approximately 4.5 million acres of cropland are affected by saline soils or saline irrigation water, with the majority of this acreage located in the western San Joaquin valley (Letey 2000). Saline soils affect crop health via several mechanisms: toxicity due to excessive uptake of certain ions, predominantly sodium and chloride; nutrient imbalance typically seen as an in-plant deficiency of potassium, nitrate, or phosphate; and drought stress caused by the more negative water potential in the soil (Ashrafi and Foolad 2012). Tomato is a moderately salt sensitive crop, suffering a yield impact in soils with an electrical conductivity higher than 2.5 dS/m, a value exceeded by many soils in the western San Joaquin valley (Hanson et al. 1999). The impact of climate change on precipitation patterns, water availability, and temperature will likely make soil salinization an even greater challenge in the future.

One possible strategy to maintain the profitability of processing tomato in areas impacted by salinization is to develop varieties able to tolerate higher levels of salt. This has been demonstrated to be effective in other crops: in wheat, a gene from an ancestral relative increased yield by 25% in saline soils when introduced into a modern variety (Munns et al. 2012). Tomato breeders and geneticists have historically leveraged diversity in tomato wild relatives for introducing new traits into domesticated tomato. For example, almost all of the disease resistance genes that have been deployed in commercial varieties were introgressed from wild species. Several wild relatives have been demonstrated to possess heightened salt tolerance, including *Solanum pimpinellifolium, S. pennellii, S. habrochaites,* and *S. cheesmanii*. In addition, genetic studies using crosses between these wild relatives and the domesticated tomato have mapped regions of the genome where the causal salt stress genes reside (Foolad 2004).

Nevertheless, the results of these genetic mapping studies have not been used in practical breeding to develop salt-resilient tomato varieties. There are several likely reasons for this. For one, many of these studies only characterized salt stress at germination or early seedling stages, although genes controlling salt stress resilience are often growth stage-dependent (Foolad 2004). For transplanted tomato production, salt tolerance at later vegetative and reproductive stages is likely more agronomically relevant. In addition, in many cases, salt stress resilience was found to be controlled by several to many genes, each of modest effect, making their utility in breeding less practical (Villalta et al. 2007). Finally, beneficial genes found in wild relatives are almost always genetically linked to additional genes with unfavorable horticultural effects, a phenomenon known as "linkage drag." Many of the populations (F2 or BC1) that have been used for salt stress mapping in the past have contained plants with 25% or 50% of their genomes on average derived from their wild parent, limiting their direct value in applied breeding.

Introgression lines (ILs) are a genetic resource consisting of a panel of inbred lines, each containing a small portion of the genome of a wild species donor in the background of an agronomically elite, domesticated parent. Recently, Chetelat et al. (2019) reported the release of a panel of ILs of *S. sitiens*, a wild tomato relative adapted to one of the harshest environments on earth, the Atacama desert of Chile. Each IL features on average 19.2 Mb of DNA, substituting approximately 2% of the domesticated tomato genome. These lines have been used to identify genes involved in morphological variation but little work has been done to date to characterize them for abiotic stress tolerance, including salt stress resilience (Chetelat, personal communication). While the salt tolerance levels of the specific *S. sitiens* parents of the ILs is unknown, it is reasonable to assume that they possess genes allowing them to tolerate high levels of salinity: populations of *S. sitiens* in the wild have been found growing in basins with visible salt deposits and with ECs as high as 96 dS/m, almost 40 times the threshold level for yield in domesticated tomato (Chetelat et al. 2008).

Given the severity of the environment to which *S. sitiens* is adapted, we hypothesized that the ILs contain genes with individual effects large enough for commercial utility in tomato breeding. In addition, as a "prebred" genetic resource resulting from almost 30 years of crossing and selection (Chetelat et al. 2019), the ILs provide a tremendous head-start for gene discovery and deployment compared to the usage of simpler mapping populations in which each plant contains a larger proportion of DNA from the wild relative.

The main goal of this project is to discover, isolate, and validate salt tolerance loci from *S. sitiens* for development of breeder-ready salt stress resilience donor lines. The first year of this project, the results of which are described below, consists of three objectives focusing on protocol development and discovery of salt tolerance in an *S. sitiens* IL population.

Objectives:

- Validate salt stress resilience of *S. sitiens* IL parents and identify discriminate salt dosage for use in screening IL population.
- Increase seed of IL population to ensure sufficient seed for further experimentation, breeding, and collaboration.
- Determine salt stress resilience levels of complete IL population and identify introgressions for future characterization and transfer.

Methodology and Results:

***Objective 1*. Materials and Methods.** In order to validate the salt stress resilience of the *S. sitiens* IL parent and identify a discriminate salt dosage for use in later experiments, we evaluated 4 *Solanum* accessions for either their fruit yield or vegetative biomass under each of five salt treatments in a greenhouse experiment. *Solanum* accessions comprised: 1) *S. sitiens* accession LA4331, one of the two *S. sitiens* parents of the IL population; 2) *S. pennelli* accession LA0716, a different wild species accession previously reported for its salt tolerance (Frary et al. 2010) and included as a check; 3) *S. lycopersicum* accession LA4354, a determinate fresh-market tomato inbred line developed by the North Carolina State University tomato breeding program and used as the domesticated parent of the IL population; and 4) SVTM9033, a processing tomato hybrid reported to demonstrate salt stress sensitivity (J. Deniz and L. Stevens, personal communication). We included only LA4331 and not LA1974, the other *S. sitiens* accession used in the development of the IL lines, since LA4331 was the donor for the majority of introgressed segments in the population and we were unable to include additional entries due to limited greenhouse space. Seed for LA4331, LA0716, and LA4354 were provided by the Tomato Genetics Resource Center (TGRC) and seed for SVTM9033 was provided by Ag Seeds.

Seeds were sown in 72-cell trays in a Cornell University greenhouse. Seed of LA4331 and LA0716 were soaked in 50% bleach prior to sowing to improve germination (60m for LA4331 and 30m for LA0716). Plants were transplanted into 2-gal pots 32 to 49 d after sowing, depending on the accession. Cornell mix was used for all accessions except LA4331, which was grown in a modified version of the desert mix recommended by the TGRC, comprised of 25% sphagnum peat, 50% perlite and 25% sand, with 0.05 lb dolomite and 0.05 lb crushed oyster shell per 3.8 cubic feet of media.

Greenhouse temperature controls targeted 75 F day / 65 F night and supplemental lighting was used to provide a 13h photoperiod. Plants were fertigated with Jack's 15-15-15 CA-MG LX fertilizer (J.R. Peters) at a rate of 200 ppm N until 53 d after sowing, at which point they began additionally receiving one of five salt treatments with every irrigation event. Treatments comprised: 1) Fertilizer only (EC ~2.5 dS/m); 2) Fertilizer plus 50 mM NaCl (EC ~7.5 dS/m); (3) Fertilizer plus 100 mM NaCl (EC ~12.5 dS/m); 4) Fertilizer plus 150 mM NaCl (EC ~17.5 dS/m); and 5) Fertilizer plus 200 mM NaCl (EC ~ 22.5 dS/m). All treatments were delivered to plants through an automated drip irrigation system. Supply irrigation water was injected with fertilizer via a Dosatron fertilizer injector, before splitting into separate lines that either ran directly to the plants in the case of the control treatment, or were injected with additional NaCl for the salt treatments. For the 7.5 EC treatment, a Mixrite 1:100 injector was used and for the 12.5 EC, 17.5, and 22.5 EC treatments, a Newtry 1:10 injector was used. Meinor two-outlet automatic water timers were used to set irrigation cycles for the lines corresponding to each salt treatment, with a separate cycle for LA4331 compared to the other three tomato accessions, due to its lower water requirement. Irrigation cycles were adjusted over the course of the experiment according to plant growth stage, and included two to four irrigation events per day, with one of the events per day long enough to achieve leaching of pots. Each pot contained one or two drip stakes, depending on the variety and its water needs.

The experiment was arranged in a split-plot design where salt treatment was the main plot and tomato accession the sub plot. Main plots were arranged in a randomized complete block design where a block corresponded to an entire greenhouse bench. Each experimental unit consisted of a pot with a single plant.

Every unique salt treatment–accession combination was replicated three times, except there were only two replicates of SVTM9033 under the 12.5 dS/cm and 17.5 dS/cm treatments due to germination issues.

Plants were not pruned of any shoots or leaves and were supported with bamboo stakes as needed. Flowers were manually vibrated daily to promote pollination. Every week, a subset of pots were tested for their pour-through EC so that over the course of the experiment, each pot was tested three times. Pour-through EC was measured by watering pots with approximately 500 ml of reverse osmosis water, collecting leachate, and measuring EC with a Hanna Instruments combined pH/EC meter.

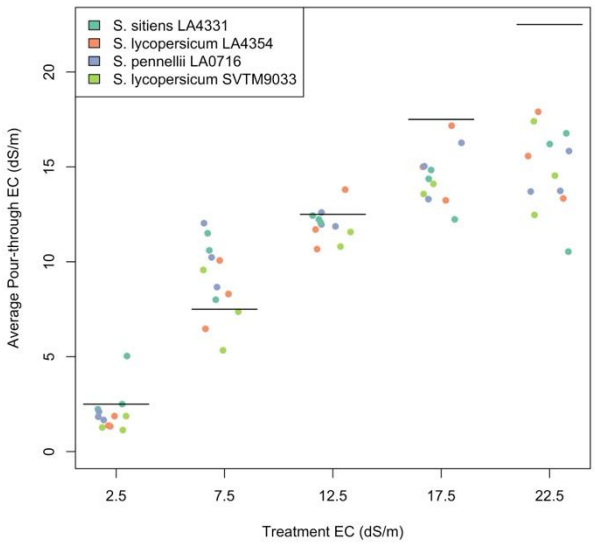

Figure 1: *Pour-through EC measurements averaged over the course of the experiment for pots under each treatment.*

Fruit from LA4354 and SVTM9033 were harvested once or twice a week at the breaker stage of ripening or beyond beginning 76 d post sowing (23 d post initiation of salt treatments). No fruit were harvested from LA0716 or LA4331 due to their small fruit size. Fruit were both weighed and counted from each pot at every harvest time point, as well as sorted into marketable and non-marketable fruit based primarily on the presence or absence of blossom end rot. At the conclusion of the experiment, 116 d post sowing (63 days post initiation of salt treatments), all fruit including green fruit were harvested and weighed from plants. All vegetative biomass above the soil surface was then bagged, dried and weighed to measure vegetative biomass.

Data was analyzed using Analysis of Variance (ANOVA) procedures for split-plot experimental designs using mixed model functions as implemented in packages lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017) in the R statistical programming language (R version 4.4.1; R Foundation for Statistical Computing, Vienna, Austria). Estimation of treatment means and comparisons were performed using the R package emmeans (Lenth 2024).

Results. Pour-through EC measurements across the experiment were significantly different according to salt treatment ($P < 2 \times 10^{-16}$), averaging 2.02 dS/cm for the 2.5 dS/cm fertilizer control, 9.01 dS/cm for the 7.5 dS/cm treatment, 11.84 dS/cM for the 12.5 dS/cm treatment, 14.27 dS/cM for the 17.5 dS/cm treatment, and 14.83 dS/cM for the 22.5 dS/cm treatment (Figure 1). There were no significant differences in pour-through EC based on *Solanum* accession ($P = 0.10$). It is unclear what accounted for the differences between pour-through EC and the EC delivered to plants through the drip system, especially in the higher salinity treatments. The 22.5 dS/cm treatment was particularly problematic, failing to reach an average pour-through EC higher than 17.9 dS/cM in any pot. As a result, data corresponding to the 22.5 EC treatment was removed and not included for any further analysis.

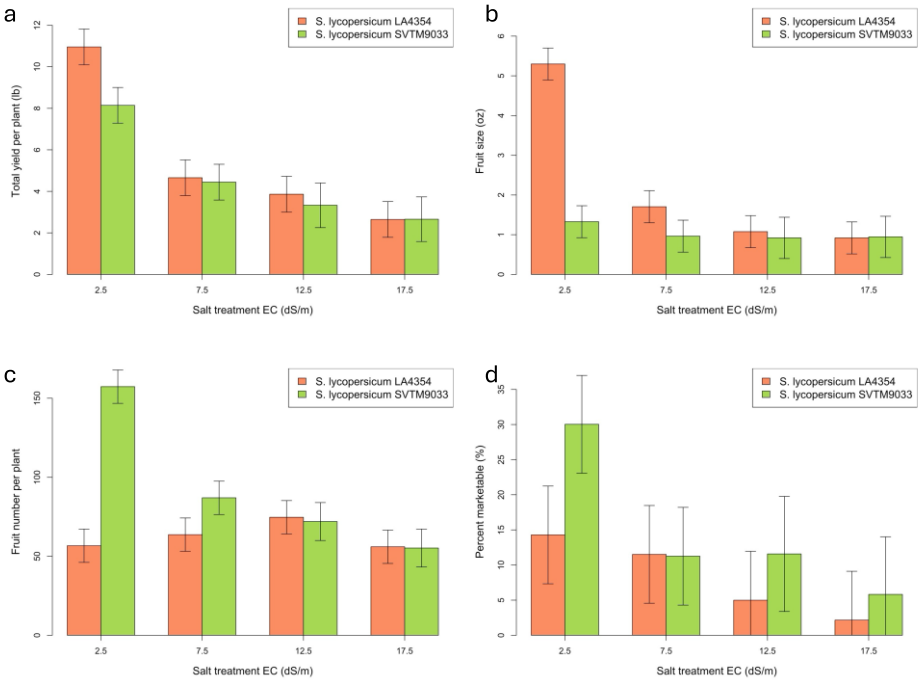

Figure 2: *Means and standard errors of LA4354 and SVTM9033 for total yield (a), fruit size (b), fruit number (c), and percent marketable yield (d) under four different salt treatments.*

For the two tomato accessions, processing hybrid SVTM9033 and fresh-market inbred line LA434, fruit yield was significantly affected by salt treatment ($P < 5.10 \times 10^{-4}$), but not by variety ($P = 0.13$) or the interaction between variety and treatment ($P = 0.24$). With increasing salt concentration, the drop in yield was the greatest between the 2.5 dS/cm control and 7.5 dS/cm treatment, decreasing 5.00 lb from an average of 9.55 lb under the control to 4.55 lb under 7.5 dS/cm (Figure 2a). In comparison, yields only dropped 0.95 lb from 7.5 EC to 12.5 dS/cm and 0.94 lb from 12.5 dS/cm to 17.5 dS/cm.

Interestingly, the yield response in the two varieties was realized through two different mechanisms, as LA4354 demonstrated a decrease in fruit size with increasing salt concentration, and SVTM9033 demonstrated a decrease in fruit number (Figure 2b and 2c). Both traits were significantly affected by salt treatment ($P = 1.38 \times 10^{-3}$ and $P = 0.04$ for fruit size and fruit number, respectively), variety ($P = 3.14 \times 10^{-3}$ and $P = 3.21 \times 10^{-4}$), and the interaction between variety and treatment ($P = 3.32 \times 10^{-3}$ and $P = 2.38 \times 10^{-4}$). Fruit sizes were low in general because measurements for fruit with blossom end rot were included.

The percent marketable fraction was low across the board, including in the control treatment, which averaged 22.16% marketable across the two varieties (Figure 2d). Percent marketable was almost entirely driven by blossom end rot, which is common in our relatively low-light conditions in the greenhouse in winter, which was when this experiment was conducted. There were no significant differences in percent marketable due to salt treatment ($P = 0.25$), variety ($P = 0.09$), or their interaction ($P = 0.36$).

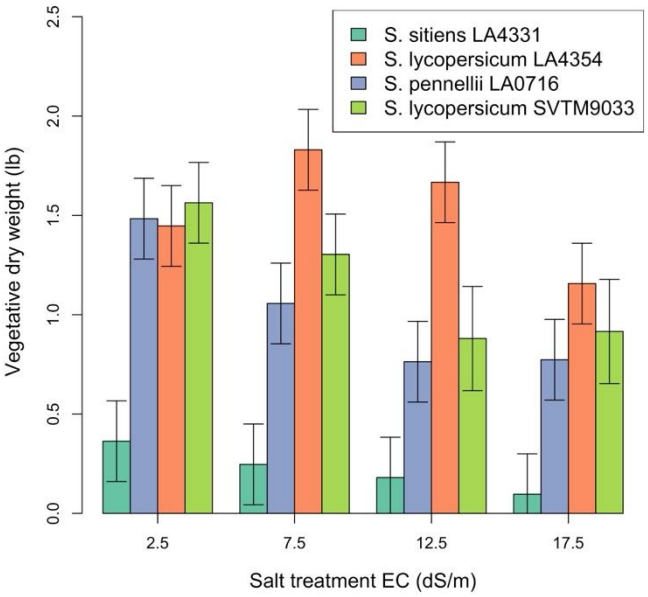

Figure 3: *Means and standard errors for vegetative dry weight for four Solanum accessions under four salt treatments.*

Vegetative biomass was compared at the conclusion of the experiment across the four *Solanum* accessions. Dry biomass weights were significantly affected by salt treatment (P = 0.03) and variety P = 4.88 x 10^{-8}), but not by their interaction (P = 0.38), indicating that the four accessions showed similar responses to increasing salt concentration. The greatest absolute decline in biomass weight between the control treatment and the highest salt treatment, 17.5 dS/cm, was seen in *S. pennellii* LA0716, with a difference of 0.71 lb, followed by SVTM9033 with a difference of 0.65 lb, LA4354 with a difference of 0.29 lb, and *S. sitiens* LA4331 with a difference of 0.27 lb (Figure 3). However, considering instead the percent decline between the two treatments in order to account for the overall differences in vegetative biomass production between the four accessions, the most severely affected accession was actually *S. sitiens* LA4331, with a 73.39% decline in dry weight from the control to the 17.5 dS/cm treatment, followed by *S. pennellii* LA0716 with a 47.87% decline, SVTM9033 with a 41.44% decline, and LA4354 with a 20.05% decline.

Objective 2. **Materials and Methods.** When we initiated this project, 39 of the 56 ILs were available from the TGRC. Of these 39 ILs, 30 carry introgressions in homozygous condition and 9 carry introgressions in heterozygous condition. Six seed of each homozygous IL and 24 seed of each heterozygous IL were sown in 72-cell trays. Tissue was collected from plants of heterozygous ILs and DNA extracted using a CTAB method. We then assayed each sample with two Cleaved Amplified Polymorphic Sequence (CAPS) molecular markers tagging their introgressions, using a subset of the published markers in Chetelat et al. (2019). Based on the assay results, one or two plants carrying the introgression for each heterozygous IL were selected and potted in 2-gal pots. For the homozygous ILs, one plant of each was potted in a 2-gal pot.

Flowers from all plants were vibrated daily to promote self-pollination. In addition, we backcrossed plants from heterozygous ILs to the domesticated parent LA4354. Seed from ripe fruit were processed following Cornell protocols, including a 9% hydrochloric acid treatment to digest fruit tissue and a 10% trisodium phosphate treatment for treating potential seedborne pathogens.

Results. Of the 30 homozygous ILs, we successfully produced >1 g seed for 14 lines and <1 g seed for an additional 13 lines. We were unable to increase any seed for 3 of the lines. Of the 9 heterozygous ILs, we produced >1 g of selfed seed for 5 lines and <1 g of selfed seed for an additional 3 lines. In addition, we produced small quantities (ranging from 2 to 39 seed) of backcrossed seed for each of 8 of the lines. We were unable to produce any selfed or backcrossed seed for 1 of the heterozygous ILs.

Objective 3. **Materials and Methods.** We proceeded to screen 27 of the introgression lines and the domesticated parent LA4354 for their salt stress resilience by evaluating them for their fruit yield under

control (fertilizer only; EC ~2.5 dS/cm) and salt stress (fertilizer plus 75 mM NaCl; EC ~10 dS/cm) conditions. The 27 ILs included in the experiment were chosen out of the 39 based on seed availability and introgression type, with none of the heteryzygous ILs included due to their additional complexity in maintaining and applying in downstream breeding. These 27 ILs included introgressed segments on all 12 tomato chromosomes, collectively covering 501.1 cM of the tomato genome, representing approximately 30% of the complete tomato genome, 32% of the coverage of the complete IL population, 63% of the coverage of the 39 available lines, and 87% of the coverage of the 30 available homozygous lines.

As in the first experiment, salinity treatments were applied with every irrigation cycle via an automatic drip irrigation system with a few modifications from the earlier experiment. A two-part fertilizer consisting of Jack's 5-12-16 and Jack's 15-0-0 Calcium Nitrate (J.R. Peters) was used at a 1:1 ratio to deliver a rate of 312.5 ppm N to both control and salt treatment plants. In addition, sulfuric acid was injected to lower the fertigation solution pH to 6.0. Fertilizer and acid were injected using Dosatron injectors and NaCl was injected into the irrigation line for plants receiving the salt treatment using a 1:10 Newtry injector. Salinity treatments were applied to plants beginning 52 d after sowing (14 d after transplant to 2-gal pots). Fruit were harvested over a 48 d period, beginning 83 d after sowing (31 d after initiating salt treatments) and ending 131 d after sowing (79 d after initiating salt treatments).

Each IL-treatment combination was unreplicated, with one plant of each IL receiving the salt treatment and another plant receiving the control treatment. The susceptible control, LA4354, was replicated three times under each treatment, although data from the third replicate was not used due to the plant receiving the control treatment demonstrating an abnormal growth phenotype. A second replicate of the complete experiment was initiated in December and is currently ongoing.

Growth conditions were the same as in the first experiment with a few exceptions. Bumblebees were used for pollination as opposed to manual vibration to achieve better fruit set. In addition, plants were pruned so as to bifurcate plants to two leaders. Accessions with a determinate growth habit were pruned of all shoots up to the first flower cluster on each leader and accessions with an indeterminate growth habit were pruned of all shoots up to the second to last flower cluster for the remainder of the experiment on each leader.

Data collection was conducted as in experiment 1. In addition, we collected soluble solids readings from up to 3 fruit per plant at two harvest time points, 109 d and 122 d after sowing. To ensure balanced data, we only retained soluble solids readings for analysis for plots which had ripe fruit harvested on both of the time points.

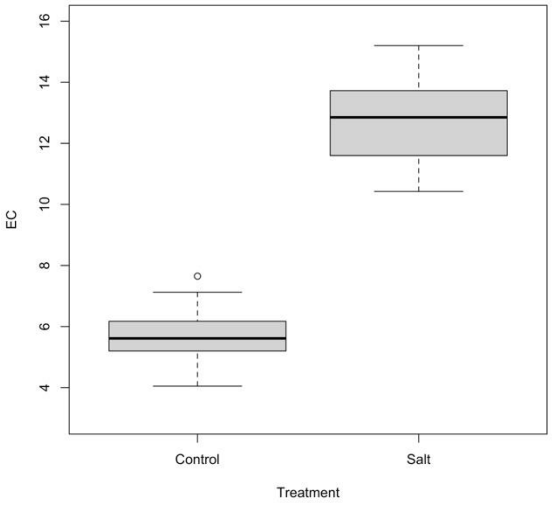

Figure 4: *Pour-through EC measurements for pots receiving the control and salt treatments averaged over the course of the IL screening experiment.*

Results. As in the first experiment, pour-through EC measurements differed from the EC of the irrigation solution delivered to plants. Pour-through ECs averaged 5.66 dS/cm in pots receiving the 2.5 EC control and 12.69 dS/cm in pots receiving the 10 EC salt treatment. Nevertheless, there was a clear separation between the two treatments in terms of their pour-through EC measurements averaged over the course of the experiment (Figure 4).

Each of the 27 ILs and LA4354 showed a yield decline under the salt treatment compared to the control (Figure 5a). LA4354 ranked 5th in terms of total yield under the control and 17th in terms of total yield under the salt treatment. In terms of the magnitude of the percent decline in yield between the control and salt treatments, LA4354 ranked 3rd with a 77.70% decline in yield. The percent decline in yield ranged from a minimum of 45.35% for LA5264 to 81.70% for LA5290 (Table 1). Variation in yield responses to salt were visually apparent among the set of accessions (Figure 6).

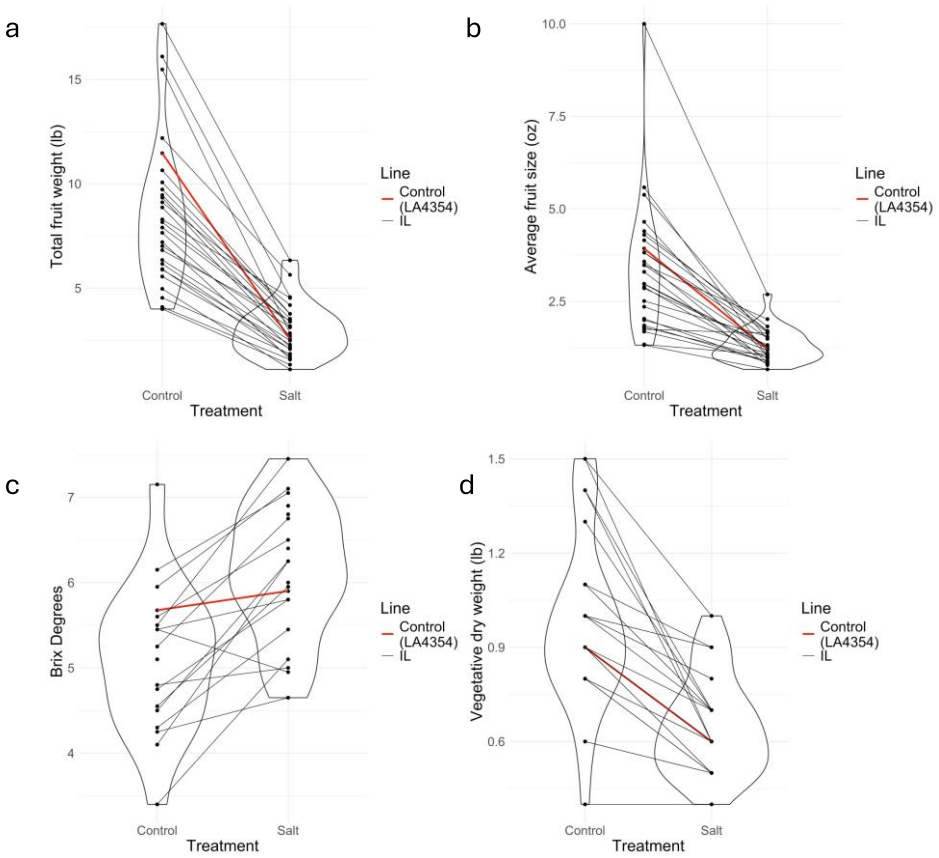

Figure 5: *Paired violin plots showing the performance of 27 ILs and their recurrent parent LA4354 for total yield (a), fruit size (b), soluble solids (c), and vegetative dry weight (d) under control and salt conditions.*

Average fruit size also declined under the salt treatment compared to the control for each of the 28 tomato accessions, ranging from a percent decline of 6.17% for LA5252 to 81.44% for LA5272 (Figure 5b). LA4354 ranked 8th for fruit size under the control, 15th for fruit size under the salt treatment, and 7th in terms of the magnitude of its decline between the two treatments.

Of the 16 accessions for which we had soluble solids data for both treatments, all but one showed an increase in Brix under the salt treatment compared to the control (Figure 5c). Of these 15 accessions, LA4354 showed the slightest increase, a 3.96% percent change from 5.68° Brix to 5.90° Brix, compared to an average percent change for the rest of the accessions of 23.06%, with a maximum of 52.44% for LA5297, which demonstrated 4.10° Brix under the control and 6.25° Brix under the salt treatment.

Vegetative biomass declined under the salt treatment compared to the control for all accessions except LA5265, which produced 0.4 lb of vegetative dry weight under both treatments (Figure 5d). LA4354 showed

an intermediate response compared to the ILS, ranking 14th in vegetative biomass under the control, 13th in vegetative biomass under the salt treatment, and 13th in terms of its percent decline in salt compared to control.

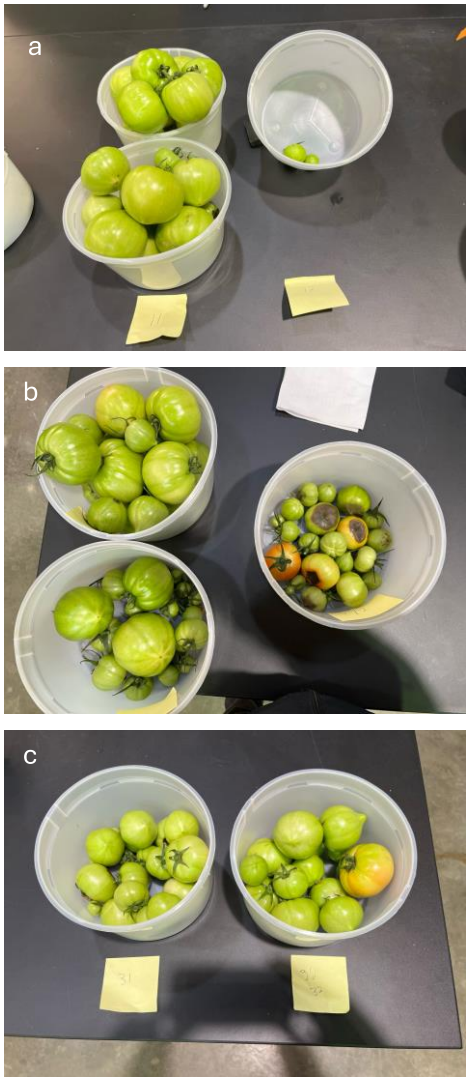

Figure 6: *Green fruit harvests at the conclusion of the experiment for ILs representing a range of degrees of salt sensitivity. Top – LA5290, control on the left and salt on the right. Middle – LA5259, control on the left and salt on the right. Bottom – LA5264, control on the right and salt on the left.*

Discussion: We successfully developed protocols to evaluate tomato plants for yield resilience under salt stress in greenhouse conditions. Unlike many existing studies on tomato salt stress—which often focus on vegetative growth after brief salinity exposures in young plants— our approach involves growing plants to maturity and assessing their relative yield. We anticipate that this method will provide more field-relevant insights. However, some challenges remain. Our salt stress treatment was limited to NaCl, whereas saline groundwater or soil in a realistic field setting would contain a complex mixture of ions including some with high potential toxicity such as boron compounds. We originally aimed to test plant responses to multiple salinity treatments representing different chemical constitutions but did not have sufficient greenhouse space to accommodate additional experiments. Additionally, we encountered issues with treatment consistency, as pour-through EC measurements systematically deviated from the irrigation supply's EC values. That being said, we were still able to apply a salt stress treatment in the second experiment that resulted in strong, consistent differences from the control, even though the pour-through ECs of pots in both treatments were consistently higher than our targets.

One of the biggest surprises from this project was the response of LA4331, the *S. sitiens* parent of the IL population, to increasing salt concentrations. Not only was its percent decline in vegetative biomass more severe than that of *S. pennelli* LA0716, it was also more severe than that of both of the tomato varieties included in the experiment. However, it is difficult to directly compare the salt tolerance of the *S. sitiens* accession to the two tomato varieties based on vegetative biomass due to their dramatically different physiologies. Over the course of the 116 d experiment, LA4331 set almost no fruit, and the small fruit that it did produce represented a negligible portion of its total biomass. The two tomato varieties, on the other hand, showed dramatic declines in fruit yield with increasing salt concentration.

The response of the ILs to the salt treatment made clear that *S. sitiens* possesses genes that contribute to salinity stress resilience in the background of domesticated tomato. All but 2 of the ILs showed a reduced percent decline under salt stress compared to their recurrent tomato parent, LA4354. In addition, all but 1

of the ILs showed a greater increase in soluble solids under salt stress compared to LA4354. That being said, it is important to note that none of the ILs demonstrated a complete insensitivity to salinity, and even the line that was least sensitive in terms of its yield response still showed a 45% decrease in yield under the salt treatment (compared to 78% for its recurrent tomato parent LA4354.

Accession	Introgression Chromosome	Introgression Position (cM)	Fruit yield - Control (lb)	Fruit yield - Salt (lb)	Percent Difference (%)
LA5264	5	0-28.9	8.29	4.53	45.35
LA5289	10	38.9-84.5	6.82	3.43	49.64
LA5265	5	8-47.8	12.19	5.64	53.75
LA5256	3	67.1-96.2	9.11	4.18	54.09
LA5298	12	73.3-99.1	7.02	3.12	55.52
LA5259	4	4-28	7.90	3.40	57.00
LA5274	7	43.4-57.3	8.16	3.40	58.39
LA5277	8	0-4.4	4.54	1.85	59.29
LA5270	6	56-78.6	6.35	2.49	60.79
LA5258	4	0-38	5.88	2.29	61.05
LA5286	9	64.2-90	4.09	1.57	61.56
LA5287	10	0-26	5.56	2.09	62.39
LA5276	7	66-74.8	10.07	3.76	62.62
LA5263	5	0-16	9.72	3.53	63.71
LA5297	12	58-99.1	17.66	6.33	64.18
LA5275	7	62-74.1	10.65	3.77	64.63
LA5262	4	87-108.1	6.16	2.08	66.33
LA5252	2	51.2-60.1	9.46	3.18	66.36
LA5257	3	67.1-105.4	4.00	1.33	66.71
LA5295	12	0-25	5.91	1.73	70.78
LA5282	9	0-23.7	9.34	2.70	71.12
LA5272	7	0	8.87	2.56	71.12
LA5246	1	47-78.9	7.65	2.18	71.51
LA5243	1	0-20.8	16.10	4.57	71.62
LA5247	1	60.6-94.5	7.20	1.61	77.63
LA4354	NA	NA	11.46	2.56	77.70
LA5254	3	9.6-70.9	4.96	1.11	77.73
LA5290	11	0-6.6	15.48	2.83	81.69

Table 1: *Introgression positions and yields for 27 ILs and recurrent parent LA4354, sorted from lowest percent different from salt to control to highest.*

The fact that increased salt stress resilience was observed in almost all of the ILs suggests that this trait is controlled in *S. sitiens* by many genes distributed across the genome. Further experimentation is needed to answer whether the effect size of any individual introgression is large enough to be commercially relevant. If not, it is possible that multiple introgressions from *S. sitiens* need to be combined in order to achieve levels of salt stress resilience that are high enough to warrant their deployment in commercial cultivars. Similar strategies involving combining multiple wild species introgressions were used to breed high-anthocyanin tomato varieties with introgressions from *S. chilense, S. lycopersicoides,* and *S. cheesmaniae* (Mes et al. 2008), as well as acylsugar-producing, insect-resistant tomato varieties with introgressions from *S. pennellii* (Mutschler 2021).

Although we are awaiting the results of a second replicate of the experiment to validate our findings, our data point to several lines as promising candidates for further characterization. Two of the top three ILs in terms of their percent decline in yield under salt stress, LA5264 and LA5265, contain overlapping introgressions on chromosome 5. It is possible that a gene or genes with strong effect on salt stress insensitivity reside in the overlapping interval between 8 and 28.9 cM. LA5265 was also one of four ILs, in addition to LA5290, LA 5243, and LA 5297, that outyielded LA4354 under both salt *and* control conditions.

The immediate next step for this project is to validate our findings by completing the second replicate of the IL screening experiment. We anticipate that the complete results will confirm 3-4 ILs as the most promising in terms of their salt stress resistance. We will then initiate several paths of research, including backcrossing introgressions to a processing tomato background and fine-mapping salt stress resistance genes, in order to further characterize these potential sources of resistance and develop lines that are ready for trialing in the field.

Acknowledgements: Thank you to Taylor Anderson (Bayer), Jonathan Deniz (TSL Seed), and Lance Stevens (Ag Seeds) for providing information on salt sensitive processing tomato hybrids and for Ag Seeds for providing seed for SVTM9033. Thank you to Roger Chetelat for advice on experimental procedures and to the TGRC for providing seed of the IL population and parents.

Other Support: This research was supported in part by start-up research funds for PI Vogel granted by the Cornell College of Agriculture and Life Sciences (approximately $20,000 for additional technician salary and greenhouse space). I am a collaborator on a pending seed grant submitted in 2024 by Len Pysh, Roanoke College, to the USDA AFRI Foundational and Applied Science Program focused on variation for salt stress tolerance within domesticated tomato. My role in the project is to provide support and feedback to the PI on greenhouse methods for measuring the relationship between salinity and fruit yield, based on experience from our current CTRI-funded project. In addition, I am expecting to leverage the results of our current project to submit a grant to the USDA AFRI Foundational and Applied Science Program, either in 2025 or 2026, on abiotic stress resistance discovery in wild tomato.

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Project Title: Decoding Resistance-Breaking Root-Knot Nematodes: A Statewide Survey and the Path to Diagnostic Tools in California's Tomato Fields

Year of Project Initiation: New Project in 2024

CTRI Funding in 2024: $33,422

Principle Investigator: Shahid Siddique, Associate Professor of Entomology Nematology, Department of Entomology and Nematology, University of California Davis.
Email: ssiddique@ucdavis.edu
Phone: 530-754-7659
Office: 4208 Storer Hall

Executive Summary:

Root-knot nematodes (*Meloidogyne* spp.) cause an estimated 2–5% annual yield reduction in California's processing tomatoes, corresponding to a loss of approximately $30–75 million per year (based on 2023 market values). These plant-parasitic nematodes are not only prevalent throughout California but also pose a major threat to agriculture worldwide. Their wide host range (about 2,000 plant species) makes them particularly difficult to control in the absence of resistant cultivars. Furthermore, ongoing efforts to reduce pesticide use place additional constraints on the available management strategies. Root-knot nematodes have a sedentary endoparasitic lifestyle. Infective second-stage juveniles (J2s) enter plant roots, establish a feeding site, and remain there while feeding and completing their development. Once mature, females lay egg masses containing 200–400 eggs, which restarts the nematode lifecycle. Under optimal conditions, these nematodes can go through up to five complete lifecycles per growing season, intensifying their impact.

Even though they are mostly microscopic, root-knot nematodes can proliferate quickly, causing visible damage such as chlorosis and reduced yield. Their feeding diverts nutrients away from leaves and fruit, and they can suppress the plant's immune system, completing their lifecycle undetected within the roots. A hallmark of root-knot nematode infection is the formation of galls (swollen masses) on the roots. These galls can leak nutrients and water from damaged cells, creating entry points for secondary pathogens such as *Fusarium* spp., *Phytophthora* spp., *Verticillium* spp., and *Sclerotium* spp. Although root-knot nematodes require a living host to survive, these secondary pathogens can kill the plant entirely. Notably, about 99% of California's processing tomato varieties contain the *Mi-1* gene, which confers resistance to several closely related root-knot nematode species within the *Meloidogyne incognita* group (*M. incognita, M. javanica,* and *M. arenaria*). This gene was bred into commercial cultivars in the 1940s from a wild tomato and remains the only known root-knot nematode resistance gene in tomato. However, overreliance on *Mi-1* has led to the emergence of isolates throughout California that can break this resistance.

Verifying resistance-breaking requires that nematodes be tested at stable soil temperatures (25–27°C). At temperatures above 28°C, Mi-1 loses effectiveness, so testing must confirm true resistance-breaking at moderate conditions. Field samples are brought to the lab, where eggs are extracted and inoculated onto resistant and susceptible tomato varieties. After 2-3 months, any isolates causing heavy galling in both resistant and susceptible varieties under stable conditions are deemed resistance-breaking. Such isolates are then purified by single egg mass isolation (taking advantage of the nematodes' asexual reproduction) and expanded into clonal populations. DNA is extracted from individual nematodes to confirm the species via sequencing. This entire process—from initial field sampling to confirmed sequencing—can span several months.

With support from current CTRI grant, our lab collected 14 root-knot nematode isolates from processing tomato fields across California between July and October 2024, corresponding to the tail end of the tomato growing season. For each of these 14 isolates, we recorded precise field coordinates (including latitude and altitude), enabling us to map their distribution and create a visualization of resistance-breaking across the state. Symptoms in sampled fields ranged from chlorosis and localized patches of dead plants to heavily galled roots, with secondary pathogens—especially *Fusarium* spp.—commonly present. All 14 isolates we collected were confirmed as true resistance-breaking. In laboratory tests at 25–27°C, both resistant and susceptible tomato varieties exhibited extensive galling. Three of these isolates have been sequenced and identified as *M. incognita*. The remaining 11 isolates are undergoing single egg mass isolation to obtain purified clonal populations, which will then be sequenced—this step is scheduled for March 2025. Each field advisor will be contacted with the results once species identification is complete.

In addition to our newly gathered isolates, the University of California, Riverside (UC Riverside) had previously collected other resistance-breaking isolates from multiple locations across the state. By combining our isolates with UC Riverside's historical samples, we aim to form a more comprehensive picture of where these resistance-breaking populations are developing. Nevertheless, the 14 isolates represent less than 1% of California's processing tomato acreage, so our current data provide an initial snapshot rather than a complete statewide assessment.

We encourage growers, Pest Control Advisors (PCAs), Cooperative Extension personnel, and industry partners to examine roots routinely for signs of root-knot nematodes. Greater sampling coverage will deepen our understanding of these resistance-breaking populations, clarify how and where they arise, and aid in developing more targeted management tactics. In the future, our lab hopes to design a diagnostic primer that can rapidly identify resistance-breaking isolates in weeks rather than months, transforming root-knot nematode diagnostics and enhancing management strategies statewide.

Introduction:
Root-knot nematodes (RKNs) cause severe damage and substantial yield losses in California's processing tomato industry, with annual losses estimated at $30–75 million. These parasitic nematodes also leave tomato crops vulnerable to secondary pathogens, leading to further crop loss. The combined impact of RKNs and secondary pathogens can be seen on a small scale in the greenhouse (**Figure 1**) and on a large scale in the field (**Figure 1**). When secondary pathogens invade already-infected roots, the roots may collapse, resulting in rot, premature plant death, and in severe cases, total crop failure.

Most processing tomato varieties carry a single dominant resistance gene called *Mi-1* (also referred to as *Mi*), introduced into both fresh-market and processing tomato cultivars in the 1940s. This gene mediates resistance against three major *Meloidogyne* species—*M. incognita, M. javanica,* and *M. arenaria*—all of which pose significant threats to growers within and beyond the tomato industry in California. However, decades of reliance on the *Mi-1* gene have led to the emergence of resistance-breaking populations of RKNs. This leaves growers defenseless when they plant tomato varieties previously considered resistant.

Current methods for identifying true resistance-breaking nematodes are time-intensive, requiring roughly three months to isolate, purify, and confirm their capacity to break *Mi-1* resistance. A lack of rapid diagnostic tools slows down management decisions and impedes our understanding of key factors such as the geographic distribution of resistance-breaking populations, their spread within fields, the frequency of their occurrence, and effective strategies for controlling them. Developing a rapid identification method—such as a diagnostic primer—would not only speed up detection but also enable us to answer these critical questions for California's tomato industry.

Figure 1: Symptoms of root-knot nematode infection. **Left**: Tomato roots infected with root-knot nematodes after two months. The root on the left also has a secondary Fusarium spp. infection, causing visible rot compared to the root on the right, which shows RKN galling but no secondary infection. Right: A processing tomato field sampled in 2024, displaying collapse of plants due to a combination of root-knot nematodes and *Fusarium* spp.

Objectives:

- **Objective 1: Field Sampling and Nematode Identification**
  Statewide Sampling**:** We will conduct sampling of processing tomato fields across California to identify and isolate resistance-breaking RKN populations.
  Identification, Sequencing and Greenhouse Assays**:** We will purify the cultures obtained from infected roots and identify the specific Meloidogyne species using Sanger sequencing. Further, we will conduct greenhouse assays on Mi-1 tomato plants to validate the resistance-breaking ability of the collected nematode samples.
- **Objective 2: Genome Comparison for Diagnostic Insights**
  We will sequence the whole genome of resistance-breaking field populations to analyze polymorphism in the *Cg-1* region. This in-depth analysis will pave the way for the development of diagnostic tools. (Note: Funding for this genomic analysis will be sourced from other grants.)
- **Objective 3: Education and Outreach**
  We will engage with growers to provide them with information based on our research findings. This will encompass educational outreach about the challenges posed by RKNs, the phenomenon of resistance breaking in tomato fields, and the introduction of effective cultural practices to mitigate these challenges**.**

Methodology and Results
Objective 1: Field sampling and Nematode Identification

To identify potential field sites for sampling, we began contacting University of California Cooperative Extension (UCCE) advisors in February 2024. Several UCCE individuals were enthusiastic about helping us locate infected processing tomato fields. Through these contacts, we were also introduced to AgSeed, a company managing multiple processing tomato fields. Monthly reminder emails were sent to UCCE advisors, providing details on our objectives—namely, the need for RKN-infected fields and the goal of characterizing collected populations. Whenever UCCE or AgSeed notified us of an infected field, we aimed to sample it within a week.

Field Sampling Protocol

Between 2–6 samples were collected randomly from each infected field. We focused on "hot spots" within the field, where large patches of chlorosis or vine decline were evident. We targeted the border zone between healthy (asymptomatic) plants and heavily infected (symptomatic) plants to capture actively growing nematode populations. For each hot spot, an individual plant was dug up, and the foliage was cut off and left in the row. Roots were shaken free of excess soil in the field before placing them in one-gallon Ziplock bags. Each bag was labeled with the date of collection and a unique sample number (one root = one sample). Samples were kept in a cooler during transport, then stored at 4°C in a walk-in refrigerator. Upon returning to the lab, we cleaned and disinfected all equipment, as well as our footwear, with MG4-Quat to prevent contamination among fields.

Greenhouse Assays for Resistance-Breaking Confirmation

In the greenhouse, we grew both resistant (*Mi-1* containing variety VFNT) and susceptible (without *Mi-1*, variety MV) tomato plants. Seeds were started in vermiculite and later transplanted into 32 oz Styrofoam pots filled with autoclaved sand; these pots had drainage holes and received a nutrient solution three times per day for three minutes each. Roots from each field sample were rinsed with water to remove debris, then pooled (combined) into one sample by cutting them into 1-inch segments. We used a 10% bleach solution (3 minutes of vigorous shaking) to free the eggs from their gelatinous egg masses. The bleach/egg solution was poured through a stack of two sieves—75 µm (No. 200) on top and 25 µm (No. 500) on the bottom—so that roots remained in the top sieve while eggs collected on the lower sieve. We rinsed away any bleach residue with cool water, then concentrated the eggs in a 50 mL Falcon tube. We enumerated eggs by pipetting five drops of 5 µL each into a Petri dish and counting them under a dissecting microscope. Only viable eggs were counted. We took the average number of eggs per drop, divided by 5 to determine eggs per microliter, then multiplied by 1,000 to obtain eggs per milliliter. Finally, we multiplied by 50 (the total sample volume, in milliliters) to estimate total eggs collected from the root sample.

Each plant was inoculated with ~15,000 eggs to avoid overcrowding. We used the equation $C\_i \times V\_i = C\_f \times V\_f$ to ensure the correct inoculum concentration (C_i = initial eggs/mL, V_i = volume to inoculate, C_f = 15,000 eggs/mL, V_f = 1 mL). Four VFNT (resistant) and two MV (susceptible) plants were inoculated per sample and maintained at 26°C in the greenhouse for three months. After this period, we washed the sand off the roots to compare galling between the VFNT (resistant) and MV (susceptible) plants. If both lines showed heavy galling, we concluded that the field population was indeed a resistance-breaking isolate. All 14 field isolates displayed galling on both plant types.

Population Purification via Single Egg Mass Isolation

For each confirmed resistance-breaking isolate, single egg masses were picked to develop purified populations. First, roots were gently rinsed to remove sand, then stained for 30 seconds in a non-toxic dye (Erioglaucine), which highlights the gelatinous egg matrix produced by female nematodes. Each stained egg mass was removed from the root with forceps and placed in an individual well of a 24-well plate containing 500 µL distilled water. Using a fine needle, we gently agitated the egg mass in water to encourage hatching. The 24-well plates were incubated at 28°C, and after two days, the newly hatched second-stage juveniles (J2s) were pipetted into resistant VFNT seedlings to produce clonal populations. Between 2–8 single egg masses successfully hatched per field isolate. These purified populations were grown under the same greenhouse conditions described above.

Objective 2: Genome Comparison for Diagnostic Insights

Although we initially considered Sanger sequencing, the plan now is to perform full-genome sequencing using high-quality DNA once each population has been fully purified. To date, we have extracted high-quality genomic DNA from 3 of the 14 isolates. The remaining 11 populations are still building up egg numbers to levels sufficient for DNA extraction, projected to be ready in March 2025. Comparisons between these newly collected resistance-breaking isolates and our lab-generated resistant-breaking strain will resume more intensively once all high-quality genomes are available.

Objective 3: Education and Outreach

Thanks to the funding from CTRI, our lab has engaged in productive discussions with industry partners and UCCE representatives, raising awareness of the economic and agronomic impact of root-knot nematodes. Over the course of the 2024 sampling season, we introduced our research to a wider audience than we had previously been able to reach. Looking ahead, we plan to expand sampling in 2025 to gain broader coverage of California tomato fields. We aim to create a more detailed map illustrating the prevalence of resistance-breaking nematodes statewide and develop a diagnostic primer to rapidly detect resistance-breaking in collected isolates. We will share our findings with the agricultural community through local field days, pamphlets for growers, and presentations at professional gatherings such as the Society of Nematology meetings, the American Phytopathological Society, and future CTRI field days. Ultimately, these data serve as a springboard for future research, and we look forward to strengthening our partnerships in the coming seasons.

Discussion:

This project aimed to collect resistance-breaking root-knot nematodes (RKNs) from processing tomato fields in California during the 2024 growing season. Our lab collected 14 isolates, while 13 additional samples were obtained in partnership with UC Riverside. All 14 isolates from our lab were confirmed to be resistance-breaking when maintained on both resistant and susceptible tomato plants at stable temperatures (<28°C). GPS coordinates for each collection site were recorded and plotted in Google Maps (Figure 2 and 3), illustrating where resistance-breaking populations were found within California.

After confirming their resistance-breaking status, each isolate was purified through single egg mass isolation, preparing them for high-quality DNA sequencing. Currently, purified cultures are being expanded, and the next step involves sending samples for whole-genome sequencing. We will use comparative genomics to better understand the mechanisms driving resistance-breaking and to explore potential solutions. Additionally, we plan to test these resistance-breaking populations on various alternative host crops to inform growers about potential management strategies.

Although most isolates originated in Yolo County, we lack comprehensive sampling coverage of California's processing tomato fields. Nevertheless, thanks to CTRI funding and growing recognition of our work at UC Davis, we have strengthened connections with both industry representatives and UCCE advisors to help identify additional field sites. In the future, we hope to form more collaborations to increase the diversity of our collected populations. A broader range of samples will enable us to make more accurate comparisons when analyzing DNA from each isolate.

Beyond mapping the statewide distribution of resistance-breaking populations, our overarching goal is to create a diagnostic primer for rapid detection in affected fields. Through ongoing engagement with growers, Pest Control Advisors (PCAs), UCCE, and the agricultural industry, we anticipate gathering a more diverse

set of isolates and ultimately improving management strategies for mitigating root-knot nematode damage in tomatoes.

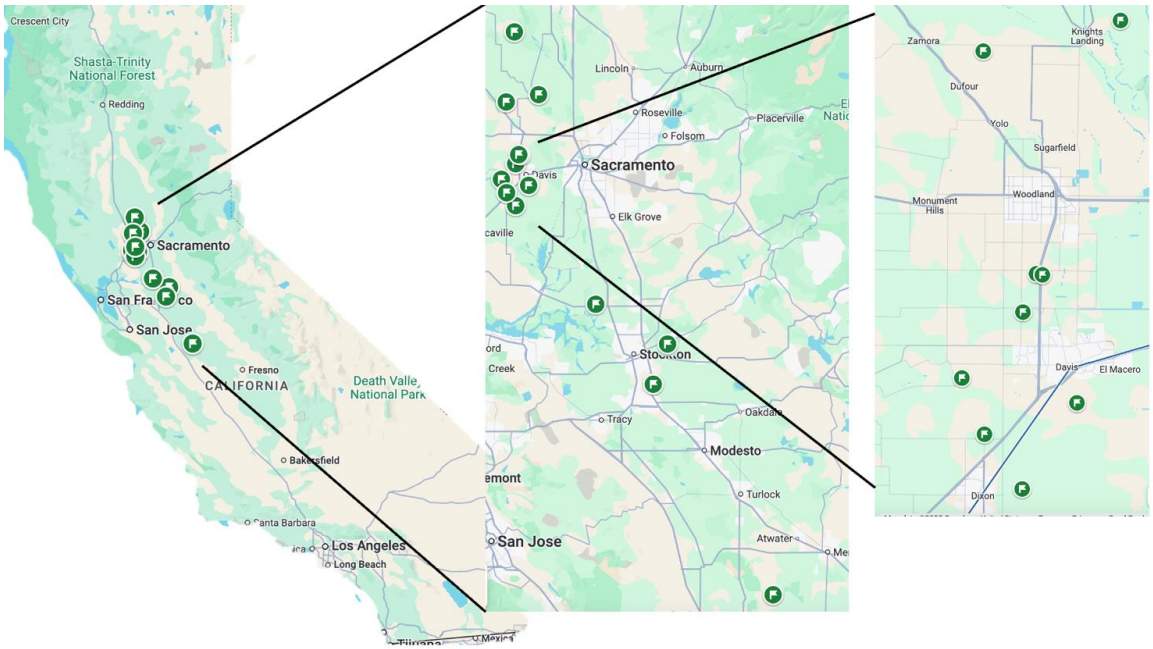


Figure 2: A statewide map of California highlighting sampling sites for root-knot nematode collection. Green flags correspond to samples collected by the Siddique Lab in 2024. Regions with a high concentration of samples are zoomed in to illustrate the local distribution of collection sites. GPS coordinates were recorded and plotted on Google Maps to produce this sampling map.

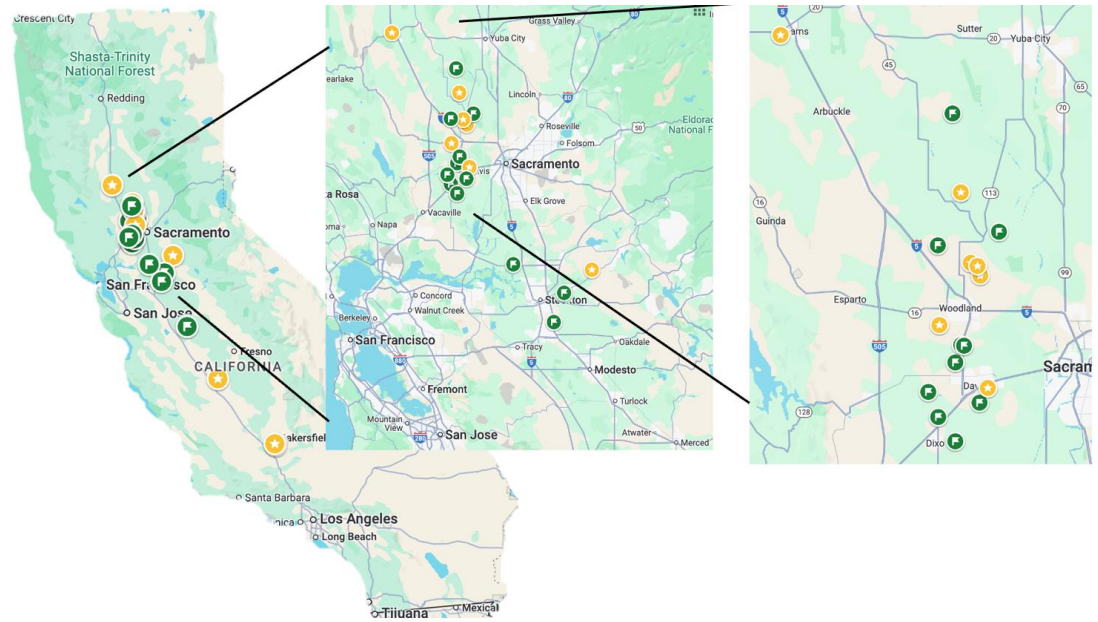


Figure 3: A statewide map of California highlighting sampling sites for root-knot nematode collection. Green flags correspond to samples collected by the Siddique Lab in 2024. Yellow stars for additional samples obtained by collaborators at UC Riverside. Each sampling site is represented as a single point (flag or star). Regions with a high concentration of samples are zoomed in to illustrate the local distribution of collection sites. GPS coordinates were recorded and plotted on Google Maps to produce this sampling map.

Acknowledgements: We extend our sincere thanks to the University of California Cooperative Extension (UCCE)—particularly Patricia Lazicki, Brenna Aegerter, and Scott Stoddard—for their invaluable assistance in locating infected fields and collecting samples. We also thank AgSeeds, and especially Ross Lopez and Erik Wilson, for identifying and granting access to field sites during the 2024 season. Furthermore, we appreciate the California Tomato Research Institute (CTRI) for funding the project, facilitating connections with growers, and raising awareness among stakeholders, which made our sampling efforts possible.

Other Support: Whole-genome sequencing for all resistance-breaking nematode isolates is being conducted with support from the USDA NIFA AFRI (Agriculture and Food Research Initiative), which enables us to perform in-depth comparative genomic analyses and advance our understanding of the mechanisms underlying resistance-breaking in root-knot nematodes.

Project title: Disease diagnosis, new pathogen monitoring, and outreach support to the California processing tomato industry

Year of Project Initiation: Ongoing

CTRI Funding in 2024: $39,915

Report Authors: Swett, C.L., Laurel Schmidt and Guy Robinson

Principle investigator: Cassandra Swett, Department of Plant Pathology, University of California, One Shields Ave., Davis, CA 95616; tel: (530) 752-3377; e-mail: clswett@ucdavis.edu

Co-PIs:
Frank Martin, Research Plant Pathologist, USDA-ARS, Salinas, CA 93905, phone 831-755-2873, email frank.martin@ars.usda.gov

Gary Vallad, Department of Plant Pathology, University of Florida, Gulf Coast Research and Education Center, email: gvallad@ufl.edu, tel: 813-419-6577

Dave Geiser, Department of Plant Pathology and Environmental Microbiology, email: dmg17@psu.edu, tel: 814-865-9773

Cooperators:

Ningxiao Li, Post doctoral researcher, USDA-ARS, Salinas, CA 93905, phone 831-755-2873; email: bioli.ningxiao@gmail.com
Brenna Aegerter, UC Cooperative Extension San Joaquin County, 2101 E. Earhart Ave. Ste. 200, Stockton, CA 95206; tel: (209) 953-6114; e-mail: bjaegerter@ucanr.edu
Patricia Lazicki, Cooperative Extension Farm Advisor, Yolo-Solano-Sacramento counties, 70 Cottonwood St., Woodland, CA 95695, phone 530-666-8732, email: palazicki@ucanr.edu
Tom Turini, Cooperative Extension Farm Advisor, Fresno county, 550 E. Shaw Avenue, Suite 210-B, Fresno, CA 93710; tel: (559) 241-7529; email: taturini@ucanr.edu
Scott Stoddard, Cooperative Extension Farm Advisor, Merced and Madera counties, 2145 Wardrobe Avenue Merced, CA 95341-6445; tel: (209) 385-7403; email: csstoddard@ucanr.edu
Zheng Wang, Farm Advisor, UC Cooperative Extension Stanislaus County, tel: 209-525-6822; email: zzwwang@ucanr.edu
Jaspreet Sidhu, Vegetable Crops Advisor, Univ. of California Cooperative Extension, Kern County, tel: 661-868-6222; email: jaksidhu@ucdavis.edu
Kamyar Aram, UC Cooperative Extension, East Bay Specialty Crops Advisor, 2380 Bisso Lane, Suite B, Concord, CA 94520, tel: (925) 608-6692, email: kamaram@ucanr.edu
Yu Chen Wang, Cooperative Extension Farm Advisor 1430 Freedom Boulevard, Suite E Watsonville, CA 95076-2796, tel: (831)201-9689; email: yckwang@ucanr.edu

EXECUTIVE SUMMARY

Tomato diseases are cumulatively among the most significant drivers of yield losses in processing tomatoes across the state, and if improperly managed, can lead to major economic losses including field abandonment. Accurate diagnosis is key to selection of effective disease-specific methods, since there are no broad-spectrum methods effective against all diseases. In collaboration with other labs, we provide diagnostic services which function for decision support; these services also function to detect new regions affected by diseases and new diseases that require management. This includes efforts to monitor for resistant breaking strains of pathogens—our efforts have thus far focused on Fusarium wilt. Part of this effort includes advancement of diagnostic and detection tools and in this year we worked to rigorously beta test a soil bioassay for Fusarium wilt. We also aim to communicate both diagnostic updates and related management information via outreach efforts.

Tomato disease diagnostics and diagnostic tool development to improve diagnosis accuracy and speed

- Diagnosed diseases for 112 tomato samples from 19 counties.
- Assisted with diagnosis of spring abiotic seedling death related to wind damage and late season abiotic decline (established abiotic etiology--cause(s) still not clear) (15% of samples abiotic).
- Detected one potentially new fungal pathogen, *Plectosphaerella (Venturia) cucumerina,* which can cause wilt, leaf necrosis, and root rot in tomato plants but is not reported in the state.
- Several detections of Alternaria stem canker caused by *Alternaria solani*—this is the first year I have seen this disease in the state although it is known to occur.
- Putative secondary root rots continue to be detected including several fungi not characterized as tomato pathogens in the state (Rhizoctonia solani, Colletotrichum black dot).
- Updated phenotype-based diagnostic results for 2023 Forl diagnoses were provided for 17 samples.
- Putative Fusarium crown and root rot was again the most commonly diagnosed disease this year (38% of samples); for the first time, in-season diagnoses was verified via new haplotyping method for 17 samples. Of these, 10 had a definitive diagnosis (6 were = non-pathogens; 4 = Forl) and 7 were ambiguous and require further phenotyping. An additional 25 are being haplotyped. ***This has reduced the number of isolates to phenotype by 60% and as such, definitive diagnosis for 60% of isolates was provided ~9 months earlier than in past years*.**
- Fusarium stem rot and decline (35% of samples) were the next most-common diagnoses. To improve diagnosis, generated over 70 genomes and conducted genome analysis to characterize F. noneumartii as a species and understand subspecies diversity. Used to identify 131 unique regions to the tomato pathogen lineage; 65 were suitable for diagnostic marker development and are undergoing in-silica validation using genomic data.
- Fusarium wilt was much less common (20% of samples); a new Fol diagnostic tool (qPCR with a new Uniq2 diagnostic region) was beta tested and found to potentially generated a lower false positive rate than the current standard method (SIX3).
- Southern blight was also commonly detected this year.

- Pure cultures were curated a ***record-breaking 173 isolates*** from diagnostic samples, for research and diagnosis.
- Trained six diagnostic interns.

***Fusarium* pathogen resistance breaking monitoring**

- Based on phenotyping Fol isolates from nine F3 fields in 2022 were either Fol race 3, Forl or non-pathogenic—race 4 has not been detected.
- Recovered Fol from 4 F3 fields in 2024; purified cultures and are reconfirming identification.
- Determined that the 2 putative Forl detections in an FR cultivar in 2023 were not resistance breaking (isolates were non pathogenic); as this was from a single cultivar (which was the same as the 2022 detections, HM5522), we hypothesize that this is a cultivar issue.
- However, in 2024 we detected putative Forl in 5 Fr cultivar fields, from four cultivars, none of which was HM5522. These cultures have been purified and are being haplotyped; putative Forl and ambiguous isolates will be phenotyped.

Outreach

- Prepared a Fusarium wilt of tomato UC ANR 8000 series article (in review, see Appendix I).
- Coordinated and presented at an in-service Vegetable Disease field day highlighting research and diagnostic updates
- Continued to work with UC IPM to develop a hard copy diagnostic guide for tomato diseases.
- Coordinated a Vegetable Disease field day (August) and a tomato disease roundtable

Needs for future work

- Continue to provide accurate diagnoses which can enable monitoring of new pathogens and changes in pathogen ecology / dispersal, while also helping growers select appropriate management tools.
- Continue to monitor for resistance breaking Fusarium pathogens.
- To improve abilities to provide accurate diagnoses that are *faster*, advance a new Fol diagnostic tool which can generate results in 3-5 days (initial validation completed in 2024).
- Continue to develop diagnostic tools for “Falciforme stem rot and decline” pathogens F. noneumartii and F. martii.
- Continue outreach efforts, specifically to hold a UC Davis Vegetable Disease field day and a Tomato Disease Research roundtable in summer 2024, develop an Fol race 4 response plan, and also to continue other leveraged outreach efforts to communicate diagnostic lab findings, new tool development information, and diagnostic training to stakeholders and stakeholder support networks.

INTRODUCTION

Tomato diseases together pose among the most significant restrictions to yield optimization statewide. Effective management of losses is hindered by frequent mis-diagnosis both in the field and in the laboratory, as well as emergence/re-emergence of diseases for which management tools are lacking. We have assisted dozens of growers who thought that they had selected the appropriate management tool(s), be it a resistant cultivar, an appropriate crop rotation or a chemical application, only to discover that it was the wrong disease they were trying to manage—losses in these cases can be devastating. To overcome these challenges, our program aims to provide highly accurate decision support to growers to both accurately diagnose the disease or diseases affecting tomato fields and identify new diseases. In addition to supporting management of known diseases, we detect new diseases every year, likely due at least in part to improved monitoring networks, more widespread movement of machines, and possibly intensive agronomic practices, as well as unpredictable, more

extreme weather events and changes in climate conditions. We also provide soil testing services for certain diseases (eg. southern blight) and are working to expand these soil testing/disease forecasting services to additional diseases. Tomatoes represent 50% of the total samples analyzed in our lab, generally totaling ~100-120 samples/yr. This service is provided without any support from the UC system. Benefits are a direct free service to growers, with additional value of pathogen monitoring and mapping, and new pathogen characterization. Through training activities we also aim to improve the services to growers offered through cooperative extension statewide networks as well as diagnostics support offered by public and private labs. In support of Fusarium wilt management, we specifically monitor for Fusarium wilt race 4, which are critical as F3 materials become more common. Starting in 2022 we have also been monitoring for possible Fusarium crown and root rot resistance breaking in three fields.

THE MAIN GOAL AND OBJECTIVES

Main goal: Our main goals are to provide accurate diagnoses to help growers select the right management strategies for diverse diseases of tomato and to monitor for new diseases in the state, including Fusarium wilt race 4. Outreach objectives aim to advance program efficacy through field diagnosis training, raised awareness of new and improved management methods, updates on emerging issues, both to stakeholders and as in-service training to the agricultural support network.

2024 Objectives:

Objective 1. Tomato disease diagnosis for grower decision support, new pathogen detection and evaluation of regional changes, and new diagnostician training via our "Diagnostics Research, Service and Training Center"

1.1 Comprehensive diagnostics including detection of multiple pathogens and strain-level identification of pathogens
1.2 Monitoring pathogen movement and new pathogen emergence
1.3 Curating and maintaining isolate cultures
1.4 Diagnostics training to the next generation of diagnosticians

Objective 2. Monitoring for resistance breaking of Fusarium diseases

2.1 Fusarium wilt race 4 monitoring in F3 cultivars

2.1.1 Pure culture and confirm Fol identity for putative Fol isolates recovered from F3 tomatoes in 2023
2.1.2 Conduct race identification of confirmed Fol isolates from F3 tomatoes in 2023 via phenotyping, for race 4 detection
2.1.3 Diagnose putative Fusarium wilt in F3 cultivar fields in 2024 and save isolates for phenotyping

2.2 Resistance-breaking Fusarium crown and root rot in Fr cultivars

2.2.1 Pure culture and confirm F. oxysporum identity for putative Forl isolates recovered from Fr tomatoes in 2023
2.2.2 Phenoptype putative Forl isolates from 2023 to determine whether there is a new resistance breaking race in the state
2.2.3 Diagnose putative Forl in Fr cultivar fields in 2024 and save isolates for phenotyping

Objective 3. Improving speed of delivering accurate tomato disease diagnoses for timely grower decision making

3.1 Developing a rapid RPA-based Fusarium wilt (Fol race 3, 4) detection tool

3.1.1 Conduct validation for a newly identified molecular-based Fol diagnostic region (TaqMan qPCR)

3.1.2 Conduct validation of RPA assay, which can detect Fol in infested plant tissue within 2 days

3.2 Develop a new rapid diagnostic tool for F. noneumartii, the primary driver of "Falciforme stem rot and decline"
3.2.1 Develop a comprehensive genomic database by adding isolates from new hosts in 2023
3.2.2 To determine whether there are multiple F. noneumartii strains in the California agroecosystem, characterize F. noneumartii pathotype diversity based on:
3.2.2.1 abilities for isolates from diverse hosts to cause disease in tomato and
3.2.2.2 somatic compatibility,
3.2.3 Use this information to identify one or more unique diagnostic region to F. noneumartii.
3.2.4 Develop TaqMan primers and probes for 1 or more diagnostic regions identified for F. noneumartii

Objective 4. Provide outreach support to enable growers to utilize diagnoses for decision making
4.1 Vegetable disease field day open to the entire community and roundtable research meeting coordinated by our lab
4.2 Grower-targeted outreach via outreach meetings
4.3 Publication of a new UC IPM field guide for tomato disease diagnosis—continue working with UC IPM for publication in early 2024
4.4 Developing of an Fol race 4 response plan, to provide growers receiving a tentative Fol race 4 diagnosis in-season

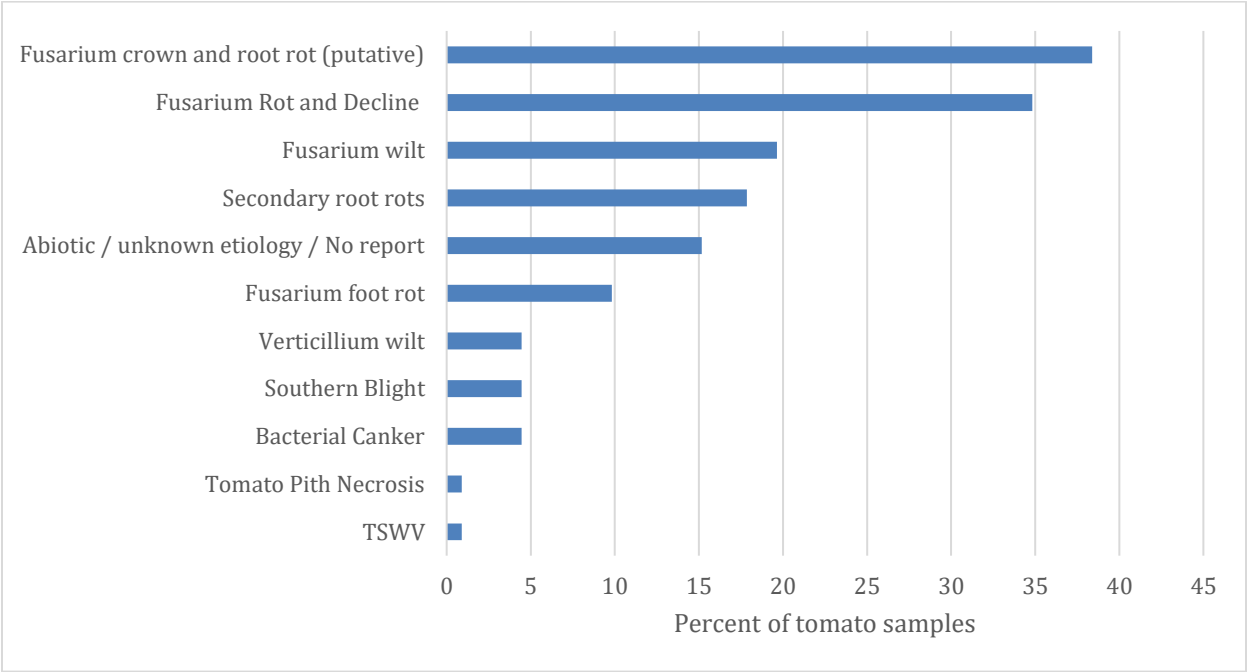
METHODOLOGY AND RESULTS

Objective 1. Provide decision support to tomato growers via our "Diagnostics Clinic, Research and Training" center

1.1 Comprehensive diagnostics including detection of multiple pathogens and strain-level identification of Fusarium pathogens.

A total of 112 tomato samples were processed and diagnosed from 19 counties. More than half of these samples had multiple diseases. The most frequently diagnosed diseases in 2024 processing tomatoes were: putative Fusarium Crown and Root Rot (43 samples), and Fusarium Rot and Decline (39 samples). Most samples came from Fresno, Yolo, and San Joaquin.

In furthering identification of Forl isolates from 2023, we phenotyped a total of 17 isolates. Of these, half were Forl. The remainder were non-pathogenic—it is possible that Forl was present, but was not successfully preserved, or alternatively that these samples were incorrectly diagnosed. This is an improvement from the 2023 false positive rate (83% of isolates) and reflects the addition of the haplotyping method prior to phenotyping. Updated reports were sent out following completion of phenotype-based analysis in late 2024. In 2024, used the new haplotyping tool post-diagnostic season on a total of 42 isolates. 17 have been haplotyped and updated diagnoses were prepared; of these, 6 were identified as non-pathogens, 4 as Forl and 7 were ambiguous and require further phenotyping. Haplotyping for the remaining 25 isolates will be completed in early 2025. Thus far, this has reduced the number of isolates to phenotype by 60% and as such, definitive diagnosis for 60% of isolates was provided ~9 months earlier than in past years.



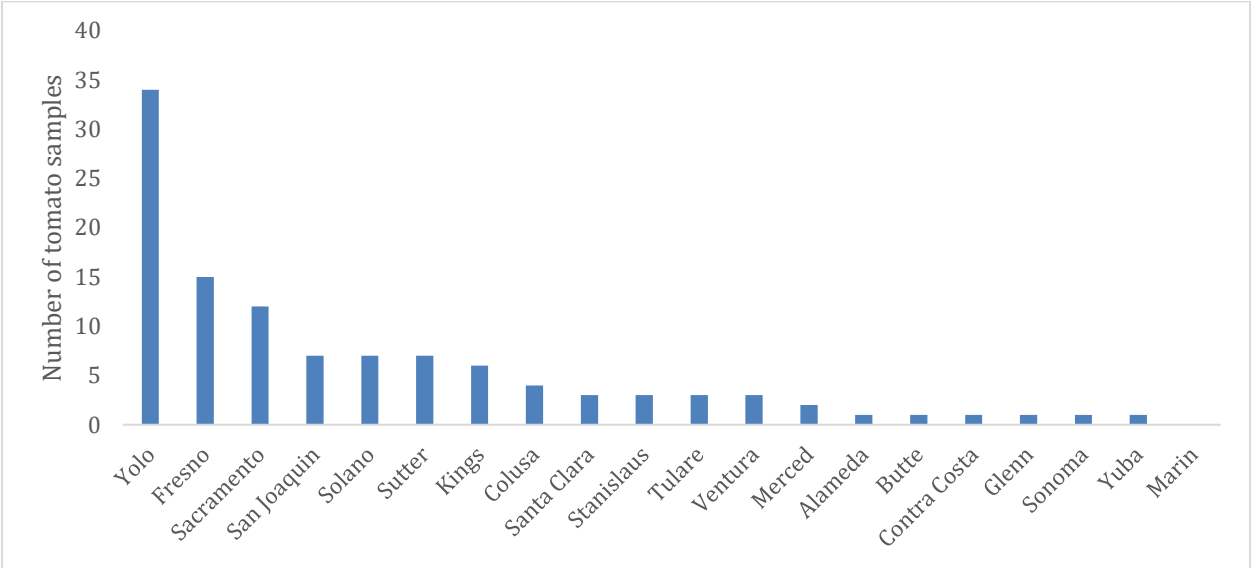


Figure 1. Percent of tomato samples diagnosed in 2024 by disease (top) and number of samples by county (bottom).

1.2 Monitoring pathogen movement and new pathogen emergence

The putatively new disease tomato collapse and root rot caused by *Plectosphaerella cucumerina* was detected for the first time this year—this disease is not known to be present in the US and cultures were saved in triplicate (note that this was only found in a fresh market tomato). This disease is reported in field grown tomatoes in Italy, Pakistan and Iran; other affected crops include peppers (where it was also found in this years detection, same field) and cucumber (Raimondo and Carlucci 2018; Alam et al. 2017; Mirtalebi and Banihashemi 2016).

New diseases detected in previous years that were again found this year include bacterial pith necrosis, F. solani sensu stricto from foot and root rot, and both Colletotrichum coccedes and Rhizoctonia solani from tomatoes with crown and root rot. We continue to monitor for these pathogens; pith necrosis isolates were provided to the Coaker lab for pathogenicity testing downstream.

Table 1. Summary of diagnoses in 2023 representing recently identified pathogens to the region

2023 tomato disease diagnoses - putative new pathogens	**No. Samples 2023**	**No. Samples 2024**
Tomato pith necrosis (*Pseudomonas corrugata)*	4	3
Rhizoctonia crown rot (*Rhizoctonia solani)*	8	1
Putative foot rot caused by *F. solani sensu stricto*	2	
Colletotrichum black dot (*Colletotrichum coccodes*)	0	3
Tomato collapse and root rot (*Plectosphaerella cucumerina*)	0	1

1.3 Curating and maintaining isolate cultures

This year we pure cultured 173 isolates from 2023 and 2024 tomato diagnostic samples. Pure culturing for isolates from 2024 diagnoses continue into 2024, with an estimated 20 additional cultures to save.

1.4 Diagnostics training to the next generation of diagnosticians

This year I trained five six diagnostic interns (two undergraduates, four technicians) on the process of diagnosis, and provided more advanced training to six additional members of my team (three graduate students, one technician, two post docs). We also trained ~15 undergraduates in components of the diagnostic process.

Objective 2. Monitoring for resistance breaking of Fusarium diseases

2.1 Fusarium wilt race 4 monitoring: diagnosis of diseases in F3 cultivars, Fol race phenotyping, publication of race emergence review and race 4 forecast summary

Over the last 7 years, we have diagnosed Fol in 32 F3 cultivar fields. Through 2021, these have all be Fol race 3, non pathogens or Forl—none have been race 4 and this was consistent in for the nine Fol detections in F3 cultivars in 2023. In 2024, we detected Fol in 4 F3 fields. Isolates are currently be pure cultured, identity as Fol confirmed for the pure culture (using diagnostic PCR) and will be phenotyped in 2025.

Table 2. Summary of results from Fol race 4 monitoring efforts 2017 to the present

	Total	Pot Fol	Fol				Forl	Non-Path
			R1	R2	R3	R4		
2017	2	2	0	0	2 (100%)	0	0	0
2018	11	11	0	0	11 (100%)	0	0	0
2019	0	0	0	0	0	0	0	0
2020	2	2	0	0	2 (100%)	0	0	0
2021	2	2	0	0	2 (100%)	0	0	0
2022	3	3	0	0	2 (66%)	0	0	1
2023	9	9	0	0	3 (33%)	0	2 (22%)	4 (44%)
2024	4	4	TBD	TBD	TBD	TBD	TBD	TBD
Total	32	32	0	0	23 (79%)	0	2 (7%)	4 (13%)

2.2 Resistance-breaking Fusarium crown and root rot in Fr cultivars

We have detected Forl in resistant cultivars for the last three years. In the past two years, this was all in one cultivar, and none of the isolates were resistance breaking, indicating that there was an issue with the R gene in that one cultivar. In contrast, this year we detected it in four new cultivars, in five different fields. It seems unlikely that this can be a cultivar issue, but it is possible that the high heat compromised resistance expression. We are pure culturing and re-testing isolates with our new haplotyping method; isolates identified as Forl will be phenotyped in greenhouse assays to determine if they are resistance breaking.

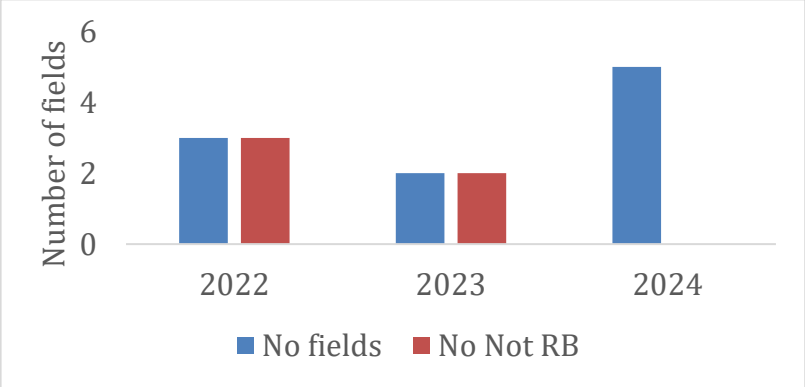


Figure 2. Fusarium crown and root rot (Forl) detections in Fr cultivars with single gene resistance to Forl and results from phenotyping trials (2022, 2023).

Objective 3. Improving speed of delivering accurate tomato disease diagnoses for timely grower decision making

Overall, the Swett lab is making strides to incorporate new diagnostic tools into our in-season diagnoses, to improve accuracy and speed. In 2024, over 60% of submitted samples used one or more new tool developed in our lab, which is both more accurate and reduces the time for diagnosis. In 2024 we beta tested Forl as a diagnostic tool, using it both in season and at the end of the season (after reports were sent out) to provide more accurate diagnoses. For those isolates which are identified as Forl or non-pathogens, we do not need to further test in phenotyping, reducing the time from one year

to three months for more accurate Forl diagnosis. We also beta tested the aforementioned Fol diagnostic region, improving diagnostic accuracy.

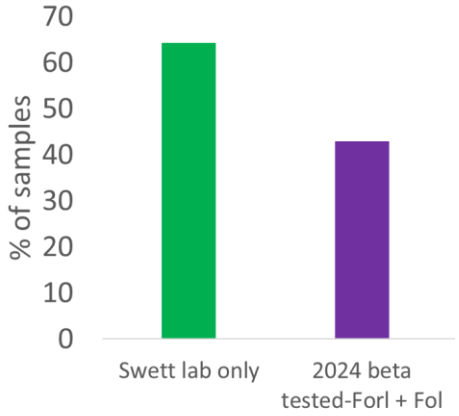


Figure 3. Number of tomato samples diagnosed with one or more new diagnostic tools currently only available in the Swett lab, and number of samples used for beta testing new tools.

3.1 Developing a rapid RPA-based Fusarium wilt (Fol race 3, 4) detection tool

In 2024 we validated and beta tested a new Fol qPCR assay. This consisted of testing both a Fol general diagnostic region (currently called Uniq2) and a Fol race 3 (lineage 3G) diagnostic region against ~40 isolates representing Fol races, Forl and non-pathogenic F. oxysporum strains. Based on this, the Fol general region was determined to have a very low false positive rate (one Forl and one non-pathogen) and the Fol race 3 (lineage 3G) region had no false positives. Unfortunately we have been unable to find a diagnostic region for Fol race 3 which also includes the second lineage (3D), so the race 3 specific region remains primarily useful for research. The Fol qPCR was beta tested against the current standard PCR region (SIX3). There was a high level of agreement between the two methods for both positive and negative results (Table 2). In some cases they did disagree, reflecting cases where the SIX3 PCR called it Fol and the qPCR method did not. We are testing these isolates to determine if they are Fol (SIX3 was correct) or not (qPCR correct). If the latter, this will indicate that the qPCR method is more accurate than the SIX3 method. These results indicate that our new qPCR-based Fol diagnostic region is more accurate that the existing SIX3 region; the qPCR method is also more efficient and less prone to ambiguity. However, it still takes 2-3 weeks for diagnosis. To transfer this to a more rapid tool, we developed RPA primers and probes and conducted preliminary validation of the RPA assay using fungal tissue. In 2025 we plan to test this new region as an RPA assay directly on plant tissue and beta test use in the diagnostic lab.

Table 3. Results of Fol qPCR-based diagnostic region beta testing in 2024, in comparison to the current standard (SIX PCR)

SIX / qPCR Result	Total	Percentage (%)
Agreement (+ result)	30	29.4
Agreement (- result)	56	54.9
Ambig / +	1	1.0
Ambig / -	9	8.8
Disagree (SIX + / qPCR -)	6	5.9
Disagree (SIX - / qPCR +)	0	0.0

3.2 Develop a new rapid diagnostic tool for F. noneumartii, the primary driver of "Falciforme stem rot and decline"

In 2024 we developed and annotated a genomic database of over 20 F. noneumartii isolates from tomato, together with over 50 other isolates closely related to F. noneumartii. Using this, we developed a 46-gene phylogenetic tree to assess the species-level relationships of these isolates and used to determine the relationship between F. noneumartii and F. martii—establishing for the first time that these are sister species. We have identified 131 putative diagnostic regions unique to the F. noneumartii group (FN-SCG 1) that causes disease in tomato. 65 of these were suitable for diagnostic marker development and we have developed primers for these regions and are currently conducting in-silica validation that these regions are both consistently present in all target isolates and absent in all non-target isolates. We hope to have a diagnostic region to test in 2025 via diagnostic lab efforts (not included in proposed research).

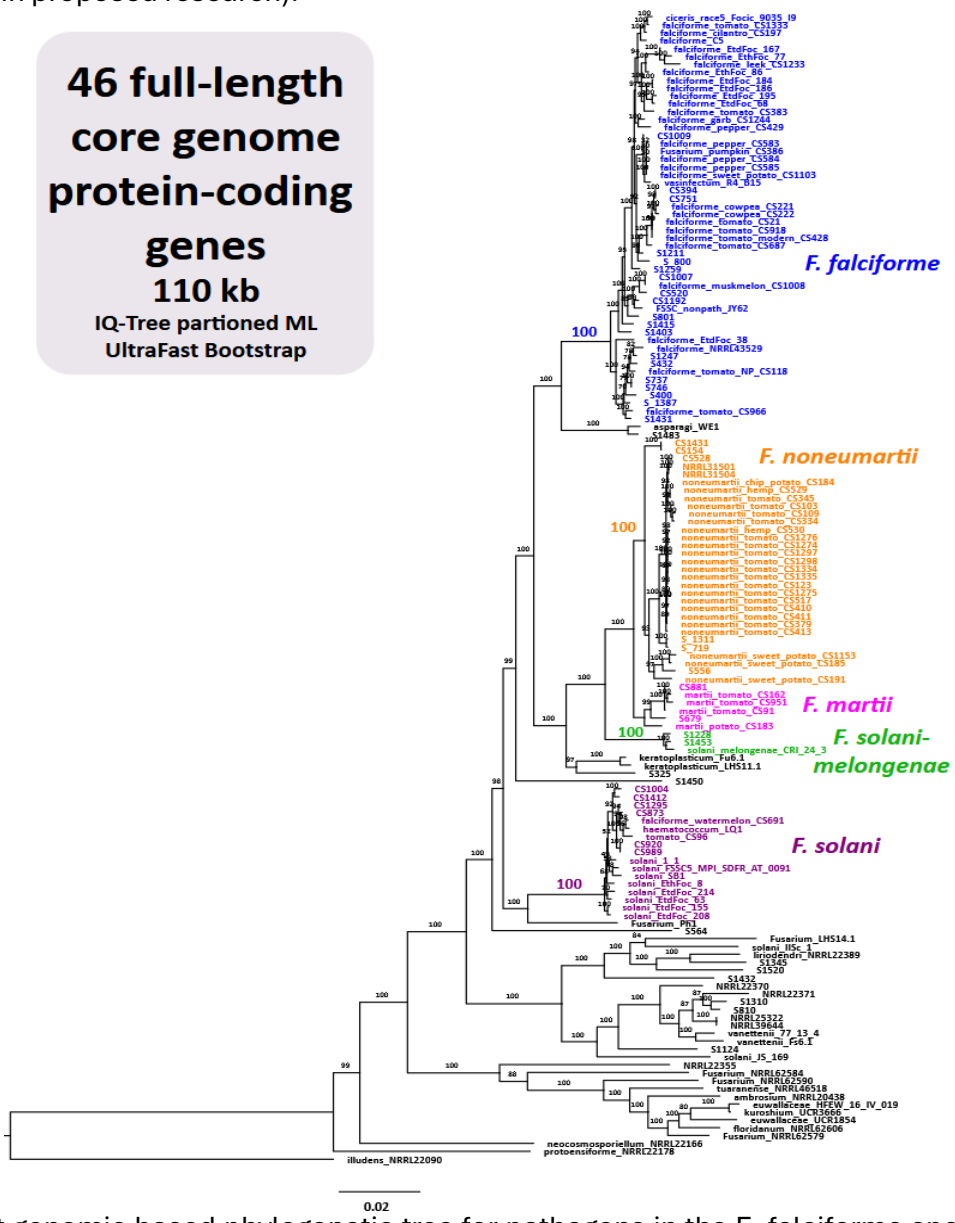


Figure 4. First genomic based phylogenetic tree for pathogens in the F. falciforme species complex, including F. noneumartii. This establishes that F. martii is a separate, sister species, to F. noneumartii and clonal groups identified with somatic compatibility analysis are consistent with phylogenetic haplotypes—the clonal group SCG FN-1 is the only group found to cause disease in tomato.

Objective 4. Provide outreach support to enable growers to utilize diagnoses for decision making

While diagnostics laboratory support is critical, the functionality of this service is dependent on having well-trained professionals (advisors, scouters, etc) capable of field diagnosis and quality sample submission. To enhance diagnostic capacities of the broader community, in 2024 I provided direct 1:1 training of three new vegetable crop advisors covering tomatoes and provided "educational diagnostics" for the new Yolo/Solano/Sacramento County advisor to enable her to more quickly learn about the diseases in her region. We hosted a field day for regional advisors and seed representatives, and a tomato research roundtable where UC Davis researchers provided updates on research to the advisor network. We also disseminated over three dozen copies of the Tomato decline and rot disease diagnosis field guide, and are working with UC IPM to get this printed as a binded set of pest ID cards. We have also completed revisions for the ANR 800 series article on Fusarium wilt management (see Appendix I) which includes an action plan if Fol race 4 is detected; this was complimented with studies in 2024 Clean Machine work which identified a wider range of effective sanitizers including StarSan, the first sanitizer we have found that is not debris sensitive.

DISCUSSION

Expected outcomes and benefits

- Growers can make effective disease management decisions for 2024/25, based on accurate diagnoses results.
- Presence of Fusarium wilt race 4 resistance-breaking and presence of Fol race 2 resistance-breaking strains assessed in the state; guidelines for disease management in the ANR 800 series article can assist with management of Fusarium wilt in F2 cultivars and rapid response if race 4 is detected.
- Awareness of new and re-emerging diseases which may need to be more closely studied for management and potentially diagnostic methods in future research efforts—for example, new bacterial and fungal diseases which continue to be detected in 2023 may have greater impact than previously thought; the newly detected *Plectosphaerella (Venturia) cucumerina* will be monitored for in future years.
- Speed of Fusarium crown and root rot diagnosis increased for a subset of samples (using haplotyping method), improving management abilities
- New Fol qPCR diagnostic tool validated and improved speed and accuracy of in-season Fol diagnoses; initial efforts in RPA validation will be continued in 2025 beta testing year.
- This information is disseminated at various outreach functions; surveys at one recent meeting indicate that of all the talks at the meeting, this was the most valuable talk for the majority of people, more so than any other talk.
- This information is disseminated through publication of a diagnostic field guide for canopy decline disorders—in prep for 2025.
- The production community has increased awareness of disease issues and management options, leading to improved disease management and reduced yield losses.

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THIS PROJECT AS LEVERAGE FOR OTHER DOLLARS

CDFA-DPR. "Reducing current and future fungicide use in California crops by providing decision support and rotation tools for managing the emerging, highly damaging Fusarium falciforme pathosystem." $598,497 total. **~$10,000** allocated to assist with tomato disease diagnostics and **$45,000** to assist with molecular diagnostic tool development = **$55,000 total.** 9/15/2023-12/31/2025. Aim: this project is looking at F. falciforme species complex pathogens across all California crops and includes diagnostic support for tomatoes.

National Plant Diagnostic Network. Secured a total of **$7,000** in NPDN funds for tomato disease diagnosis.

UC Davis GSR fellowship. Fall 2024 (new GSR). New graduate student. Funds will help cover 5% time allocation to tomato disease diagnosis. **$4,000**

UC Davis TAship. Spring 2024 (Myles Collinson). Funds will help cover 5% time allocation to tomato disease diagnosis and field day set up and coordination. **$4,000**

UC Davis TAship. Fall 2024 (Annika Briggs). Funds will help cover 5% time allocation to tomato disease diagnosis and field day set up and coordination. **$4,000**

Additional leveraged funds from other grants (various). Covering lead diagnosticians (post-docs, technicians). **$7,000**

UC Davis extension funds. Summer 2022. Funds will help cover long term truck rental, used for farm calls. **$500**

APPENDIX I

ANR 8000 series

Fusarium wilt of tomato: resistant cultivar-based management and other integrated management opportunities to reduce disease losses and both prevent and respond to new race emergence

Cassandra Swett, Brenna Aegerter, Kelley Paugh, Johanna Del Castillo, Beth Hellman, Samuel Hutton, Kacey Zimmerman, Justine Beaulieu

Documented in over 30 countries, Fusarium wilt caused by *F. oxysporum* f. sp. *lycopersici* (Fol) is a major driver of yield losses in tomato worldwide, with significant impacts in California processing tomato production. Tomato cultivars with single gene resistance comprise the keystone of integrated disease management. However, resistance efficacy has been compromised by the emergence of two successive resistance breaking races, race 2 and race 3. Presently, control of Fol race 3 relies primarily on F3 cultivars containing the *I3* resistance gene, together with crop rotation and chemical management methods.

The resistance breaking timeline as a crystal ball—predicting when I3 resistance will break

Understanding the timeline for resistance breaking can help predict the duration of single gene utility and inform resistance breaking race monitoring programs.

Fol race 1

- Fol race 1 has been known to be the cause of Fusarium wilt for over 130 years (1886-1899) and has been in California since the 1940s.
- Resistance to Fol race 1 (conferred by the *I* gene) was discovered in 1939 and resistance was deployed in California (F cultivars) around 1959 (~ 15 years after pathogen detection).
- Fol race 1 resistance remained durable for ~11 years, when Fol race 2 emerged.

Fol race 2

- Fol race 2 was detected in California in 1970.
- The resistance gene (*I2*) had already been identified (1955) and resistant (F2 or FF) cultivars were being used in California by 1975, with widespread use within 10 years.
- Fol race 2 resistant cultivars remained effective for ~12 years, when race 3 emerged.

Fol race 3

- Fol race 3 was detected in the state around 1987. This race remained in the Sutter Basin for several decades with minimal economic impacts on the industry outside of this region.
- Fol race 3 became a management priority statewide when it moved outside the Sutter Basin (2003-2019); it currently occurs across all processing tomato producing counties in the Central Valley.
- Race 3 resistance (I3 gene) was identified prior to race 3 detection in the state (~1980) and resistant (F3) cultivars were available for use in California by 2009; with fruit quality and yield issues, widespread adoption did not start until 2016.
- As of 2024, many F2 cultivars are still preferred if the field is thought to be free of race 3, and several processors have a very limited selection of F3 cultivars.
- Fol race 4 has not been detected in California, and F3 cultivars are effective for management of Fusarium wilt as of 2024.

Fol race 4

- Fol race 4 is not known to be present anywhere in the world as of 2024.
- Based on the past resistance-breaking timeline, race 4 is anticipated to emerge with 12 years of widespread F3 cultivar use, between 2022 and 2028.
- From the time race 4 is found, it will likely be several years before resistant cultivars are identified.

Table 1. Summary of Fol race emergence, resistance deployment and resistance breaking

Event	Fol race			
	Race 1	**Race 2**	**Race 3**	**Race 4**
Detected in CA	1940s	1970	1987	**Anticipated 2022-2028**
Resistance gene	I	I2	I3	none known
Years to initially find R gene	50 years	10 years	5 years	na
Resistance initially found	1939	1955	1980	na
Years from detection to resistance use in CA	15 years	5 years	22-29 years	Unknown
Resistant cultivars used in CA starting in	1959	1975	2009-2016	na
Years effective in CA	**11 years**	**12 years**	**Anticipate ~12 years**	**na**

What to do if a new resistance breaking (RB) race of Fol is detected?

Early resistance breaking race detection and containment will be critical to preserving efficacy of existing resistance while new resistant materials are developed and deployed. There are several key components to resistance breaking race containment:

1. Prevent movement into new fields: field equipment sanitation
    - Physical cleaning and sanitizers provide the most effective means of control
    - Effective sanitizers for Fol include peracetic acid and quaternary ammonium
    - BMPs for equipment sanitation: https://swettlab.faculty.ucdavis.edu/extension
2. Do not plant the field to tomatoes again, if you think you have a resistance breaking race
3. Monitor for resistance breaking. As California (and particularly the Sacramento Valley) is thought to be a site of origin for race emergence, this region should be aggressively monitored. Submit possible RB cases to a diagnostic lab for testing. Detection and rapid response to resistance-breaking race emergence depends on accurate and timely diagnosis.

If you are a diagnostician: how should you test for a new resistance breaking race?

1. Traditional laboratory diagnosis of Fol using morphology-based methods can provide results quickly, but morphology cannot be used to accurately identify species or to provide any indication of formae specialis or race identity.
2. New molecular-based diagnostic methods can provide a more rapid means to identify Fol in resistant cultivars.

3. Since Fol race 4 has not yet been identified anywhere in the world, there is no race specific diagnostic test. Thus, in-planta-based methods are necessary for accurate race identification.
4. In-planta identification methods vary in plant age, inoculation method, and duration needed for symptom development, with results in two to twelve weeks, depending on the method. Use of laboratory assays and seedlings have a high false positive risk; 8-week assays with mature plants provides the most reliable means to identify races.

Co-management of Fol races with resistant cultivars

While Fol race 3 is currently the top priority for management, Fol races 1 and 2 also still exist in the state, although these races are generally only found in fresh market tomatoes, some of which lack resistance. The F3 designation for cultivars only means that the cultivar has the I3 resistance gene and some F3 cultivars being grown do not have I1 or I2 genes. There is some limited published evidence that the I3 resistance gene conveys some resistance to races 1 or 2, but this relationship has not been clearly defined. Growers should be aware that when planting F3 tomatoes with only the I3 gene to fields with race 1 or 2, it is possible that these races will cause disease.

A new challenge: sometimes Fusarium wilt still develops in resistant cultivars

Fol has been detected in nearly two dozen F3 processing tomato fields since 2017. There can be three explanations for Fusarium wilt race 3 occurring in race 3-resistant fields

1) The emergence of a resistance breaking strain of Fusarium wilt, which would be called race 4
2) Seed lots sometimes have off-type individuals which might not carry the I3 gene; this off type rate is supposed to be kept below 2% of a seed lot
3) The I3 gene is present, but its expression is compromised by severe stress such as high salinity or other factors such as presence of other diseases (e.g. root knot nematode). The rate of compromised plants is normally low but can occasionally be as high as 30% of individuals (Table 1).

We have not found race 4 in California; thus susceptible plants are due to off-type or compromised vines.

Table 2. Fusarium wilt development in Fol race 3-resistant (F3) cultivars grown in a field artificially infested with Fol race 3 (two trial years).

Cultivar	Year 1	Year 2	Both years
H1310	0% ± 0%	36.7% ± 17.6%	18.3% ± 11.4%
N6428	0% ± 0%	10% ± 6%	3% ± 3%
HM58801	0% ± 0%	3% ± 3%	1.6% ± 1.6%

Management of stress can help reduce risks of disease development in resistant cultivars

Studies suggest that stresses can allow Fol race 3 to cause wilt in resistant cultivars. Tomatoes are being grown in salt-affected soils across several counties and soil salinity has been shown to increase Fusarium wilt development in resistant cultivars. Soil testing before planting can help to avoid planting to saline fields. If a field has high salt levels, leaching can help to reduce salinity levels if there is adequate drainage. Other stresses that may influence Fusarium wilt development include water stress associated with reduced irrigation inputs and heat stress. These can be managed to some extent by increasing water inputs, especially during heat waves. Certain cultivars appear to be more sensitive to stress than others and develop Fusarium wilt at higher rates under stress (Table 2)—avoiding cultivars with low stress tolerance can also help to manage Fusarium wilt.

Integrated management opportunities for Fusarium wilt

Given challenges with Fol race 3 disease development in resistant cultivars, and continued use of F2 cultivars, integrated management continues to be important. Further, continued use of Fusarium wilt-suppressive methods can reduce the risk of race 4 emergence.

Crop rotation

Duration of rotation: Rotate out tomatoes with fallow or non-supporting crops (see below) for at least 9 months and ideally at least 3 years to reduce pathogen loads and disease

Composition of rotation: Fallow is the optimal rotation; crops comparable to fallow include most legumes (lima bean, garbanzo, fava bean) and grain crops (corn, rice), as well as onions and cotton.

Avoid disease enhancing crops: Disease enhancing crops include sunflower, pepper, muskmelon, pumpkin, and wheat. Broccoli, arugula and hairy vetch (cover crop) should be avoided.

Chemical control

Fumigation: Spring fumigation is more effective than fall fumigation. The fumigant KPam has demonstrated efficacy in reducing Fusarium wilt at high rates (30 gal/A) but not lower rates (15 gal/A).

In season chemigation via buried drip: Applications need to be made at 2-to-3-week intervals starting at planting, for the first six weeks after planting. Chemicals with efficacy include Velum One and Miravis (most effective but not currently on label for chemigation).

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Project Title: Developing an integrated management strategy for Fusarium ("Falciforme") stem rot and decline (FRD) in processing tomato utilizing cultivar-based management, crop rotation, and targeted weed management.

Year of Project Initiation: 2018

2024 Funding: $59,590

Authors: Myles Collinson, Cassandra Swett and Brenna Aegerter

Principal Investigator: Cassandra Swett, Extension specialist for vegetable and field crop diseases, Department of Plant Pathology, University of California, One Shields Ave., Davis, CA 95616; tel: (530) 752-3377; e-mail: clswett@ucdavis.edu

Co-PI: Myles Collinson, Graduate Student Researcher, Department of Plant Pathology, University of California, One Shields Ave., Davis, CA 95616; tel: (858) 922-1992; e-mail: mcollinson@ucdavis.edu

Cooperative Extension Co-PIs:

Brenna Aegerter, Farm Advisor, UC Cooperative Extension San Joaquin County, 2101 E. Earhart Ave. Ste. 200, Stockton, CA 95206; tel: (209) 953-6114; e-mail: bjaegerter@ucanr.edu
Zheng Wang, Farm Advisor, UC Cooperative Extension Stanislaus County, tel: 209-525-6822; email: zzwwang@ucanr.edu
Tom Turini, Farm Advisor, UC Cooperative Extension Fresno County, 550 E. Shaw Avenue, Suite 210-B, Fresno, CA 93710; tel: (559) 375-3147; email: taturini@ucanr.edu
Patricia Lazicki, Cooperative Extension Farm Advisor, Yolo-Solano-Sacramento counties,2780 E Gibson Rd., Woodland, CA 95776, email: palazicki@ucanr.edu

Collaborators:

Brad Hanson, Professor**,** Cooperative Extension Weed Specialist, Department of Plant Sciences,University of California, One Shields Ave., Davis, CA 95616; tel: (530) 752-8115; e-mail: bhanson@ucdavis.edu
AgSeeds, Ross Lopez and Lance Stevens 28 N East Street, Woodland, CA 95776, ross@agseeds.com, Lance@agseeds.com
TS&L Seed Company, Scott Picanso, PO Box 359, Woodland, CA, 95776, Scott@tslseed.com

Main goal: Develop an effective integrated management toolkit for Fusarium stem rot and decline in processing tomato.

Executive Summary:

Over the last four years, we have identified economically impactful species associated with Fusarium Stem Rot and Decline (FRD) and established an integrated disease management tool kit to reduce losses in processing tomato and other annual crops rotated with tomato. Our work has included chemical management, cultivar-based management, crop rotation guidelines, crop avoidance guidelines, and targeted weed management.

This year we finalized population studies for the FRD complex. When this project was initiated, it was unclear what the species break down of the fungal pathogens associated with FRD was and which species

were driving observed economic losses. Over the last 4 years we have established that *F. noneumartii* and *F. martii* are the primary drivers of FRD and that *F. falciforme* is a closely related species that drives the historical disease Fusarium Foot Rot. Of these three species, *F. noneumartii* and *F. martii* are associated with an economic loss in processing tomato and so have been the primary target of our work while *F. falciforme* is not associated with economic losses. Specifically, *F. noneumartii* appears to be slightly more virulent than *F. martii* and is more commonly recovered, thus initial studies focused on this species.

Each year we expand cultivar recommendations for FRD based on UC Davis controlled trials and commercial trials across the state. The UCD controlled trials act as a key stone, allowing us to compare results from across the state to confirm that cultivar recommendations are consistent throughout. This year, we tested 18 cultivars in the UCD controlled trial, aiming at cultivars that are increasing in use. 2024 cultivar disease levels ranged from less than 30% of plants dead or declined to almost 90%. Diagnostic support was also provided to chemical management trials conducted by both Lazicki and Aegerter, evaluating FRD management, together with root knot nematode. Results of those trials are provided by indicated PIs.

We have carried out 4 years of controlled rotation trials, starting with warm season rotations (summer to summer) and expanding to cool season rotations (winter to summer). In warm season trials, we evaluated rotations including sunflower, safflower, melon, cotton, corn, pepper, garbanzo bean as well as weedy and chemical fallow and have found no rotation crops that actively reduce disease levels in processing tomato (as compared to a chemical fallow baseline) but there are rotation crops that are higher risk than others. A sunflower or safflower rotation, for example, led to a twofold increase in vine decline incidence as compared to a corn, melon, or cotton rotation. Warm season trials were repeated twice across 3 years to confirm consistency of results. In cool season rotation trials, we evaluated rotations including broccoli, romaine, alfalfa, garlic, onion, carrots, vetch, parsley, wheat, spinach, fava, and mustard and saw a wider range of disease response in tomato after rotation treatments. Some crops (such as cilantro) led to a lower level or vine decline in tomato (~30% of plants) than a winter fallow treatment while others (such as wheat and vetch) led to very high levels of disease in toamto (almost 100% of plants dead or declined). Cool season trials have been repeated twice thus far, but due to the wide range of disease response, we are planning to repeat it a third time.

In addition to controlled trials, we also carried out commercial field rotation trials. These trials were initiated in 2019 with the identification of naturally infested FRD fields. We have then been following these fields over the years as the growers rotate through different crops. This year, three fields were planted to tomato; one of which went through a tomato, wheat, safflower, tomato rotation. This field saw a two-fold increase in vine decline after this rotation regime. This corroborates the controlled trials that showed wheat and safflower to be high risk rotations for FRD.

Through greenhouse trials and field trials leveraged from the rotation trials, we have been able to characterize the host range of *F. noneumartii* outside of processing tomato. These trials have shown that sunflower, safflower, potato, pepper, hemp, carrot, lettuce, and cilantro are hosts while wheat, vetch, mustard, alfalfa, barley, spinach, and fava are non-hosts to *F. noneumartii*. Potato, cilantro, and lettuce appear to be economically impacted by *F. noneumartii* and so should not be planted in fields known to have FRD.

Introduction:

Fusarium Stem Rot and Decline (FRD) is a severe disease of processing tomatoes that continues to spread across the state. It is the second most reported disease of processing tomatoes (after Fusarium crown and root rot). Symptoms include rot of the roots, foot, crown, and stem, extending as high as 20 cm above the soil line. In the canopy, foliar symptoms begin as chlorosis and deformity of young leaves that gradually progresses to whole branches and premature vine decline. The premature decline exposes fruit to the sun prematurely, leading to sun damage and yield losses; up to 60%.

When this disease first emerged as a problem, it was unclear what species were driving disease. Initially, through diagnostics, isolates from diseased tomato plants were identified as '*Fusarium falciforme*' based on molecular identification and thus the disease was referred to as 'Falciforme'. We eventually determined that this was actually a group of closely related species within the Fusarium Solani Species Complex (FSSC). This was broken down into three species: *Fusarium noneumartii, Fusarium martii,* and *Fusarium falciforme sensu stricto*. It was still unclear which of these species were associated with the severe plant decline and economic impact observed in the field. Through controlled field trials, we determined that *F. noneumartii* and *F. martii* are the primary drivers of economic losses and *F. falciforme* is, at least in tomato, weakly pathogenic, leading to no yield losses or vine decline in the field. As *F. noneumartii* is the most commonly recovered species from diseased fields, we have focused on it for developing management tools.

Over the last four years of this project, we have worked to establish an integrated management toolkit for FRD, focusing on *F. noneumartii*. This includes chemical management, cultivar-based management, crop rotation guidelines, crop avoidance guidelines (for crops other than tomato), and targeted weed management guidelines. As part of cultivar-based management guidelines, we have also partnered with industry breeders, independent of CTRI funding, to provide screening services and tools so that they can advance development of new, more FRD resistant cultivars.

Methods and Results:

Objective 1. Developing F. noneumartii-tolerant cultivar recommendations

1.1. UC Davis cultivar field trials. Cultivar screenings were initiated at the UC Davis Plant Pathology research station in April 2024, plant death was evaluated 4 weeks pre harvest, and the trial was harvested on September 4th, 2024. The trial was laid out as a randomized complete block design with 3 blocks, 18 70ft plots per block. Within these plots, we established a 25ft monitoring plot from which canopy symptoms and yield data were collected. A sampling of symptomatic plants was collected for diagnostics to confirm *F. noneumartii* was the cause of crown rot and decline.

Vine decline and death was high in this trial, likely due to the high inoculum load in the field and the high summer temperatures. Overall, yield and vine decline followed an inverse trend with the cultivar with the lowest incidence of vine decline having the highest yield and vice versa (with the exception of HM4521 which had the third highest incidence of plant death (~75%) but still yielded well (~30 tons/acre)). SY0275 was the top performer with the lowest incidence of plant death (50% lower than the susceptible check) and highest yield (~45 tons/acre). SVTM09032 was our susceptible check and had the highest incidence of plant death (~90%) but second lowest yield (~12 tons/acre). Only one cultivar (N6475) had lower yield (~10 tons/acre).

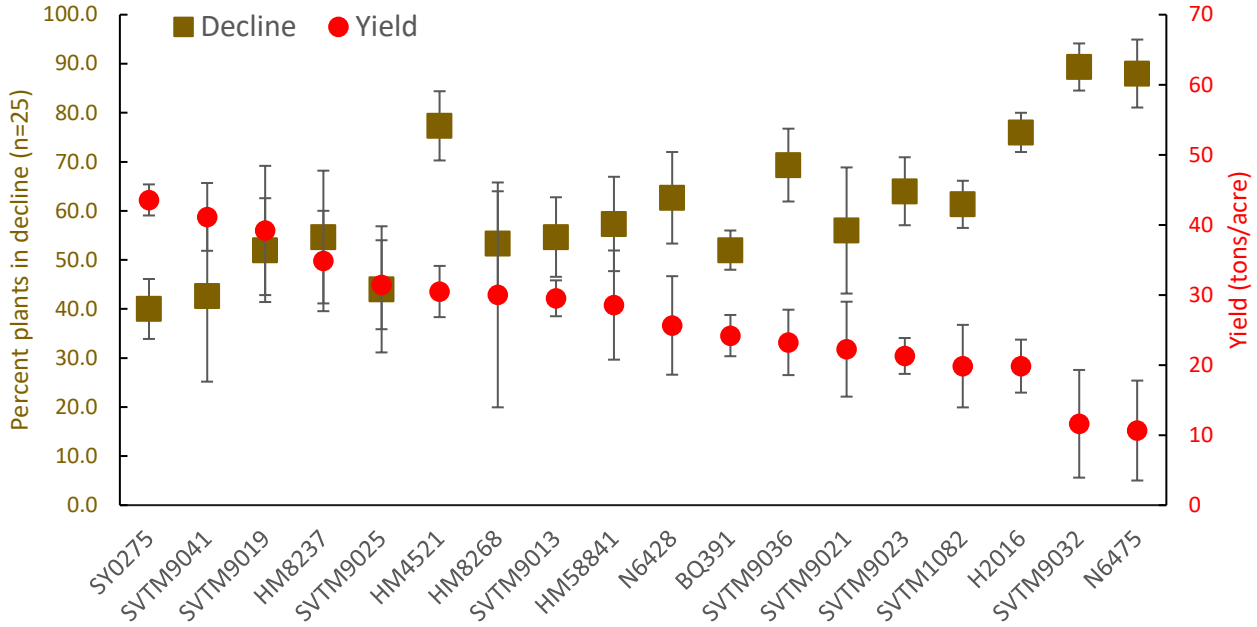

Figure 1: *UC Davis controlled cultivar trial. Incidence of plant death 4 weeks pre harvest (brown) and total yield (tons/acre, red). SY0275 (previously BQ275) was a top performer.*

1.2. On-farm cultivar screenings.

Table 1. Details of on-farm variety trials evaluated in 2024; all trials established by AgSeeds.

County	planting date	evaluation date	harvest date	#cvs	#rep	FRD pathogens lab confirmed	other diseases
Colusa	27-Mar	2-Aug	8-Aug	24	3	*F. noneumartii*	Southern blight
Sutter	27-Mar	2-Aug	5-Aug	24	3	*F. noneumartii* and *F. martii*	
Yolo	2-Apr	2-Aug	9-Aug	24	3	*F. martii*	Fusarium wilt in F2 cultivar
Sutter	12-Apr	14-Aug	19-Aug	24	3	*F. noneumartii*	Fusarium wilt and foot rot
Yolo	16-May	20-Sep	20-Sep	24	3	*F. martii*	Fusarium wilt, bacterial canker, possible Fusarium crown & root rot
San Joaquin	2-May	18-Sep	23-Sep	24	3	*F. martii*	Southern blight, bacterial canker, anthracnose
San Joaquin	2-May	27-Aug	no yield data	24	3	*F. noneumartii* confirmed in 2023	Southern blight
San Joaquin	21-May	27-Sep	no yield data	24	3	*F. noneumartii* confirmed in multiple previous years	Southern blight

Trial details are in table 1, including diagnostics conducted by the Swett lab. All fields were 60" single row bed configuration and sub-surface buried drip irrigation. Plot length varied by the trial, but was generally 75 to 100 ft, with three replicates of each entry. The primary disease metric that we are using is advanced decline (percentage of plant dead or nearly dead) just prior to harvest. Symptomatic plants were sampled near harvest and submitted to the Swett lab for laboratory diagnosis. For the yield trials, plots were machine harvested using standard grower practices. For one location, we additionally have a sort out of a 5-gallon fruit subsample to determine cull rates. From the eight evaluated, six are presented here - two were eliminated because there was too much disease pressure from other diseases in the trial.

Figure 1 combines the results on performance of the 24 common entries in the six trials, with the entries listed in order of average yield in these trials. Varieties exhibiting the fewest plants with advanced vine decline include HM 8237, LS 0681 and SVTM 9040. The highest yielding varieties often had low vine decline, but there were some exceptions such as SVTM 9041 and HM 58841 which yielded well despite moderate levels of vine decline (both have EFH trait). The varieties with the highest rates of vine decline were always associated with lower yield, although several of these varieties are known to be high yielding in the absence of disease.

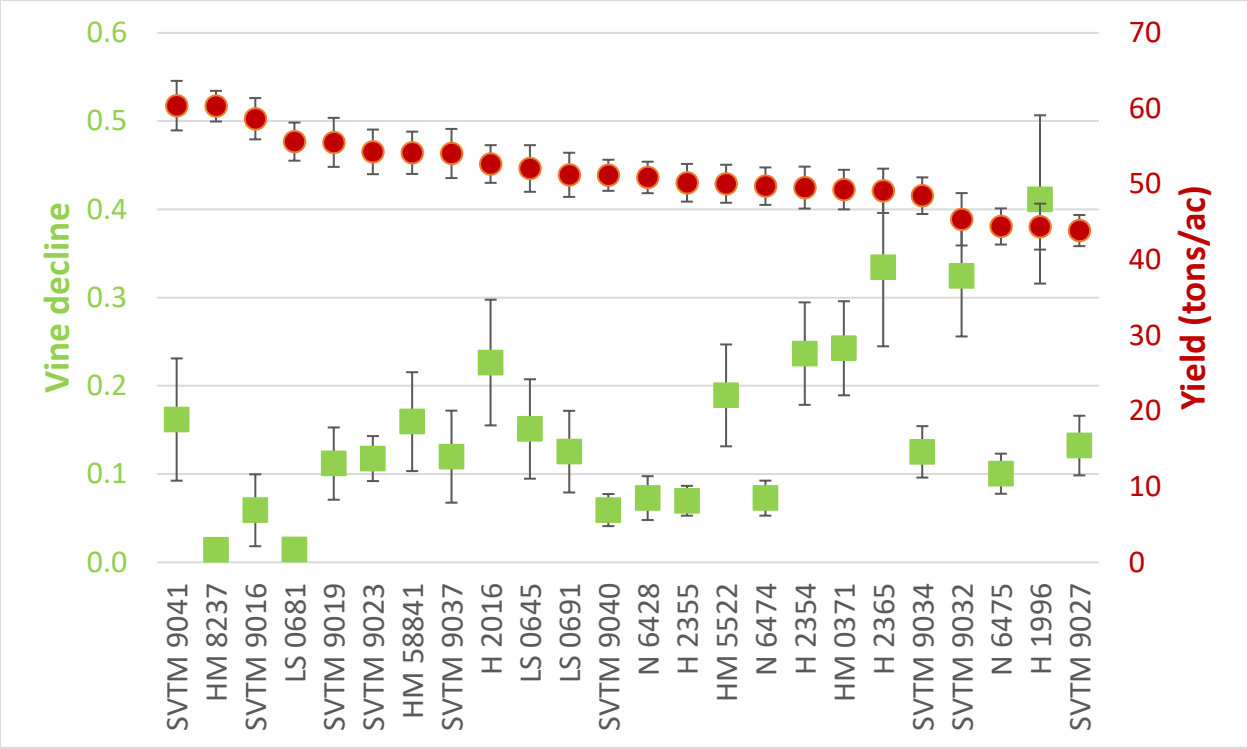

Figure 2. Mean of six locations of AgSeeds variety trials in commercial fields with disease pressure from Fusarium stem rot and decline (FRD). Error bars represent the standard errors of 18 observations (3 replicates x 6 locations).

1.3 Complete analyses of past trial data.
Analysis completed for all 2024 trials and integrated into the multi-year cultivar trial tables. Results from San Joaquin trials submitted for publication in May 2024 as a Plant Disease Management Report; results from UCD and Yolo/Colusa trials in prep as PDMRs for May 2024.

Objective 2. Providing support to regional chemical management trials for F. noneumartii
2.1 Provide diagnostic support for chemical efficacy trials.
Diagnostic support was provided for chemical management trials for FRD as reported by Lazicki and Aegerter.

Objective 3. Developing crop rotations to minimize F. noneumartii inoculum load build up and subsequent losses in tomato
3.1 Assessing host status of *F. noneumartii* in cool season rotation crops: Greenhouse trials to assess host status of cool season crops began in spring of 2024 and each ran for roughly 13 weeks. Trials were laid out in a randomized complete block design with three blocks, 6 plants per crop per block. Crops were either inoculated with a spore suspension of *F. noneumartii* (3 plants per block) or mock inoculated with water (3 plants per block). Crops were monitored for symptoms for 12 weeks and then evaluated for canopy and rot symptoms. Symptomatic plants were sampled for diagnostics to confirm causal agent of rot. Above ground biomass from all plants was collected, dried down for 2 weeks, and weighed to characterize effect of inoculation on overall plant health and growth.

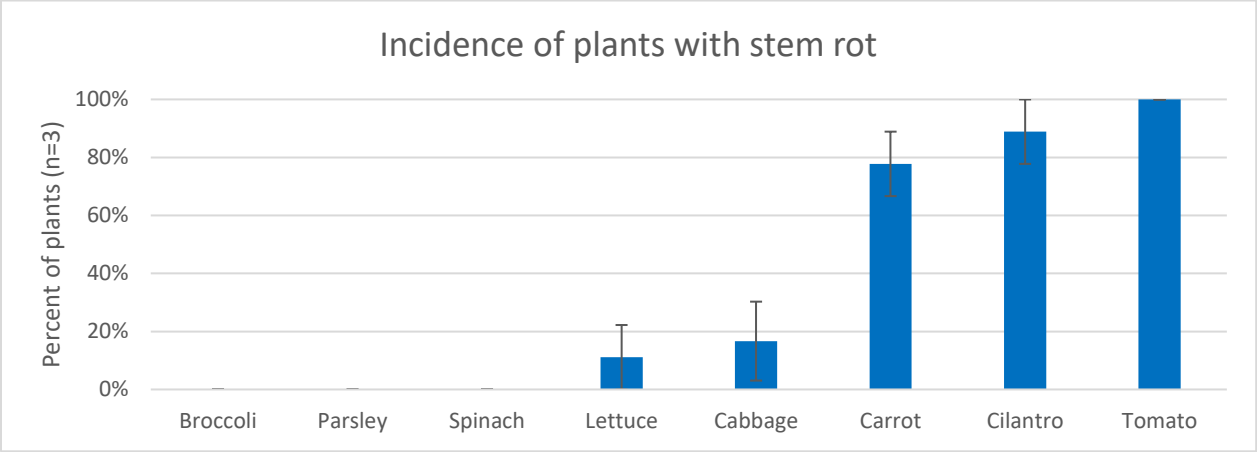

***Figure 3**: Percent of plants with stem rot in cool season crops tested in greenhouse trials.*

***Figure 4**: Crown and stem rot symptoms in cool season crops. A. Lettuce, B. Carrot, C. Cilantro.*

Carrot, lettuce, and cilantro developed the most severe symptoms after inoculation with *F. noneumartii*. These crops not only developed severe rot but also plant death. *F. noneumartii* was recovered from inoculated lettuce, carrot, cilantro, cabbage and tomato checks. Cabbage had very small lesions and no canopy symptoms. Broccoli, parsley, and spinach appear to be non-hosts.

Host range of *F. noneumartii* was also characterized in a controlled field trial. The field trial was laid out as randomized complete block design with 3 block, each containing 15 70 ft plots. The field was naturally infested, and additional inoculum was injected in December 2023. In spring of 2024 through to the summer, 10 plants per crop plot were randomly pulled and evaluated for canopy and rot symptoms. Each

crop was evaluated based on when it reached maturity and would be harvested normally (garlic and onion for example were not mature until July 2024). Any symptomatic plants were saved for diagnostics to confirm causal agent of disease.

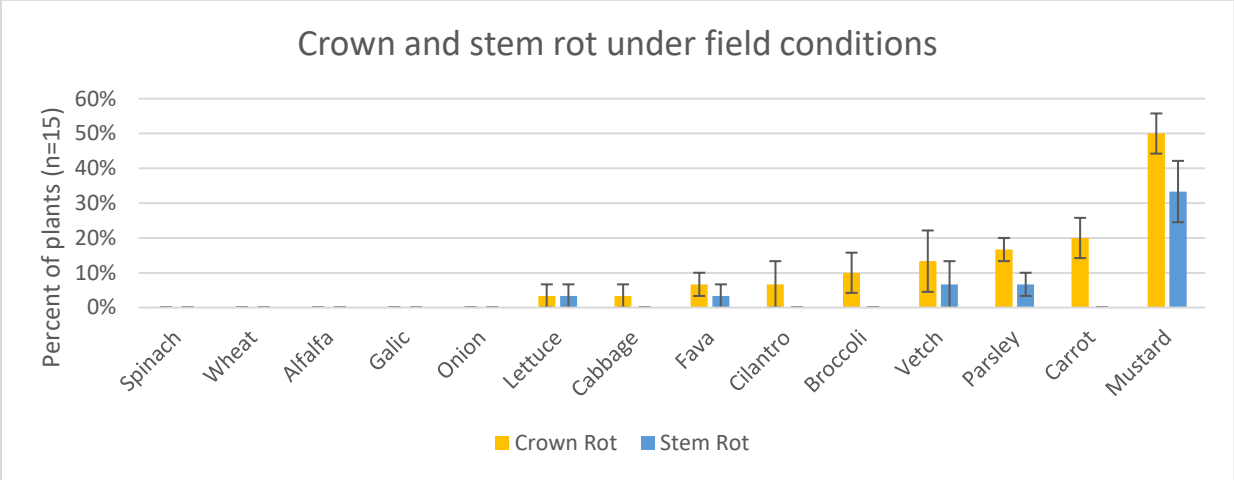

***Figure 5**: Percent of plants with crown and stem rot in cool season crops evaluated under field conditions.*

***Figure 6**: Crown and stem rot in cool season crops grown under field conditions in a FRD infested Field. From left to right: carrot, parsley, mustard*

As seen in previous years, symptoms were milder in crops grown under field conditions. Parsley, carrot, and mustard had some mild crown and/or stem rot (20-50% of plants evaluated) but we were not able to recover *F. noneumartii* from any symptomatic plants. No crops had obvious canopy symptoms in the field.

3.2 Developing winter crop rotations to minimize *F. noneumartii*- driven losses in tomato: After the above cool season host range evaluations in the field were complete (mid-March 2024), the crops were incorporated, taking care to keep each crop and its residues in the plot where it was grown. The field was left fallow for 1 month before being planted to tomatoes. Beds were prepped per industry standard with buried drip and pre-plant herbicide treatments. The winter rotation trial was laid out in the same design as described in Obj 3.1. After the tomatoes had established, we measured out 15ft monitoring plots for canopy evaluations. Disease incidence and severity were measured 2 weeks pre- and at harvest.

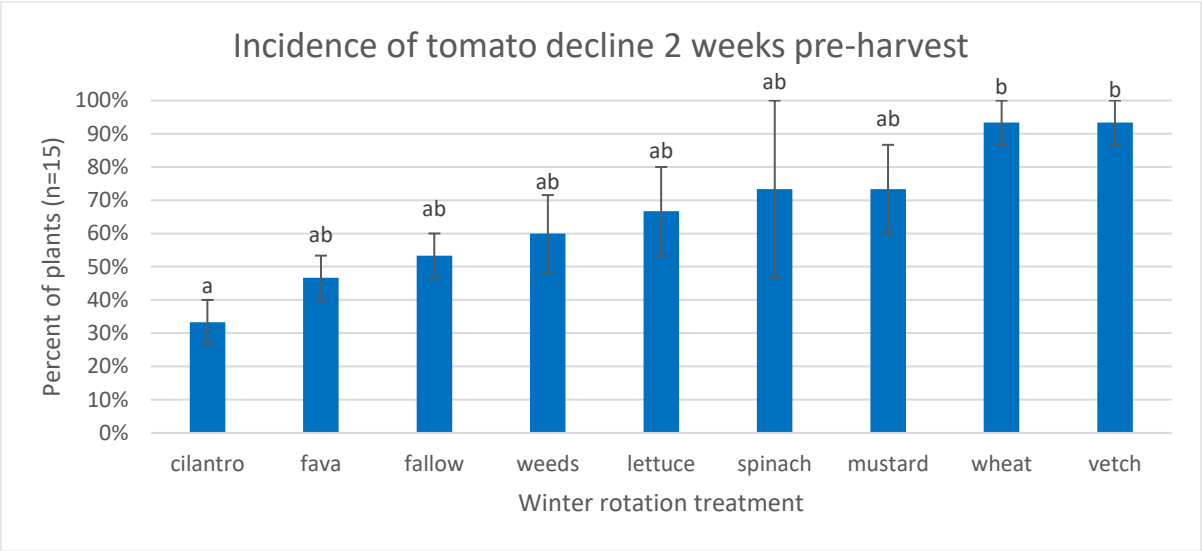

***Figure 7**: Incidence of decline in tomato after different cool season rotation treatments 2 weeks pre harvest. Plots previously planted to cilantro had significantly lower decline than plots planted to wheat and vetch (p=0.029)*

Winter rotation treatments had a significant effect on disease development in tomato. Cilantro plots had the lowest incidence of decline (~30% of plants in decline) while wheat and vetch plots had the highest (>90% of plants in decline). No treatments were significantly different from the chemical fallow control.

In addition to measuring disease development in tomato, we also characterized crop residue decomposition rates for each cool season crop during the summer. Crop residues from each crop were gathered in March, dried down at ambient room temperature, and shredded into 2in pieces. Mesh bags were then filled with different crop residues, weighed, and buried in the field in the same plots that each crop had been grown in. At the end of the summer, the bags were removed, dried, and reweighed and change in mass calculated.

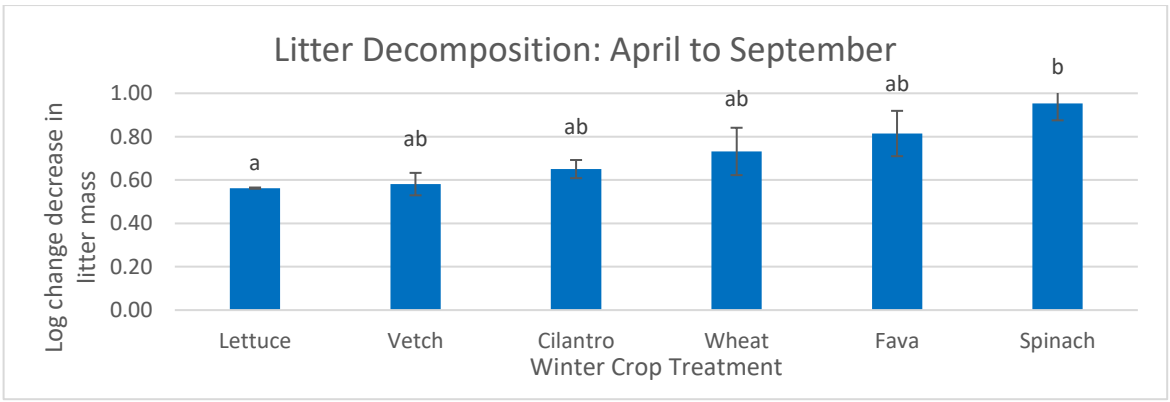

***Figure 8**: Litter decomposition rate for cool season crop residues over the summer (April-September). Spinach decomposed significantly faster than lettuce (p=0.0384).*

Crops varied in decomposition rates with lettuce decomposing significantly slower than spinach. Vetch, cilantro, wheat, and fava all decomposed at different rates but where not significantly different.

3.3 Multiyear crop rotation assessments in commercial fields: This summer we continued to follow 5 fields across Yolo and San Joaquin counties. Only these were planted to tomato this year, one of which was after a multi-year non-tomato rotation. Decline in these fields was evaluated by counting the number of plants in decline in 8 randomly placed 100ft transects at harvest.

			Percent of plants in decline		
Rotation	**Location**	**Years**	**Pre-Rotation**	**Post-Rotation**	**Change**
Tomato, Sunflower, Alfalfa, Alfalfa, Tomato	Yolo (1)	2019-2023	17.5% ± 5%	27.71% ± 6.79%	36.85%
Tomato, Cucumber, Tomato	San Joaquin (2)	2020-2022	25.9% ± 5.6%	11.49% ± 4.76%	-25.41%
Tomato, Wheat/Teff, Safflower, Tomato	San Joaquin (3)	2021-2024	15%	29.4% ± 1.8%	96%
Tomato x2	San Joaquin (4)	2022-2023	8.24% ± 1.69%	4.02% ± 1.55%	-51.20%
Tomato x3	San Joaquin (2)	2022-2024	11.49% ± 4.76%	0.15% ± 0.15%	-98.69%

***Figure 9**: Summary of commercial fields used in the rotation trial. San Joaquin site 3 was the only field to be planted to tomato after a multi-year non-tomato rotation.*

Of the three fields planted to tomato in 2024, only one was after a multi-year non-tomato rotation. This field went through a wheat/teff and safflower 2-year rotation and saw an almost 2-fold (+96%) increase in vine decline. This field was severely affected by FRD. The other two fields were planted to tomato after repeatedly being in tomato. Interestingly, these fields both saw a reduction in vine decline incidence.

Objective 4. Assessing host status of summer and winter weeds for developing targeted weed management guidelines

4.1 Assess host status of warm and cool season weeds in controlled replicated field study: Due to low weed diversity, methodology for evaluation of host status in summer weeds was altered to a survey approach, selecting symptomatic weeds for evaluation. We identified 6 cool season weeds and 3 warm season weeds growing in our infested FRD field. Plants were randomly pulled and evaluated for presence of rot. Symptomatic plants were taken for diagnostics to confirm causal agent. Cool season weeds were evaluated from January to April 2024, warm season weeds were evaluated from June to September 2024.

Family	**Genus, Species**	**Common Name**
Boraginaceae	*Amsinckia sp*	Fiddleneck
Brassicaceae	*Raphanus raphanistrum*	Wild Radish
Geraniaceae	*Erodium*	Filarees
Fabaceae	*Medicago polymorpha*	Burr Clover
Brassicaceae	*Capsella bursa-pastoris*	Shepards Purse
Montiaceae	*Calandrinia menziesii*	Redmaids
Solanaceae	*Solanum nigrum*	Black Nightshade
Amaranthaceae	*Amaranthus albus*	Tumble Pigweed
Amaranthaceae	*Amaranthus blitoides*	Prostrate Pigweed

***Figure 10**: Weeds identified in F. noneumartii host range surveys*

No cool season weeds had rot or disease symptoms. All warm season weeds identified had rot ranging from mild to severe (whole crown/stem rotten). We were able to recover *F. noneumartii* from all symptomatic warm season weeds.

4.2 Evaluate the effect of winter weedy fallow on disease development in processing tomatoes: As part of the cool season rotation study, we included weedy fallow plots to evaluate the effect of a winter weedy fallow period on disease development in tomato. Plots were allowed to go weedy from fall of 2023 to mid-March of 2024. They were then incorporated and planted to tomato 1 month later. Beds were prepped per industry standard with buried drip and pre-plant herbicide treatments. Disease in tomato was evaluated based off incidence or rot and canopy decline 2 weeks pre- and at harvest.

Plots left to chemical fallow over the winter had a 73% increase in vine decline compared to the plots left to weedy fallow. This result was not significantly different, however.

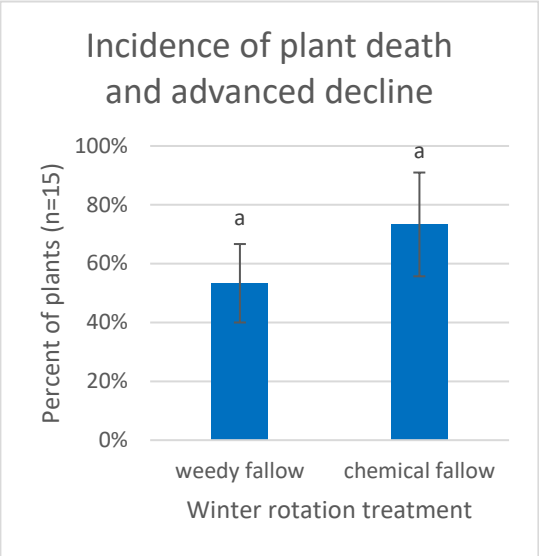

Figure 11: Incidence of plant decline in tomatoes grown in plots left to either winter weedy fallow or chemical fallow

4.3 Survey grower fields with known *F. noneumartii* infestation for symptomatic weeds and confirm disease causal agent: We surveyed three grower fields. Weed pressure was low this year and so we collected 2 species of weeds for evaluation (black nightshade, and prostrate pigweed). We were able to recover *F. noneumartii* from both species of weeds, which aligns with the results of our controlled studies.

Objective 5. Conduct F. falciforme population studies to determine whether management practices will be broadly effective against diverse genetic groups and develop diagnostic tools

To assess Obj 5.1-5.2, we repeated last summer's multi-isolate field trial. This year we included a total of 6 isolates (plus a non-inoculated control) across all three species in the susceptible processing tomato cultivar SVTM9032. For each species, we included two representative isolates. We used previous greenhouse assay data to select a more virulent isolate and a less virulent isolate to help us further characterize the disease complex.

Treatment	Virulence	Isolate	Species	Cultivar
T1	Low	CS870	*F. noneumartii* (m)	SVTM9032
T2	High	CS109	*F. noneumartii* (m)	SVTM9032
T3	Low	CS162	*F. martii*	SVTM9032
T4	High	CS91	*F. martii*	SVTM9032
T5	Low	CS918	*F. falciforme*	SVTM9032
T6	High	CS966	*F. falciforme*	SVTM9032
T7	na	na	Non	SVTM9032

***Figure 12**: Treatment description with CS isolate number, species designation, and virulence designation*

The trial was designed as a randomized complete block design with 3 blocks, 9 70ft treatment plots per row (2 of the 9 plots were planted as non-inoculated buffer plots). The field used was a clean, fumigated field with no other known pathogens present. Beds were prepped per industry standard with buried drip and pre-plant herbicide treatments. Transplants were dipped in a spore suspension of the appropriate isolate and then hand transplanted into their respective plot. Great care was taken to not cross contaminate isolates or treatments, including sanitizing all tools and boots, changing gloves between treatments, and assigning people to specific isolate treatments when doing field work so they did not move between treatments.

Once plants were established, 15 plant monitoring plots were marked out. These were used to monitor canopy symptoms and protect a section of each plot for harvest.

Disease development was characterized in three ways: on a rating scale to account for minor differences in canopy symptom development, incidence of decline, and rot ratings. Disease evaluations took place 6 weeks, 4 weeks, 2 weeks pre-, and at harvest. On September 5th, the monitoring plots were harvested to quantify overall yield impact, and a sample of the fruit was sorted to quantify fruit quality impact.

5.1 Characterize within lineage diversity and potential non-pathogens within the *F. falciforme* species complex: As described above, we included a more and less virulent isolate for each species (selected based on previous greenhouse virulence trials) to determine if virulence trends held up under field conditions.

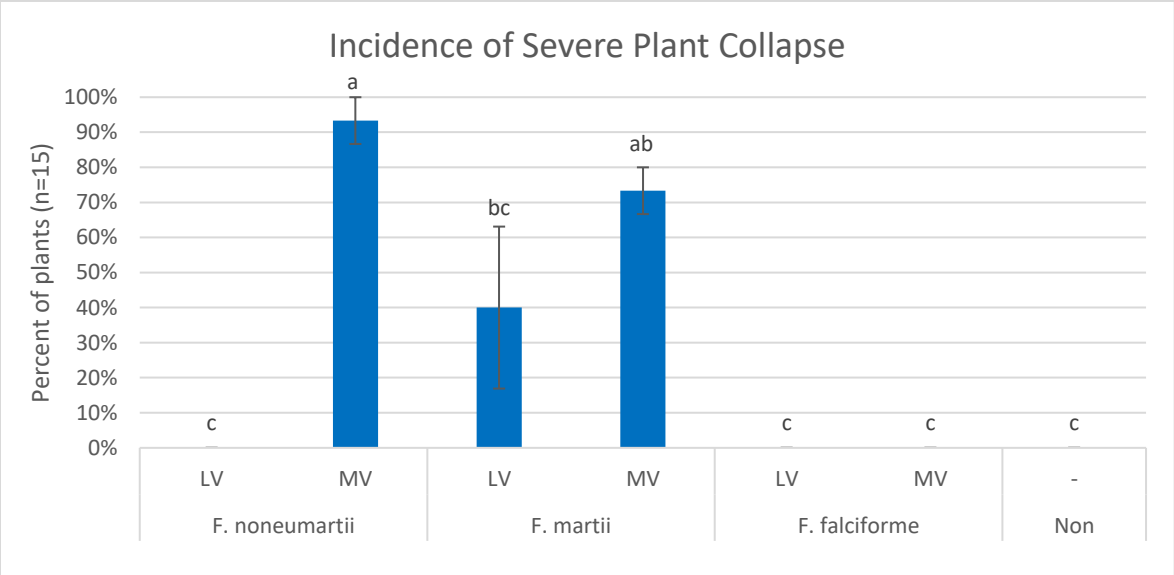

***Figure 13**: Percent of plants dead and declined at harvest. LV = less virulent, MV= more virulent. P<0.001*

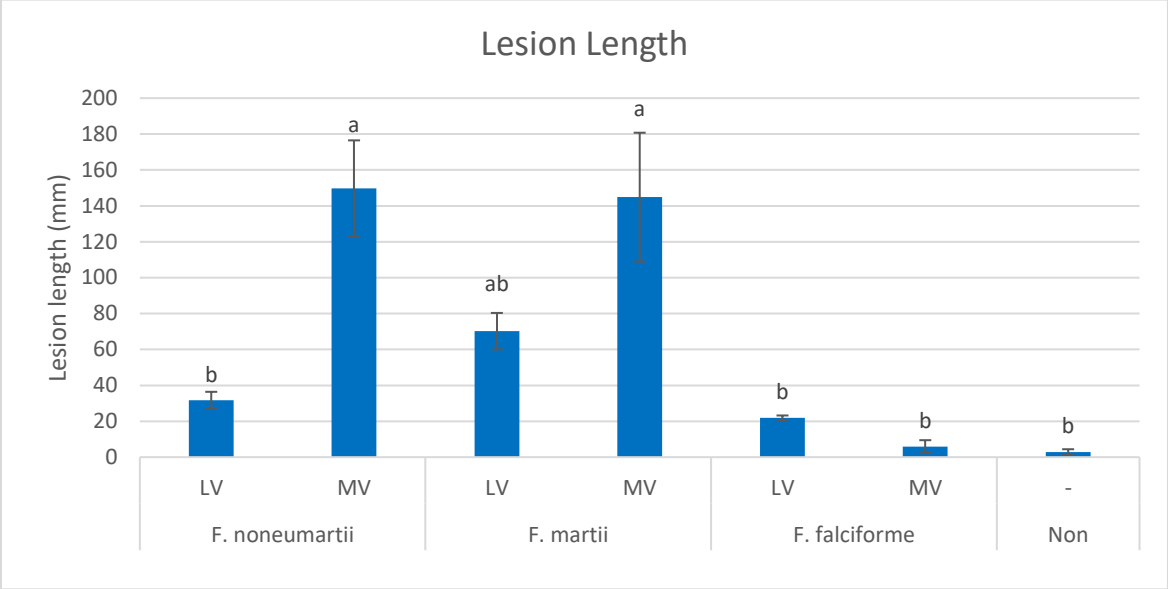

***Figure 14**: Lesion length at harvest. LV=less virulent isolate, MV=more virulent. P<0.001*

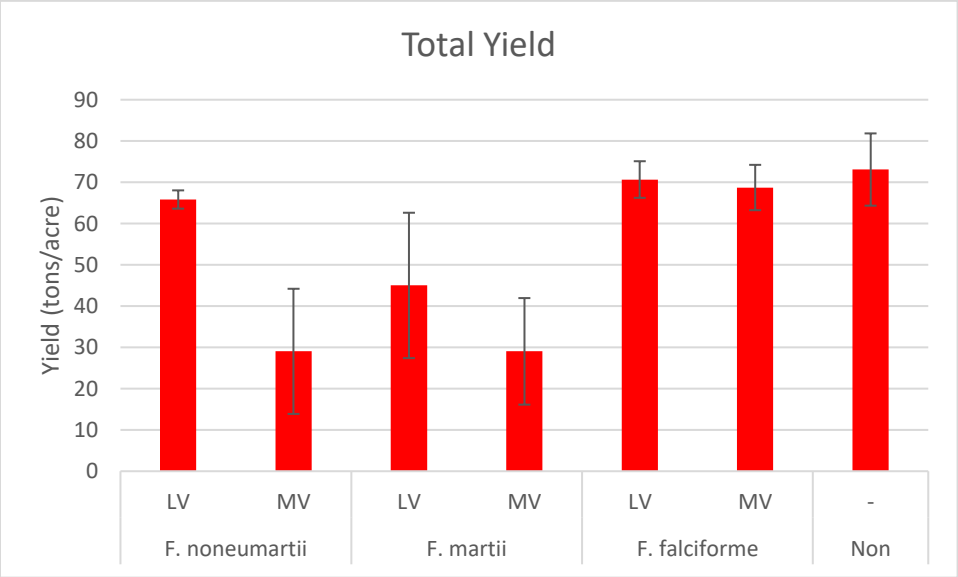

***Figure 15**: Total yield for plots inoculated with a more and less susceptible isolate of either F. noneumartii, F. martii, or F. falciforme as well as a non-inoculated plot. LV=less virulent isolate, MV=more virulent.*

As seen in greenhouse trials, the less virulent isolate for each species led to a lower incidence of plant decline, smaller lesions, and smaller yield impact than the more virulent isolate. *F. falciforme* did not differ between isolates as neither isolate caused disease levels or yield losses significantly different than the inoculated plot. The less virulent isolate for *F. noneumartii* did not lead to severe disease as expected but this is likely due to an issue with the inoculation process and not related to the virulence of the isolate.

5.2 Determine whether the *F. falciforme* species complex lineage *F. falciforme sensu-stricto* contains isolates which impact tomato production: To determine *F. falciforme* is an economically impactful species and thus needs management tools, we gathered yield data from plots inoculated with either *F. noneumartii*, *F. falciforme*, or mock inoculated non).

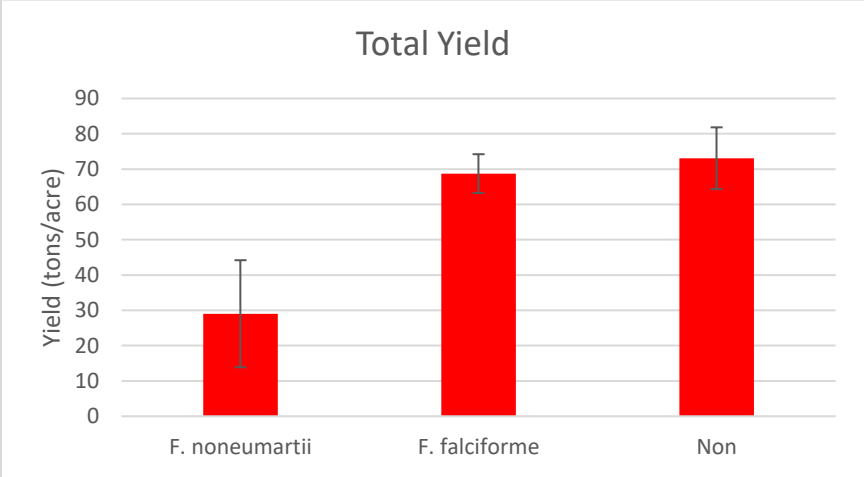

***Figure 16**: Total yield for plots inoculated with either F. noneumartii or F. falciforme as well as a non-inoculated plot. LV=less virulent isolate, MV=more virulent.*

Plots inoculated with *F. falciforme* were almost indistinguishable from, and lead to similar yields as, the mock inoculated plots (non). In comparison, plots inoculated with *F. noneumartii* had an over 2 fold reduction in yield. This indicated that *F. falciforme* is not a management concern for processing tomatoes.

5.3 Determine whether cultivar susceptibility profiles are similar across the members of the *F. falciforme* species complex causing vine decline and yield loss: Greenhouse trials to compare susceptibility profiles of *F. noneumartii* and *F. martii* are still underway. The greenhouse trial was laid out as a randomized complete block design with three blocks, each containing 9 cultivars (6 plants each) inoculated either with *F. noneumartii*, *F. martii*, or a mock inoculated control. Cultivars were selected based on field trials so that we included 3 susceptible cultivars, 3 moderately resistant cultivars, and 3 highly resistant cultivars.

5.4 Determine whether the different *F. falciforme* lineages have similar host range and thus can be managed with the same crop rotation strategies: Greenhouse trials to compare disease host ranges of *F. noneumartii* and *F. martii* were started in spring of 2024 and ran through to late fall of 2024. We tested a suite of host (cilantro, cabbage, potato, sunflower) and non-host (alfalfa, garlic, cotton, melon) warm and cool season crops (based off previous host range trials). Each greenhouse trial was arranged as a randomized complete block design with 3 blocks, each containing 4 plants per crop per treatment. Crops were inoculated with a spore suspension of either *F. noneumartii*, *F. martii*, or a mock inoculated control. Each trial also included tomato as a known host positive control. Canopy symptoms, rot, and lesion length were evaluated approximately 13 weeks later. Any symptomatic plants were collected for diagnostics to determine causal agent of disease. We also collected, dried, and weighed all above ground biomass from each plant to quantify over al plant health and growth.

***Figure 17:** Rot in cilantro caused by F. martii (left) and F. noneumartii (right)*

***Figure 18**: Potato crown and stem rot (left) and canopy decline (right) caused by either F. martii (FM) or F. noneumartii (FN). Non-inoculated as a control (Non)*

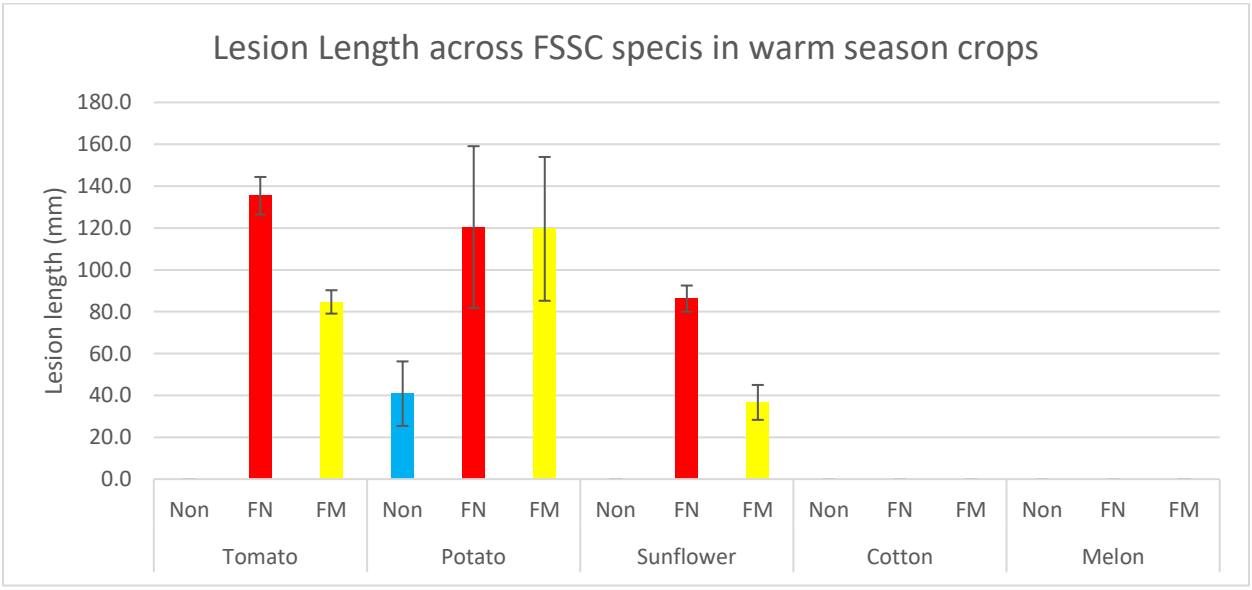

***Figure 19**: Lesion length in warm season crops inoculated with either F. noneumartii (FN) or F. martii (FM) as compared to the non-inoculated plants of that crop (Non). There were some non-inoculated potatoes that had rot.*

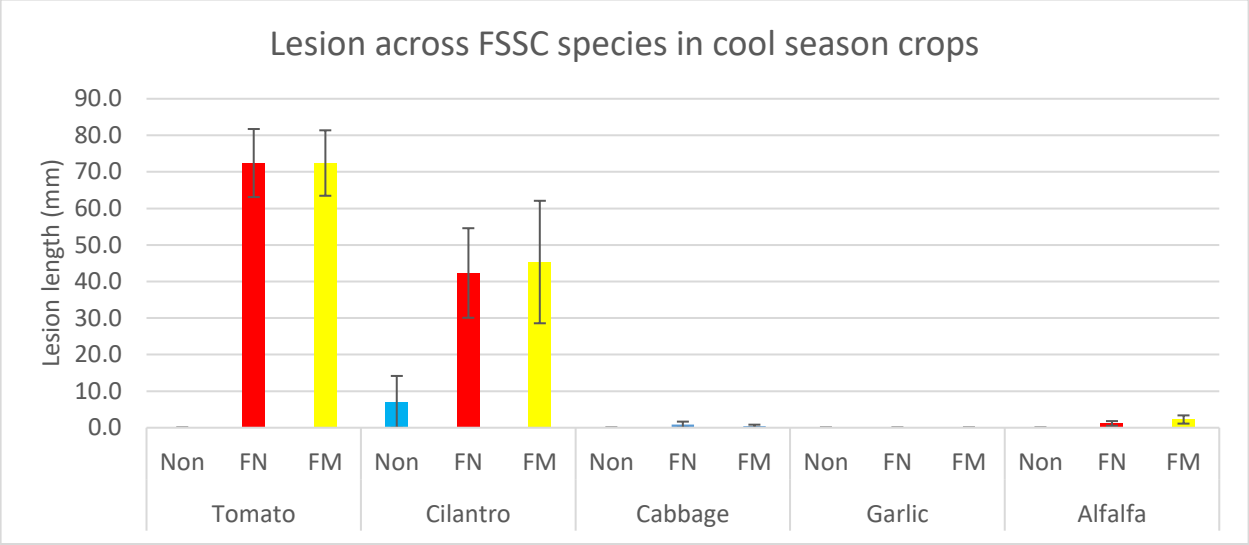

***Figure 20**: Lesion length in cool season crops inoculated with either F. noneumartii (FN) or* F. martii *(FM) as compared to the non-inoculated plants of that crop (Non)*

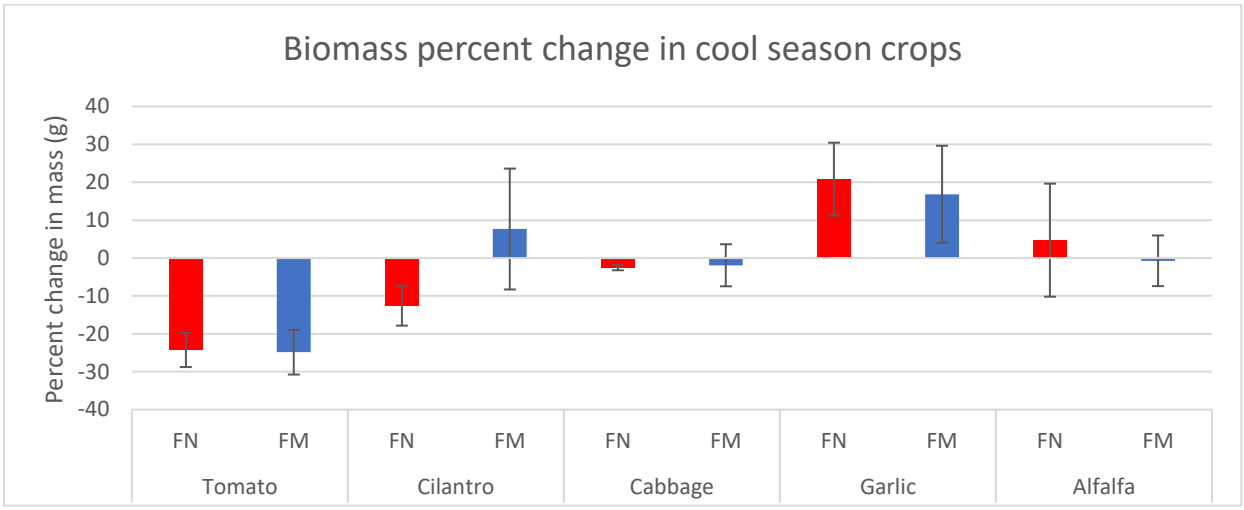

***Figure 21**: Percent change in biomass of cool season crops inoculated with either F. noneumartii (FN) or F. martii (FM) as compared to the non-inoculated plants of that crop*

Host crops (cilantro, potato, sunflower, tomato) all developed similar lesion lengths when inoculated with either *F. noneumartii* or *F. martii* while all crops that were non-hosts to *F. noneumartii* were also non-hosts to *F. martii* suggesting these two pathogens have similar host ranges. Biomass was only affected in tomato for the cool season crops. Biomass data for warm season crops is still underway.

Discussion:
Cultivar Trials

Results from the controlled UC Davis cultivar trial suggest that SY0275 (previously BQ275), SVTM9041, and SVTM9025 are top performers in terms of vine decline while N6475, SVTM9032, H2016, and HM4521 are poor performers. Interestingly, HM4521 had one of the highest incidences of vine decline (77% of plants) but was in the top 6 cultivars for yield suggesting it was still able to yield well despite severe vine decline (highly tolerant).

We continued to expand upon *F. noneumartii* host range studies this year. As we gather more data on a wider range of crops, a pattern has emerged for *F. noneumartii* host range. It appears that the pathogen

can affect a wide range of crops but only a few are severely affected to the point of plant death or economic loss. For example, in warm season crops, sunflower, safflower, potato, pepper, garbanzo, hemp and pumpkin all develop crown and/or stem rot when inoculated with *F. noneumartii* but only sunflower and potato appear to be killed or show canopy symptoms. This has also been observed in cool season crops where cilantro, carrot, lettuce, and cabbage all developed some amount of rot but only carrot and cilantro were severely affected (cilantro was one of the most severely affected crops, leading to complete plant death soon after inoculation). A grower should thus not plant these severely affected crops (sunflower, potato, cilantro, carrot) in fields infested with FRD as they may see an economic impact. The other milder hosts are still important, however, as they could cryptically increase inoculation loads in fields infested with FRD without showing canopy symptoms.

As we have now evaluated a wide range of crops and weeds across many families, we have been able to get a better idea of family-level patterns for host range. It appears that the Solanaceae and Asteraceae family contain many key hosts of *F. noneumartii*, such as tomato, pepper, nightshade, and sunflower, safflower, sow thistle, and horseweed. Interestingly, while previous host range work (Nakayama, K. and Aoki 2010, Romberg, M. K., and Davis, R. M. 2007, and Sagara, D. S. 2004) suggested that legumes (fava, kidney beans) are common hosts, we have not seen that in our work. We have seen however that these two crops commonly develop a physiological darkening of stem tissue in greenhouse trials not associated with a pathogen and this may have been mistaken for stem rot in previous studies. We have tested fava, vetch, and alfalfa, all of which did not develop disease symptoms. Other families, including the grass (corn), mallow (cotton) and cucurbit (melon) families appear to include species which are non-hosts.

As warm season crop rotation trials were completed last year, we focused on cool season rotation trials this year. A winter rotation program resulted in a large range of disease development. In the tomato plots previously planted to different winter rotation/cover crops we observed plant mortality and decline ranging from 30% to and as severe as 95% of plants. Cilantro led to the lowest levels of disease (~30 of plants in decline) in tomato while wheat and vetch lead to the highest (~90% of plants). A chemical fallow treatment (no crops or weeds) lead to a ~50% incidence of plant decline in toamto which was higher than a cilantro or fava winter rotation, but differences were not significantly different. As seen in previous years, host status does not seem to be as predictive of rotation risk as in warm season crops. Cilantro, which is a severely affected host of *F. noneumartii* appears to be the lowest risk rotation while wheat and vetch, which are clear non-hosts, are high risk. This aligns with results from last year where an alfalfa rotation (another non-host crop) lead to the highest levels of disease in tomato. This suggests there may be other factors associated with these crops which may be contributing to rotation risk.

Multi-year commercial rotation trials have been useful to further evaluate rotation regimes in FRD infested fields. Thus far, commercial rotation programs have corroborated our controlled trial findings, suggesting that crops such as wheat, sunflower, safflower, and alfalfa are high risk rotations while cucurbits (specifically cucumber) are low risk rotations.

In the duration of this project, we have furthered our understanding of the pathogen make up of this disease complex and how it fits together with the overall Fusarium Solani Species Complex (FSSC). When we first started this work, we believed that there was one primary pathogen, *F. falciforme*. We later identified an additional and highly virulent species to be contributing to disease: *F. noneumartii*. Two years ago, we characterized a third virulent species: *F. martii*. This year we finalized population studies and have confirmed that F. noneumartii and *F. martii* are both highly virulent, economically impactful species and are of concern for the processing tomato industry. *F. falciforme sensu stricto*, on the other hand, is a weakly pathogenic species and is not associated with economic losses in processing tomato. We have thus narrowed our focus to *F. noneumartii* and *F. martii* for development of management tools.

As part of a dual-species management approach, this year we initiated multi-species host range studies. Thus far our rotation and host range studies have focused on *F. noneumartii* but as *F. martii* is also an

economically impactful pathogen, it is important that we confirm management tools designed around *F. noneumartii* are also effective for *F. martii*. This year we focused on expanding the host range and cultivar studies to include *F. martii*. Multi-species host range studies suggest that crops that are hosts to *F. noneumartii* are also hosts to *F. martii* and visa versa for non-hosts. We observed similar disease levels in cilantro, sunflower, and potato when inoculated with either *F. martii* or *F. noneumartii*. Similarly, we saw no disease develop in crops designated as non-hosts (such as garlic, alfalfa, cotton, and melon) when inoculated with *F. martii*.

Next Steps

As new cultivars continue to be released and cultivar preferences continue to change, updated and expanded cultivar trials remain important to provide growers and industry leaders with up-to-date cultivar recommendations for use under FRD pressure. The controlled UC Davis cultivar trials also are vital for interpreting statewide commercial cultivar trials and to confirm results are consistent statewide with a single pathogen field trial.

Host range trials are in their final phase. Warm season crop host range trials are complete and cool season trials need one more repeat to confirm results. With our suite of crops tested we will be able to provide growers with a well-defined list of crops that are affected by FRD, whether severely or cryptically as discussed above. We also plan to continue to expand host arrange studies to *F. martii* so that rotation and crop avoidance guidelines can be used to holistically management all impactful members of FRD. By utilizing greenhouse *F. martii* host range studies, we hope to avoid field-based *F. martii* rotation trials as we can extrapolate crop response to these pathogens.

Warm season crop rotation trials are complete and cool season trials are in their final year. Moving forward we plan to combine all data from these multi-year trials and develop crop rotation guidelines for FRD management. In addition, we plan to utilize soil organic matter and nutrition studies to develop predictive risk guidelines for crops. As discussed above, some crops appear to be non-hosts but are high-risk rotations and vice versa, suggesting there may be other crop attributes that are connected to rotation risk.

Acknowledgments: We would like to acknowledge the generous cooperation of Harlan Farms, AgSeeds and their grower cooperators, TS&L, and HM Clause. We appreciate the hard work of UCD farm managers Bryan Pellissier and Alexa Sommers and the Swett lab members who assisted with experiments and diagnostics support.

This project as leverage for other dollars:

CDFA-DPR. "Reducing current and future fungicide use in California crops by providing decision support and rotation tools for managing the emerging, highly damaging Fusarium falciforme pathosystem." $598,497 total. ~$15,000 for objectives on this project. Collinson, student and technician salary support; supplies.

California League of Food Producers. $28,519. Collinson salary support. Developing additional information that can be used to develop more resistant cultivars for FRD.

Seed Companies—FRD screening services. $25,000. Collinson salary support. To evaluate commercial and pre-commercial lines for FRD resistance in high throughput greenhouse screenings.

AgSeeds. **~$10,000.** Transplant donation for all field trials.

Western Sustainable Agriculture and Education (WSARE); $29,999 to continue work on developing rotation guidelines for FRD and furthering soil health management; ~$10,000 leveraged for this project period. Collinson, technician and student salary support.

TA-ship-Collinson. Spring quarter, covering tuition ($5,000) and salary ($23,750).

BUDGET SUMMARY

	Responsibility/ purpose	% time on project	Requested Funds 2023
Personnel (salary)	GSR (Collinson-PhD student)-rotation, UCD CV assist, lineage management, PDMR co-author, FA CV trial asst	25%	22,667
	Undergraduate assistant	27%	12,160
	GSR (Collinson-PhD student)	25%	510
Personnel (fringe)	Undergraduate assistant	27%	274
Supplies and Equipment	Lab supplies		2,800
	Plant growth supplies		800
	In state travel for surveys in commercial fields		300
Travel	Greenhouse space rental		800
Other (specify)	Sequence analysis		800
	UC Davis Field Station recharge ($1,100/A x 2A)		2,200
	2 quarters GSR tuition (Collinson)		10,012
Total Costs			53,323

Yield performance from 27 variety trials from 2019 to 2024. Six sites on UCD campus, all others in commercial fields with confirmed Fusarium stem rot and decline. Summary excludes varieties that are no longer in the top 50 statewide. Those highlighted in green have had the highest yield. Yield is normalized to the average from each trial, so a value of 1.18 indicates that variety yielded 18% higher than the trial average yield.

Variety	# of Trials	Normalized Trial Yield	2024 Loads Rank (PTAB)	Notes about FRD tolerance/susceptiblity and fruit holding traits
HM8237	15	1.18	2	Tolerant, EFH
SVTM9025	5	1.15	13	Moderately tolerant with EFH, Forl resistance
SVTM9041	11	1.13	40	low to moderate rates of vine decline, EFH
SVTM9016	17	1.11	1	Tolerant, EFH
HM58841	19	1.08	4	Fairly tolerant of FRD, although occassionally does decline, good yields in problem fields, EFH, susceptible to Fus wilt race 3
LS0681	6	1.08	---	new variety from Lark Seeds, low vine decline
N6428	25	1.06	9	Tolerant, EFH
SVTM9019	16	1.06	11	Tolerant, EFH
SVTM9037	17	1.06	22	low to moderate vine decline, EFH
H5608	5	1.05	23	did well at most locations, moderately tolerant
SVTM9023	10	1.03	15	susceptible to vine decline, but has EFH and yields decently
H2016	12	1.01	5	moderately susceptible to FRD, but manages to yield decent in problem fields
SVTM9036	12	1.01	26	high yields under low disease pressure and has EFH, but very susceptible to vine decline
HM5522	15	1.00	6	susceptible to FRD and Fus wilt race 3, although yields decently despite decline, Forl resistance
HM8268	9	1.00	7	low vine decline at 7 locations, yields decent
LS0645	6	1.00	---	new variety from Lark Seeds, moderate vine decline
H2355	6	0.98	---	low to moderate vine decline
SVTM9040	14	0.98	---	variable rates of vine decline
HM0371	10	0.97	---	moderately susceptible to FRD, Forl resistance
N6474	6	0.97	25	low vine decline at most locations, EFH
H2354	6	0.96	---	susceptible to vine decline
SVTM1082	4	0.96	17	low decline at 3 of 4 sites
SVTM9013	6	0.96	20	variable vine decline rates
SVTM9021	4	0.96	30	moderately susceptible to FRD, Forl resistance
SVTM9034	10	0.96	28	low to moderate vine decline, late early variety
H2365	6	0.95	---	susceptible to vine decline, EFS™
SVTM9038	3	0.95	---	susceptible to vine decline, EFH
H1662	9	0.91	16	variable performance, did well at 1 location, medium to poor at others
BP74	9	0.90	31	variable performance, did well at 3 locations, medium to poor at others
H1996	12	0.90	3	susceptible to vine decline, but has EFS™ trait
SVTM9032	12	0.89	18	susceptible to vine decline but has Forl resistance, early variety
N6475	10	0.88	21	low vine decline at 3 of 4 sites, EFH
SVTM9027	6	0.86	8	relatively low vine decline, but not high yielding

Project Title: Evaluation of Insecticide Programs in Processing Tomatoes for the Management of BCTV and TSWV Vectors and Viruses

Year of Project Initiation: 2024

Amount of funding requested from CTRI for this year: $7,715

Principle Investigator:
Thomas A. Turini
University of California Ag and Natural Resources
Fresno County Cooperative Extension
550 E. Shaw Avenue, Suite 210-B
Fresno, CA 93611
taturini@ucanr.edu
559-375-3147

Executive Summary:

Beet curly top virus (BCTV), which is vectored by beet leafhopper, and *Tomato spotted wilt virus (TSWV)*, which is primarily transmitted by Western flower thrips in California, occur annually in California, occasionally causing massive economic losses. In work previously sponsored by CTRI, *BCTV* incidence was significantly lower in treatments that included insecticides within two modes of action that include anthranilic diamides [Verimark (cyantraniliprole)] and neonicotinoids [Admire (imidacloprid), Platinum (thiamethoxam) and Venom (dinotefuran)]. Verimark was consistently effective when applied to transplants 72- to 24-hours before planting, but did not demonstrate efficacy when drip-injected. Neonicotinoids were effective applied in transplant water or injected into the sub-surface drip irrigation systems. However, on 1 January 2024, The Department of Pesticide Regulation adopted regulations restricting use of nitroguanidine neonicotinoid insecticides, which include imidacloprid, thiamethoxam, and dinotefuran as well as all commercial products containing those active ingredients. This substantially limits usage, which included prohibiting application of any regulated neonicotinoid after flowering, which is approximately two weeks post-plant depending upon transplant age and environmental conditions. Because Verimark must be applied pre-transplant and may have a 28- to 35-day residual, and neonicotinoids must be applied pre-bloom and may have 21-day residual under heavy pressure, there are no registered insecticides with experimental verification of efficacy in reducing BCTV incidence for protection after approximately 35-days post-transplant. Infection at that stage of crop development can occur and substantially reduce yields.
The focus of this work was to evaluate ability of insecticides registered for mid-season use to reduce *BCTV* incidence within an IPM program. This project is particularly relevant in consideration that during season with very high pressure, mid- and late-season has caused economic damage and in areas with a history of losses, neonicotinoids used beyond bloom are a common part of the pest management program.

Insecticide programs were compared at the University of California West Side Research and Extension Center in Fresno County in a four-replication randomized complete block experiment in which programs that included Verimark-transplant treatments or pre-bloom Admire applications were followed by foliar programs that included rotations of insecticides, which included Assail (acetamiprid), Exirel (cyantraniliprole), Mustang Max (Zeta-cypermethrin) and Sivanto (flupyradifurone) at four-, six- and/or eight-weeks post-plant. This trial was designed to complement an IR-4 trial that focused on early
Due to low disease pressure, the results of the 2024 study were inconclusive. However, the issue remains critical and similar studies should be conducted in 2025.

Introduction:

CTRI supported work conducted in Fresno County showed that a systemic insecticide program using neonicotinoids (IRAC Group 4A) such Admire and Platinum provides significant reduction in curly top. In addition, Verimark (cyantraniliprole, IRAC Group 28) transplant treatments consistently and significantly reduced BCTV incidence.

On 1 Jan 2024, use of neonicotinoid insecticides in California were restricted. California Department of Pesticide Regulation released 'Text of Final Regulation' Neonicotinoid Pesticide Exposure Protection Section 6990 that reduces both the maximum allowable rate of restricted neonicotinoids per season and limits applications to pre-bloom stage of crop development. Total allowable rate of any combination of neonicotinoid insecticide, or one material applied to both soil and foliage is not to exceed 0.172 lbs active ingredient per acre per season. Before this regulation, it was permissible to use up to 0.38 lbs imidacloprid, 0.172 lbs thiamethoxam and 0.263 lbs dinotefuron per acre per season and it could be applied regardless of the presence of bloom. A single active ingredient applied either to the soil or to the foliage may be applied at the previous label rates. However, prior to the regulation, neonicotinoids were used in sequence through the season to reduce transmission of *BCTV* when there was risk of beet leafhopper migrations at later stages of crop development.

The issue is that efficacy of the available materials under the new regulations does not provide a potential for use of a tool with documented, consistent effect in reducing the incidence of beet curly that will protect the plants after approximately five weeks post plant. It is unlikely that Verimark would have efficacy 45 days after application, and the neonicotinoids that were applied pre-bloom are also unlikely to have efficacy under heavy pressure five weeks post-plant, which is likely to be approximately 31 to 35 days after the latest possible application before bloom.

In view of the limitations on the use of neonicotinoid insecticides, the efficacy of alternative insecticides that are less likely to be subjected to regulatory pressures, such as Assail (acetamiprid a neonicotinoid unaffected by the regulation) and Sivanto (flupyradifurone, IRAC Group 4D) and the use of Exirel (cyantraniliprole, IRAC Group 28) as a foliar equivalent to Verimark will be evaluated in programs to bridge the mid-season gap in protection from BCTV that is left by the neonicotinoid restrictions.
Although impact of neonicotinoids on Western flower thrips has not been documented in local studies, additional materials should be evaluated given the seriousness of the issue with *TSWV* in this this production area and the absence of highly efficacious insecticides or insecticide programs. Therefore, *TSWV* incidence was recorded.

Main Goal and Objectives:

To generate efficacy data of insecticide programs allowable under current regulations that mitigate the risk of loss due to BCTV and TSWV.

- Evaluate effect of alternative insecticides on beet curly top disease incidence in processing tomatoes.
- Evaluate effect of alternative insecticides on Western flower thrips population densities and tomato spotted wilt virus incidence in processing tomatoes.

Methodology and Results:

Commercially produced transplants (H5608) were mechanically planted into 60-inch beds with sub-surface drip tape injected at a 10-inch depth into Panoche clay-loam soil at the University of California West Side Research and Extension Center on May 23. Because there is greater risk of infection under low

to moderate pressure before the canopy closes due to leafhopper behavior, the transplant spacing was placed at 16-inches rather than the typical 12-inch spacing between plants.
The experimental design was a randomized complete block with four replications. Each plot was a single bed x 60 ft. Experimental plots were separated by one untreated, planted row and there was a 10 ft planted buffer within the row between plots.

Application details are as follows: Verimark was applied to the transplants on May 22 at a rate of the equivalent of 13.5 fl oz per acre based on a plant density of 6534 plants per acre. Each 192 cell tray was treated with 12 ml Verimark in 275 ml water. All materials applied through the sub-surface drip irrigation were injected with generator-powered electric metering pumps (A-1600 FlexFlo® Peristaltic Pump Blue and White Industries, Huntington Beach, CA) over 30 minutes, which was followed by 2 hours of additional irrigation time. Dyne-Amic 0.25% v/v was included in all foliar tank mixes. On the 21 Jun, sprays were directed; applied with a CO2-pressurized back-pack sprayer at 30 psi with two TeeJet 8003EVS nozzles 19-inches apart at an equivalent volume of 20 gallons per acre. On 5 and 23 July, broadcast applications were made with CO2-pressurized back-pack sprayer at 30 psi with three TeeJet 8003EVS nozzles 19-inches apart at an equivalent volume of 30 gallons per acre.

transplant trt (22 May)[z]	pre-bloom drip (6 Jun)[y]	4th week post-plant (21 Jun)	6th week post-plant (5 Jul)	8th week post-plant (23 Jul)	BCTV incidence (%)			TSWV (%)	
					13-Jun	26-Jun	31-Jul	26-Jun	31-Jul
Verimark 13.5 fl oz					0.51	1.47	1.96	0.00	0.99
Verimark 13.5 fl oz	Admire Pro 10.5 fl oz				0.00	0.00	0.00	0.00	1.42
Verimark 13.5 fl oz		Beleaf 4.2 oz drip[y]	Exirel @20.5 fl oz foliar[x]		1.01	1.94	2.45	0.00	2.01
Verimark 13.5 fl oz		Beleaf 4.2 oz drip	Beleaf 4.2 oz drip	Exirel @20.5 fl oz/a foliar	0.00	0.48	0.00	0.00	0.00
Verimark 13.5 fl oz		Beleaf 4.2 oz drip	Beleaf 4.2 oz foliar	Exirel @20.5 fl oz/a foliar	0.00	0.00	0.00	0.00	1.00
Verimark 13.5 fl oz		Assail 30SC 3.4 fl oz			0.00	0.00	0.00	0.00	2.02
Verimark 13.5 fl oz		Mustang Mx 4 fl oz DF[w]			0.00	0.00	0.49	0.00	1.96
Verimark 13.5 fl oz		Sivanto 28 fl oz drip			0.00	0.51	0.51	0.00	0.97
Verimark 13.5 fl oz		Sivanto 9 fl oz foliar	Sivanto 9 fl oz foliar	Sivanto 9 fl oz foliar	0.00	0.00	0.50	0.00	2.50
	Admire Pro 10.5 fl oz		Assail 30SC 3.4 fl oz flr		0.00	1.68	1.68	0.00	0.50
	Admire Pro 10.5 fl oz		Mustang Mx 4 fl oz foliar		0.50	0.99	1.49	0.00	0.57
Untreated control					0.61	0.61	1.22	0.00	4.18
Probability					0.348	0.399	0.438	NS	0.408

[z] Verimark was applied to transplants on 22 May at a per acre equivalent of 13.5 fl oz.

[y] All drip-injected materials were applied with electric metering pumps over 30 minutes, which was followed by 2 hours additional irrigation.

[x] Broadcast foliar treatments were applied with a CO_2-pressurized back-pack sprayer at 30 psi in the equivalent of 30 gallons per acre with Dyne-Amic surfactant 0.25% v/v.

[w] Directed foliar treatments were applied with a CO_2-pressurized back-pack sprayer at 20 psi in the equivalent of 30 gallons per acre with Dyne-Amic surfactant 0.25% v/v.

Discussion:

Disease through the production area was relatively low in 2024. Although *beet curly top virus* was present in the trial, it remained at very low levels throughout the season, so treatment differences were not present. *Tomato spotted wilt virus* was also present in the field but did not increase until very late in the season.

The impact of a post-bloom ban on applications of neonicotinoid insecticides poses a serious limitation in avoiding economic loss in the event of a year with high population densities of beet leafhoppers carrying *BCTV*. Neonicotinoids have demonstrated efficacy in reducing incidence of this virus. Currently, under the California Department of Pesticide Regulation Neonicotinoid Pesticide Exposure Protection Section 6990 the prohibition of post-bloom applications, no insecticides that have shown efficacy in reducing BCTV incidence in tomato experimentally can be used to protect against mid-season infection that will weaken or kill plants and reduce yields.

In 2025, similar studies should be conducted.

Other Support: Support from Bayer Crop Science ($3000) and FMC ($6,000) was received for this work. In addition, another study to evaluate insecticides applied at earlier stages of crop development for effect on BCTV incidence was supported by IR-4 ($15,000).

Project Title: Statewide surveillance for virus diseases in processing tomatoes and investigation of unusual curly top and spotted wilt disease outbreaks in the Northern Counties

Year of Project Initiation: Ongoing

CTRI Funding in 2024: $39,000

Principle Investigator: Robert L. Gilbertson
Department of Plant Pathology,
University of California-Davis,
1 Shields Ave. Davis, CA 95616
Phone: 530-752-3163 FAX: 530-752-5674 e-mail: rlgilbertson@ucdavis.edu

Co-Principle Investigator: Neil McRoberts, Department of Plant Pathology University of California-Davis e-mail: nmcroberts@ucdavis.edu

Cooperators:
Tom Turini, UCCE Farm Advisor
Fresno County
e-mail: taturini@ucdavis.edu

Patricia Lazicki, UCCE Farm Advisor
Yolo, Solano and Sacramento Counties
e-mail: palazicki@ucdavis.edu

Other UCCE Farm Advisors, PCAs and growers
Diane Ullman, Entomologist
Department of Entomology and Nematology University of California-Davis
e-mail: deullman@ucdavis.edu

Executive Summary:

This is an ongoing project to provide rapid virus surveillance and diagnostics for viral diseases of processing tomato in California, and research on the disease biology and development of integrated management approaches. More than 10 viruses can affect processing tomatoes in California, but **curly top disease (CTD) and tomato spotted wilt disease (TSWD) are most important,** with exotic viruses such as tomato brown rugose fruit virus (ToBRFV) also a concern. **In 2024,** the overall incidence of virus disease in processing tomatoes in California was relatively low based on results of our field surveys, the reduced number of samples received for virus testing, and no requests for field visits for virus disease outbreaks. A similar situation was observed in 2023, and this was associated with cool wet winter and spring weather, and this was also the situation in 2024. It is possible these cool wet conditions may reduce populations of overwintering insect vectors. In 2024, CTD was not observed in fields in the Northern production area and very low beet leafhopper (BLH) numbers were captured on yellow sticky cards (YSCs) and BCTV was not detected in any of these BLHs. In Fresno the disease was present at low levels, consistent with results of YSC monitoring and virus testing of BLHs. TSWD appeared at the end of April-early May in the North and Fresno. However, spread of the disease was low in the North, whereas it was substantially greater in Fresno, though much of this was late infection and did not impact yield. TWSV strain typing revealed samples from Fresno infected with the resistance-breaking (RB) TSWV YPT strain, samples from Colusa and Sutter with the new CPN strain and those from Yolo infected with both strains often in mixed infection. Interestingly, in the mixed infections, levels of CPN were consistently greater than those of YPT, suggesting competition between strains. We also performed a mechanical inoculation screening of the top 13 processing tomato varieties with CPN and YPT and found that all varieties were infected with both strains, YPT and CPN were equally pathogenic and symptom development varied among varieties. In 2024, we again ran the

thrips generations degree-day (DD) model, with blogs for the Northern production area prepared by Patricia Lazicki and for other areas by Neil McRoberts. This allows growers to target the Gen 2 and 3 thrips to slow down TSWV spread. Finally, we maintained a strong outreach effort in terms of getting this information out to our in different ways and as quickly as possible.

Introduction

Processing tomatoes grown in California can be affected by more than 10 viruses, with the importance and prevalence varying depending on the year, location and other factors. The two most important viral diseases of processing tomatoes are tomato spotted wilt caused by tomato spotted wilt virus (TSWV) and curly top caused by beet curly top virus (BCTV). The situation with spotted wilt has become more problematic with the emergence of the resistance-breaking strain of TSWV (RB-TSWV) in Fresno, Kings and Merced Counties (Batuman et al., 2017). This makes the dominant Sw5 resistance gene no longer effective. Moreover, to date, no effective source of resistance to this RB-TSWV strain has been identified. With the detection of RB-TSWV in Yolo, Colusa, and Sutter Counties in 2022, there is the chance it becomes established and makes the Sw5 gene less effective in this area. In addition, there is the potential for introduction of exotic viruses such as the quarantine pathogen ToBRFV and virus-like agents such as viroids like potato spindle tuber viroid.

The establishment of the RB-TSWV strain in major processing tomato counties indicates that the virus is effectively surviving in between tomato crops. However, our results indicate that weeds are not an important means of overwintering in Fresno County. Bridge crops definitely can be a way for the virus to overwinter, and this is well-known, and efforts have been taken to minimize these sources of inoculum. What is still not clear is whether viruliferous adult thrips, emerging from viruliferous pupae overwintering from in the soil, can infect newly transplanted tomato plants with TSWV. We previously demonstrated that adult thrips are commonly emerging from soil collected from tomato fields having high thrips populations and spotted wilt. However, we did not conduct experiments to prove whether some of the emerging adults are carrying RB-TSWV. We believe this is highly likely but needs to be experimentally established.

The new exotic tobamovirus ToBRFV has caused major losses in tomato production in protected culture in Canada, Europe, the Middle East, Mexico and the United States. The virus also has disrupted the flow of seed and fruit. Like many tobamoviruses of tomato, ToBRFV is mainly a problem of protected agriculture, where plants are touched frequently. In addition, processing tomatoes in California have rarely had outbreaks of tobamoviruses in the past, regardless of whether the variety had resistance or not. For these reasons, we have told growers that ToBRFV is unlikely to become an economically important virus in processing tomatoes in California and that has been the case to date. However, the virus has spread rapidly and overcame the Tm-2^2 resistance gene, so it is important to monitor for the appearance of the virus in California.

There is more of a need than ever to address the virus problem as a whole and to have the flexibility to rapidly address any type of outbreak that appears and to provide information and management option. We believe we bring 1) the necessary experience and knowledge, 2) the needed diagnostic tools and testing, 3) an extensive network of Farm Advisors, growers, PCAs and industry personnel that allow us to implement the viral surveillance system. We feel that the system will benefit growers by providing rapid diagnosis and up-to-date information on detection and management of current and new or emerging viruses of processing tomatoes in California.

Objectives:

- Continued statewide surveillance program for providing accurate and rapid diagnosis, detection, and recommendations for virus diseases of processing tomatoes in California to growers, PCAs, Farm Advisors and stakeholders in 2024

- Intensive monitoring of processing tomato fields in the Northern Counties for (i) beet leafhoppers and curly top disease and (ii) thrips and spotted wilt disease in 2024

- Continue to run the degree day (DD) model to predict the time of appearance of thrips generations for more effective application of insecticides to slow spread of TSWV

- Determine the nature and severity of spotted wilt symptoms induced by (i) the Fresno RB TSWV-CA-YPT and (ii) the new Sutter-Colusa RB TSWV-CA-CPN

- Maintain a strong outreach effort toto provide timely reporting of results and recommendations

Methos and Results

Objective 1. Continued statewide surveillance program for providing accurate and rapid diagnosis, detection, and recommendations for virus diseases of processing tomatoes in California to growers, PCAs, Farm Advisors and stakeholders in 2024

In 2024, CTD was virtually absent in the North, with no samples received or collected (Table 1**). In Fresno, CTD levels were higher (trace to <5%), but still very low for this potential CTD hotspot area.** This also was consistent with results of tests conducted on BLHs collected from yellow sticky cards (YSCs) from around fields in Fresno (low populations and carrying little or no BCTV). **Thus, CTD caused little to no economic loss in 2024, like 2023.**

Incidence of TSWD in 2024 differed in the North and Central production areas. A somewhat **similar situation was observed for TSWD in 2024, with relatively low incidences (0-<5%) in the North,** including in areas that were heavily affected by TSWD in 2023. This **was despite the early detection of TSWV in a tomato sample with TSWD symptoms received from Yolo County on May 2, 2024. However, levels of TSWD in the monitored fields in 2024 were (somewhat unexpectedly) substantially lower (trace to<5%) compared with 2023.** Further evidence for this comes from i) most of the spotted wilt samples in the North were collected during our surveys, ii) we made no field visits for TSWD in the North in 2024 and iii) our field survey in Sutter/Colusa counties conducted on September 10 revealed very low levels of TSWD.

In the **Central production area** TSWD was first observed around the end of April in known hotspot areas like Canua Creek. Unlike the situation in the North, the TSWD spread more rapidly in the Central production area and thrips management was recommended in part based on DD model predicting emergence of the 2nd and 3rd generation adult thrips. Representative samples of these outbreaks were provided by samples were provided by Tom Turini early (5/14) and late (9/13) and these were all infected with the YPT RB TSWV strain (Table 2). This suggests the new CPN strain has not spread to Fresno County. Importantly, like 2023, **the low level of virus disease in processing tomatoes was associated with cooler and wet spring weather conditions** (Fig. 1).

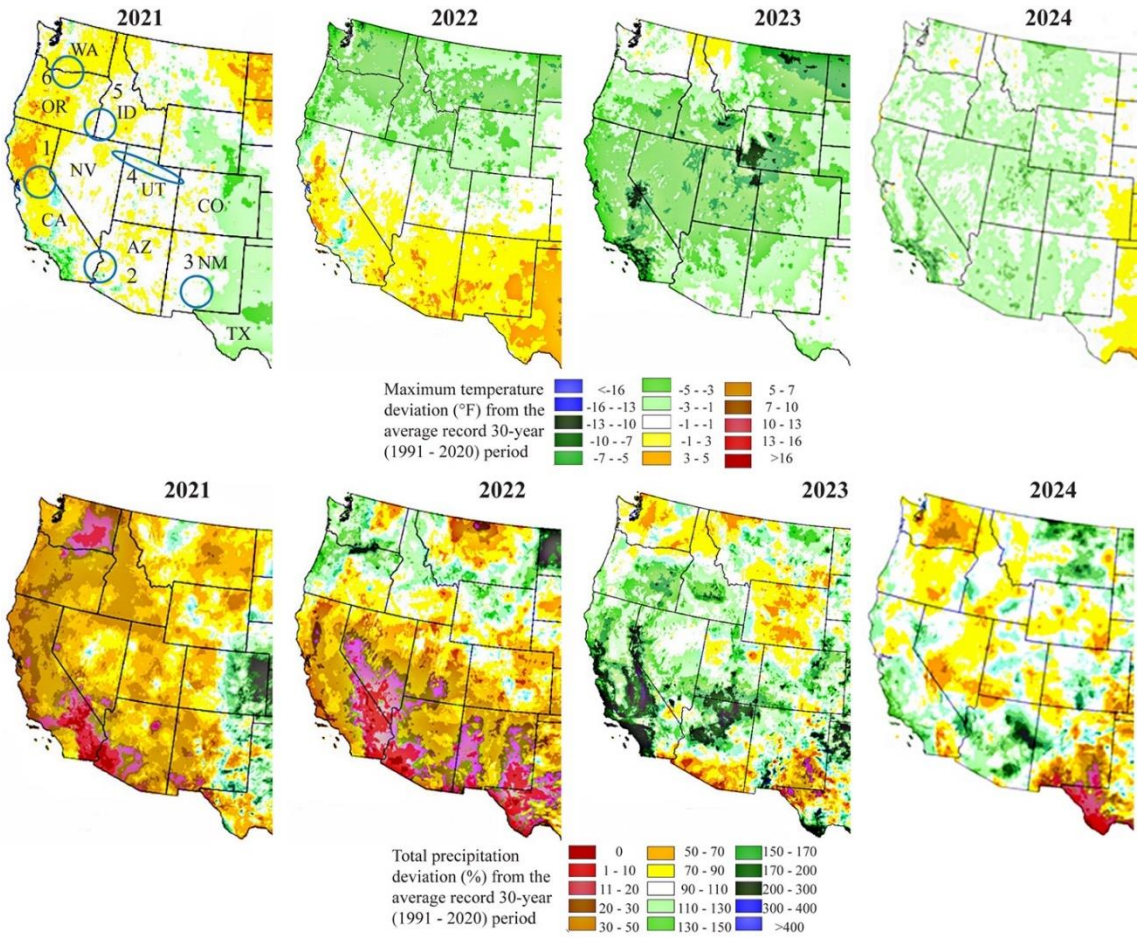

Fig. 1. Maps of the Western United States showing the deviations (or anomalies) of maximum daily temperature (MT) (upper panel) and total precipitation (TP) (lower panel) for spring (March, April and May) weather conditions in 2021, 2022, 2023 and 2024.

Objective 2. Intensive monitoring of processing tomato fields in the Northern Counties for (i) beet leafhoppers and curly top disease and (ii) thrips and spotted wilt disease in 2024

For the intensive monitoring objective, co-PI Patricia Lazicki identified four representative processing tomato fields, three in Yolo County (Esparto [Es], Winters [WI] and Zamora [Za]) and Solano (Dixon {Di]). These fields were monitored for BLHs and thrips with YSCs and for symptoms of virus disease by conducting field walks in multiple locations in each field. were identified for monitoring with YSCs for BLHs and thrips and for viral disease symptoms. Cards were exchanged and fields surveyed on five dates (May 1 and 22, June 5 and 19 and July 8) and a late season survey of fields in Sutter and Colusa counties was conducted on 10 September.

No CTD and low BLH populations. Because of the cool wet spring weather conditions in 2024 (Fig. 1), transplanting was delayed and there also was a **transplant problem** involving stem damage/weakness, sometimes resulting in small, stunted bushy plants that resembled CTD but all tested negative for BCTV infection. Otherwise, **no plants with CTD symptoms were observed in any of the fields during any of the surveys**. Results of YSC monitoring revealed **low BLH populations** (0-4 BLHs/card) across the five sampling dates (Fig. 2A). Furthermore, these BLHs **tested negative for BCTV** in PCR tests. These results also were consistent with not receiving CTD samples for testing or requests for fields visits for CTD in the North in 2024.

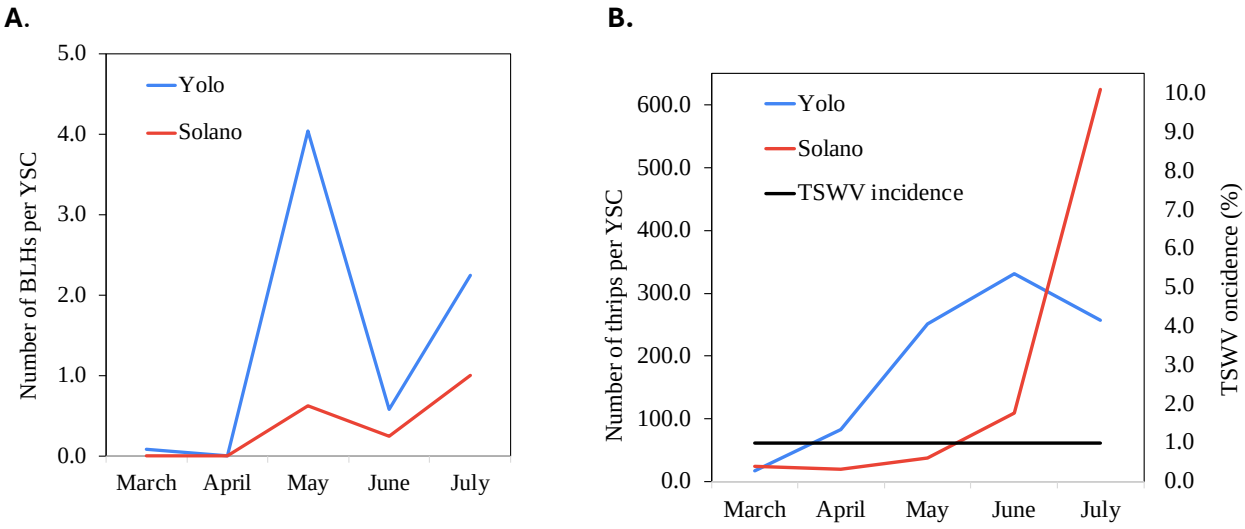

Fig. 2. A. Population of beet leafhoppers (BLH) detected on yellow sticky cards (YSCs) placed around the 4 monitored fields in Yolo County in 2024. **B**. Populations of adult thrips captured on these YSCs (blue and red lines) and incidence of spotted wilt disease in these fields (black line).

The YPT and CPN RB TSWV strains were **detected in monitored fields in late May-early June, but did not spread much, despite high populations of thrips detected on YSCs (Fig, 2B)..** The incidence of TSWD in the four monitored fields was very low (tract to <1%), like other fields in the North as mentioned above. TSWD was not observed in the plants of any field in the first survey (5/1/24) and **was first detected in a small number of plants from the Zamora and Esparto fields in the next survey (5/22).** For the June 5 and 19 surveys, **TSWD was detected in a small number of plants from the Dixon field (Solano County), and in a cluster of ~20 plants (hotspot) in the Winters field.** However, TSWD levels **remained very low** in the Zamora and Esparto fields, where it had been detected ~1 month previously. Moreover, **this was despite the observation of high populations of thrips on YSCs** (~200/card) and over multiple sampling dates (Fig. 2B). In addition, an outbreak of Southern wilt caused by Sclerotium rolfii was observed in one corner of the Zamora field, whereas foliar yellowing symptoms with Fusarium infection was observed in the Esparto field and complicated the visual identification of plants with TSWD. Results of the final survey of these fields, conducted July 8 and a late season survey of processing tomato fields in Colusa and Sutter counties **further showed that levels of TSWD in the North in 2024 were substantially less than those observed in 2023** (Macedo et al., 20024).

Distribution of TSWV resistance-breaking strains in 2024 was like 2023 with new CPN strain only detected in the North. As we have documented in previous CTRI reports and a recent publication (Macedo et al., 2024), the first tomato RB strain detected in processing tomatoes in California was the YPT strain. Which first appeared in Fresno County in 2016 and spread to the Northern production area in 2021. In 2023, a second tomato RB TSWV strain, CPN, appeared in Colusa and Sutter counties and had spread to Yolo County. Both the YPT and CPN strains are very pathogenic (virulent) on tomato varieties carrying the Sw-5b resistance gene (Macedo et al., 2024). Using specific RT-PCR tests for these strains, we can monitor for their spread and relative importance.

New CPN RB strain persisted in the North but did not spread to the Central production area. The results of our testing of samples of TSWD for these RB TSWV strains in spotted wilt samples collected in 2024 is presented in Table 2. First, only the YPT RB TSWV strain was detected in the samples from the Central production area (Fresno, Merded and Stanislaus counties), whereas both the YPT and CPN strains were detected in the Northern production area. Furthermore, more CPN strain was detected in samples from Colusa and Sutter, whereas more YPT strain was detected in samples from Yolo County. This is like what was observed in 2023 and shows the CPN strain is persisting in the North and continues to effectively compete with the YPT strain.

The large number of samples from Yolo County that were infected with both strains (43, Table 2) shows that both strains are well-established and can readily co-infect plants in the field, Interestingly, based on the relative RT-PCR signal generated from plants co-infected with the CPN and YPT strains, we **consistently observed that the CPN strain had stronger signals that those of the YPT strain** (Fig. 3, compare lanes with black and red arrows). This suggests that in co-infected plants CPN is accumulating higher levels of virus and possibly outcompeting the YPT strain. However, in the absence of CPN, the YPT strain accumulated to levels similar to CPN, **showing both strains effectively infect and accumulate in processing tomato varieties with the Sw-5b gene**.

Table 2. Number of samples per county where the RB-TSWV variants were detected in samples collected in 2024.

		TSWV-RB strains				
County	**CPT**	**FPT**	**YPT**	**CPN**	**MIX (YPT/CPN)**	**Total**
Colusa*	0	2	4	6	0	12
Sutter*	0	0	5	19	0	24
Yolo*#	0	2	28	8	45	83
Stanislaus	0	0	3	0	0	3
Fresno	0	0	44	0	0	44
Merced	0	0	8	0	0	8
Total	0	4	92	33	45	174

*Northern counties

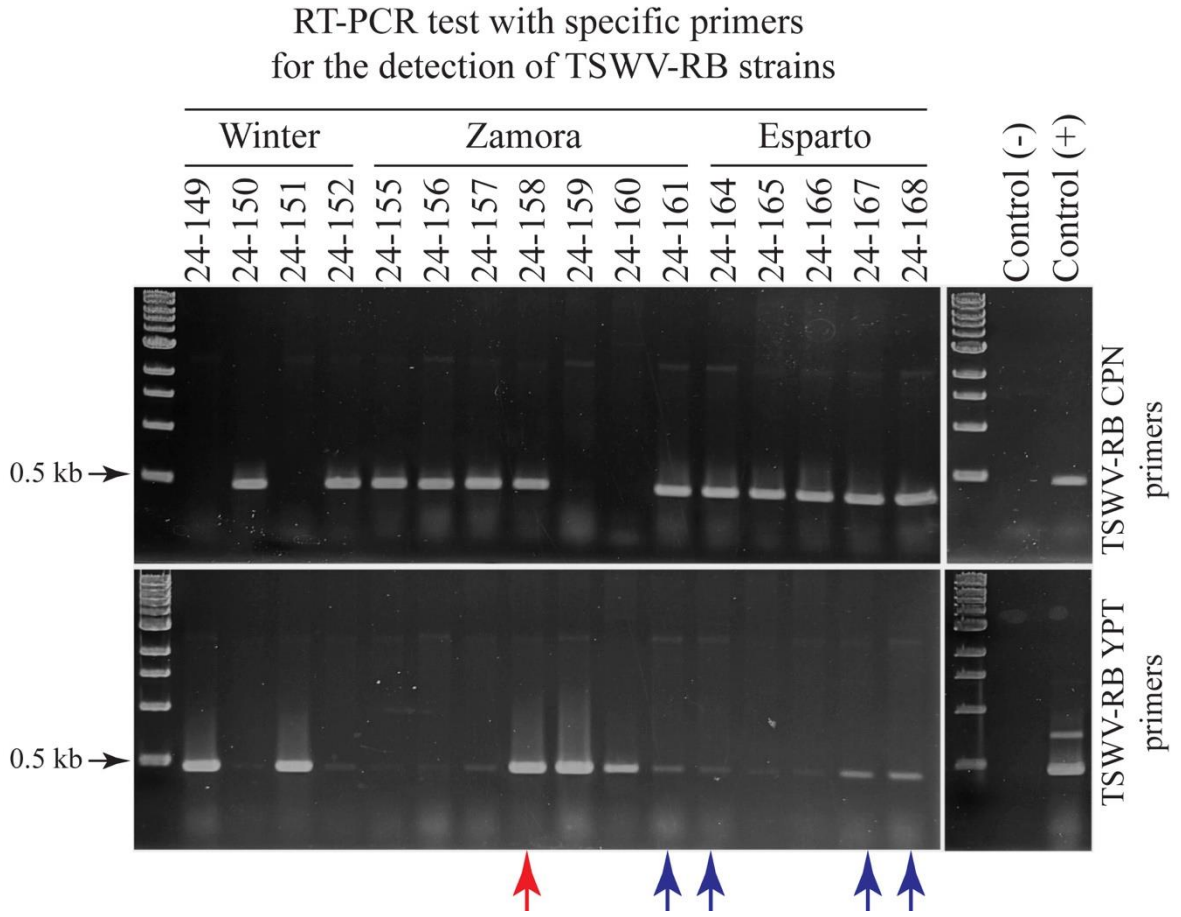

Fig. 3. RT-PCR detection of the resistance-breaking (RB) tomato spotted wilt virus (TSWV) strain CPN (top) and YPT (bottom). Blue arrows show four co-infections where accumulation of YPT is lower than CPN and red arrow shows a co-infection where levels of YPT and CPN are similar.

Analysis of the cytochrome oxidase I gene (mtCOI) of thrips from YSCs in our monitored fields may provide insight into low TSWD spread: Many were onion thrips. Identification of insects, including vectors of plant viruses, can be difficult because i) it can be based on hard to see taxonomic characters, ii) recovery of insects from YSCs makes it difficult to use these characters, iii) insects may exist if different color forms and iv) exotic insects are being introduced more frequently. The identification of the sequence of the mtCOI gene as a useful taxonomic tool taken together with the availability of many genome sequence of insects has allowed mtOOI sequences to be used as a rapid means to identify insects. We have now developed methods for rapid extraction of insect DNA, including those recovered from YSCs, sequencing and comparison with the GenBank to rapidly identify insects. As part of the development and testing of this method we evaluated numerous insects, including thrips recovered from the YSCs from our monitored fields. First, we presumed that the thrips caught around our monitored tomato fields in Yolo County were *Frankliniella occidentalis*, the Western flower thrips and excellent vector of TSWV. However, as shown in Table 3, the majority of the thrips tested were identified as *Thrips tabaci*, the onion thrips, which prefers onions and is not a good transmitter of TSWV. Thus, *T. tabaci* was detected from cards from 3 or the 4 monitored fields and for the months of April, May and June (Table 3). Thus, these preliminary results suggest that a substantial portion of the thrips captured on the YSCs around our monitored fields were *T. tabaci*, which might explain why the TSWV in these fields did not spread rapidly.

Table 3. PCR amplification of the Cytochrome C oxidase subunit 1 gene (COI) and BLAST analysis of the nucleotide sequence of thrips collected from YSCS (yellow sticky cards) in 2024.

County	Field	Date of collection	BLAST results		
			Scientific Name	% of similarity	Accession number
Yolo	Esparto	04/19/24	*Frankliniella occidentalis*	87.06%	OR136224.1
	Winter	04/19/24	*Thrips tabaci*	93.31%	OR136280.1
	Winter	05/01/24	*Thrips tabaci*	93.19%	OR136282.1
	Esparto	05/01/24	*Thrips tabaci*	98.19%	OR136280.1
	Esparto	05/22/24	*Thrips tabaci*	99.04%	OR136282.1
	Zamora	06/19/24	*Thrips tabaci*	93.15%	OR136282.1
	Esparto	06/19/24	*Thrips tabaci*	97.23%	LC650834.1
	Zamora	07/08/24	*Frankliniella occidentalis*	85.38%	OR136254.1
Solano	Dixon	07/08/24	*Frankliniella occidentalis*	90.88%	OR136224.1

Objective 3. Predict thrips generations with the DD model and detect TSWV in thrips emerging in the spring.

Effective control of western flower thrips (*F. occidentalis*), the vector for TSWV, requires sprays be timed to when populations have started to increase but are not yet very large, typically the 2nd and 3rd generations in a growing season, but not population numbers. Because thrips generations are highly temperature-dependent, a growing degree day (DD) prediction model developed for appearance of thrips generations using data from weather stations in multiple locations in California corresponding to areas where processing tomatoes are grown.

In 2024, we ran the thrips DD model through the ANR website: http://ucanr.edu/sites/TSWVfieldriskindex/Thrips_Population_Projections/. Based on our knowledge of the biology of the TSWV-thrips interactions, it is recommended to target 2-3rd generation adults for insecticide treatment, to slow the spread of TSWV, with the urgency to spray increased if TSWV has also been detected in the field. To make this model more accessible and provide interpretation and recommendations, we provided a series of blogs throughout the period in which TSWV infection can lead to economic loss in processing tomatoes (March-August) in these locations. Thus, we published regular region-specific model predictions for thrips generation cycles, complemented by farm advisor/PCA/grower field observations. (for example, https://ucanr.edu/blogs/ThripsTSWVYoloColusa/index.cfm). In this blog, In the blog for Yolo, Solano, and Sacramento County, maintained by UCCE Vegetable Crops Advisor Patricia Lazicki, we also include results from yellow sticky-card monitoring in known TSWV hot spots in Yolo and Solano Counties. In 2024 nine blog reports were published from March through June, with greatest frequency in April and May when thrips approach the target management stage.

Objective 4. Determine the nature and severity of spotted wilt symptoms induced by (i) the Fresno RB TSWV-CA-YPT and (ii) the new Sutter-Colusa RB TSWV-CA-CPN variants in the major processing tomato varieties grown in 2023

In 2023, outbreaks of TSWD in some fields in the Northern production area were characterized by unusually severe symptoms including very strong yellowing and purpling and necrosis of leaves, ringspots on stems and minimal symptoms on fruits. It was initially thought these symptoms were caused by the new CPN variant. However, our 2023 field survey in Fresno revealed similar symptoms in some fields suggesting that these symptoms might be related to the genotype of the variety to these RB TSWV strains.

To further investigate the processing tomato genotype X RB TSWV strain we were kindly provided seeds of 13 top processing tomato varieties planted in 2024 and these along with our positive control cv. Glamour were individually mechanically inoculated with the YPT and CPN RB TSWV strains and plants maintained in a greenhouse. A PCR test was used to confirm that all 13 processing tomato cultivars possess the Sw-5b resistance gene for TSWV. Three weeks following inoculation the plants were rated according to a 1-4 scale with 1=no symptoms, 2=mild symptoms (mosaic curling), 3=severe (mosaic, yellowing, vein purpling and stunting) and 4=very severe symptoms=extreme stunting, yellowing, and necrosis and dieback. The results are presented in Table 4 and revealed overall disease ratings of 3.4 for the YPT strains and 3.3 for the CPN strain. The varieties had similar ratings for the two strains. It was noted that in some varieties inoculated with the CPN strain infectivity was substantially reduced (Table 4). Furthermore, based on the mean disease ratings Based on the ratings and infectivity, three groups of varietal responses were recognized: i) least severe symptoms (2.6-3.2) and reduced infectivity of CPN, ii) severe symptoms and higher rates of infectivity, and iii) very severe symptoms and high rates of infectivity. Finally, there was a group of three varieties and the positive control Thus, once the Sw-5b gene was broken, the response of the underlying genotypes varied from moderate to severe. This type of variation to TSWV infection of genotypes underlying the Sw-5b gene has been previously noted by Turini et al., 2023), and varieties with lower rating suggested for planting in hotspot areas. Our results are also consistent with the notion that it was the very severe symptom response in some varieties grown in the Norther and Central production areas that was responsible for the unusually severe TSWD symptoms observed in 2023.

Table 4. Disease rating of 13 top processing tomato tomato varieties inoculated with two TSWV-RB strains

	CPN strain		YPT strain	
Variety	**mDR[a]**	**%[b]**	**mDR**	**%**
HM 8237	2.6	45	3.2	95
SV 9016	2.8	37	3.2	79
SVTM 9027	3.0	79	3.4	85
H 1996	3.1	53	3.1	95
HM 5522	3.1	47	3.2	85
SVTM 9019	3.2	85	3.5	100
HM 7103	3.2	85	3.7	90
N 6428	3.3	90	3.5	95
HM 58841	3.4	83	3.0	50
SV 9023	3.5	60	3.5	100
SV 9013	3.8	85	3.3	100
H 2016	3.8	65	3.6	100
SVTM 9032	3.9	100	4.0	100
Glamour	3.7	100	3.7	100
Average	**3.3**	**70.3**	**3.4**	**90.3**

[a] average of disease rating > 1

[b] percentage of plants with rating > 1/total inoculated plants)

Objective 5. Maintain a strong outreach effort to provide timely reporting of results and recommendations to Farm Advisors, growers, PCAs, and industry personnel.

During 2024, we continued our outreach efforts, as indicated below.

- Presentation at the South Sacramento Valley Processing Tomato Production Meeting, January 9, 2024.Wednesday,
- Presentation at Vegetable Crop Pest Management meeting held at Westside Field and Extension Center, March 7, 2024.
- Presentation at Napa Processing Tomato Meeting November 11, 2024
- Continued to provide detailed reports together with our diagnostic tests of processing tomato samples submitted for testing for possible virus infection.

Discussion:

In 2024, we received another cool wet winter-spring climate and, consistent with 2023, the observation of no CTD in the North and very low BLH populations is further evidence that it was the hot dry windy conditions of 2021 and 2022 that facilitated the unusual CTD outbreaks caused by BCTV-SpCT. This may indicate that these cool wet conditions are unfavorable for survival of overwintering BLHs, which are desert insects. The finding that CTD also was relatively low in Fresno, a host-spot for the disease, lends further support to the concept that the cool wet weather is detrimental to BLH survival.

In terms of TSWD, the finding that all samples tested from the North and Fresno were infected with RB TSWV was not unexpected as nearly all cultivars possess the Sw-5b gene. The strain typing results for 2024 were similar to 2023 and showed YPT is still prevalent in Fresno, where it first emerged. There was no evidence CPN spread to Fresno. On the other hand, CPN was the predominant strain in Colusa and

Sutter, where this strain emerged in 2023. Again in 2024, Yolo County is the place where the two strains overlap, and both are important. Thus, these results indicate both strains persisted effectively in the North and are likely to continue to be important. The observation that CPN outcompetes YPT in mixed infections based on semi-quantitative RT-PCR, is consistent with some CPN isolates being highly aggressive and virulent. The reason why CPN is outcompeting YPT, which itself is quite virulent, may relate to replication efficiency or ability to colonize the plant faster.

In the screening of the 13 commercial varieties (all of which were confirmed to possess the Sw-5b gene) YPT and CPN showed similar virulence and both induced severe symptoms. After overcoming the Sw-5b resistance, a range of responses to TSWV infection were observed, indicating the underlying genotypes vary is degree of susceptibility. These results further show that CPN and YPT are important players in TSWD in the Northern production area.

We continue to encourage growers and PCAs to use the that the DD model because it provides very useful information in terms of when to best target thrips early in the season. This is critical because it can delay both thrips population development and spread of TSWV. In 2024, we were fortunate Patricia Lazicki wrote the blogs for the Northern production area. Ultimately, we hope this becomes an important part of growers management of thrips and TSWV.

Fhe slow spread of TSWV infields in Yolo County was unexpected, particularly because were we trapping very high numbers of thrips on the YSCs surrounding our monitored fields. Insight into this may have come from the results of the rapid insect identification method indication that ~66% of these thrips were *T. tabaci,* the onion thrips. These thrips feed on plants in the onion family and are not good vectors of TSWV. Thus, this finding provides an explanation for why TSWV did not spread faster in the North in 2024.

Acknowledgements.
We would like to recognize the many processing tomato growers that allowed us to conduct surveys in their fields and the growers and PCAs that provided samples of curly top and spotted wilt symptoms for testing.

Project Title: Controlling in-row weeds with post plant applications of pre-emergent herbicides

Year of Project Initiation: 2024

CTRI Funding in 2024: $6,925.00

PI: Scott Stoddard, UC Cooperative Extension. 2145 Wardrobe Ave., Merced, CA 95341. 209-385-7403 csstoddard@ucanr.edu

EXECUTIVE SUMMARY

Trials were conducted at the UC West Side Research and Extension Center (WSREC) and in commercial fields in LeGrand and Merced to evaluate weed control within the plant row with post plant applied pre-emergent herbicides incorporated with a finger weeder in processing tomatoes. The herbicides evaluated were rimsulfuron (Matrix), pendimethalin (Prowl), napropamide (Devrinol), and metribuzin (Sencor). The herbicides were applied in a banded application as a directed spray two weeks after transplanting then incorporated to a depth of about 2". Comparison treatments included Matrix herbicide with incorporation, finger weeder cultivation without herbicides, and an untreated control. Compared to the untreated control, a post plant application of Matrix provided 61% - 75% broadleaf weed control at 4 weeks after transplanting, whereas the finger weeder without herbicides provided 15% to 75% control. Combining herbicides with the finger weeder generally improved weed control to 84% - 94%. As weed control improved, there was a commensurate reduction in the number of hand weeding time. Post plant but incorporated Prowl, Sencor, and Matrix were very effective in Merced and at WSREC. Crop injury from the herbicide sprays or crop damage from the finger weeder was minimal at all locations and not significantly different from the untreated control.

INTRODUCTION

A standard pre-emergent herbicide program for processing tomatoes in California would be Treflan/Prowl + Dual Magnum (trifluralin/pendimethalin + metolachlor) pre-plant incorporated followed by Matrix (rimsulfuron) applied in a band over the top at 7 – 10 days and again at 14 – 17 days after transplanting. Grass specific herbicides, such as clethodim, are applied post emergence as needed. While the preemergent herbicides can be very effective, one of the challenges with their use is the need for incorporation, either mechanical or with sprinklers. Incorporation before planting is both efficient and effective, but often the movement of soil during planting/transplanting moves the soil and herbicides, resulting in little weed control in the plant row. The result is that hand weeding is often still required even when Matrix has been applied.

Hand weeding or robotic cultivators can be used to control weeds in the plant row. However, another alternative is the finger weeder, a simple mechanical cultivator capable of removing weeds from the plant row. The system uses interlocking rubber fingers to remove small weeds in the plant row once transplants are established. Unfortunately, finger weeders are effective only if the weeds are very small, which limits their use to a very short period during the cropping season. However, finger weeders should be able to safely incorporate preemergent herbicides back into the plant row, which would greatly expand the amount of time they could be used. Finger weeders have the added benefit of being simple, reliable, and inexpensive as compared to robots currently available.

OBJECTIVES

1. Evaluate weed control, time, and costs associated using finger weeders to incorporate preemergent herbicides as part of a conventional weed management program in a commercial processing tomato field in central CA and at the UC Westside Research and Extension field station (WSREC).
2. Evaluate pre and post herbicide programs in comparison to mechanical finger weeders.

METHODS

This trial began on May 20, 2024, at the UC Westside Research and Extension Center (WSREC), near Five Points in Fresno County. A second location was also added on May 18, 2024, in a commercial field near Merced. The objective of both trials was to evaluate weed control, crop safety, and costs associated with using a finger weeder to incorporate pre-emergent herbicides within the plant row after planting as part of a conventional weed management program. Herbicide treatments were Prowl + Dual (pendimethalin + metolachlor) at 1.5 pints per acre pre-plant incorporated; rimsulfuron (Matrix) 2 oz/A post, rimsulfuron + halosulfuron (Matrix + Sandea) 2 oz/A + 1.0 oz/A, napropamide (Devrinol) 4 lbs/A post, Prowl (pendimethalin) 1.5 pts/A post, and Sencor (metribuzin) at ½ lb/A post. The Dual + Prowl at WSREC was applied 1 day before transplanting and incorporated mechanically with a rotary plow to a depth of about 3", but at the commercial field in Merced no PPI herbicides were used. An untreated control was used at both locations.

At WSREC, a tank mix of Prowl 1.5 pts/A + Dual at 1.5 pts/A was applied to select plots on preformed beds using a backpack CO_2 sprayer with TeeJet 8002 nozzles and 40 gpa water volume, then incorporated to 3" using a power rotary cultivator. Tomato cultivar SV9023 was transplanted 1 day later, on May 21, 2024, using cone mechanical transplanters on 18" spacing and 250 gpa water. After transplanting, the field was irrigated via subsurface drip irrigation for the remainder of the season. On June 4 (two weeks after transplanting) the trial was cultivated using the finger weeder using the 14" fingers at 3.5 mph. Immediately prior to cultivation, applications of Matrix, Matrix+Sandea, Devrinol, Prowl, and Sencor were made as a band application on either side of the plant row to minimize contact with foliage, using a band adjusted rate of 50% of broadcast. Herbicides were applied with a CO_2 backpack sprayer at 38 psi with a single Tee Jet 8002 flat fan nozzle. Application rate was about 25 gpa. Spray swath was about 24". Band applications were used since the cultivation width of the finger weeder was only about 1 foot down the center of the bed. A listing of all treatments is shown in Table 1.

Plot size for the herbicide and finger weeder treatments was 1 bed (60") by 60 ft. The experimental design was a RCB with 4 replications. Not all post-applied herbicide treatments were incorporated so that herbicide treatments could be compared with and without incorporation. Weed ratings were made 2, 4, and 6 weeks after using the finger weeder to incorporate herbicides. Ratings were based on a subjective scale using a 0 – 10 system, where 0 = no weeds and 10 = 100% weed cover in the treated area of the plot. The plots were hand weeded 6 weeks after treatment and times recorded.

The trial area was drip irrigated using Netafim Streamline Plus 7/8" 15 mil tape with a flow rate of 0.23 gph with 12" emitter spacing. The tape was injected to a depth of about 10" in the center of the bed. Irrigation scheduling was set to match crop Et using data from the CIMIS station located on the station. Irrigation run times began at 2 days per week and peaked at 32 hours per week after 60 days. Nitrogen fertilizer as UAN32 was applied every 2 weeks through the drip tape for a total of 140 lbs N/A. Plants were treated with Verimark insecticide on the day of transplanting and imidacloprid insecticide was injected through the drip

1 month after transplanting for virus management. All plots were treated with a foliar application of Quadris fungicide about one month before expected harvest. Yield estimates were made by hand harvesting 10 ft from the center of each plot in 2 of 4 reps on 9-Sept-2024. Plants were cut at the base and fruit shook into a large rubber garbage can. Not all plots were harvested due to the high amount of stink bug damage in the trial that resulted in most fruit with mold. Therefore, PTAB samples were not taken. Data were analyzed using the analysis of variance procedure for a randomized block design to compare all treatments to each other and as a two-way randomized block design where the factors were post-transplant pre-emergent herbicides (yes/no) and finger weeder cultivation (yes/no).

This second trial began on May 18, 2024, in a commercial field near Merced, in Merced County (George Seasholz, Seasholz Farms). Processing tomato variety HZ1996 was transplanted on May 6 on 60" centers and 12" plant spacing. No pre-emergent herbicides were used in this location, as the grower's standard method was cultivation and Matrix herbicide POST applied. Matrix (rimsulfuron) herbicide was applied using the growers spray rig on May 17, while the other herbicide treatments were applied by hand on May 18. The initial herbicide applications were made using the same methods and rates as at WSREC, using a backpack CO_2 sprayer and a single wand to make a directed-spray band application. Rates were adjusted at this location to ½ of the broadcast rate. The plots were then cultivated with the finger weeder to incorporate herbicides (Figure 1). Plot size for the herbicide treatments was 1 row (60") by 50 ft; the cultivation treatments were the same. The experimental design was a RCB with 4 replications. Weed ratings were made at 2, 4, and 6 weeks after herbicide treatments were incorporated. Hand weeding times were recorded on July 17 at 6 weeks after treatment. was done on This field was hand weeded using a contract crew and therefore no weeding times were recorded. Yields also were estimated by harvesting 10 ft from the center of the plot on two of the reps. A summary of treatments and site information is shown in Table 1.

The LeGrand was hand weeded shortly after the treatments were applied, and no weed control data could be collected.

Figure 1. The finger weeder at the Merced location, showing shanks and paddle wheels in front of the finger wheels to improve herbicide incorporation.

Table 1. Herbicide and cultivator trial information and treatments, Merced County 2024.

	Trial 1: WSREC	Trial 2: commercial field
Location	UC WSREC	Merced
	Five Points (Fresno County)	Quinley Rd, south of Dickenson Fer
Cooperator		George Seasholtz, Seasholtz Farms
Soil	Cerini clay loam	
Variety and plant date	SV9023, transplanted May 22, 2024. 1 row, 12" (7400 plants/A)	HZ1996, May 6, 2024. 12" spacing
Plot size	Herbicide plots: 1 bed (60") x 60 ft	Herbicide: 1 bed x 50 ft
	Cultivation plots: 1 bed x 60 ft	Cultivation: 1 bed x 50 ft
Irrigation	SSDI on 60" beds	SSDI on 60" beds
Herbicide incorporation	power mulcher PPI and finger weeder on June 4 where noted	no PRE, all post emergence with Matrix or finger weeder
Weed evaluation		
Harvest	9/10/2024 by hand 10 ft	Aug 26 by hand, 10 ft
days	112	112

Treatments:	WSREC Herbicides	POST FW	*timing pre/post*
1	UTC	no	---/---
2	No PPI, finger weeder POST	yes	none/June 4
3	No PPI, Dual + Prowl 1.5 pints/A + FW	yes	none/June 4
4	No PPI, Matrix 4 oz/A + NIS	no	none/June 4
5	No PPI, Matrix 4 oz/A + Sandea 2 oz/A + NIS	no	none/June 4
6	Dual + Prowl 1.5 pts/A PPI + FW POST	yes	May 21/June 4
7	Dual + Prowl 1.5 pts/A PPI + Matrix 4 oz/A POST	no	May 21/June 4
8	Dual + Prowl 1.5 pts/A PPI + Matrix 4 oz/A + FW	yes	May 21/June 4
9	Dual + Prowl 1.5 pts/A PPI + Devrinol 4 lbs/A + FW	yes	May 21/June 4
10	Dual + Prowl 1.5 pts/A PPI + Prowl 1.5 pts/A + FW	yes	May 21/June 4
11	Dual + Prowl 1.5 pts/A PPI + Sencor 0.5 lbs/A + FW	yes	May 21/June 4

FW = Steketee Finger Weeder with 14" fingers
POST Herbicide treatments applied as directed spray at 50% band rate
Herbicide treatments applied at 25 gpa with 8002 nozzles
RCB design with 4 reps

Treatments:	Seasholtz Farms Herbicides	POST FW	*timing*
1	Matrix 4 oz/A POST (grower std)	no	5/17/24
2	UTC	no	---
3	Prowl 1.5 pts/A + FW POST	yes	18-May
4	Devrinol 4 lb/A + FW POST	yes	18-May
5	Sencor 0.5 lbs/A + FW POST	yes	18-May
6	No PPI, FW POST	yes	18-May
7	Matrix 4 oz/A POST fb Dual + Prowl 1.5 pts/A + FW	yes	May 17 and 18

FW = Steketee Finger Weeder with 14" fingers
POST Herbicide treatments applied as directed spray at 50% band rate
Herbicide treatments applied at 25 gpa with 8002 nozzles
RCB design with 4 reps

RESULTS

Weed pressure at the WSREC location was dominated by a mix of broadleaf annual weeds, but annual morning glory and volunteer melons were especially bad at this location; other weeds included field bindweed, pigweed, purslane, and nightshade. There were very few grasses at this location, and no grass herbicides were applied. Therefore, weed control ratings were limited to broadleaf weeds.

Used alone, the finger weeder worked well at this location, significantly reducing weed pressure at 2, 4 and 6 weeks after treatment (WAT) as compared to the untreated control (Table 2). For example, at 4 weeks after treatment, weed control averaged across all herbicides was 53.0%, but 85.5% when herbicides + the finger weeder was used (Figure 3). Herbicides by themselves also significantly improved weed control on all evaluation dates (Table 2 and Figure 3) as compared to the untreated control. When the herbicides were used in conjunction with the finger weeder, weed control was further improved. All of herbicides worked well when incorporated, with over 80% weed control at 4 weeks after application (Figure 4). Hand weeding times and subsequently hand weeding costs were lowest in the Matrix and Devrinol treatments when combined with the finger weeder incorporation, at 6.5 and 6.8 hours per acre per person. While there was only slight and temporally crop damage from the herbicides, no significant crop stand differences were observed between those treatments with the finger weeder as compared to those that were not cultivated.

At the Merced location, no pre-plant incorporated herbicides were applied, so the comparison treatments were a standard application of Matrix at 4 oz/A and an untreated control. Main weeds at this location were hairy and black nightshade, redroot pigweed, and puncture vine. Some plots also had barnyardgrass, but overall weed pressure was less than the WSREC location. Weed control and weed counts at 2, 4, and 6 WAT were significantly better as compared to the untreated control (Table 3), Ranging from 15 to 90% (Figure 5), however, the finger weeder only treatment (no PPI herbicides and no post plant herbicides) was not significantly different than the untreated control, and at harvest had even more weeds. Herbicides again caused little to no crop phytotoxicity in the first two weeks after application. Hand weeding was done on July 17. Hand weeding times were significantly reduced from 25.2 hours/A to about 8.7 hours where herbicides were combined with the finger weeder; the best treatment was Prowl + finger weeder, though the over-the-top application of Matrix without incorporation also did very well at this location.

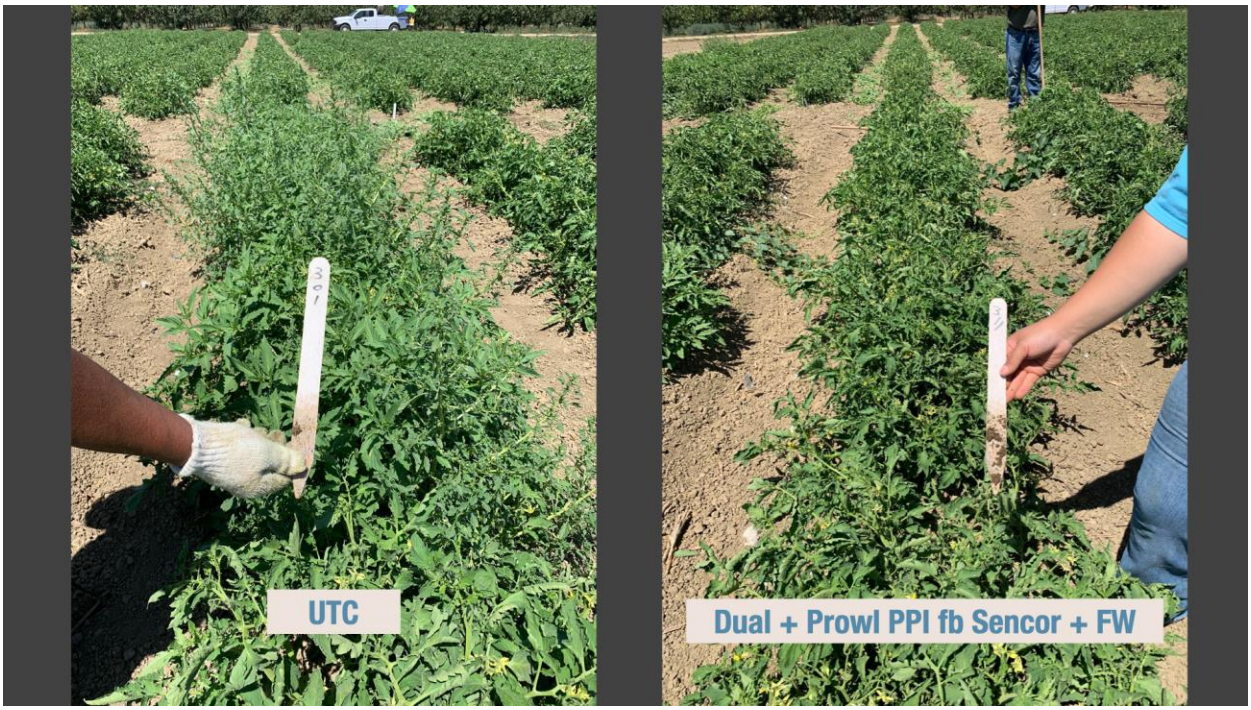

Figure 2. Weed control 4 weeks after transplanting, WSREC 2024.

DISCUSSION

The significant improvement in weed control when Devrinol, Prowl, and Dual were incorporated with the finger weeder is not surprising, as these herbicides need water or mechanical incorporation to work effectively. Though not required on the label, Matrix and Sencor also showed improved control when combined with the finger weeder. The finger weeder is a cultivator that allows this incorporation to occur in the plant row after transplanting or crop emergence. At the WSREC location, the finger weeder by itself reduced the number of broadleaf weeds in the plant row by about half as compared to doing nothing. This same effect was not observed at the commercial field in Merced, where the finger weeder caused an increase in the number of weeds emerged in the plant row. This may have occurred because the main weed at this location was black and hairy nightshade, which emerged later in the growing season, and the mixing action caused by the finger weeder stimulated germination. However, when the finger weeder was combined with the pre-emergent herbicides evaluated in these trials, weed control was further significantly improved another 20 – 30%. In general, the cultivation x herbicide interaction was significant at the WSREC trial, which indicates that the finger weeder worked better with some of the pre-emergent herbicides than others.

ACKNOWLEDGEMENTS.

Many thanks to George Seasholtz with Seasholts Farms in Firebaugh, CA, and the crew at the UC WSREC for their help and cooperation. This research was funded by a grant from the California Tomato Research Institute.

Table 2. Weed pressure, hand weeding time, and treatment yields. WSREC, 2024.

	Herbicide treatment			4-Jun	18-Jun	2 WAT	crop	2-Jul 4 WAT		hand	16-Jul 6 WAT		10-Sep	est
treatment	PRE	POST	FW POST	weeds/plot	plants/A	weed score	phyto	weed score	% control	hrs/A	Weed score	% control	lbs/10 ft	tons/A
1 Untreated Control (UTC)	no	no	no	155.8	7477.8	8.0	0.0	8.8	6.5	51.7	7.0	21.8	8.4	8.0
2 No PPI, finger weeder POST	no	no	yes	---	7550.4	1.8	0.3	3.3	74.8	20.4	1.5	93.8	24.3	14.9
3 No PPI, Dual + Prowl 1.5 pints/A + FW	no	yes	yes	---	6969.6	1.5	0.5	2.3	86.4	11.0	1.0	96.3	43.4	23.2
4 No PPI, Matrix 4 oz/A + NIS	no	yes	no	151.3	7151.1	2.5	0.5	4.3	60.8	29.8	3.8	67.3	35.3	19.7
5 No PPI, Matrix 4 oz/A + Sandea 2 oz/A + NIS	no	yes	no	188.5	8094.9	4.0	0.0	4.3	61.0	28.1	3.0	78.3	21.0	13.5
6 Dual + Prowl 1.5 pts/A PPI + FW POST	yes	no	yes	---	7586.7	1.5	0.0	2.5	84.5	8.0	2.0	89.1	53.8	27.8
7 Dual + Prowl 1.5 pts/A PPI + Matrix 4 oz/A POST	yes	yes	no	33.5	6497.7	2.0	2.3	2.5	83.6	15.4	2.0	89.1	60.9	30.9
8 Dual + Prowl 1.5 pts/A PPI + Matrix 4 oz/A + FW	yes	yes	yes	---	6679.2	0.8	0.3	2.3	85.6	6.5	1.0	96.3	29.2	17.1
9 Dual + Prowl 1.5 pts/A PPI + Devrinol 4 lbs/A + FW	yes	yes	yes	---	7804.5	2.5	0.5	2.5	84.5	6.8	1.5	93.8	26.2	15.7
10 Dual + Prowl 1.5 pts/A PPI + Prowl 1.5 pts/A + FW	yes	yes	yes	---	8094.9	0.8	0.0	1.5	93.8	7.4	1.0	96.3	26.2	15.7
11 Dual + Prowl 1.5 pts/A PPI + Sencor 0.5 lbs/A + FW	yes	yes	yes	---	7514.1	1.3	0.0	2.0	89.1	7.4	1.3	93.5	34.2	19.2
			Average		7401.9	2.4	0.4	3.3	73.7	17.5	2.3	83.2	33.0	18.7
			LSD 0.05		ns	1.4	0.8	1.2	14.8	16.0	1.7	19.3	---	---
			CV, %		12.0	38.9	139	25.7	13.9	63.1	51.2	16.1	---	---
			No POST herbicide		7535.9	3.8	0.1	4.8	55.3	26.7	3.5	68.2		16.9
			POST applied herbicides		7347.1	1.9	0.5	2.7	80.6	14.1	1.8	88.8		19.4
			t-test		ns	***	ns	***	***	***	***	***		---
			No post plant cultivation		7303.6	4.1	0.7	4.9	53.0	31.3	3.9	64.1		18.0
			Finger weeder POST		7463.3	1.4	0.2	2.3	85.5	9.6	1.3	94.1		19.1
			t-test		ns	***	ns	***	***	***	***	***		---
			Finger weeder X herbicide interaction			***	ns	***	***	*	***	***		---

WAT Weeks After Treatment

Weed Score 0 - 10 scale. Subjective scale. 0 = no weeds/no crop phytotoxicity, 1 = 2.5%, 2 = 10%, 3 = 21%, 4 = 35%, 5 = 50%, 6 = 65%, 7 = 79%, 8 = 90%, 9 = 97.5%, 10 = all weeds or total crop loss

% control based on weed score rating using arcsin transformation.

LSD 0.05 = Least significant difference at the 95% confidence level. Means within a column greater than this amount are not significantly different. --- not enough data to calculate.

CV% = coefficient of variation.

t-test: *, **, *** significant at 0.05, 0.01, and 0.001 respectively

CTRI 2024 Full Reports - In-Row Weeds - Stoddard

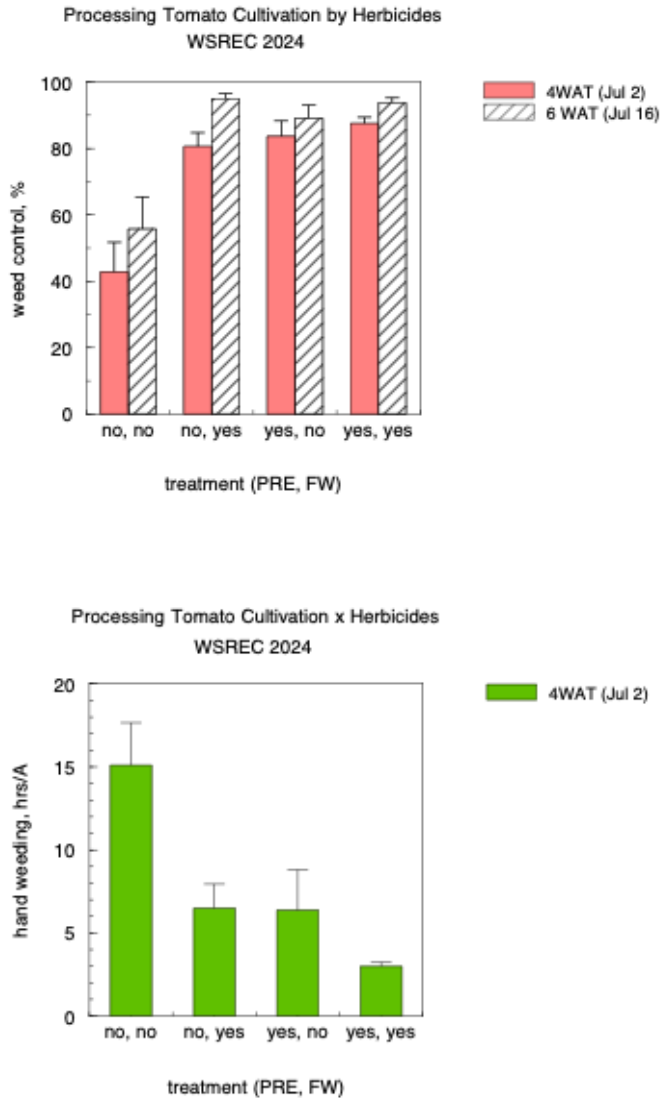

Figure 3. Weed control at WSREC comparing the main effect of herbicide and finger weeder cultivation at 4 and 6 weeks after transplanting (top), and corresponding hand weeding time (bottom).

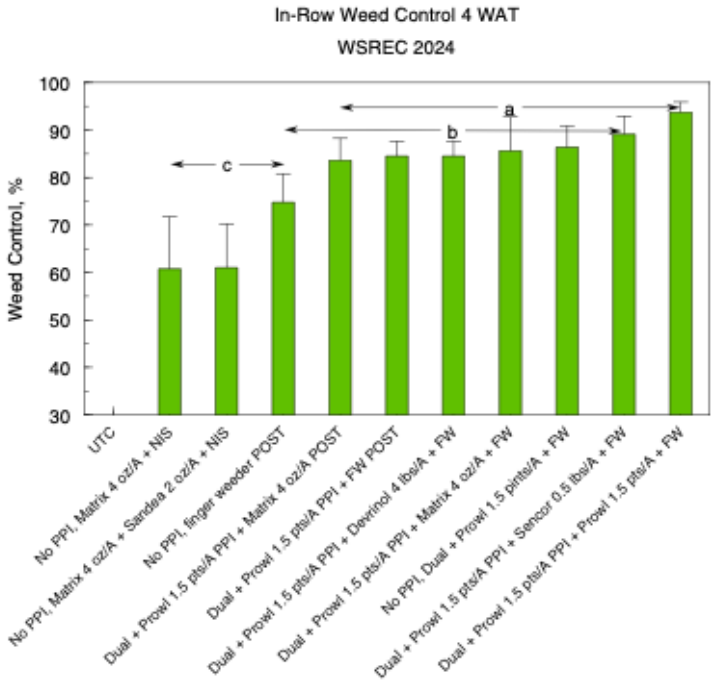

Figure 4. Weed control by treatment at 4 weeks after transplanting, WSREC 2024. Untreated (UTC) is shown for comparison but not included in the statistical analysis.

Table 3. Weed pressure, hand weeding time, and treatment yields. Merced, 2024.

Treatment	3-Jun 2 WAT Plants/A	weeds/plot	28-Jun 4 WAT weed score	weeds/plot	% control	17-Jul 6 WAT hand weed, hrs/A	10-Sep weed score	% control	est yield tons/A	PTAB Color	SS	pH
1 Matrix 4 oz/A POST (grower std)	7144	8	0.8	2.8	74.5%	5.0	2.3	83.5	56.6	20.8	4.9	4.48
2 Untreated control (UTC)	7449	32	3.8	20.5	0.0%	25.2	6.8	0.0	57.8	21.3	4.9	4.58
3 Prowl 1.5 pts/A + FW POST	7405	2	0.5	4.8	76.3%	2.8	3.3	74.8	49.5	21.3	5.0	4.48
4 Devrinol 4 lb/A + FW POST	7405	6	2.3	10.8	39.0%	15.9	6.5	30.0	58.0	19.0	4.9	4.47
5 Sencor 0.5 lbs/A + FW POST	7405	2	0.8	3.0	86.6%	10.9	2.8	79.1	61.8	20.3	5.1	4.47
6 No PPI, FW POST	7536	17	4.0	27.0	15.3%	28.3	6.8	0.0	51.6	19.8	4.6	4.48
7 Matrix 4 oz/A POST fb Dual + Prowl 1.5 pts/A + FW	7449	2	0.3	2.3	89.0%	5.3	1.8	91.0	53.6	20.3	5.1	4.50
Avg	7399	9.8	1.8	10.1	54.4%	13.3	4.3	51.2	55.5	20.4	4.9	4.49
LSD 0.05	145.2	12.8	2.2	14.6	45.4%	12.1	2.9	34.0	---	---	---	---
CV, %	1.6	107	84	97	47.5	60.7	44.8	38.5	---	---	---	---

WAT Weeks After Treatment

Weed Score 0 - 10 scale. Subjective scale. 0 = no weeds/no crop phytotoxicity, 1 = 2.5%, 2 = 10%, 3 = 21%, 4 = 35%, 5 = 50%, 6 = 65%, 7 = 79%, 8 = 90%, 9 = 97.5%, 10 = all weeds or total crop loss

% control based on weed score rating using arcsin transformation.

LSD 0.05 = Least significant difference at the 95% confidence level. Means within a column greater than this amount are not significantly different. --- not enough data to calculate.

CV% = coefficient of variation.

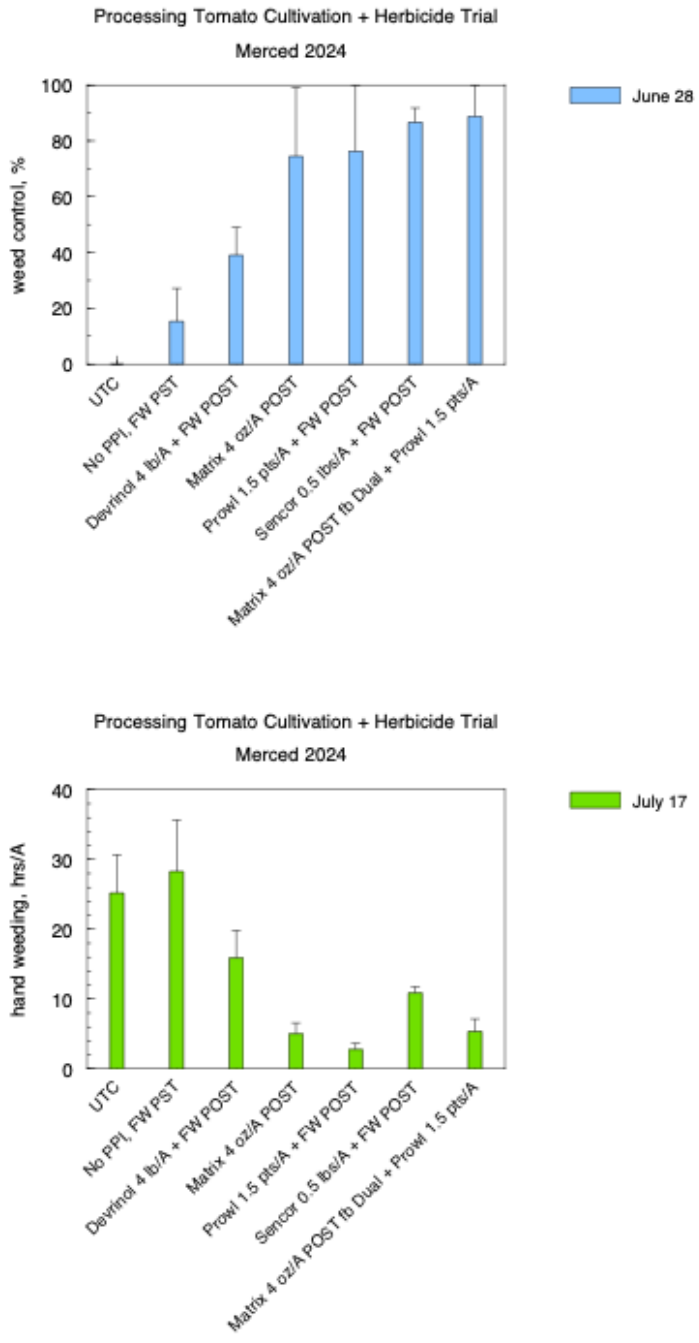

Figure 5. Weed control by treatment at 4 weeks after transplanting, Merced 2024 (top), and corresponding hand weeding times (bottom).

2025 RESEARCH - PROJECT LIST

CTRI GROWER FUNDED RESEARCH: 2025			
2025 TOTAL AFTER FINAL BOARD DECISIONS & COST SHARING: $757,178			
Broomrape Containment, Control and Management		**Research Lead**	**Institution**
2020 Start	Broomrape: Devt. of Long Term Mgmt. Options: CA Commercial Field Conditions & Contained Research Facility Ongoing Work	Brad Hanson	UC Davis
2021 Start	Developing best equipment sanitation practices for broomrape and other high-profile soil borne pathogens; to mitigate field-to-field spread* - CLFP Co-Funding	Cassandra Swett	UC Davis
2022 Start	Determining the population structure of *Phelipanche ramosa* and *Orobanche aegyptiaca* field detections in California	Adam Schneider	UW-LaCrosse
2022 Start	Developing Tomato Lines Resistant to Branched Broomrape, a Critical California Pest	Neelima Sinha	UC Davis
2022 Start	Inducible Suberin for Tomato Drought Tolerance (root architecture)	Siobhan Brady	UC Davis
2023 Start	Training and Refinement of Broomrape Detection Model using Satellite Imagery	Alireza Pourezza	UC Davis
2025 Start	Targeting Strigolactone Receptors in Branched Broomrape (*Phelipanche ramosa*)	Marco Burger	Salk Institute
2025 Start	Broomrape resistant, climate resilient tomato rootstocks for grafted processing tomatoes	Ryan Lefers	iyris
2025 Start	AgCeption In-Field Broomrape Detection System Development* - CDFA Broomrape Board Co-Funding	Chris Laudando	L&A
Agronomic/Water/Nutrient Management			
2025 Start	Exploring causal factors for the yield gap between “new” and “old” tomato fields	Patricia Lazicki	UC Extension
Germplasm and Variety Development			
1991 Start	C. M. Rick Tomato Genetic Resource Center	Roger Chetelat	UC Davis
2024 Start	Leveraging germplasm resources for genetic discovery and deployment of salt stress resilience	Greg Vogel	Cornell
2025 Start	Engineering broad and durable disease resistance: Genome Editing to modulate susceptibility genes against Fusarium wilt in tomato.	Daniel Rodriguez-Leal	University of Maryland
2025 Start	Marker-trait association study to develop DNA markers for RB-TSWV resistance in tomatoes	Reza Shekasteband	NCSU
Insect & Invertebrate Management			
2025 Start	Evaluation of management programs for Consperse stink bug management	Tom Turini	UC Extension
Pathogen Management			
2017 Start	Disease diagnosis, pathogen movement / emergence monitoring, new pathogen ID and F4 monitoring for the CA processing tomato industry	Cassandra Swett	UC Davis
2018 Start	Developing an integrated mgmt. strategy for F. falciforme vine decline in processing tomato, including co-management with Fusarium wilt	Cassandra Swett	UC Davis / UC Extension
2023 Start	Integrated solutions to address the issue of fusarium stem rot and vine decline (FRD) in processing tomato* - USDA IR-4 Funded	Patricia Lazicki	UC Extension
2025 Start	Evaluating the potential of Trichoderma biofungicides as preventative measurements for Fusarium stem rot and vine decline in processing tomato	Zheng Wang	UC Extension
2017 Start	Viral Diagnostics* - CDFA BCTV Control Board Co-Funding	Robert Gilbertson	UC Davis / UC Extension
2024 Start	Evaluation of Insecticide Programs in Processing Tomatoes for the MGMT of BCTV and TSWV Vectors and Viruses	Tom Turini	UC Extension
Weed Control and Management			
2025 Start	Evaluating chemigated rimsulfuron herbicide on field bindweed suppression in processing tomatoes	Scott Stoddard	UC Extension

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