ANNUAL RESEARCH REPORT

for

2023

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TABLE OF CONTENTS

INTRODUCTION

Welcome, this report marks the 55th year of continuous crop research sponsored by the contributing members of the California Tomato Research Institute (CTRI).

The primary function of the CTRI is to identify production challenges and opportunities and to fund projects which research and development can address. Funding is through tonnage assessments ($0.12/paid ton in 2023) from its voluntary grower members. Decisions are governed by its Board; made up of growers. With the aim of building and maintaining an effective, robust and dynamic research agenda CTRI management promotes durable coalitions between growers, allied industry and researchers. Since 1968, when the CTRI was founded, over 750 research projects have been supported. These projects have primarily focused on improving field production, particularly in the areas of: pest management (350+ projects); variety development, pre-breeding and variety evaluation (150+ projects); agronomics (150+ projects); market development and process quality (75+ projects); and automation (25+ projects). Figure 1 charts our long running research categories over time.

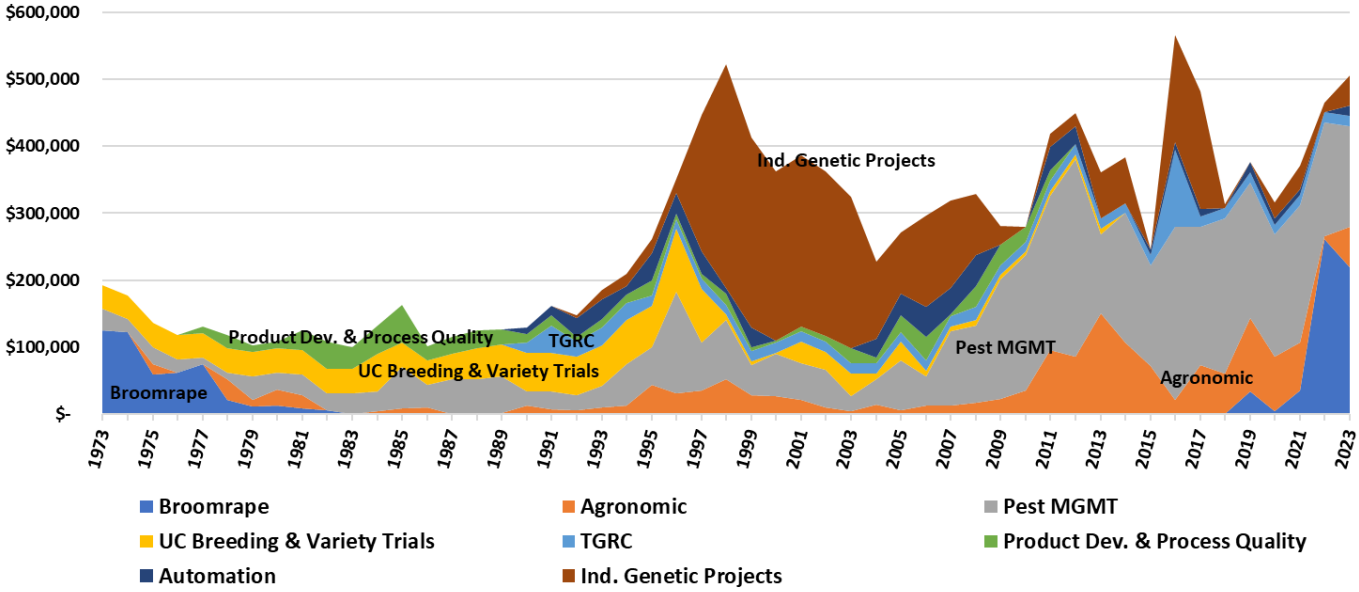


Figure 1. Expenditure by category through time (1973-2023)

As evidenced by a membership which represents over two thirds of the paid tons in 2023 (see Figure 2) and 55 years of historical expenditures, the CTRI has invested significantly (over 13.7 million USD) into the future of the processing tomato industry in California. These investments have come not only in the form of short term projects with results which can be immediately implemented in commercial fields (side-by-side crop protection product testing as an example) but also in the form of long term projection of industry need (continued annual TGRC commitment). Past experience highlights the reality that there is significance in not only what the CTRI chooses to fund from year to year but also in how we, alongside the industry, leverage those findings in two key ways: 1. To make the in-field changes which will continue to drive the industry forward incrementally and 2. To maintain and build the network of growers, processors, allied industry and researchers globally to cultivate and extend the next idea which will give us more than incremental change.

In the following pages we report on these efforts from 2023.

Additional resources for growers and allied industry can be found by joining the industry email alert system by filling out the sign up form found here: https://bit.ly/CTRIemails.

Please do not hesitate to direct any and all questions related to this report or the work of the Institute to Zach Bagley at zach@tomatonet.org or 530-405-9469.

MEMBERSHIP & ASSESSMENT HISTORY

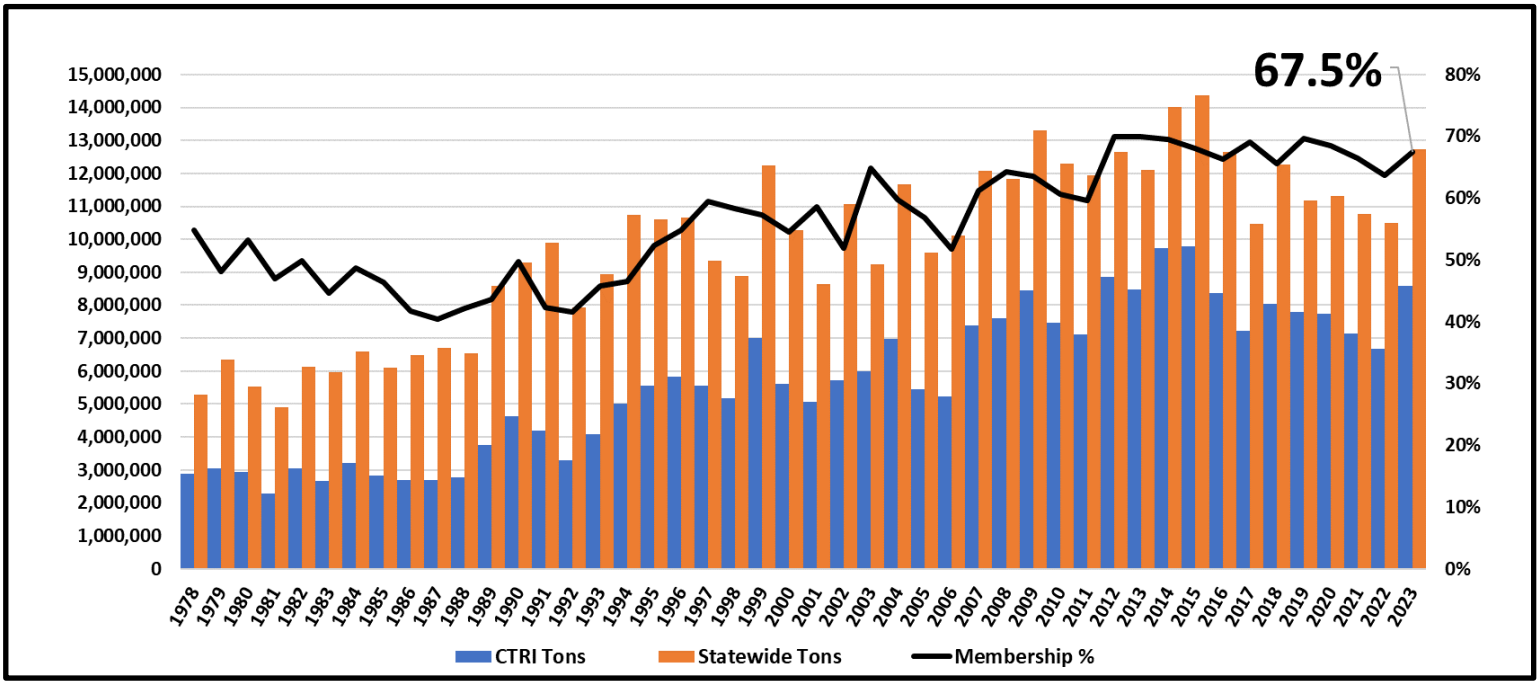
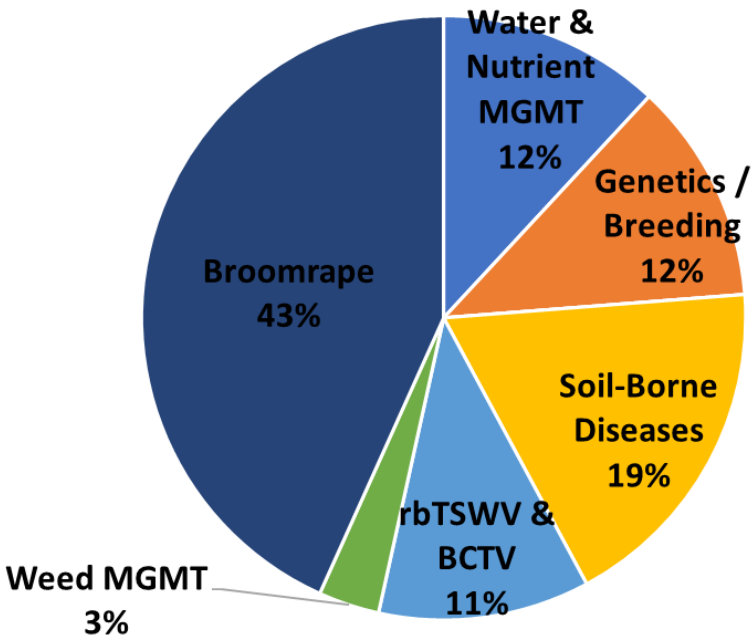


Figure 2. Membership & Assessment through time (1978-2023)

2023 RESEARCH - DOLLAR ALLOCATION

2023 Actual Allocations			
Category	**Funding**		**%**
Agronomic	$	60,312	12%
Genetics	$	59,984	12%
Bacterial	$	-	0%
Southern Blight	$	-	0%
RKN	$	-	0%
Fusarium et.al.	$	93,015	18%
BCTV	$	28,650	6%
TSWV	$	28,650	6%
Weed MGMT	$	16,531	3%
Broomrape	$	219,021	43%
Other Insect Pests	$	-	0%
TOTALS	**$**	**506,163**	**100%**



California Tomato Research Institute

2023 RESEARCH - PROJECT LIST

2023 CTRI FUNDED RESEARCH PROJECTS

2023 TOTAL FUNDING: $506,163

Broomrape Containment, Control and Management		**Research Lead**	**Institution**	**$219,021**
2020 Start	Broomrape: Devt. of Long Term Mgmt. Options: CA Commercial Field Conditions & Contained Research Facility Ongoing Work	Brad Hanson	UC Davis	$ 53,807
2020 Start	Broomrape: Devt. of Long Term Mgmt. Options: Chilean Commercial Field Conditions *	Brad Hanson	UC Davis - Chile	$ -
2021 Start	Developing best equipment sanitation practices for broomrape and other high-profile soil borne pathogens; to mitigate field-to-field spread	Cassandra Swett	UC Davis	$ 29,972
2022 Start	Determining the population structure of Phelipanche ramosa and Orobanche aegyptiaca field detections in California	Adam Schneider	UW-LaCrosse	$ 9,816
2022 Start	Developing Tomato Lines Resistant to Branched Broomrape, a Critical California Pest	Neelima Sinha	UC Davis	$ 60,475
2022 Start	Inducible Suberin for Tomato Drought Tolerance (root architecture)	Siobhan Brady	UC Davis	$ 18,836
2023 New	Detection of Broomrape Infestations with Remote Sensing *	Alireza Pourezza	UC Davis	$ 22,432
2023 New	Screening of a VOC Sensor to Identify Broomrape Infestations *	Cristina Davis	UC Davis	$ 23,683
Agronomic/Water/Nutrient Management				**$ 60,312**
2022 Start	Adapting the 'CropManage' weather-based irrigation decision-support tool – Year 2: Further Investigation of Pulse Irrigation *	Zheng Wang	UC Extension	$ -
2023 New	Evaluation of materials to mitigate negative effects of salinity and high temperatures on yields of processing tomatoes	Tom Turini	UC Extension	$ 20,312
2023 New	Climate Smart Mgmt. Innovations for Improved Soil Quality, and Productivity of CA Processing Tomatoes	Amelie Gaudin	UC Davis / UC Extension	$ 30,000
2023 New	KPAM in Soils with RKN AND Fungal Challenges - Impacts on Yield and Disease Severity	Patricia Lazicki	UC Extension	$ 10,000
Germplasm and Variety Development				**$ 59,984**
1991 Start	C. M. Rick Tomato Genetic Resource Center	Roger Chetelat	UC Davis	$ 15,000
2021 Start	Marker-assisted breeding for polygenic tomato spotted wilt resistance in tomatoes	Reza Shekasteband	NCSU	$ 44,984
Insect & Invertebrate Management				
2011 Start	Evaluation of Alternative Nematicides for the Control of Root-Knot Nematodes of Processing Tomatoes - with addition of novel biocarriers *	Jaspreet Sidhu	UC Extension	$ -
Pathogen Management				**$150,315**
2017 Start	Disease diagnosis, pathogen movement / emergence monitoring, new pathogen ID and F4 monitoring for the CA processing tomato industry	Cassandra Swett	UC Davis	$ 33,958
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	$ 49,690
2020 Start	Developing an integrated mgmt. strategy for F. falciforme vine decline in processing tomato, including co-management with Fusarium wilt	Brenna Aegerter	UC Extension	$ 9,367
2021 Start	Further monitoring of beet leafhopper and curly top virus in processing tomato fields in Stanislaus County – year 2 with updated evaluation plans *	Zheng Wang	UC Extension	$ -
2023 New	Addition of Fluazinam (Omega) Fungicide to Southern Blight trials *	Jaspreet Sidhu	UC Extension	$ -
2023 New	Jump starting BCTV awareness and pro-active management in the Sacramento Valley	Robert Gilbertson	UC Davis / UC Extension	$ 57,300
Weed Control and Management				**$ 16,531**
2023 New	Evaluation of new automated weeders in processing tomatoes	Steve Fennimore	UC Extension	$ 16,531

* Denotes a project which is ongoing with the review and confidence of the CTRI, but with 100% outside funding

* Denotes a project which is ongoing with the review and confidence of the CTRI, with 50% co-funding from the CLFP

FIELD AND CRF RESEARCH TOWARDS BRANCHED BROOMRAPE MANAGEMENT

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

Year of Project Initition: 2019-ongoing

Executive Summary:
Branched broomrape is an invasive noxious weed that has been increasingly reported 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 along with regulatory barriers present in California. Work began in 2019 evaluating an existing herbicide program developed in Israel based upon two ALS-inhibitor herbicides: sulfosulfuron and imazapic. In 2021, research pivoted from imazapic to imazamox due 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 24c 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 a standards. In addition to this herbicide trial, a randomized variety trial was conducted in the same broomrape infested field. Three varieties were included in the randomized trial, and an additional variety was screened in an unrandomized demo trial. These cultivars included HM8237, SVTM 9016, and SVTM 9019. The dodder-resistant cultivar H9553 was planted and evaluated against HM58841 in the unrandomized demo trial. In addition to in-field trials, greenhouse studies were conducted in 2023 to evaluate existing cultivars and precommercial cultivars for sensitivity to branched broomrape. Along with these trials, a synthetic strigolactone germination stimulant was evaluated for potential seedbank depletion in the greenhouse.

Over several years, tomatoes in plots treated with chemigated imazamox and imazapic have suffered minor to severe crop injury, and chemigated imazamox will not be pursued further due to lack of crop safety. Chemigated rimsulfuron alone and paired with sulfosulfuron at all rates and timings significantly reduced broomrape emergence. This supports data from 2022 and is very promising given the recently aquired 24c SLN label for chemigated Matrix SG. Future research will continue to refine and optimize this

registered chemigation treatment, as well as combining it with other promising herbicides. A first-in-the-US evaluation of foliar maleic hydrazide indicated promising results for reducing broomrape parastism. Discussions are underway with the registrant and this treatment will be pursued in the future alone and paired with other herbicides. There does not appear to be a difference in sensitivity to branched broomrape among available commercial cultivars. Results from the greenhouse and field study did not yield vast differences in broomrape emergence among cultivars. A first-in-the-world evaluation of a synthetic strigolactone soil treatment for stimulating gemination of branched broomrape had positive results in greenhouse assays and this research is being scaled up to pots of soil with the intention of moving to the field in 2024 or 2025.

Introduction:

Branched broomrape *(Phelipanche ramosa)* is an invasive noxious parasitic plant that has been reported in commercial tomato fields in recent years in Yolo County, California. Broomrapes are native to the Mediterranean basin and the biology of broomrape makes its control via conventional weed control practices very difficult. Broomrape is an obligate parasite with seeds that only germinate after receiving a chemical signal from a suitable host plant. After germination, it quickly attaches to the host roots via a specialized structure known as a haustorium and draws all of its nutrients and water from the host. The above ground portions of the plant lack chlorophyll and quickly produce a large amount of minute seed, which are highly persistent in the soil seedbank. It has a wide crop host range and can parasitize plants from the Solanaceae, Asteraceae, Brassicaceae, and various other crop plant families. Branched broomrape *(Phelipanche ramosa)* is an "A-listed" noxious weed in California and growers that report branched broomrape in their fields face strict regulatory and quarantine measures.

Work to validate existing broomrape management programs developed in Israel for California conditions began in 2019 and has continued to the present. These programs, known as PICKIT, are decision support systems that utilize growing degree days to inform applications of herbicides for Egyptian broomrape control in Israeli processing tomatoes (Eizenberg and Goldwasser). The Israeli PICKIT program relies on two chemistries: preplant incorporated (PPI) sulfosulfuron paired with in-season chemigated imazapic (Eizenberg and Goldwasser). Projects in 2019 and 2020 included crop safety and efficacy trials focusing on imazapic; however, imazapic faces significant regulatory hurdles in California and efforts to evaluate imazamox instead began in 2021.

With the support of CTRI, field projects were conducted during the 2021-22 growing season in Chile and during 2022 in California. Both research teams addressed chemigation and planting date objectives. In California, two chemigation crop safety studies were conducted in a non-infested tomato field near Davis, CA and an efficacy study was conducted in a broomrape-infested commercial tomato field near Woodland, CA. This Woodland study included evaluations of chemigated imazamox and rimsulfuron alone and paired with preplant incorporated sulfosulfuron, as well as an alternate planting date and several different grafted and non-grafted tomato varieties.

Main Goal and Objectives:

The goal of this research is to develop programs for management of branched broomrape in California processing tomato. Many of the chemigation and equipment sanitation objectives funded by CTRI in recent years will continue for 2-3 more years with new CDFA-SCBG funding; however, additional follow up or exploratory work, particularly in the quarantine greenhouse, cannot be accomplished with the CDFA-SCBG project due to the budget maximum and limited scope of work flexibility. The primary focus of this proposal is chemical approaches to managing broomrape in the field, evaluation of tomato cultivars, greenhouse research related to seed sanitation and germination stimulation, and as a

foundational program to support and facilitate research being conducted by other groups with separate funding.

The specific objectives of the 2023 project were to:

A. Field:
   A1. Further evaluation of sulfosulfuron PPI treatments supplemented with a limited number of imazamox or imazapic treatments in order to validate previous performance and crop safety data generated in California and Chile. (built on 3 previous seasons)
   A2. Systematic evaluation of sulfosulfuron PPI treatments supplemented with various rimsulfuron chemigation programs based on recent 24c label to be in effect during 2023. (built on limited research in 2022)
   A3. A large-plot demonstration of 2-3 promising chemigation treatments will be conducted with a newly identified cooperator. (carryover objective from previous year)
   A4. Evaluate the impacts of three tomato transplanting dates on broomrape emergence in California. (follow-up on 2022 project; coordinated with a 2023 Chile project)

B. Contained Research Facility (CRF):
   B1. Conduct a systematic screening of commercial tomato cultivar sensitivity to broomrape parasitism in the quarantine greenhouse at UC Davis. (built on pilot study in 2022)
   B2. Develop system for assay of seedling plants for broomrape parasitism as a preliminary screening tool. (new objective)
   B3. Pot assays with rootstocks and/or cultivars with reported broomrape resistance. [collaborative with industry] (new objective)
   B4. Pilot broomrape studies with synthetic strigolactone compound with reported activity on striga but unknown impact on broomrape. [from KAUST] (new objective)

Methodology and Results:

A: Field

A1: An efficacy trial evaluating PPI sulfosulfuron treatments paired with chemigated imazamox and imazapic treatments was conducted in spring/summer of 2023 in the branched broomrape infested Yolo County commercial tomato field. The trial evaluated PPI sulfosulfuron paired with chemigated imazamox and PPI sulfosulfuron paired with chemigated imazapic in a randomized complete block design with four replications. Each plot consisted of a 120-foot-long bed with a buried dripline in the center of each bed at a depth of 9-10 inches. The trial was transplanted on May 21, 2023, with HM 58841 planted in a single-line with 12” spacing. Treatment applications were made from early June to late June (Tables 1, 2). Preplant incorporated (PPI) and foliar treatments were applied with a CO_2 backpack sprayer and 3-nozzle boom while chemigated treatments were mixed in a 3-liter bottle and injected directly into the dripline of individual plots using CO_2. Visual injury and broomrape emergence data were collected weekly from mid July to mid August; yield data was not collected due to variability in the crop as a result of injury from herbicide and recurring scouting activity.

This year, both chemigated imazamox and imazapic treatments resulted in unacceptable levels of injury on tomatoes. Previous trials conducted in 2021 and 2022 experienced injury with chemigated imazamox at higher rates (19.2 g ai/ha), however, there was never injury observed in plots treated with chemigated imazapic. Due to the severe level of injury, there was no broomrape emergence in any of the plots treated with chemigated imazamox or chemigated imazapic (Table 2).

A2: An efficacy trial evaluating PPI sulfosulfuron treatments paired with and without chemigated rimsulfuron were applied in the branched broomrape infested Yolo County commercial tomato field. The trial evaluated PPI sulfosufulfuron alone and chemigated rimsulfuruon alone and paired with PPI sulfosulfuron at several different rates and timings, and PPI sulfosulfuron paired with foliar and chemigated rimsulfuron. In addition, a new postemergence experimental treatment of foliar maleic hydrazine was evaluated at two rate regimes. The trial was transplanted on May 21, 2023, with HM 58841 planted in a single-line with 12" spacing. Treatment applications were made from early June to late June (Tables 1, 2). Preplant incorporated (PPI) and foliar treatments were applied with a CO_2 backpack sprayer and 3-nozzle boom while chemigated treatments were mixed in a 3-liter bottle and injected directly into the dripline of individual plots using CO_2. Visual injury and broomrape emergence data were collected weekly from mid July to mid August; yield data was not collected due to variability in the crop as a result of injury from herbicide and recurring scouting activity.

None of the treatments resulted in injury on tomatoes throughout the trial period (data not shown). There was significant broomrape emergence throughout the trial area and there was signficiant reduction in emergence from control in all treatments (Table 2). There were not significant differences in broomrape emergence among treatments. Broomrape emergence ranged from 28 clusters (control 1) to 2 clusters (treatment 6)(Table 2). There appears to be a slight numerical reduction in broomrape emergence in the treatment applied according to a growing degree day schedule versus the calendar schedule (days after treatment). Growing degree day schedule treatments were applied earlier than calendar treatments (Table 1). Future research will investigate application timing differences.

A3: A large-scale demonstration study was not conducted this year, as the newly identified cooperator was not able to provide the planned trial site and farming. With the 24c registration of chemigated rimsulfuron approved for the 2023 season and rapidly widely adopted, this objective will not be pursued further unless new herbicide targets are identified. We anticipate trying to gather anonymous anecdotal information about the perceived efficacy of chemigated Matrix following the 2023 harvest season.

A4: The impacts of transplanting date were not able to be evaluated this field season. The exceptionally wet spring did not allow an early planting and the grower cooperator and transplant service were not available for the mid-season planting date. The entire trial was planted on the proposed late planting date of May 21, 2023. A comparison of several commercial cultivars was evaluated in a replicated plot demo at the grower trial site instead.

Three processing tomato cultivars commonly planted in the Sacramento area were mechanically transplanted in a single row with 12" spacing on May 21, 2023. The cultivars included SVTM 9016, SVTM 9019, and HM 8237. The trial was arranged in a randomized complete block design with eight replications. In addition to this randomized trial, two additional cultivars were compared in an unreplicated demonstration. Two rows of each cultivar were mechanically transplanted in a single-line with 12" spacing on May 21, 2023. The cultivars that were compared were HM 58841 and H9553, the latter known for possessing dodder (*Cuscuta spp.*) resistance. Both trials were watered and fertilized following commercial practices. When the crop reached commercial maturity, the trial was scouted once and branched broomrape clusters were flagged and recorded.

There was broomrape emergence in every rep of every variety planted. There were no significant differences in broomrape emergence among varieties (Table 3). Broomrape emergence by cultivars is as follows: SVTM 9016 had 16 clusters on average, SVTM 9019 had 22 clusters on average, and HM 8237 had

13 clusters on average (Table 3). The two rows planted with HM 58841 had 8 and 9 clusters per 120 ft plot, while the rows planted with H9553 had 4 and 12 clusters per plot (data not shown). Due to the logistical limitations, we were only able to plant two rows of H9553. While statistical inferences cannot be made, there does not appear to be substantial differences in broomrape emergence between H9553 and HM 58841.

B: Contained Research Facility (CRF)

B1, B3: The greenhouse screening trial was initiated in early 2023. Tomato varieties from the 2021 top 20 PTAB list and other cultivars of interest from industry partners (Table) were seeded into 1”x1” plug trays on February 16, 2023, and transplanted into 4”x4” pots on March 16, 2023. H9553 was seeded later on March 20, 2023, directly into the inoculated 4”x 4” pot. Branched broomrape seed was preconditioned for 10 days prior to transplant. During transplanting, tomato plants were inoculated with preconditioned branched broomrape seed. To inoculate transplants, preconditioned seed was thoroughly mixed into two liters of soil. A small amount of this infested soil was placed into the hole into which tomato seedlings were transplanted. The trial was arranged in a randomized complete block design with three single-plant replications of each variety. Branched broomrape emergence was first observed on May 19, 2023 (~2 MAT) and was recorded twice weekly throughout the remainder of the trial.

All of the varieties were infested with branched broomrape in every replication (Table 4). The first emergence was on May 19, 2023 (685 GDD) and the last recorded emergence was on June 13, 2023 (946 GDD). The first emergence was observed on SVTM 9023, and the last emergence was also observed on SVTM 9023 and Heinz 9553 (Table 4). The timepoint with the highest broomrape emergence was on June 7, 2023 (880 GDD). Average growing degree days of first broomrape emergence by variety were calculated and are presented in Table 4.

B2: Two small scale assay methods based on polyethylene bags or clear beverage cups for seedling parasitism by branched broomrape is currently being developed and evaluated. (Figure 2). This method has dramatically improved experimental throughput and helped with space limitations in the quarantine greenhouse. Current experiments include evaluations of non-tomato hosts and the effects of rimsulfuron on broomrape attachements on seedling tomatoes.

B4: Two synthetic strigolactone products developed by researchers in Saudi Arabia for management of striga (witchweed), a root parasite of maize and sorghum, were imported into the US and tested for the first time on branched broomrape. Results from Petri dish germination experiments in the quarantine greenhouse indicate promising results of the experimental products for stimulating germination of branched broomrape seed, comparable to GR24, the laboratory standard (Figure 3). This work will be repeated in vitro and scaled up to pot experiments with soil and tomato host plants. We anticipate moving this to the field in 2025 if results warrant (and if research approvals can be obtained and sufficient chemical imported into the US).

Discussion

Results from 2023 confirm results from 2022, indicating that chemigated rimsulfuron has a positive impact in reducing broomrape emergence. Chemigated rimsulfuron alone and paired with sulfosulfuron at all rates and timings had significantly lower broomrape emergence than controls. Future research will focus on refining these applications and investigating application timing to optimize this approved treatment for California growers. Results from this season’s trial on foliar maleic hydrazide are promising. Maleic hydrazide significantly reduced broomrape versus controls at both protocols. Future research will seek to confirm these results and work toward developing registration support data if the registrant is willing to

pursue registration in California for branched broomrape control. After several field experiments, chemigated imazamox does not appear to have reasonable crop safety on tomatoes and will not be pursued further. There does not seem to be a vast difference in broomrape sensitivity among current commercial cultivars. Data from the greenhouse study and field study did not indicate significant differences among cultivars in broomrape emergence. Future field chemigation research will focus on fine-tuning rimsulfuron protocols and "stacking" treatments for an integrated approach to managing branched broomrape.

Acknowledgments
We would like to acknowledge and thank those who collaborated on this project. This project would not have been possible without the extremely generous work of Eric Schreiner and Schreiner Bros. Farming. Without their efforts over the years, none of the broomrape field research to date would be possible.
Thank you to Gene Miyao for being a collaborator on the broomrape effort since 2019, Patricia Lazicki for her recent collaboration on the broomrape effort, the Juan Carlos Galaz at the UC Davis Chile Life Sciences Innovation Center and his group for his work on the broomrape effort in the southern hemisphere, and members of the Hanson lab for their invaluable help in the field and greenhouse on various aspects of this project. We would like to also acknowledge and thank AgSeeds Unlimited for transplants, California Transplanting for transplanting, and Wilbur Ellis Woodland for material support.

This project as leverage for other dollars
Branched and Egyptian broomrape are the largest threat to the processing tomato industry and allied specialty cropping systems in California in decades. This project serves as the applied solutions touchstone project that coordinates with several closely-related equipment sanitation, plant physiology and host genetics research projects or proposals developed by collaborators. Several of these projects are also supported by CTRI (e.g. Swett and Hanson, Sinha and Brady, Schneider) but are interconnected with this project due to access to broomrape plants in the field or in quarantine greenhouse, broomrape seeds, or experience with the weed. Hanson and Swett and collaborators were awarded $472,997 from the CDFA Specialty Crop Block Grant Program in November 2022 to continue and expand upon the herbicide management and sanitation projects initially funded by CTRI. Swett and Hanson also were awarded funding from California League of Food Processors for broomrape-related equipment sanitation research. Hanson's collaborators at the UC Davis Chile Life Sciences Center are in discussion with Chilean industry partners to fund additional research on broomrape management based on work initiated with CTRI funding in 2020 and 2021; if this is funded, we anticipate continuing our California-Chile collaboration to enhance the California objectives. Lastly, Hanson is a collaborator on a $520,013 proposal to Foundation for Food and Agricultural Research (FFAR) led by Sinha and Brady for their work on developing broomrape-resistant tomato lines. Together, investment by CTRI into broomrape management research is likely to leverage at least four-fold more funding over the next several years.

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Table 1. Application dates from a 2023 broomrape efficacy trial conducted near Woodland, CA

Preplant incorporated	5-May
Transplant	21-May
Post-rimsulfuron	31-May
Chemigation: 400 growing degree days	12-Jun
Chemigation: 500 growing degree days	16-Jun
Chemigation: 600 growing degree days	20-Jun
Chemigation: 700 growing degree days	23-Jun
Chemigation: 800 growing degree days	30-Jun
30 days after transplant	14-Jun
50 days after transplant	11-Jul
70 days after transplant	5-Aug
Maleic Hydrazide- 100 growing degree days	31-May
Maleic Hydrazide- 200 growing degree days	5-Jun
Maleic Hydrazide- 400 growing degree days	12-Jun
Maleic Hydrazide- 700 growing degree days	23-Jun
Maleic Hydrazide- 1000 growing degree days	5-Jul

Table 2. Treatments from a 2023 broomrape efficacy study conducted near Woodland, CA.

	Treatment	Active Ingredient	Rate	Application	Timing
1	Control 1				
2	Control 2				
3	Sulfosulfuron solo	Sulfosulfuron	37.5 g ai/ha	PPI	
4	Rimsulfuron solo 4x GDD	Rimsulfuron	1 oz product	POST; CHEM	400, 600, 800 GDD
5	Rimsulfuron solo 4x DAT	Rimsulfuron	1 oz product	POST; CHEM	30, 50, 70 DAT
6	Sulfosulfuron	Sulfosulfuron	37.5 g ai/ha	PPI	
6	Rimsulfuron 4x1oz	Rimsulfuron	1 oz product	POST; CHEM	400, 600, 800 GDD
7	Sulfosulfuron	Sulfosulfuron	37.5 g ai/ha	PPI	
7	Rimsulfuron 3x1.3oz GDD	Rimsulfuron	1.33 oz product	CHEM	400, 600, 800 GDD
8	Sulfosulfuron	Sulfosulfuron	37.5 g ai/ha	PPI	
8	Rimsulfuron 3x1.3oz DAT	Rimsulfuron	1.33 oz product	CHEM	30, 50, 70 DAT
9	MH Protocol 1	Maleic hydrazide	400 g ai/ha	FOLIAR	100, 200, 400, 700, 1000 GDD
10	MH Protocol 2	Maleic hydrazide	270 g ai/hax2, 540 g ai/ha x3	FOLIAR	100, 200, 400, 700, 1000 GDD
11	Sulfosulfuron	Sulfosulfuron	37.5 g ai/ha	PPI	
11	Imazapic	Imazapic	4.8 g ai/ha	CHEM	400, 500, 600, 700, 800 GDD
12	Sulfosulfuron	Sulfosulfuron	37.5 g ai/ha	PPI	
12	Imazamox	Imazamox	9.6 g ai/ha	CHEM	400, 500, 600, 700, 800 GDD

PPI: preplant incorporated, POST: post emergence, CHEM: chemigated, GDD: growing degree days, DAT: days after transplant

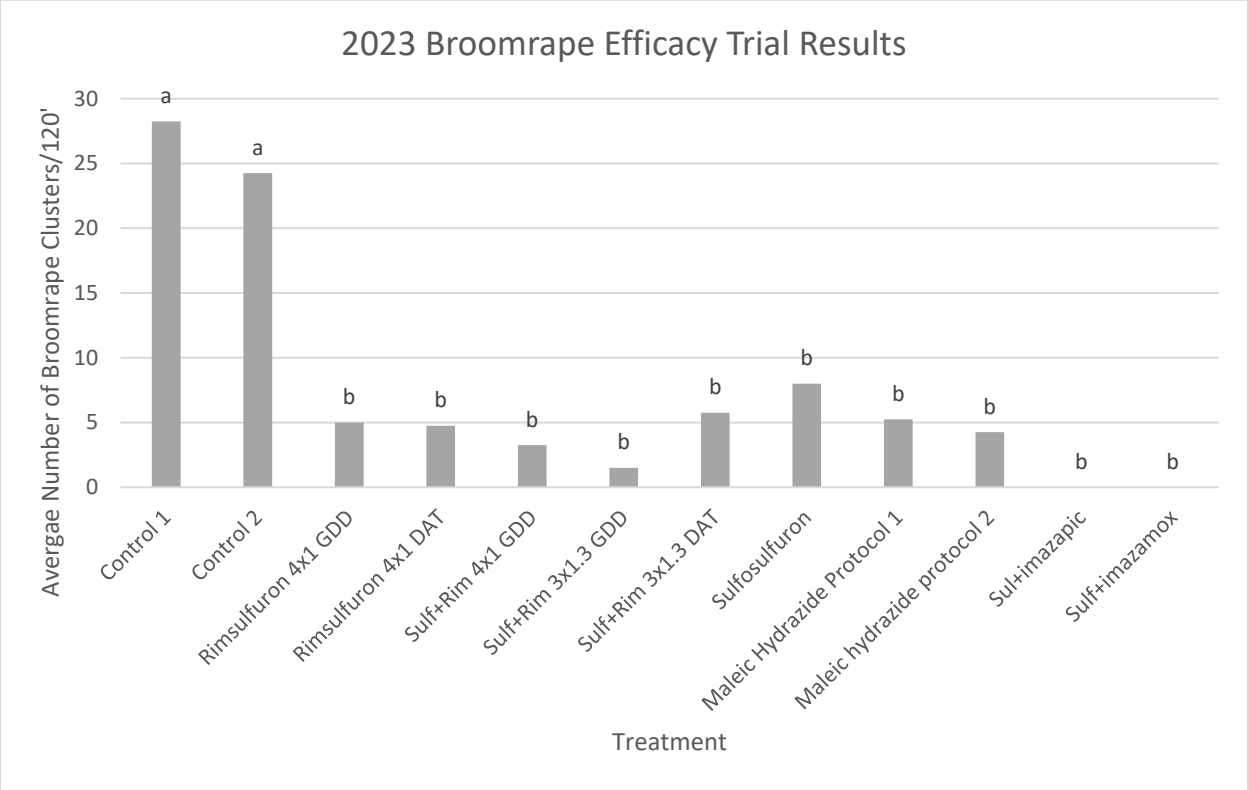

Figure 1. Average number of branched broomrape clusters per 120-ft plot by treatment across four replications in an infested tomato field in Yolo County, CA. **Sulfosulfuron + imazapic and sulfosulfuron + imazamox treated plots were severely injured and the lack of broomrape emergence in these plots is a result of that injury.*

Table 3. Varieties and average number of broomrape clusters per 120’ plot in a 2023 screening study conducted near Woodland, CA.

Variety	Clusters
SVTM 9019	22
SVTM 9016	16
HM8237	13

Table 4. Tomato variety list and average growing degree day for first observed broomrape emergence in a 2023 variety screening trial in the CRF.

Number	Variety	Average GDD	Number of Replicate Pots Parasitized
1	N 6428	805	3/3
2	Heinz 1662	805	3/3
3	HM 58841	892	3/3
4	BP 13	849	3/3
5	SVTM 9013	832	3/3
6	SVTM 9016	860	3/3
7	HM 5235	835	3/3
8	SVTM 9024	809	3/3
9	HMX 4909	880	3/3
10	Heinz 1015	860	3/3
11	SVTM 9025	725	3/3
12	SVTM 9011	860	3/3
13	Heinz 1428	880	3/3
14	N 6434	849	3/3
15	SVTM 9023	806	3/3
16	BQ 403	774	3/3
17	Heinz 5706	880	3/3
18	BQ 413	798	3/3
19	DRI 0319	860	3/3
20	BQ 273	849	3/3
21	Syngenta	849	3/3
22	Seminis DynaFort	805	3/3
23	Seminis MultiFort	924	3/3
24	Heinz 5508	829	3/3
25	Heinz 9553	924	3/3

GDD: growing degree days

Figure 2: *Observing broomrape attachment and tubercle formation on tomato plant in reduced-scale double-potted growth system. This should have potential utility for additional crop screening, tomato cultivar screening, and germination stimulator research in the quarantine greenhouse.*

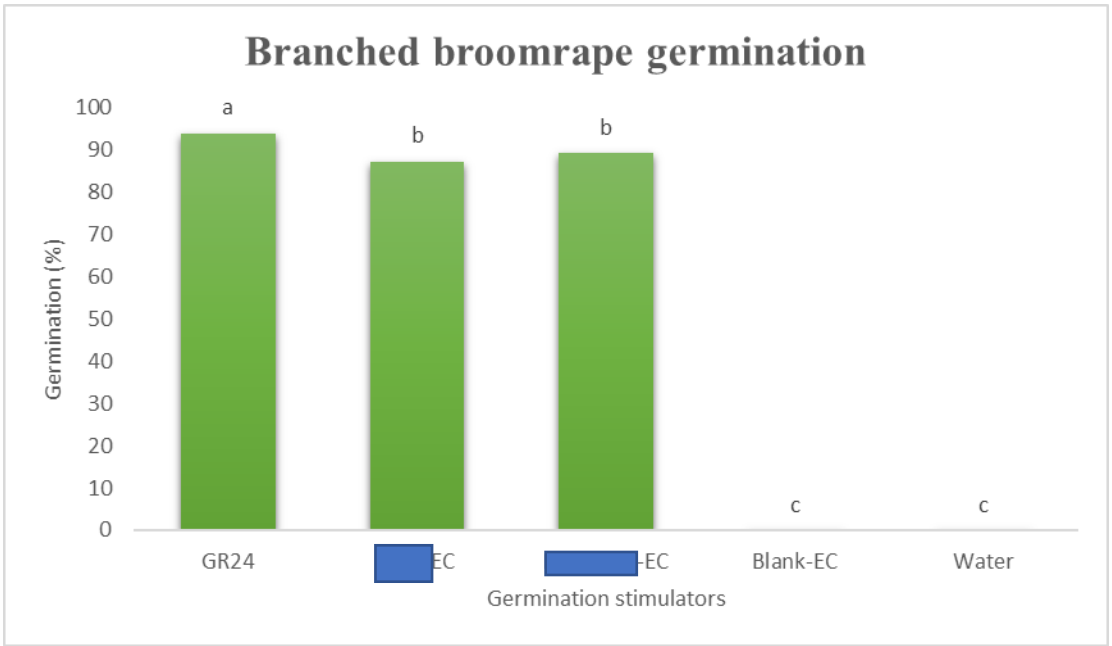

Figure 3: *Comparison of three germination stimulators, GR24 (commercial laboratory strigolactone) and two experimental synthetic strigolactone products and the formulation control for branched broomrape seed germination. Data are means ± SE (n = 8), and treatments with various letters differ significantly according to one-way analysis of variance (ANOVA) ($p < 0.05$).*

DEVELOPING BEST EQUIPMENT SANITATION PRACTICES FOR ERADICATION OF BRANCHED BROOMRAPE AND OTHER HIGH-PROFILE SOIL BORNE PATHOGENS; TO MITIGATE FIELD-TO-FIELD SPREAD

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Authors: Swett, C.L., Hanson, B., Hosseini, P., Zimmerman, K., and Beaulieu, J.

Year of Project Initiation: 2021

Executive Summary

Key take homes:

- Commercial QAC sanitizers all have similar efficacy.
- Studies indicate that QAC compounds rapidly lose efficacy against broomrape and Fol in contact with soil; studies on the effects of plant material are underway.
- The on-harvester studies of debris load-QAC interaction indicate that while debris loads less than 1” may not fully inhibit QAS efficacy, levels over 1” can reduce QAC efficacy by 50%.
- Peracetic acid was not effective against broomrape.
- Comprehensive, bilingual outreach efforts are being utilized to enable behavioral change.
- As work progresses, many new questions and knowledge gaps are emerging.

Specific outcomes and benefits are:

- Growers and canneries have access to recommendations and consultation support for effective in-season and off-season harvester sanitation, which can also be applied to other equipment to reduce the risk of spreading broomrape.
- These methods also work to reduce spread of soil borne pathogens. This includes Fusarium wilt, which is at high risk of resistance breaking—equipment sanitation will provide the only means to prevent spread of a new race in the years immediely following introduction.
- BMPs are improved upon, providing new information on a wider range of sanitizer options, specifically peracetic acid, which is certified for organic use.
- BMPs are improved upon, providing new information on debris management targets.

- Researchers learn about barriers to effective sanitation as well as innovations developed by grower and processor operations, which could provide critical information for overcoming cleaning time and other challenges.

What's next for this project:

- As QACs are only moderately effective in the high debris load environment of field equipment, identification of other effective products that are less debris sensitive would help improve existing BMPS. Thus in 2024 we plan to evaluate the efficacy of a wider range of products against broomrape and efficacy in the presence of debris.
- In addition to broomrape, there are cases where sanitation is needed for other soil borne pests, and there may be sanitizers less effective against broomrape which are, nevertheless highly effective against other pathogens. Thus in 2024, we plan to develop a table summarizing the efficacy of a wider range of sanitizers against the pathogens causing Fusarium wilt, and southern blight, bacterial canker.
- Studies are needed to expand risk assessment analyses to a wider range of field equipment to more cohesively address and manage the risk of broomrape spread within the agricultural system.

Introduction:

This project focuses on equipment sanitation practices to reduce the potential spread of branched broomrape (*Phelipanche ramosa*, aka *Orobanche ramosa*) among processing tomato fields and production regions. This proposal merges previous ongoing equipment sanitation critical control point assessments for microbes (Swett) with preliminary studies of sanitizer efficacy on broomrape seed viability (Hanson). The primary goal is to reduce the risk of spreading branched broomrape seed to new sites within affected counties or spread to new counties, to mitigate economic impacts of field quarantine in the short term and the impacts of broomrape on tomato production once established in a field. The secondary goal is to simultaneously mitigate losses from emerging soil borne pathogens by preventing establishment in uninfested fields.

The efficacy of quaternary ammonia (compounds and products) under harvester sanitation-relevant time frames will be addressed using a combination of laboratory dose-response assays and a biometric approach using Fusarium loads as a proxy for broomrape seed on commercial tomato harvest equipment. Quaternary ammonia sanitizers will be tested for efficacy against broomrape seeds and Fusarium pathogens of tomato. In this proposal we emphasize Fusarium wilt (F. oxysporum f. sp. lycopersici-Fol), to provide tools to both mitigate spread of race 3 into uninfested fields and provide a protocol which can be immediately implemented if/when race 4 is detected; methods effective against Fol will also be tested on F. falciforme. This initial set of laboratory experiments will serve the dual function of establishing products with efficacy on seed and spore sterilization and the relative sensitivity of broomrape seed compared to Fusarium spore survival, in order to develop a biometric proxy using Fusarium propagules to estimate broomrape seed survival. Subsequently, using this biometric analysis, we evaluate harvester efficacy of different sanitation practices for broomrape and pathogen removal in replicated field

trials. To rapidly enable growers and custom harvest operations to adapt sanitation practices in response to research findings, Swett and Hanson will produce a BMP handout for sanitation methods and host a harvester sanitation demonstration field day at UC Davis.

Our work addressed the overarching problem of inadequate baseline information on existing practices, limitations and feasible options on equipment sanitation. Accordingly, the long-term goal of this work was the provision of cost-benefit information on effective sanitation practices together with recommendations on best management practices. At a time when sanitation concerns are heightened, we developed both a collaborator network and baseline information on sanitation barriers and practices which can be leveraged for downstream proposals to state and/or federal programs to garner support for longer-term efficacy studies.

Main Goal and Objectives:

The main *Goal* and *Objectives* of the funded project: develop programs to 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 nematodes and pathogens.

Obj. 1: Determine debris removal threshold targets using a combination of laboratory assays (broomrape seed and Fusarium spores) and studies with commercial tomato harvest equipment (biometric analysis).
Obj 2. Evaluate dose-dependent efficacy of peracetic acid against broomrape seed and Fusarium spores at different exposure durations.
Obj 3. Conduct an after-season harvester sanitation efficacy survey and consultant service.
Obj 4. Outreach to rapidly enable the tomato production community to mitigate spread of broomrape and other soil borne pests.

Methodology and Results:

<u>Objective 1</u>: Evaluating efficacy of quaternary ammonia compounds in killing branched broomrape seed, plus representative soil borne pathogens of tomato

1.1 Effect commercial sanitizers against broomrape and subsequent effect of <u>soil</u> debris-QAC interactions on efficacy in reducing broomrape seed viability

Methods. Branched broomrape seeds were collected in 2022 from a tomato research field infested by branched broomrape in Woodland, CA. The experiments were done within the Contained Research Facility (CRF) of the University of California, Davis in 2023. We evaluated the effectiveness of three commercially available sanitizers containing quaternary ammonium compounds (QACs), namely MG4-Quat (Mg4), Flo-Quat (FQ), and Cleaner QT-185 (CQT). These assessments were carried out through a 1-minute exposure period, encompassing nine distinct concentrations: 0 (deionized water), 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, and 2.5% (v/v) of deionized water.

Branched broomrape seeds (around 40-50 seeds) were placed in 2 ml Eppendorf tubes and soaked in 1 ml of the specific dose of each chemical. Once the appropriate exposure time had elapsed, the seeds were subjected to a rinsing process using distilled water, facilitated by a cell strainer (Pluristrainer Mini, 70 µm).

To introduce soil and plant components into the solution, we employed a fine sieve (63 microns) to sift regular soil from the Davis fields. This resulted in particles finer than both broomrape seeds and the filter mesh. Tomato seedlings were dried at 25°C for four days and subsequently crushed and sifted through the same sieve. A specific quantity of soil or plant powder was then added to 1 ml of the sanitizer solution. This examination was conducted for 1-minute exposure, utilizing the recommended QAC dose of 1% w/w. The soil and plant materials were tested at four distinct concentrations: 0%, 10%, 30%, and 50% w/v for soil powder and 0%, 2%, 8%, and 16% w/v for plant powder. These concentrations were categorized as low, medium, and high levels of concentration, respectively. The rinsing process was conducted as previously described.

Following this rinsing procedure, the seeds were transferred onto filter papers (Whatman paper) situated in Petri dishes with a diameter of 60 mm. To all Petri dishes, 1 ml of deionized water was added and dishes were sealed with parafilm and kept at 25 °C in darkness for ten days within an incubator (Isotemp Incubator, Fisher Scientific), constituting the pre-conditioning phase. A 10-5 M solution of GR24 (strigolactone analog) was created by combining Milli-Q water, and subsequently, 1 ml of this solution was introduced into the Petri dishes. The Petri dishes were then resealed with parafilm and placed in a dark incubator set at 25 °C for 10 days.

All Petri dishes were unsealed and left to air dry for one day. Subsequently, images were captured using a microscope-mounted camera. Germinated and non-germinated seeds were counted manually from the images. Seed mortality data were analyzed using statistical software package R. One-way analysis of variance (ANOVA) with Least Significant Difference (LSD) multiple range test was used for analyzing the effect of treatments on broomrape seed mortality data. For dose-response analysis, a three-parametric log-logistic function was used to the seed germination of branched broomrape to the concentration of chemicals.

Results:

a. Commercial sanitizers. The result showed that commercial sanitizers (Mg4, CQT, and FQ) containing a mix of several quaternary ammonium compounds as their active ingredients demonstrated remarkable effectiveness in preventing the germination of branched broomrape seeds. We observed that these sanitizers reduced the germination of branched broomrape seeds within a short time. The decrease in broomrape seed viability was noticeable at concentrations as low as 0.05% v/v. At the recommended concentration of 1%, no germination was observed in the Mg4 and FQ treatments, indicating the potent inhibitory effect of those solutions on the seeds.

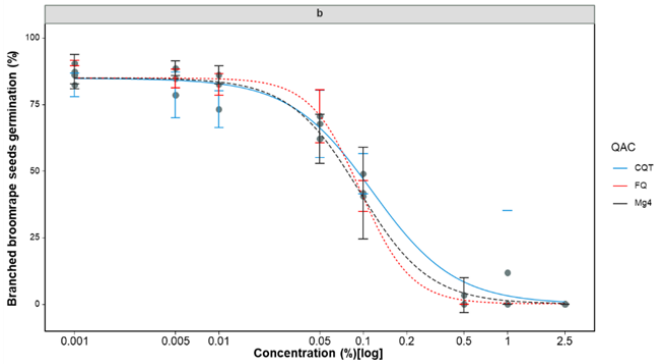

Figure 1. Dose–responses curves of branched broomrape seed germination in response to doses of commercial sanitation agents for 1 minute. Abbreviation: Mg4: MG 4- Quat, CQT: Cleaner QT-185, FQ: Flo-Quat Sanitizer.

b. Commercial sanitizers + soil debris. The three QAC sanitizers demonstrated the capability to prevent the germination of branched broomrape seeds when applied at a 1% v/v concentration, the recommended dose. However, it was observed that the efficacy of these chemicals was compromised when soil was introduced into the solutions. The mortality rate of branched broomrape in the absence of soil within the solution was found to be 100%, but this rate declined to less than 20% across all soil-containing treatment conditions. Statistical analysis did not reveal any significant differences among the three soil concentration levels, which were set at 10%, 30%, and 50% w/v.

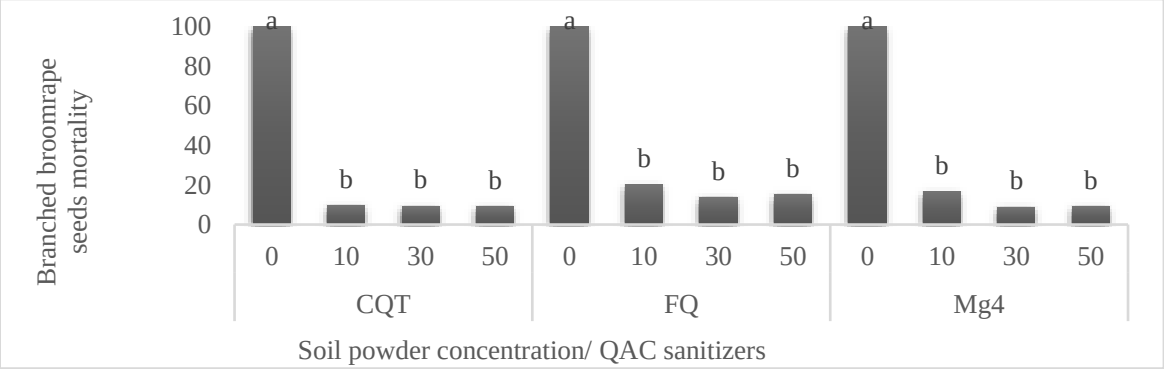

Figure 2: Branched broomrape seed mortality with QAC sanitizers in the presence of soil powder for a 1-minute exposure duration. Abbreviation: Mg4: MG 4- Quat, CQT: Cleaner QT-185, FQ: Flo-Quat Sanitizer. Data are means ± SE (n = 3), and treatments with various letters differ significantly according to one-way analysis of variance (ANOVA) ($p < 0.05$).

c. Effect of exposure duration on soil debris-QAC product interactions. For the next phase, our objective was to assess the potential influence of altering exposure duration and introducing a surfactant into the solution. To address this, we conducted another experiment involving three exposure durations: 1, 5, and 10 minutes with or without surfactant. The surfactant was Lansurf AEP63 (manufactured by Lankem) and, we used one QAC sanitizer, Mg4, and 1 soil concentration, 10% w/v. The results indicated that varying exposure duration and incorporating a surfactant had no discernible effect on the outcome. The sanitizer was rapidly deactivated in the presence of soil, leading to little or no inhibition of broomrape seed germination.

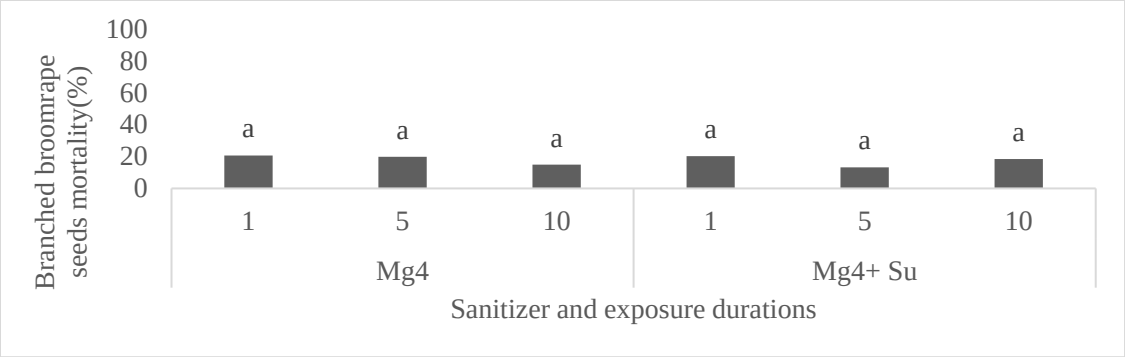

Figure 3: Branched broomrape seed mortality with Mg4 sanitizer in the presence of 10% soil powder for 1, 5, and 10 minutes exposure duration and with or without surfactant. Abbreviation: Mg4: MG 4- Quat, Su: Lansurf AEP63. Data are means ± SE (n = 3), and treatments with various letters differ significantly according to one-way analysis of variance (ANOVA) ($p < 0.05$).

d. Effect of QAC concentration on soil debris-QAC interactions

In the third phase of our study, our primary goal was to evaluate the potential impact of increasing sanitizer concentrations. To achieve this objective, we conducted an additional experiment encompassing three higher QAC concentrations: 2%, 4%, and 8% v/v. We used all three QAC sanitizers, Mg4, FQ, and CQT, and a constant soil concentration of 10% w/v. The findings from this experiment revealed that elevating the sanitizer concentration to 8% in the case of FQ completely inhibited broomrape seed germination in a 10% w/v soil condition. CQT at 8% v/v and FQ at 4% v/v showed a 90 and 65 percent reduction in broomrape seed germination, respectively, in the presence of 10% w/v soil (Figure 3).

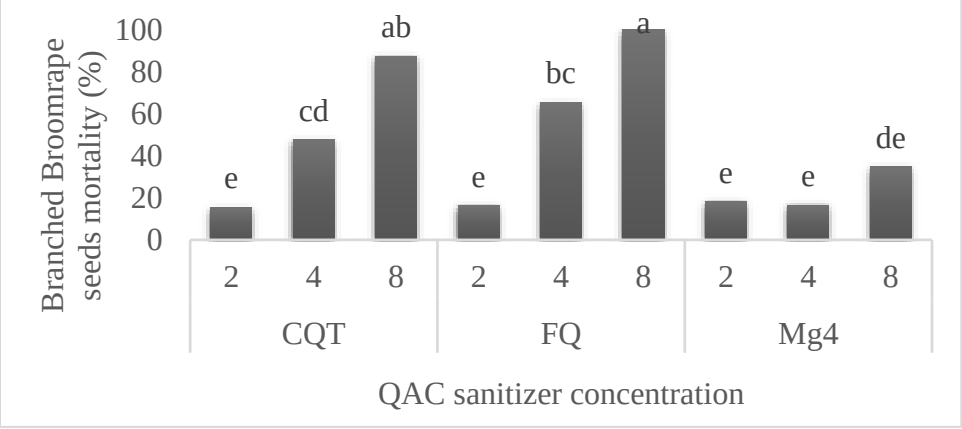

Figure 4: Branched broomrape seed mortality with increasing sanitizer dose in the presence of 10% soil powder for 1 minute exposure duration. Abbreviation: Mg4: MG 4- Quat, CQT: Cleaner QT-185, FQ: Flo-Quat Sanitizer. Data are means ± SE (n = 3), and treatments with various letters differ significantly according to one-way analysis of variance (ANOVA) ($p < 0.05$).

1.2 Effect of <u>plant</u> debris-QAC interactions on efficacy in reducing broomrape seed viability

Methods. The experiment was conducted in a manner consistent with previous trials, involving four steps: sanitation, pre-conditioning, seed viability assessment, and imaging. Notably, in this iteration of the project, soil and plant powder were introduced into the seed sanitation process

to directly assess the impacts of various types of debris on sanitizer efficacy. To introduce soil and plant components into the solution, we employed a fine sieve (63 microns) to sift regular soil collected from a field near Davis. This resulted in particles finer than both broomrape seeds and the filter mesh so that sanitizers and debris could be rinsed away while broomrape seeds were retained and could be evaluated in subsequent germination assays. For the plant debris experiment, leaf and stem material from tomato seedlings was dried at 25°C for four days and subsequently crushed and sifted through the same sieve. The same process was used for actual soil and plant debris collected from various surfaces of fruit transport trailers at a tomato processor and tomato harvesters at the conclusion of the 2023 harvest season. Following the methodology from the previous experiment, varying concentrations of plant powder (2%, 8%, and 16% w/v) were incorporated into 1% v/v solutions of three QAC sanitizers. The outcome of this experiment revealed that plant powder, similar to soil, can neutralize the sanitizer's effectiveness.

We ran another dose-response assessment for soil and plant debris at higher sanitizer concentrations and 1 minute exposure duration. In this experiment, a range of sanitizer concentrations 90, 1, 2, 3, 4, 5, 6 and 8% v/v), of all three sanitizers (Mg4, FQ, and CQT) were subjected to a single low-concentration of soil (10% w/v) or plant (4% w/v) debris. The repeated experiment focused on plant and soil debris collected from the trailer and harvester.

Results. The ED_{50} parameter represents the sanitizer concentration that resulted in a 50% reduction in branched broomrape seed germination (Table 1). This parameter varied between plant and soil but remained relatively consistent between the two plant sources and the two soil powders. Our previous research on QAC sanitizer efficacy in no-debris situations suggested ED50 values ranging from 0.09 to 0.01% v/v. In the current experiments with either plant debris mixed with QAC sanitizers, the ED50 values were in the 3-4% v/v range. When soil debris was introduced, this ED50 parameter increased to 4-6 % v/v (Table 1).

Table 1. Estimated ED50 parameter, the effective dose (% v/v), resulting in 50% reduction in branched broomrape seed germination. Calculated using the three-parameter log-logistic models used to describe the branched broomrape seed germination responses to increasing doses of the three QAC sanitizers.

	Collecting place	Mg4	FQ	CQT
Soil	Field	6.59 (2.01)	4.62 (0.22)	5.66 (1.06)
	Debris from trailer	4.7 (0.49)	5.16 (0.57	6.27 (1.45)
Plant	Field	3.45 (0.12)	3.47 (0.14)	3.54 (0.14)
	Debris from trailer	3.32 (0.13)	3.47 (0.15)	4.46 (0.54)

Values in the parenthesis are standard errors.

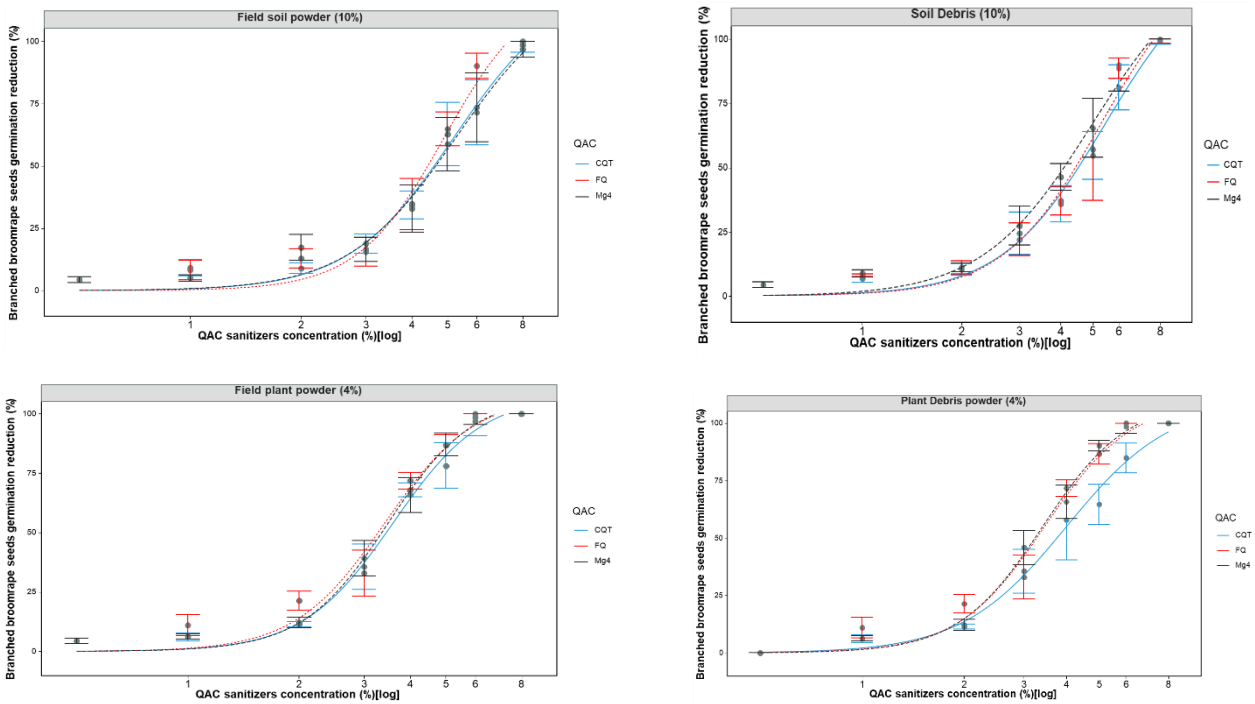

Figure 5. Dose–response curves of branched broomrape seed germination reduction in response to doses of three QAC sanitizers under 1 minute exposure durations. Abbreviation: Mg4: MG4-Quat, FQ: Flo-Quat, and CQT: Cleaner QT-185.

1.3 How do plant debris-QAC interactions influence efficacy in killing Fusarium oxysporum f. sp lycopersici spores?

Methods. Plant debris was dried, ground, then sifted 63-micron sieve. Two different debris concentrations were prepared: 1% w/v (10 mg/ml) and 4% w/v (40 mg/ml). 8% debris had an interaction with the spores even without sanitizer and was removed from further studies. Debris was added to a sterile glass slide and dried before the Fol spore suspension was added. 0% debris, just the spore suspension, was used as the control. Next, either 1% v/v of a commercial QAC or water was added to the slide and allowed to sit for 1 minute before it was neutralized. The neutralized solution was then plated and the resulting colonies were counted 3-5 days later. Percent change was calculated comparing the 0% and 1% QAC for each plant debris concentration. MG 4-Quat, Flo-Quat, and Cleaner QT-185 were the three commercial sanitizers tested.

Results. Plant debris reduced QAC efficacy in all commercial sanitizers (Figure 1). 4% plant debris seemed to have the most impact in the MG 4Quat experiment so we focused on that concentration for Flo-Quat and Cleaner QT-185. Out of the three sanitizers, Flo-Quat appeared to be the most effective against 4% plant debris, with a 72% spore reduction with debris compared to 100% reduction with no debris. MG 4-Quat and Cleaner QT-185 were much less effective with spore reductions of 42.2% and 22.4% respectively. While Flo-Quat may still be somewhat effective against plant debris, it is clear that efficacy is affected by plant matter and

may not be the best sanitizer to use against agricultural equipment with high debris loads. Of note, soil-QAC interactions tests are also underway, with results anticipating in early 2024.

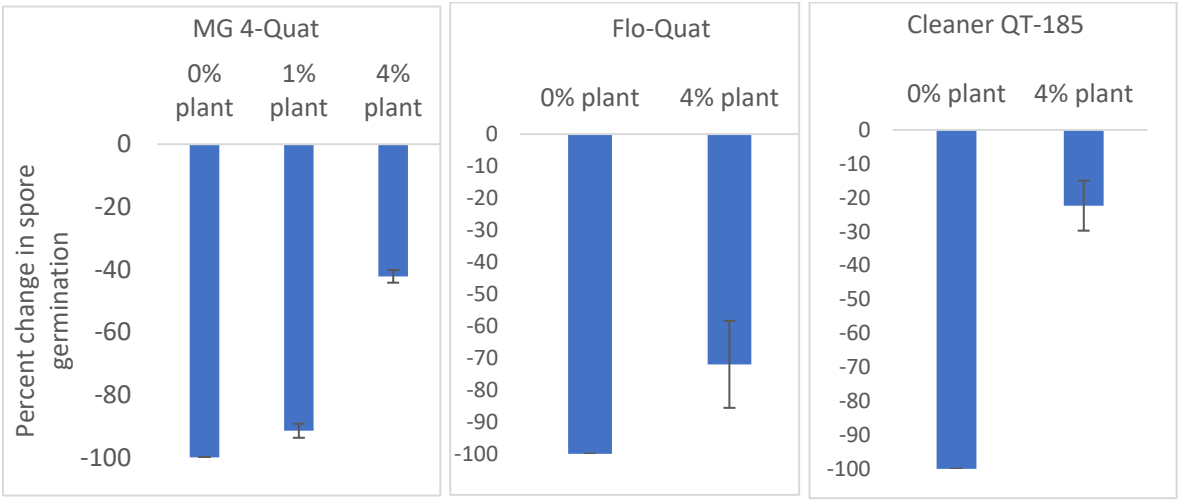

Figure 6. Percent change in Fol spore germination in response to three commercial QACs in the presence of plant debris. Plant debris significantly reduced efficacy of all QACs tested.

1.4 Harvester trials-effect of different endogenous debris loads on sanitizer efficacy (backpack spray)

Methods. We coordinated with three tomato processing operations to determine the effect of soil and plant debris loads on cleaning efficacy. With all collaborators, we rated soil and plant debris loads on various surfaces before and after the operation's cleaning and/or sanitizing procedure. Soil and plant debris loads were rated based on two different numerical scales (1-7 and 1-5 for soil and plant debris, respectively). We also evaluated microbial loads via swabs taken from each surface; diluted swab washes were assayed within two days for total *Fusarium* colonies.

Table 2. Numeric ratings and corresponding soil and plant debris load descriptions

Numeric Rating	Soil debris load	Plant debris load
1	Clean (no obvious soil)	Clean
2	Light dust/soil	Patchy distribution <1 mm
3	Uniform dust w/aggregation <1 mm	Uniform distribution <1 mm
4	Patchy distribution >1 mm	Some areas (but not all) >1 mm
5	Uniform distribution >1 mm, <1 in.	Most of area >1 mm
6	Patchy distribution >1 in.	NA
7	Uniform distribution >1 in.	NA

With two operations, debris loads were rated on six trailer surfaces (outer tire, inner tire, support bar, bucket side, mud flap, and axle) before and after a cleaning/sanitizing step. Operation A cleaned surfaces with hose water and Operation B moved trailers through a drive-thru system that sprayed surfaces with a detergent and sanitizer combination. At Operation C, we rated

debris loads on trailers using a categorial system (Low, Medium, or High) based on the numerical scale used in Operations A and B before and after applying the QAC MG4 (1%).

Table 3. Categorical soil and plant debris load categories and their corresponding original numeric ratings.

Original Numeric Rating	Soil debris load categories	Plant debris load categories
1	Low	Low
2		
3	Medium	Medium
4		
5		High
6	High	NA
7		NA

Results. Overall, at low and medium debris loads, the sanitizer remained moderately effective, with ~90% reduction in propagule loads (data combined). However, at the high debris load treatment, sanitizer efficacy was reduced to 48%, indicating that high debris greater than 1 inch thick can be inhibitory to sanitizer efficacy. Although evaluated in this study, QAC applications at high volume and hiher pressure may be able to partially overcome the high debris load effect by simultaneously reducing debris loads and applying a sanitizer. There was some effect of surface type on the efficacy of the sanitizer. When normalized, sanitizer efficacy on medium levels of debris were similar on both surface types. However, at the higher debris levels it appears that sanitizers were less effective on metal (37% microbe reduction) vs. rubber (50% reduction) surfaces.

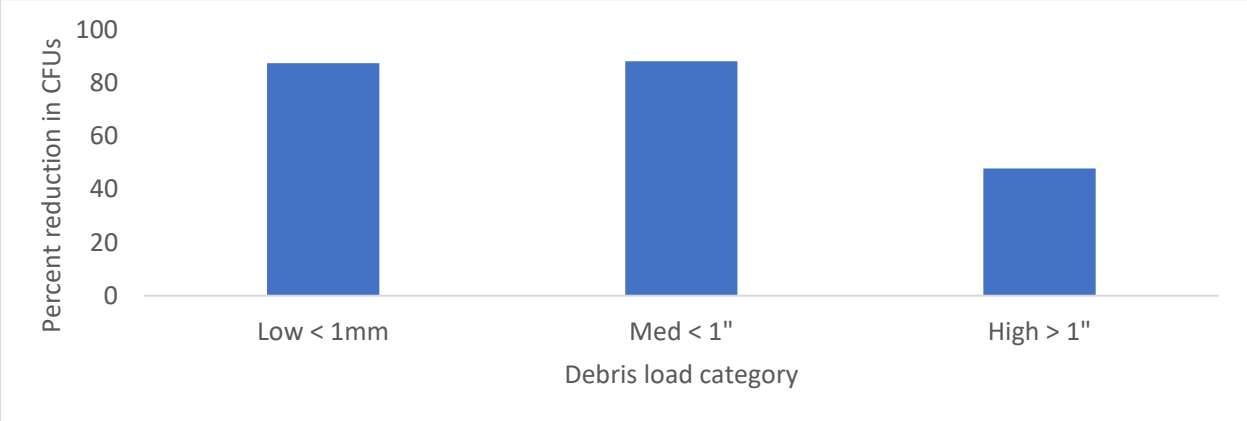

Figure 7. Effect of different debris treatments (low, medium and high) on sanitizer efficacy (percent reduction in propagule loads)

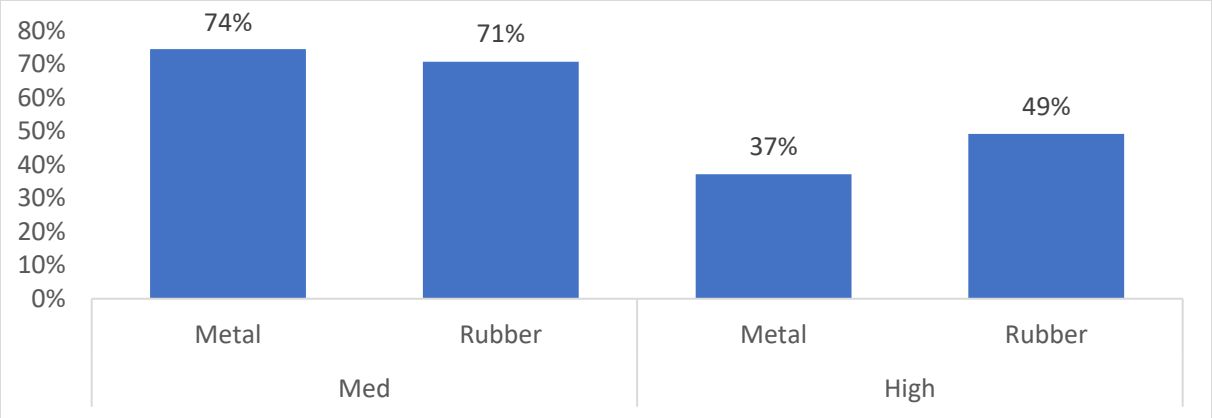

Figure 8. Effect of different surface types on the debris load-QAC interaction at the medium and high treatments (microbe loads too low to detect an effect at the low levels).

Obj 2. Evaluate dose-dependent efficacy of peracetic acid against broomrape seed and Fusarium spores at different exposure durations.

Methods. We assessed the effectiveness of peracetic acid in diminishing the viability of broomrape seeds, employing methodologies consistent with prior research. This investigation encompassed a range of peracetic doses and a 1-minute exposure duration. Specifically, we examined seven concentrations: 0% (utilizing distilled water as the control), 0.05%, 0.1%, 0.2%, 0.5%, 1%, and 2.5% (v/v) in distilled water. The entirety of these treatments was executed at the Contained Research Facility (CRF) at UC Davis. Seed germination data were subjected to a dose-response analysis. A three-parametric log-logistic function best describes the seed germination of branched broomrape in relation to the concentration of chemicals.

Results. Peracetic acid exhibited limited efficacy in reducing the viability of branched broomrape seeds. Even when applied at its highest concentration (2.5%), over 65% of the seeds successfully germinated (Figure 1). The parameter "e" signifies the concentration at which a 50% reduction in germination occurs, and our model predicts this value to be 9.63% for peracetic acid (Table 1). In practical terms, this means that a peracetic acid concentration of 9.63% would render only 50% of broomrape seeds non-viable. Given its relatively low effectiveness, we opted to exclude peracetic acid from further experimentation.

Table 3. Estimated parameter values for the three-parameter log-logistic models used to describe the branched broomrape seed germination responses to a several concertation of peracetic acid over a 1-minute exposure duration.

Sanitizer	Parameter estimates (±SE)			
	b*	u*	e*	RMSE#
Peracetic acid	1.05 (0.54)	87.14 (1.63)	9.63 (7.83)	6.23

‡ Values in the parenthesis are standard errors.

Root mean square error

*In the model, b represents the steepness of the inflection point, u is the upper limit, i.e., maximum seed germination when the dose of the ammonium compound is zero, and e is the dose that produces a germination response half the u value.

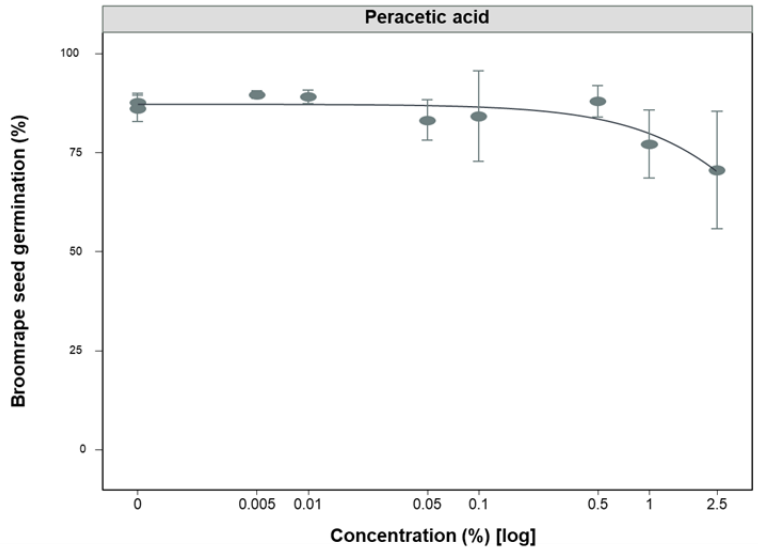

Figure 9. Dose–responses curves of branched broomrape seed germination in response to doses of peracetic acid. A three-parameter logistic model (Eq. 1) was used fitting the model to the data. Lines are fitted values, and solid circles indicate observed germination averaged (n=3). Error bars indicate 95% confidence intervals. Model parameter estimates are shown in Table 1.

Obj 3. **After-season harvester sanitation efficacy survey and consultant service.** *Progress*. We have identified two collaborating growers and coordination efforts are underway; this study will be conducted in spring 2024 (no cost extension).

Obj 4. **Outreach**

We updated the harvester sanitation BMP in winter 2023, with another revision planned for winter 2024 that includes information from the studies discussed above and related CDFA-funded projects. We held a dual English-Spanish outreach meeting in spring (June 22, 2023; with 30 attendees), with special guest speaker Dave Viguie. We provided English and Spanish version of the BMPs and broomrape identification guides at this meeting, and have disseminated via the Swett lab website and CTRI newsletters; we assisted Patricia Lazicki in edits for her field day synopsis for the regional Yolo County ANR newsletter. Further, we have met with three collaborating canneries and two collaborating growers to provide evaluation-based consultations of equipment cleaning methods, with planned follow up consultations = in winter.

As a result of this work, there are reported increased use of field equipment cleaning by growers and canneries for broomrape as well as other soil-borne pests both in the Sacramento Valley and nearby San Joaquin County. Consultations have indicated inconsistency in use of sanitizers as part of cleaning procedures, which is likely limiting efficacy of existing methods in mitigating broomrape spread.

Task	Deliverable	Status
Update BMPs	We plan to update BMPS in winter 2023 and again in fall 2023, integrating additional information from laboratory and field studies	Yes-v 1.2
	Fall BMP revisions aim to include debris load reduction threshold information, information on new products, as well as a new section on off-season equipment cleaning.	In prep
Offer an outreach meeting in the field for operators	English-based outreach event targeting operators, consultants, and regulators, to provide updates on BMPs and get input on challenges.	Hosted June 22, 2023. 30 participants, including 10 as part of Spanish translated session
	Provide printed guides for broomrape identification and Sanitation BMPs (English + Spanish)	Included
	Outreach article published summarizing field day topics (Author: P. Lazicki)	Published online July 2023

Discussion:
This project builds on previous funded by CTRI and CLFP and addresses several key questions related to managing and reducing the spread of these economically damaging pests of processing tomato in California, emphasizing branched broomrape management. In line with CTRI priorities, this project emphasizes control of broomrape spread, while also leveraging this work to provide solutions to the high priority pathogens Fusarium wilt race 3 and *F. falciforme* (priority 1). This project specifically addresses **priority 6**, The development and extension of sanitation best management practices that can be widely adopted and will minimize the transmission of Broomrape, other weed seeds, and soil borne pathogens between fields via equipment. Metrics of success here will be: efficacy, time, and cost.

As there has never been a concerted effort to develop best equipment sanitation practices for soil borne pest communities in any California crop (to our knowledge), and no recent studies for broomrape sanitation, we anticipate that this project will have high impact on tomato growers. Based on PI needs assessments, there is at present no single more critical pest management gap in California agriculture.

Specific outcomes and benefits are

- Growers and canneries have access to recommendations and consultation support for effective in-season and off-season harvester sanitation, which can also be applied to other equipment to minimize risk of spread.
- These methods also work to reduce spread of soil borne pathogens. This includes Fusarium wilt, which is at high risk of resistance breaking—equipment sanitation will provide the only means to prevent spread of a new race while new resistant cultivars are being developed.

- BMPs are improved upon, providing new information on a wider range of sanitizer options, specifically peracetic acid, which is certified for organic use.
- BMPs are improved upon, providing new information on debris management targets.
- Researchers learn about barriers to effective sanitation as well as innovations developed by commercial processing operations, which could provide critical means to overcoming cleaning time and other challenges.

What's next for this project

- As QACs are only moderately effective in the high debris load environment of field equipment, identification of other effective products that are less debris sensitive would help improve existing BMPS. Thus in 2024 we plan to evaluate efficacy of a wider range of products against broomrape and efficacy in the presence of debris.
- In addition to broomrape, there are cases where sanitation is needed for other soil borne pests, and there may be sanitizers less effective against broomrape which are highly effective against other pathogens. Thus in 2024, we plan to develop a table summarizing the efficacy of a wider range of sanitizers against the pathogens causing Fusarium wilt, and southern blight, bacterial canker.
- Studies are needed to expand risk assessment analyses to a wider range of field equipment to more cohesively address and manage broomrape spread within the agricultural system.
- Effects of foam treatment on sanitizer efficacy in reducing propagule loads requires further study. Studies of QAC applications at high volume and pressure are also needed to determine if we can overcome QAC inhibition at high debris loads.
- Active distribution of BMPs and continued outreach, together with innovations on the side of industry, are needed to help improve harvester sanitation practices. In 2024, this includes releasing videos on a new YouTube channel.

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.

This project as leverage for other dollars:

In 2021 we were able to leverage CTRI support to get matching funding from CLFP. This project is providing the baseline data and collaborator base that was previously identified as necessary for proposal submission to state and federal agencies (identified via past proposal efforts). We are also leveraging this data for a new CLFP proposal for 2024/25 as well as a proposal to the NIFA-Methyl Bromide Transitions Program.

POPULATION STRUCTURE AND INVASION HISTORY OF *PHELIPHANCHE RAMOSA* FIELD DETECTIONS IN CALIFORNIA

Project Leader and any Co-PIs: Adam Schneider, Ph.D., Assistant Professor, University of Wisconsin- La Crosse,

Year of Project Initiation: 2022

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 used a population genetic approach using 13 regions of the genome called “microsatellites” that commonly show variation within and between populations of the same species. Previous research on European populations of this species had identified a set of 20 gene regions, but only 13 were found to be suitable for the Californian samples. A total of 76 samples, which could be verified to have come from separate plants and passed all data quality checks, come 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 2022 (“Historical Samples”). Genetic data was then analyzed in five 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.

In summary, these analyses show no clear signatures of multiple origins. Therefore, the most likely explanation for the multiple *Phelipanche* outbreaks over time is the persistence of seeds. However, these patterns could also be due to the signal in the data being undetectably weak(e.g., repeated origins from a very similar genetic pool). A key limitation to this study -- but also opportunity for future research--is the lack of comparable genetic data from tomato-parasitizing populations outside of California. To what extent do California branched broomrape strains differ from non-Californian strains that parasitize tomato, and can this provide further insight into the invasion biology of this species?

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 from high-quality producers and not sourced from infected areas.

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 1950. 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.

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 *Goal* and the *Objectives* under that goal:

Goal:

- 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.

Objectives/Timeline:

1. Survey California herbaria for historical collections of *P. ramosa*
2. Extract DNA from contemporary and herbarium specimens
3. Validate genetic loci and published amplification protocol
4. Amplify genetic regions of interest
5. Data analysis.

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 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 *(2022: Yolo Co.)*	Site 01	Private Farm 1	8	2.53	0.33 / 0.40	0.28
	Site 02	Experimental Farm	19	2.86	0.25 / 0.53	0.54
	Site 09	Private Farm 2	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 Juaquin 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 2-4)*

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.

Data Analysis and Results *(Objective 5)*

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 form ("SeedCo2022", 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.

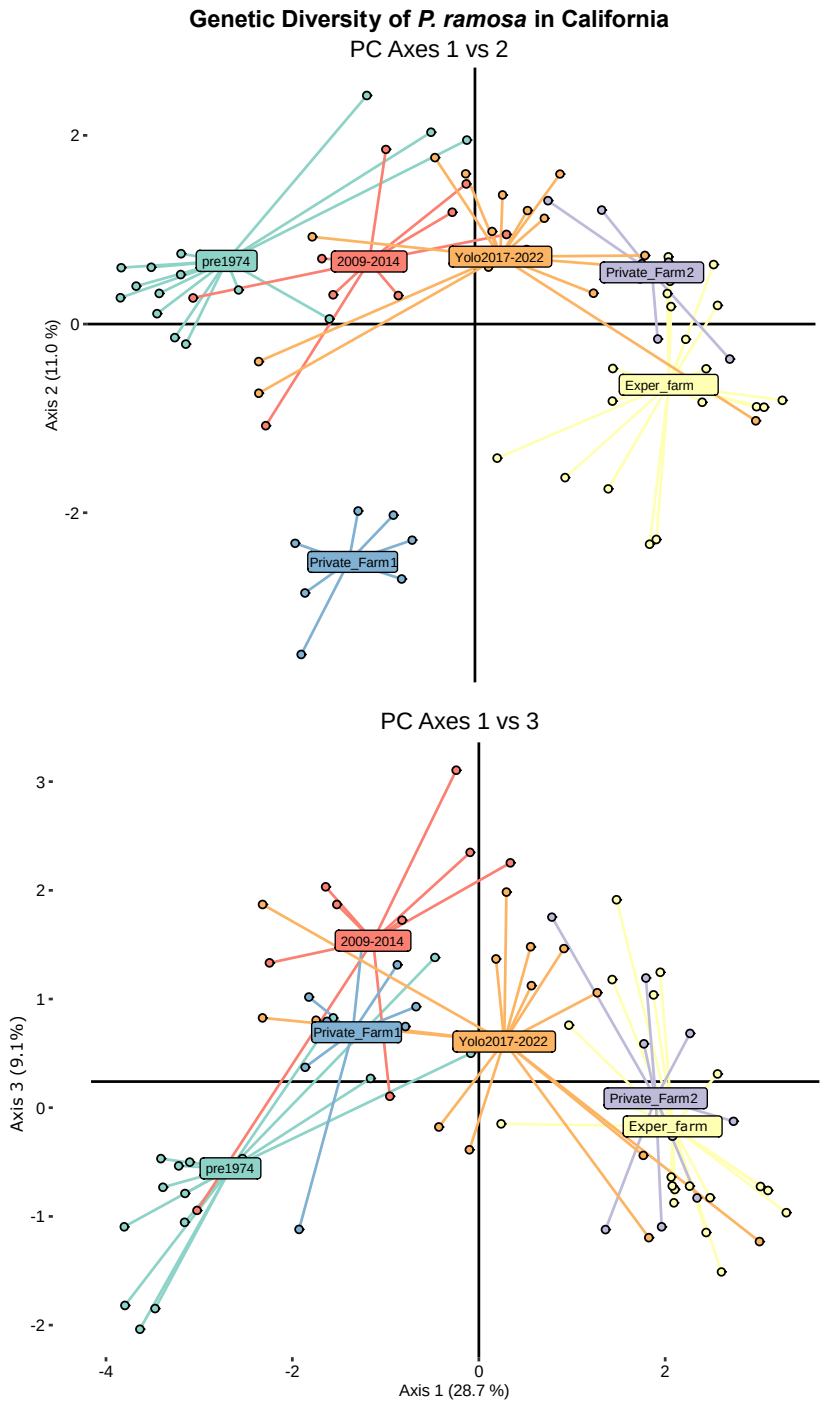

Figure 1. Principal Component Analysis showing genetic variation of Californian samples of branched broomrape, *Phelipanche ramosa*. Plots show the first two ordination axes[1] (top) and first and third axis (bottom), collectively representing nearly 50% of the genetic variation. Points represent individual 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).

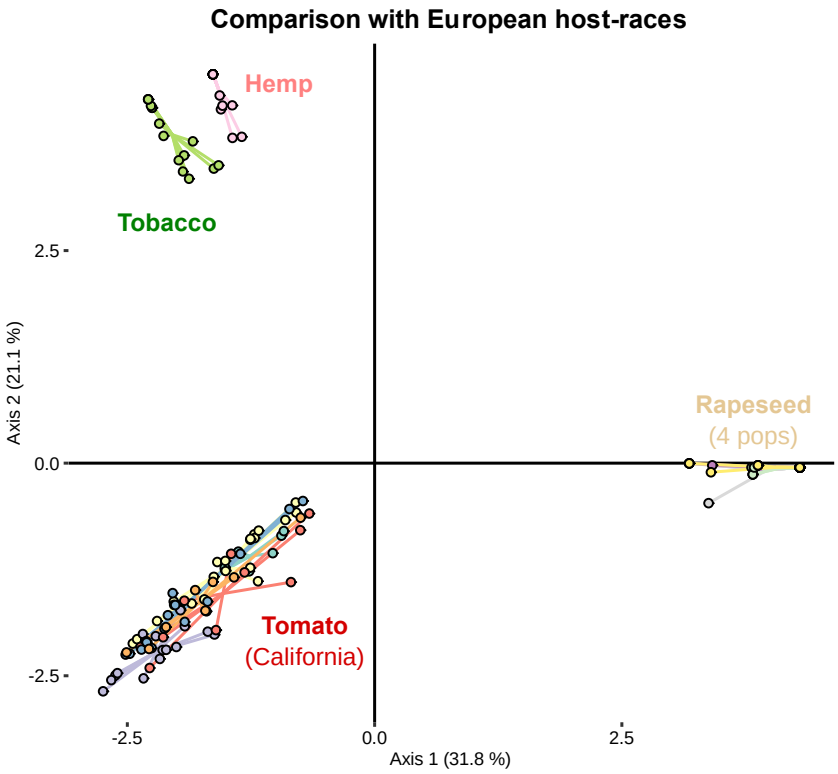

Figure 2. Principal component analysis comparing genetic diversity of tomato-parasitizing branched broomrape in California with six populations parasitizing other hosts. Data from tobacco, hemp, and rapeseed from Le Corre et al. 2014.

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.

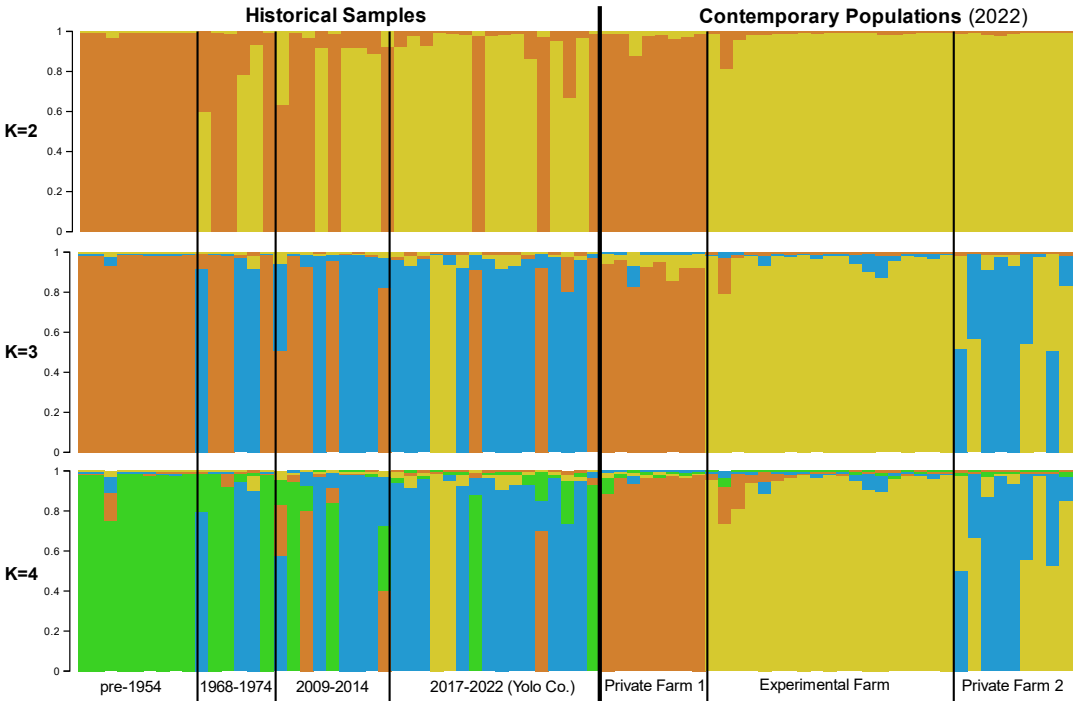

Figure 3. Population structure analysis of Californian branched broomrape for a pre-determined number of subgroupings (K=2, 3, or 4). Each column represents one of 76 genotypes ordered by either time-bin (Historical Samples), or by sampling location (Contemporary Populations), and colored based on the 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.

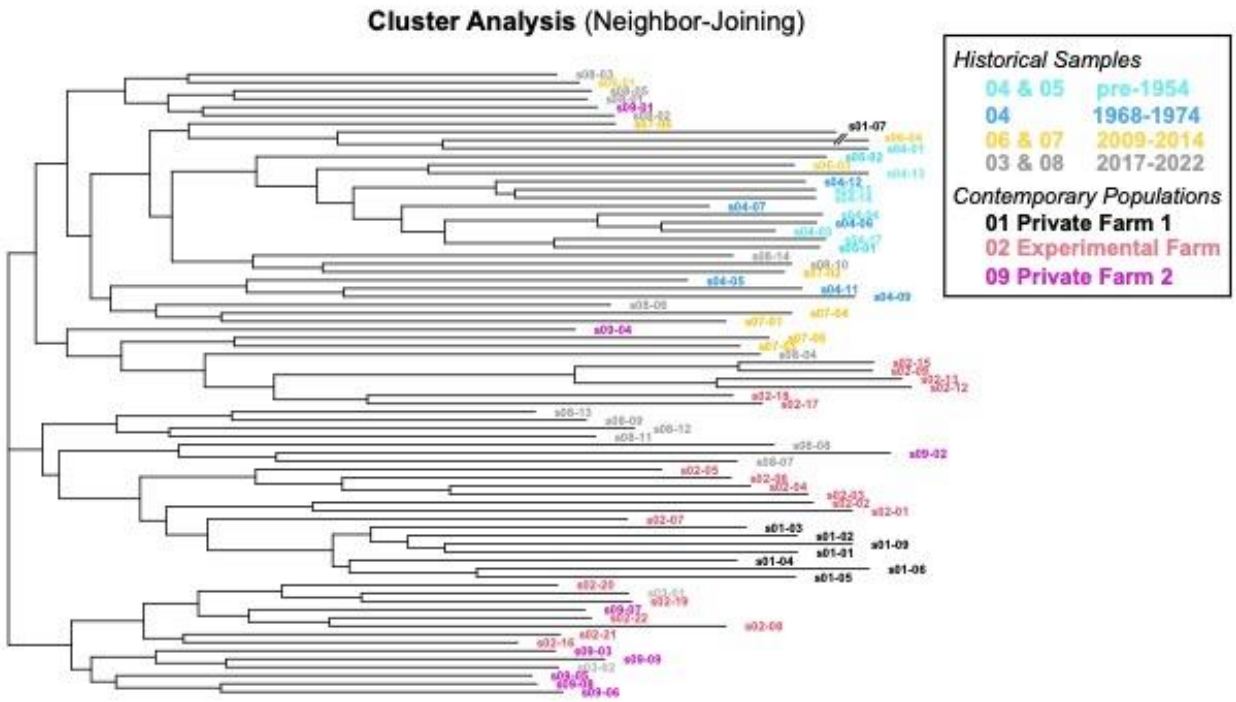

Figure 4. Unrooted neighbor-joining tree showing genetic similarity of 76 branched broomrape samples from California. Each sample is color-coded based on the groupings in Table 2, which mostly corresponds to the sample number. Branch length of sample s06-04 reduced for compactness.

Discussion:

The central question of this research is: "**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 (Fig. 1-4) supports a close relationship among all branched broomrape reported in California. **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). By contrast, changes in genetic profile of samples over time seem to be more consistent with gradual change, perhaps through genetic drift (Fig. 1). However, the alternative hypothesis of multiple origins cannot be completely excluded, and additional sampling or data analysis may be warranted.

European populations of branched broomrape, including those parasitic on tomato, are reported to be exceptionally inbred, meaning that individual populations are more genetically uniform (Stojanova et al. 2019). Moreover, tomato-parasitizing plants comprise at least two large genetic groups globally (Stojanova et al. 2019), so if introductions had come from both of these groups, it should be detected.

If a single introduction, is it reasonable for branched broomrape seed to persist for over 40 years in California? 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.

Limitations/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.

Other limitations of this study can be overcome by future research. Additional sampling from tomato-parasitizing populations outside of California would help contextualize the variation found within the state. For example, showing populations outside of California are more similar to some Californian samples than others would provide strong evidence of a more complex population history. While obtaining the data from La Corre et al. (2014) for use in Figure 2, the authors alerted me of a much more expanded study, to be published soon, that could provide additional necessary European data along with the data from Stojanova et al. (2019). As far as I know, South American populations of *P. ramosa* have not yet been studied.

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.

Acknowledgements: Thanks to the private landowners who allowed sampling from their property for the contemporary populations, and to Alison Colwell (UCD) and Genevieve Walden (CDFA) who provided access to herbarium specimens and permission for destructive sampling. Zach Bagley (CTRI) 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 one-year $8646 grant from the University of Wisconsin-La Crosse to compare the *Phelipanche ramosa* in California with broomrape populations in Texas that have been called *P. ramosa*, but may actually be the close relative *Phelpanche nana. P. nana* is not officially reported from the United States but is also a potential agricultural threat. This research leverages the *P. ramosa* collections collected in this project and will compare them with other invasive broomrape elsewhere in the United States.

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Developing Tomato Lines Resistant to Branched Broomrape, a Critical California Pest

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Year of Project Initiation: 2022

Executive Summary:
Branched broomrape has recently re-emerged in tomato fields across several counties in California's Central Valley. These fields are pivotal to the state's agricultural economy, as California was responsible for producing over 90% of the 12 million tons of tomatoes in the United States in 2018. The challenges associated with eradicating broomrape emphasize the urgent need for better understanding of its biology and parasitic behavior for the development of broomrape-resistant tomato varieties. An urgent research gap exists on how tomato cellularly and molecularly responds to the broomrape. In the past two years, our efforts funded by CTRI, have enabled us to establish robust assays aimed at addressing this critical knowledge gap while laying the foundation for the development of an effective strategy for protecting California's vital processing tomato industry. We have developed and optimized protocols for co-cultivating commercial tomato lines with broomrape in multiple systems. These methods allow for generating robust gene expression data sets as well as overtime monitoring of broomrape parasitism on tomato roots which was not possible with the traditional soil-based infection assays.

i) We developed protocols for co-cultivating commercial tomato lines with broomrape in a soil-based rhizotron system located at the UC Davis Contained Research Facility (CRF) and conducted transcriptome profiling. These proof-of-principle experiments demonstrate that tomato does indeed change gene expression in response to branched broomrape attachment. This data set was reduced in scope due to the space limitations introduced by working in large rhizotrons within a very limited space available to our group in the CRF. In parallel, to better understand the intricate interactions occurring during broomrape parasitism within its natural environment, we further collected tomato root tissue that was infested with branched broomrape from a field in the Yolo County for gene expression profiling.

ii) We developed new co-cultivation methods aimed at minimizing space requirements and maximizing screening of multiple tomato cultivars with adequate replication. Now we can robustly identify genes expressed at the earliest point of infection, which will be the best targets for editing for resistance. We also established a plate-based *in vitro* system to screen tomato hairy root mutants for infection. With this system we can identify genes that control resistance within 3 months after introduction of the edited constructs. This work, as well as our interconnection with other teams on the UC Davis campus was recently highlighted in: https://www.ucdavis.edu/food/news/parasitic-weeds-threaten-tomato-plants-on-california-farms

iii) We will leverage our established protocols to finalize transcriptome profiling of tomato commercial lines to identify a maximum of 5 candidate gene for CRISPR mutagenesis in tomato hairy roots, followed by screening of these lines for parasite resistance. These results will form the foundation for the development of stable transgenic tomato lines that are resistant to broomrape infestation.

Introduction:

The California processing tomato industry, a critical component of the state's agricultural economy is currently facing a threat from the parasitic plant branched broomrape (*Phelipanche ramosa*). This parasitic species poses a challenge due to its ability to attach to the roots of tomato plants, extract essential nutrients, and thus significantly reduces crop yields. The gravity of this issue extends beyond the immediate economic losses and would have long term implications for tomato industry if not dealt with promptly. The majority of global efforts has traditionally focused on chemical treatments with less attention to molecular and genetic strategies to combat broomrape. Despite advancements in chemical strategies, the industry continues to experience substantial losses, highlighting a clear need for other sustainable solutions. Our team has focused on advancing our understanding of the host-parasite interaction at a genetic and molecular level, a relatively under-researched field in the battle against broomrape. By developing and refining novel co-cultivation assays, transcriptome profiling and employing cutting-edge CRISPR-Cas9 technology, our molecular strategy aims to uncover the genetic basis of broomrape resistance to reduce reliance on chemical control methods. This approach will also offer an alternative, sustainable and long-term solution to broomrape threat by genetically engineering resistant tomato cultivars.

The main *Goal*s and *Objectives* of project:

1- Characterization of gene expression changes in tomato roots from four different commercially available cultivars in response to *Phelipanche* infestation
2- Identification of genes (minimum 5, maximum 10) that are central to the parasitism response based on gene clustering and network-based analyses
3- Field-based confirmation of the genes identified
4- CRISPR mutagenesis of five genes using hairy root transformation and testing these hairy roots to identify lines demonstrating resistance to branched broomrape
5- Production of stable transgenic *Phelipanche*-resistant tomato lines with CRISPR mutagenesis in identified genes

Methodology and Results:

1. The Controlled Research Facility (CRF) is the only place on the West Coast of the USA where we can grow branched broomrape. We initially used large pot-based soil rhizotrons for co-cultivation and gene expression analysis in early and late broomrape attachments to tomato roots. However, the limited space available to our group in the CRF made setting up experiments in these rhizotrons challenging, as they have a large footprint and could not be used effectively to set up multiple biological replicates for our experiments (Figure 1A). Based on experiments in these rhizotrons we obtained preliminary RNAseq data that showed one Heinz cultivar (H9553 – which also showed resistance to Cuscuta (Jhu et al., 2022) had the most significant differentially expressed genes which are candidates for parasite resistance. One of the most differentially expressed genes encodes a pectin-acetyltransferase (*Solyc08g014380*) and taken along with other genes such as a non-specific lipid transfer protein (*Solyc10g075110*), extracellular

chitinase (*Solyc02g082930*), pectin lyase superfamily gene (*Solyc05g005170*), a pectin esterase

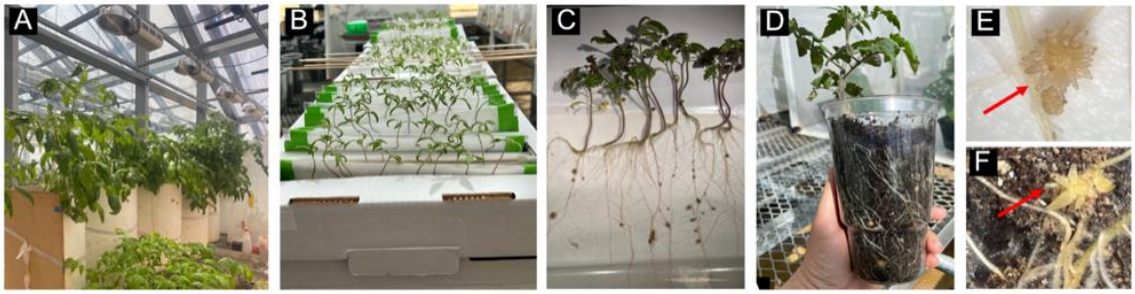

Figure 1. Tomato branched broomrape co-cultivation in (A) Large pot rhizotrons; (B, C) Germination bags; (D) Small container (E, F) Parasite attachments in bags (E) and containers (F). Red arrows point to parasite attachments.

(*Solyc04g080530*), and laccases (*Solyc05g052360* and *Solyc05g052370*), we hypothesize that cell wall remodeling is a key part of the parasitism process. However, we were concerned about the robustness of this data due to low replication forced by space constraints. To address these, we spent considerable effort to develop a new co-cultivation method in germination bags. This system requires much less space and multiple bags/plants can be assayed with a very small footprint. We now have successfully used this to induce broomrape infestation of tomato roots (Figure1B,C,E). Our germination bag system is allowing us to do large, replicated experiments and test multiple stages of broomrape infestations to get robust gene expression data. Using this system, we have completed the RNA-Seq library preparation for both control and infected samples across four commercially available tomato varieties. We are currently in the RNA-Seq data analysis phase, with the objective of identifying candidate target genes for subsequent CRISPR mutagenesis.

In collaboration with the Hanson lab, we have also developed a small, clear container tomato-broomrape interaction assay system for soil-based assays (Figure 1D,F).

We have also utilized the germination bag system to quantify the rate of broomrape attachments in the 4 commercial tomato lines. Briefly, 15 plants from each cultivar were germinated and subsequently transferred into 3 germination bags. Plants were grown in the bags while supplied with modified Hoagland solution for an extra 10 days to develop at least 2 true leaves. At this stage, each bag was opened and each plant was inoculated with 50 pre-conditioned broomrape seeds. Plants were co-cultivated with broomrape in the bags in the CRF greenhouse with their roots covered. Plants were monitored over time (~10 days) for broomrape tubercle formation on the tomato roots as a sign of parasite attachment. Our results show different attachment rates in different cultivars. Heinz cultivar (H9775) – had the least number of attachments compared to other genotypes with a statistically significant difference compared to HM-885 (Figure 1G).

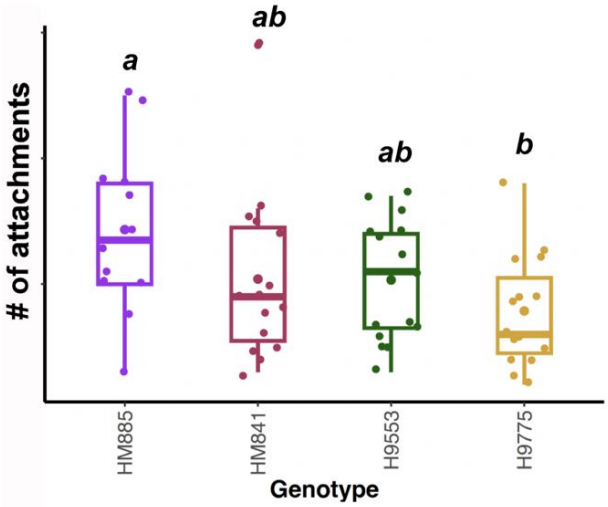

Figure 1G. Quantification of parasitic attachments, represented by the number of tubercles formed, across four tomato cultivars. Statistical analysis was conducted using a one-way ANOVA with post-hoc Tukey HSD test to determine significant differences between cultivars. Distinct lindicate groups that are statistically significantly different from each other (n=15).

2. We expect candidate gene identification to be finalized shortly after the RNA-seq data analysis is completed (end of January 2024).

3. Since field samples are precious, we have held off on analyzing these until we have robust gene expression data.

4. Tomato hairy root transformation (Ron et al., 2013) will provide an efficient way to test whether mutations of our candidate genes confer resistance to branched broomrape. In order to test identified genes for conferring resistance to branched broomrape in CRISPR mutagenized hairy roots, we required a robust assay method. However, broomrape seedlings and tomato hairy root cultures cannot be grown together on the same medium as broomrape requires a low nutritive medium to germinate and be infectious, while hairy roots require rich media. To allow roots in culture to be infected by broomrape seedlings, we have recently developed an in vitro split-plate method that combines two media: one rich medium that supports the hairy root culture, and the other a non-nutritive medium that provides favorable environment for broomrape germination and infection (Figure 2). Hairy roots supported by the rich medium grow into the second low nutrient medium, where they stimulate broomrape seed germination and are infected by the emerging broomrape seedlings. This system has enabled us to successfully identify infection of hairy roots by broomrape on plates (Figure 2A-C). We have also used these attachments to study more closely the attachment points between broomrape and tomato roots and to determine if vascular connections are made between the parasite and host (a sign of successful parasitism, Figure 2 D,E). In addition, since cell wall barriers could be one way to prevent parasite haustorium penetration, these methods will allow us to assay the contact points between host and parasite for presence and nature of such barriers. To investigate the role of lignin in resistance to broomrape, we utilized a mutant tomato hairy root line with increased lignification in the cortical cell layers, developed by the Brady group (Figure 3A). Co-cultivation of these mutant hairy root lines with pre-conditioned broomrape seeds was conducted using our established split-plate method. Plates were monitored for tubercle formation/attachments. Attachments were harvested and subsequently sectioned and stained with basic Fuchsin for presence of lignin. Our observations suggest that the mutant lines display an increased lignin deposition at the points of broomrape attachment (Figure 3B,C,D) compared to control lines without broomrape infection. Interestingly, the deposition of additional lignin in cells near and around the attachment site appears to inhibit the characteristic xylem bridge (red arrow heads in Figure 2E) formation between host and parasite, suggesting that excess lignin disrupts the establishment of vascular

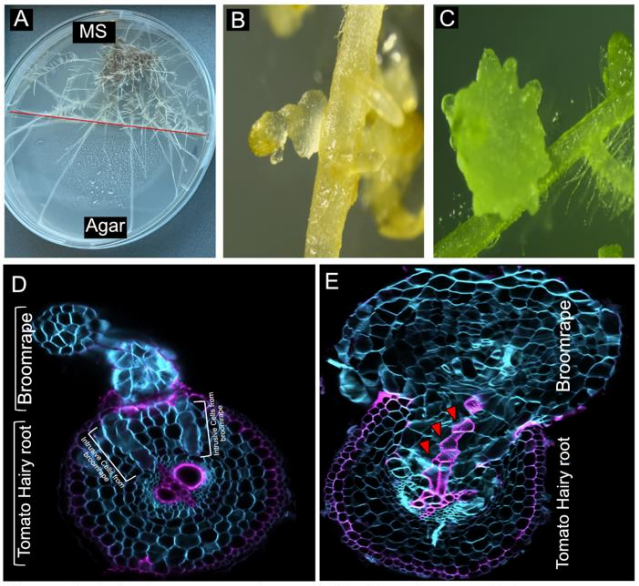

Figure 2. Split-plate system for co-cultivation of tomato hairy roots and broomrape. (A) Plate with divider containing both medium (B) Tubercle formation in an early attachment (D) late attachment in hairy roots. (D,E) Cross section of tomato hairy roots with attachments; stained with DY-96 a general cell wall stain (cyan) and Fuchsin (pink) for lignin. An early attachment indicating intrusive cells from haustorium invading host cortex cells (D) A More developed attachment with established xylem bridge and vascular connection between the host and parasite (Red arrows).

connection. This is particularly evident in more mature attachments where there is a lack of fuchsin staining where a typical xylem bridge is expected.

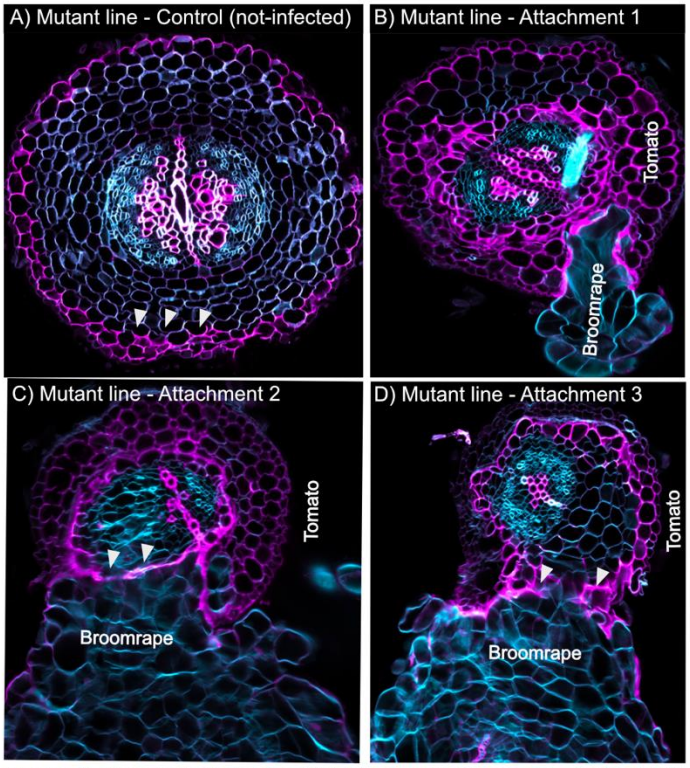

Figure 3. Tomato hairy root mutant lines corss sections - stained with Basic Fischsin for lignin (pink) and Direct-yellow 96 (general cell wall stain - magenta). Extra lignin deposition in multiple cell layers in B,C and D upon broomrape infection. Arrow heads indicate extra lignin in the mutant lines in the cortex cells (A) and close to attachment site in B,C and D.

Acknowledgements: We thank HM Clause for providing us with some grower preferred lines that we are using in our analyses.

This project as leverage for other dollars: With data generated from this CTRI funded project we have a commitment from CDFA to provide us with funds for the next 1.5 years. We are working with CDFA to get approvals and obtain funding in the amount of ~$150,000.

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Inducible Suberin for Tomato Drought Tolerance

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- 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

Year of Project Initiation: 2023 (for Year 2)

Executive Summary:
Water availability is a pressing concern in the State of California, and expected to further worsen due to climate change in the near future. New strategies and tools are required in order to maintain the yield of processing tomatoes in California, which accounts for 90% of national tomato production. As part of the CTRI long-term strategic planning, our project investigates the effect of enhanced suberin deposition in tomatoes as a strategy to improve yield in adverse environmental conditions. Suberin is a biopolymer that accumulates in the tomato root exodermis and whose deposition is correlated with drought tolerance and resistance to a number of pathogens.

As part of our initial research proposal (Year 1, 2022), we screened HM Clause commercial germplasm. We identified commercially relevant lines with increased suberin content. We also generated transgene constructs for targeted increased suberin deposition taking advantage of newly identified transcriptional regulators of suberin deposition within the tomato root exodermis (Canto-Pastor et al. 2024, Nature Plants). In Year 2 (2023), we generated the transgenic lines in the standard laboratory cultivar (M82) and HM Clause relevant processing lines. Second generation (T1) lines have been genotyped. We have phenotyped the M82 transgenic lines and have validated an increase in suberin deposition in the root. Phenotyping of the HM Clause transgenic lines is currently on-going. Our current goal is to quantify the degree to which increased suberization is able to drive drought tolerance in both the greenhouse and the field as well as generate hybrids with the HM Clause-modified germplasm. Fruit yield will be directly quantified according to processing industry standards. If increased suberin is able to drive gains in yield under these conditions, then we will work with HM Clause to use available variation in breeding populations or by CRISPR-based gene editing to fix this trait for commercial purposes. Overall, we accomplished the expected goals for the first and second year, and are in good standing to complete the initial proposed work for the next year.

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* impregnation of a 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, Cantó-Pastor 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., 2024), 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 research from our group, to parasitic plant (*Striga hermonthica*) infection of *Sorghum bicolor* (Kawa et al., 2022). Finally, suberin is also linked to higher shelf-life of agriculture products including tubers (Boher et al. 2013) and tomato fruits (Lara, Heredia, and Domínguez 2019) *via* its related biopolymer cutin which is produced by much of the same biosynthetic pathway. ***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 eleven transcriptional regulators of suberin biosynthesis (unpublished). Seven of these transcription factors are also osmotic stress-inducible regulators of suberin biosynthesis, one of these is used in the research activities described here.

Our proposal focused 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 applied our knowledge gained from basic research at the UC Davis on genes that regulate suberin production to generate lines with increased suberin production and we are trying to determine the degree to which they can confer resilience to water stress, 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.***

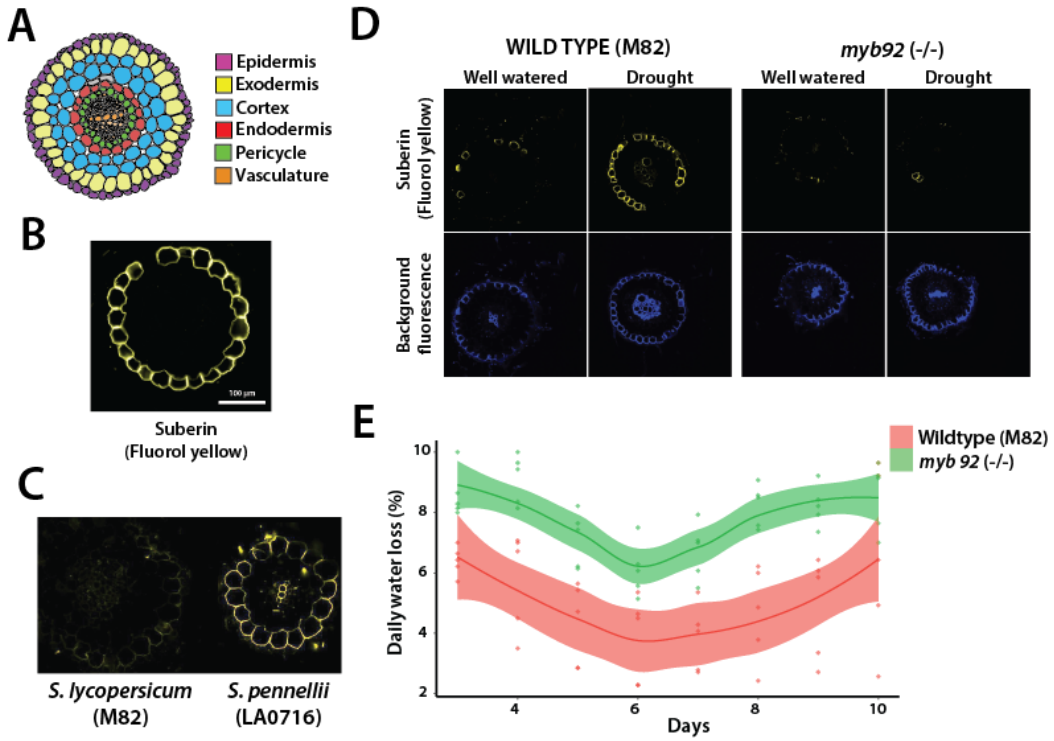

Figure 1: The suberized exodermis in the tomato root and its transcriptional regulation. (A) Root cell type organization in tomato roots. **(B)** Cross-section of tomato roots stained with fluorol yellow. Yellow = suberin deposition in the exodermis. **(C)** Comparative deposition of suberin under normal 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.

The main *Goal* and *Objectives* of the funded project:

Main Goal: *Increase suberin production in roots to enhance drought tolerance in tomato* (Fig. 2A)
Aim 1. *Generate exodermal and drought-inducible tomato with targeted increased suberin production.*
Aim 2. *Determine the influence of increased root suberin on tomato plant growth and yield in control and water deficit conditions.*

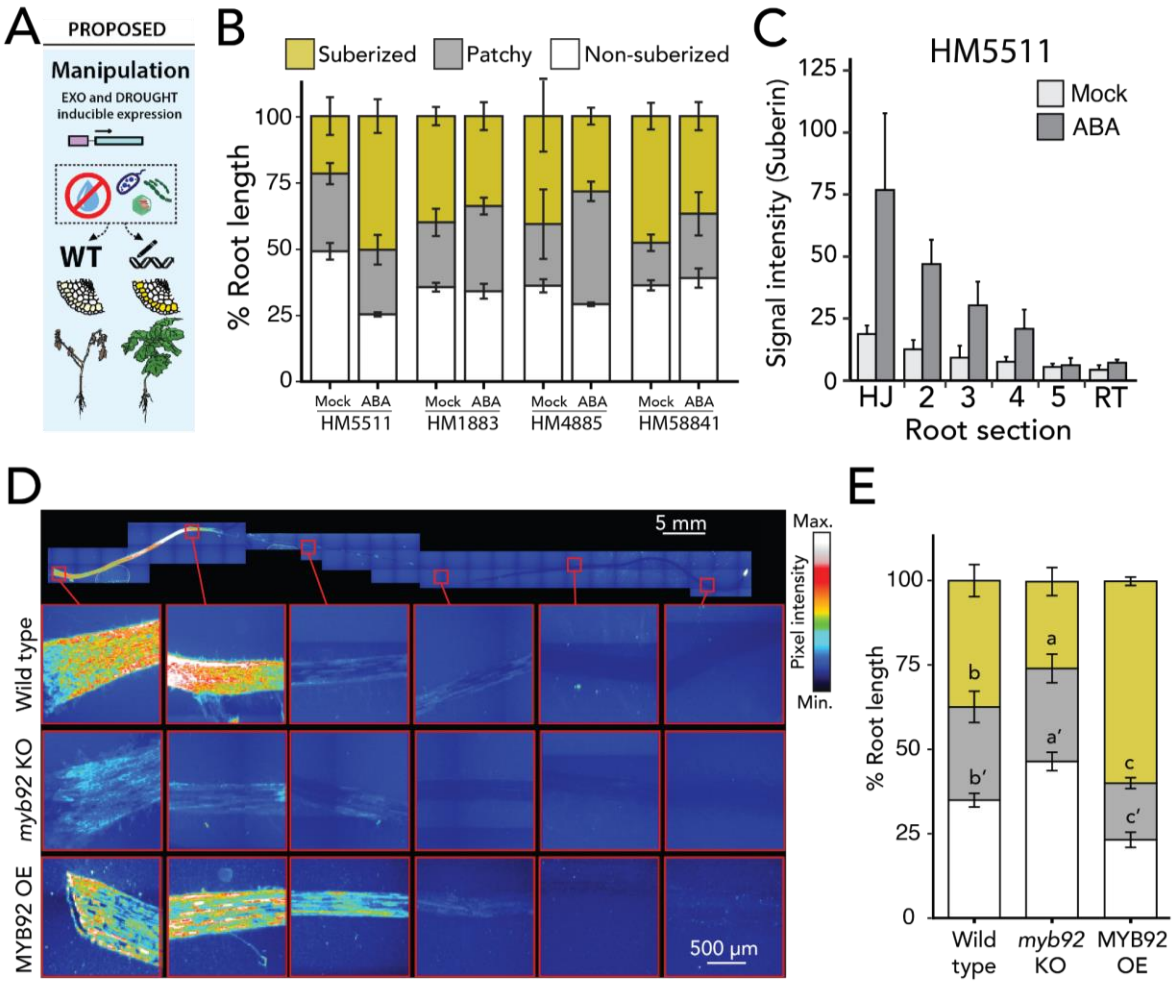

Figure 2. Progress summary of the proposal from Years 1 and 2. YEAR 1: (A) Graphical overview of the project goals; (B) Developmental stages of suberin deposition of HM Clause lines treated with mock or 1 µM ABA for 48 h. Zones were classified as non-suberized (white), patchy suberized (gray) and continuously 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. YEAR 2: (D) FY staining for suberin in 7-day-old wild-type, *slmyb92* Knock-Out (KO) mutant and 35S:MYB92 Over Expressor (OE) line. Whole-mount staining of primary roots across different sections (bottom). (E) Developmental stages of suberin deposition from these lines. Letters indicate significant differences (one-way ANOVA followed by a Tukey HSD test, adj p-value < 0.05).

Methodology and Results: Note the progress on your goals and objectives to date against the benchmarks that were laid out in your approved proposal, objective by objective.

Aim 1. Generate exodermal and drought-inducible tomato with targeted increased suberin production

1a. Test variation in suberin content in commercial germplasm. **COMPLETED in Y1.**

We tested the suberin drought inducibility of the HM Clause varieties to assess the ideal lines to be transformed. Roots were stained with fluorol yellow and quantified using the EVOS instrument available in the UC Davis Genome and Biomedical Sciences Facility (Figure 1B&C). Additionally, roots were cross-sectioned using a Vibratome and stained with basic fuchsin to determine differences in lignin deposition (Figure 1D). Overall, line HM5511 was found to have a moderate level of suberin compared to the other lines, and it was the most inducible line in suberin deposition under ABA stress (as a proxy for drought) (Figure 1B). 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.

1b. Generate tomato lines with increased suberin within the exodermis and upon water deficit. **COMPLETED in Y2.**

Since processing tomato varieties are hybrids, and given the results in Aim 1A, we chose to generate additional suberin overproducer lines 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**). In order to more precisely control the levels of suberin, we generated two different lines – (i) *ASFTpro*:MYB92 (which should increase suberin only in drought conditions), and (ii) *RAB18pro*:MYB92 (which should increase suberin only in drought conditions. The cloned promoters drive the expression of SlMYB92 (Solyc05g051550), a transcription factor necessary for suberin synthesis in tomato under control and drought-stress conditions (Canto-Pastor et al., 2024). All constructs (ASFTpro:SlMYB92, RAB18Apro:SlMYB92, and 35Spro:SlMYB92) were then introduced in *Agrobacterium tumefaciens* EHA105 for stable transformation. Our choice of which commercial variety to transform these constructs into was dependent upon conversations with HM Clause, which were not completed until July 2022. Therefore, in the meantime, we generated 35S:MYB92 lines with the UC Davis Transformation Facility in M82 as a proof of principle. These first T0 plants were received starting January of 2023. Since then, we have genotyped and progressed these lines to T1, and have obtained T2 seeds. We have demonstrated that the 35Spro:MYB92 transgenic lines (35Spro is a strong promoter that drives expression in nearly all cells of the plant) have more suberin in the root (Figure 2D-E). In addition, we are currently isolating RNA from the roots to ensure increased expression of the transcription factors of interest and fluorol yellow will be used to determine the abundance of suberin as described in aim 1A under both control and water deficit. While we are currently still screening more lines to select the best candidates, we have fulfilled our Year 2 objective of generating lines with increased suberin, and are in good standing to complete this third year of the proposed project.

Aim 2. Determine the influence of increased root suberin on tomato plant growth and yield in control and water deficit conditions. ***ONGOING - YEAR 3 PROJECT.***

2a. Test the influence of induced suberin production on plant growth and photosynthetic parameters under control and drought conditions.

2b. Test the influence of increased suberin on plant yield and quality under control and drought conditions in the field.

2c. Test the influence of increased suberin production on nematode and branched broomrape infection.

We conducted a drought proof-of-principle experiment to benchmark our conditions and quantification of physiological parameters (Cantó-Pastor et al., 2024). In conjunction with an additionally funded CTRI project, we developed a mid-throughput and higher-throughput assay to test for branched broomrape infection in the Controlled Research Facility a.

Discussion:

The experiments funded in this CTRI project will provide a proof-of-principle to demonstrate that increased suberin will result in many beneficial traits (water limitation and pathogen tolerance). Our goal is to generate lines with enhanced suberin that will, consequently, become high performing cultivars under water-limited conditions. We will need input with CTRI partners as to the best strategy to implement these findings in a commercially relevant manner that will be acceptable to the consumer and grower. One potential approach, if increased suberin can drive gains in yield under these conditions, then we can use available variation in breeding populations to fix this trait for commercial purposes and/or CRISPR gene editing approaches to increase suberin deposition as accepted by the USDA regulatory services.

Acknowledgements:
We thank several members of HM Clause who provided significant input into the choice of germplasm for the generation of these suberin over-producing plants as well as a strategy for generating hybrids in the shortest time possible to fit the project time frame, particularly Shantel Martinez. We also thank Zach Bagley for his input.

This project as leverage for other dollars:

1. We have received an NSF-PGRP funded project ($3,100,000; 10/2021-09/2025) to in part explore how suberin levels will interact with a symbiotic organism (arbuscular mycorrhizal fungi) to further mitigate the tomato drought response.
2. We received a $10,000 award from Bayer Halo-Ag to contribute to generation of these lines with increased suberin.
3. We collaborate with Neelima Sinha via a postdoc Mona Gouran to screen these lines for branched broomrape tolerance.
4. We submitted an FFAR SeedingSolutions proposal to use these lines and others to identify genes that are critical for branched broomrape resistance and to generate resistant lines ($208,000).
5. $150,000 in funds have been granted by the California Department of Food and Agirculture to explore the link between branched broomrape resistance and cell wall barriers. We will use these lines described here as genetic material.
6. Using funds from a just completed USDA project, we have determined a link between N levels and suberin. These suggest an additional trait which could be mediated by increased suberin - increased nitrogen use efficiency. We submitted a USDA grant proposal (requesting $800,000) regarding this topic in August 2023.

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REMOTE SENSING FOR EARLY DETECTION OF BRANCHED BROOMRAPE IN TOMATO

Project Leader and any Co-PIs: Alireza Pourreza (Associate Professor of Cooperative Extension, UC Davis, Department of Biological and Agricultural Engineering), Mohsen B. Mesgaran (Assistant Professor, UC Davis, Department of Plant Sciences)

Cooperating Personnel: Hanan Eizenberg (Newe Ya'ar Research Center, ARO, Israel)

Year of Project Initiation: 2023

1. Executive Summary:

1.1. Why the Work Was Undertaken:

Branched broomrape (*Phelipanche ramosa* (L.) Pomel) presents a critical threat to the tomato industry in California, where it causes significant economic losses. The need for effective management strategies is crucial, particularly in light of the limitations of existing control measures. Our research aimed to develop a remote sensing methodology for early detection of broomrape infestation, a key to mitigating its impact.

1.2. Relevance to Operations:

Our findings are directly relevant to tomato growers, particularly in California. The ability to detect broomrape infestations at its early invasion stage allows for more targeted, less costly, and effective management strategies, reducing the reliance on broad-spectrum herbicides and minimizing crop damage and economic loss.

1.3. Methodology:

The research encompassed leaf and canopy-level analysis using advanced remote and proximal sensing techniques. We employed a variety of tools, including handheld spectrometers, drones equipped with multispectral and hyperspectral cameras, and LiDAR technology on multiple occasions during the growing season. Machine learning models like Adaptive Boosting were utilized to accurately classify healthy and infected plants.

1.4. Findings and Conclusions:

- Leaf-Level Analysis: This showed that the shortwave part of the spectrum was most informative for distinguishing between healthy and broomrape-infected plants.
- Canopy-Level Analysis: NIR reflectance values indicated some differences but lacked the accuracy seen in leaf-level analysis. Integrating 3D radiative transfer modeling improved understanding but highlighted the complexities of canopy-level data.
- Machine Learning Models: Demonstrated high accuracy in early detection, with Adaptive Boosting performing exceptionally well.

1.5. Recommendations:

- Early Detection: Implementing remote sensing techniques for early detection of broomrape can help with its containment and the risk of its spread.

- Satellite Imagery: Future work should explore combining drone imagery with satellite data to detect and map broomrape-infested fields.

1.6. Impact on Individual Operations:

Implementing these recommendations can significantly impact tomato operations by:

- Reducing the economic losses due to broomrape infestations.
- Avoid planting host crops if a neighboring field is infested.
- Reducing herbicide use through precision treatment of infested areas only.

1.7. Need for Future Work:

There is a need for continued research, particularly in refining canopy-level analysis and integrating these techniques into existing agricultural practices. Exploring the combination of drone and satellite imagery to achieve high-resolution images in the shortwave part of the spectrum and further developing machine learning models will be crucial in advancing broomrape management strategies.

This executive summary encapsulates our comprehensive approach towards early detection and management of branched broomrape in tomato crops, offering insights and directions for future research and application.

2. Introduction:

Branched broomrape (*Phelipanche ramosa*) poses a significant threat to the tomato industry, particularly in California. As an obligate parasitic weed, it has been re-emerging in tomato fields across several counties in California, a state renowned for producing over 90% of the United States tomatoes (Osipitan et al., 2021). The infestation by this parasite not only results in quarantine and crop destruction but also leads to substantial economic losses for growers. In severe cases, yield reductions due to broomrape can range from moderate to as high as 80%, depending on various factors such as infestation level, host, and environmental conditions (Osipitan et al., 2021).

Control measures for branched broomrape have been challenging. Studies have explored various management practices, including chemical treatments and sanitation measures. For instance, ammonium compounds have shown effectiveness in preventing branched broomrape seed germination, highlighting the importance of sanitation in controlling this weed (Hosseini et al., 2022). New approaches like silicon dioxide nanoparticles have also been investigated to reduce broomrape infection in tomatoes by fortifying the cell wall and modulating reactive oxygen species homeostasis (Madany et al., 2020).

In California, the re-emergence of branched broomrape in tomato fields calls for urgent early detection, containment, and effective management strategies. Our team's work over the past year has contributed to this field by focusing on the early detection of broomrape in tomato plants using remote and proximal sensing. This approach is vital as it provides a proactive measure to identify and manage broomrape infestations before they cause extensive damage. Early detection is crucial for containing this pest at its early stages of invasion, thus minimizing the economic impact on the tomato industry in California.

Building on the research by Atmson (2022), which demonstrated the use of hyperspectral imaging in detecting subtle differences in the reflectance spectra from sunflower leaves, our study aimed to apply similar techniques to tomato plants for early detection of branched broomrape infestation. We focused on measuring the reflectance of healthy and infested tomato leaves at various growth stages, specifically

every 200 growing degree days. This research was conducted at both the leaf and canopy levels of tomato plants.

3. The main *Goal* and the *Objectives* under that goal:

3.1. Main Goal:

Our primary goal is to develop a remote sensing methodology for early broomrape infestation detection in tomato crops by understanding how light interacts with tomato leaves and canopies. We will integrate optical leaf models such as PROSPECT PRO (Féret et al., 2021) and photon tracing models such as LESS (Qi et al., 2019) to Integrate them into Radiative Transfer Modeling (RTM) and generate 3D models incorporating realistic spectra and tomato plant structures. These models will be combined with RTM and explainable Machine Learning (ML) algorithms for enhanced accuracy.

3.2. Specific Objectives:

- Determine the impact of broomrape on tomato leaf traits, structure, and spectral reflectance (Objective1)
- Develop a mechanistic approach for retrieving tomato plant traits at the canopy level (Objective2)
- Develop a remote sensing approach for early detection of broomrape infection at the canopy level using multispectral imagery (Objective3)

These objectives direct our research toward achieving broomrape early detection capabilities for tomato growers.

4. Methodology and Results:

4.1. Data Collection:

Our data collection was strategically timed to commence at 200 Growing Degree Days (GDD), with subsequent data collections occurring at intervals of 200 GDD each. This systematic approach ensured that we captured broomrape infestation's progression at the tomato plants' critical growth stages. The data collection process included:

4.1.1. Leaf-Level Data Collections:

Handheld Spectrometer Measurements: Four sessions were held to measure leaf reflectance using a handheld spectrometer, covering a range of 400nm to 2500nm. This provided detailed insights into the spectral properties of individual leaves.

Fluorescence Imaging: Cross-leaf microscopy was conducted using fluorescence imaging to examine potential cellular changes in leaves due to broomrape parasitism.

4.1.2. Aerial Data Collections:

RGB Data: Conducted seven sets of aerial collections using RGB imagery to capture high-resolution color images of the tomato farm.

Multispectral Data: Six aerial collections using a multispectral camera to capture data in specific wavelength bands, including blue, green, red, red-edge, and near-infrared (NIR).

Hyperspectral Data: Six aerial hyperspectral data collections were performed to capture a wide spectrum of light for each pixel in the image, providing detailed information about the health of the plants.

Our hyperspectral camera, the PIKA L, covered a spectral range of 400nm to 1000nm and had a spatial resolution of 1.8319 nm.

LiDAR Data: Six aerial LiDAR data collections to generate precise three-dimensional information about the shape and structure of the tomato plants and the surrounding environment.

4.1.3. Ground Data Collections:

We conducted four comprehensive ground-truthing data collection. These involved detailed inspections and data gathering from the farm to validate and correlate with the leaf-level and aerial data findings.

Figure 1 illustrates the tomato field in Woodland, Yolo County, California, where infected plants are marked with flags, providing a visual reference for the spatial distribution of data collection points. This comprehensive data collection approach, encompassing both aerial and ground-level analyses, was crucial for accurately assessing the impact of broomrape infection on tomato plants and validating our remote sensing data.

Figure 1. A tomato field in Woodland, Yolo County, California, was used in this study. Flags indicate tomato plants infected with branched broomrape.

4.2. Leaf-Level Data Analysis:

Spectral reflectance data were collected from two leaves of 300 plants at 585, 897, 1216, and 1568 GDD following tomato planting. Almost half of these plants were broomrape-free, while the remaining were parasitized (flagged). Figure 2 presents the spectral reflectance of healthy and broomrape-infected leaves at these four growth stages. This visual representation highlights the spectral differences between

healthy and infected leaves, emphasizing the specific parts of the spectrum where these changes are most pronounced.

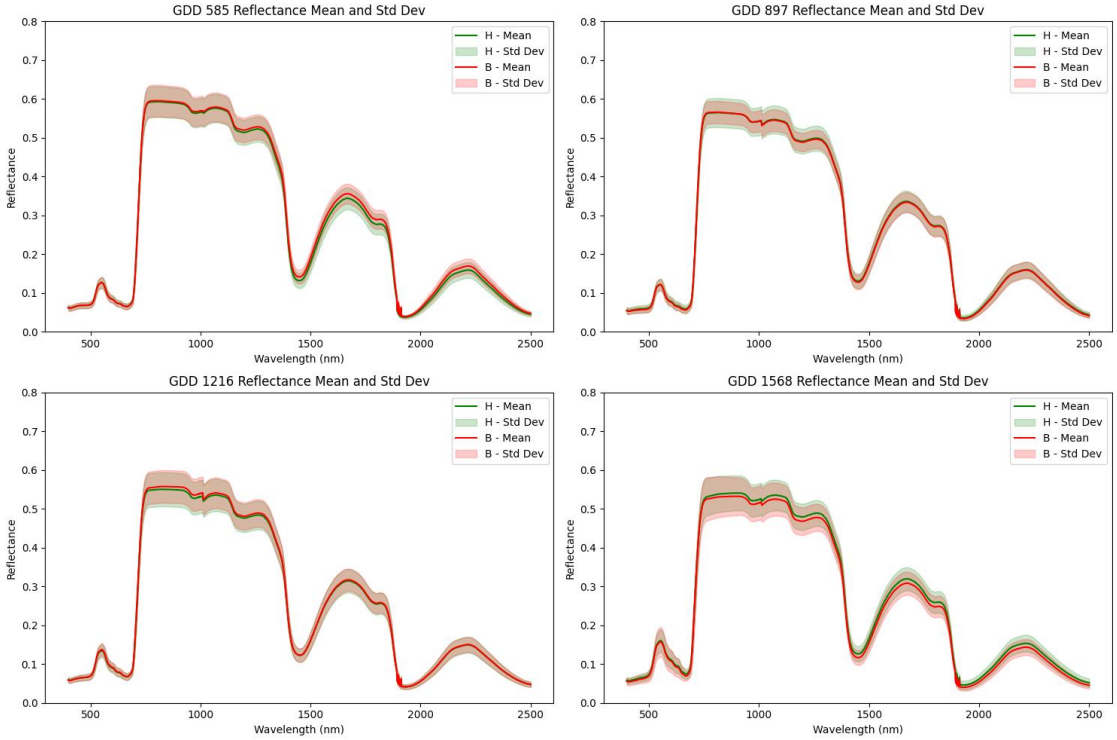


Figure 2. Mean and standard deviation of spectral reflectance for both healthy and broomrape-infested leaves

To further our understanding of the impact of broomrape infestation on tomato plants, we visualized the normalized mean absolute difference in reflectance between healthy and infected leaves based on the healthy class. This analysis was pivotal in identifying the key wavelengths that exhibit significant differences due to infestation (Figure 3).

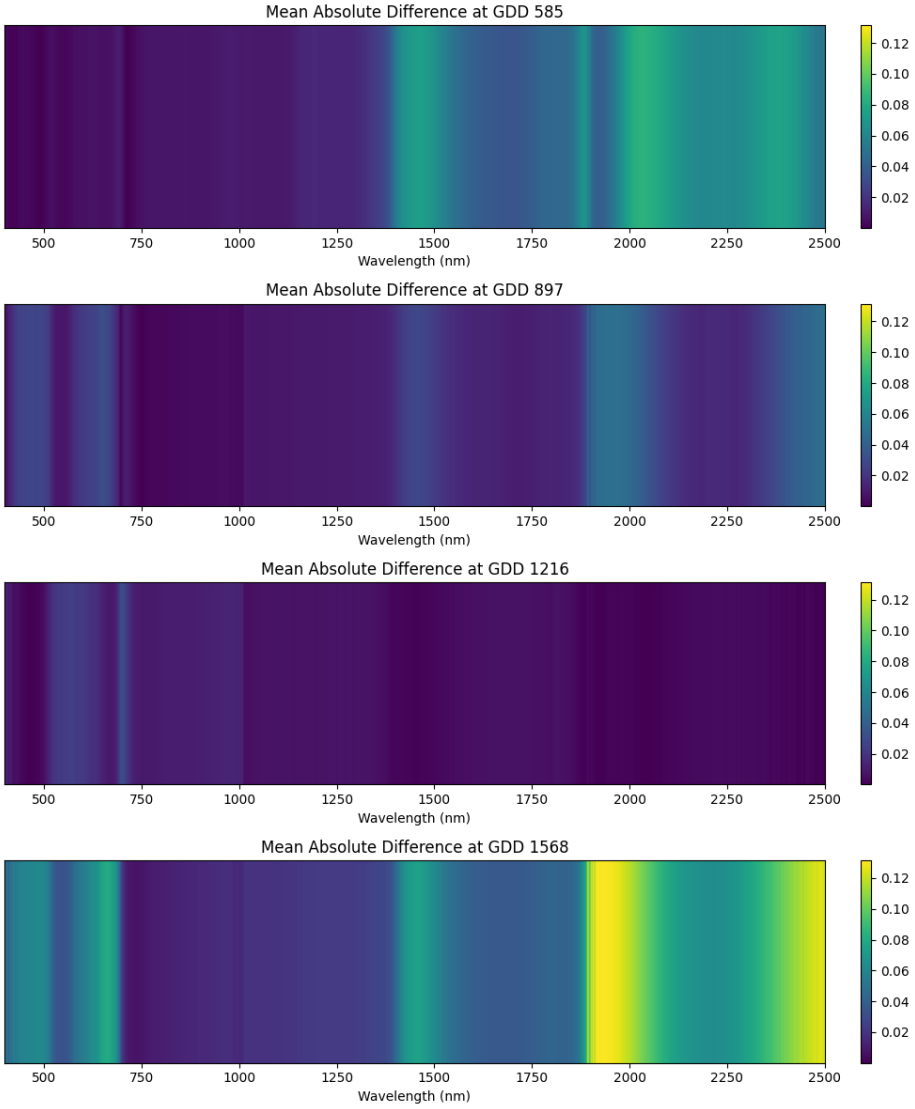


Figure 3. The mean absolute difference in reflectance between healthy and infected leaves normalized by the healthy class

4.2.1. Machine Learning Methods:

We explored a variety of advanced machine-learning models to detect broomrape infection at the leaf level. Our dataset underwent preprocessing before being subjected to 17 different machine-learning models. These included Linear Discriminant Analysis (LDA), Principal Component Analysis combined with Logistic Regression (PCA with Logistic Regression), Support Vector Machine (SVM) with Linear, Poly, and RBF kernels, Random Forest, XGBoost, Adaptive Boosting (AdaBoost), Gradient Boosting Machine (GBM), Light GBM, CatBoost, Extremely Randomized Trees, Decision Trees, K-nearest Neighbors (KNN), 1D Convolutional Neural Networks (1D CNN), Hyperparameter-tuned CNN, and Naive Bayes.

Of these methods, Adaptive Boosting, a tree-based machine learning model, exhibited the best performance across all GDDs. Figure 4 in our report showcases the confusion matrix for the test dataset, revealing AdaBoost's promising results in accurately classifying healthy and broomrape-infected leaves.

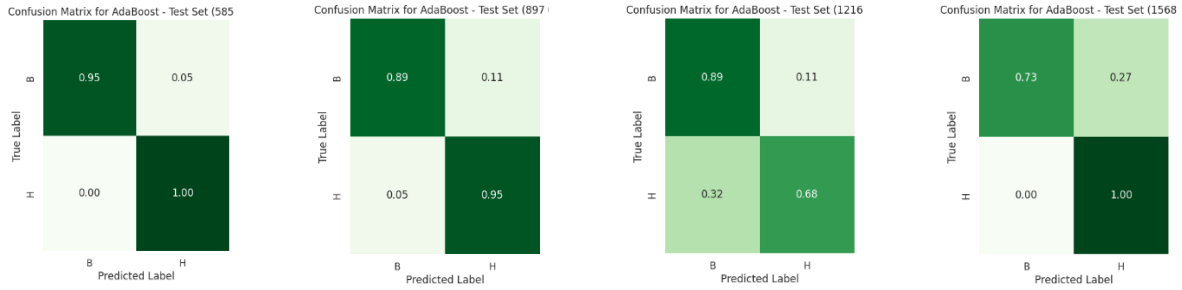


Figure 4. The confusion matrix for the adaptive boosting classification model was applied to data collected at different GDDs following tomato planting.

Averaged over the four sampling dates, the model successfully classified healthy leaves with 91% accuracy and correctly identified 86% of leaves infected with broomrape. This level of accuracy demonstrates the model's effectiveness in early detection. However, these results are based on a single-year study, while for a robust analysis, independent data collected over multiple years/sites is needed.

Furthermore, one of the key strengths of Adaptive Boosting, particularly when dealing with hyperspectral data with many features, is its ability to discern the most influential bands for classification. Figure 5 illustrates how the Adaptive Boosting model pinpointed specific bands across the spectrum's visible, infrared, and shortwave parts to make accurate classifications. This insight is invaluable for understanding the spectral characteristics critical for distinguishing between healthy and infested leaves.

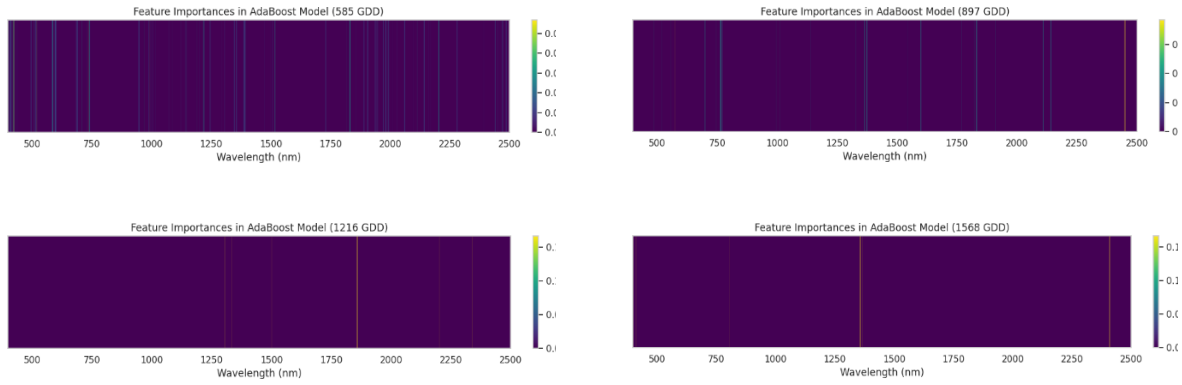


Figure 5. Feature importance assessment in the Adaptive Boosting model to identify the most discriminating spectral bands at different sampling dates.

Analysis of feature importance, as shown in Figure 5, reveals that the most critical bands for differentiating healthy and broomrape-infested leaves are predominantly in the shortwave part of the spectrum. This finding remains consistent across all evaluated GDDs (585, 897, 1216, and 1568).

4.2.2. Leaf Micro Cross-Section Analysis:

A study identified anatomical differences in leaf cross-sections between sunflowers infected with *Orobanche cumana* Waller and control plants. The air space in the mesophyll of *O. cumana*-infected plants was about 10% greater than that in non-infected ones (Cochavi et al. 2017). We counted the number of cells per area unit in randomly selected regions within each image. We found that cell density in the mesophyll of non-infected plants is ~5% less than in infected plants. However, this is only for the data from the second sampling date.

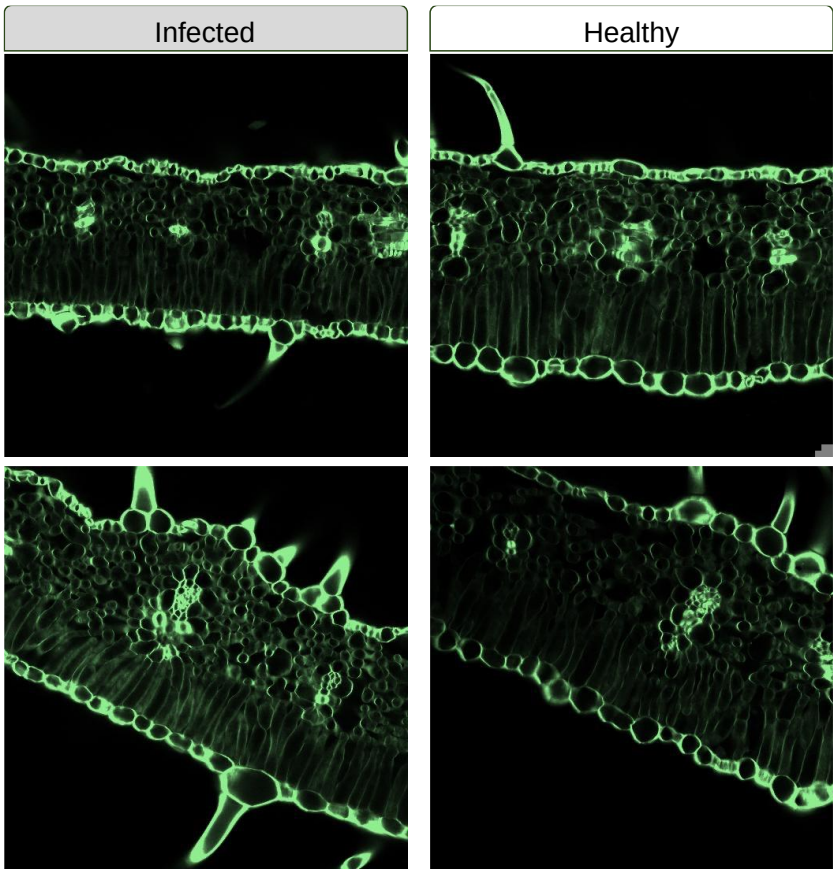


Figure 6. Cross-section microscopic images of leaves from healthy and broomrape-infected tomato plants.

4.3. Canopy-Level Data Analysis:

We collected aerial data at the canopy scale at key growth stages: 71, 325, 574, 880, 1196, and 1556 GDDs. Utilizing RGB, multispectral, hyperspectral imagery, and LiDAR, these collections aimed to detect broomrape infestation on a larger scale and correlate our findings from leaf-level analysis to the canopy level.

4.3.1. RGB and Multispectral Canopy-Level Data Analysis:

For aerial RGB imagery throughout all growth stages, we employed a DJI Phantom 4 RTK drone. The RGB imagery was instrumental in mapping the tomato farm and locating our flagged target plants.

We used a DJI Matrice 210 drone with an Altum-PT camera for multispectral imagery. This sensor captured images in blue, green, red, red-edge, near-infrared, panchromatic, and thermal bands. Figure 9 in our report presents an orthomosaic image of the targeted tomato farm, showcasing RGB and multispectral aerial imagery. This visual representation helps in understanding the spatial distribution and condition of the farm at a canopy level.

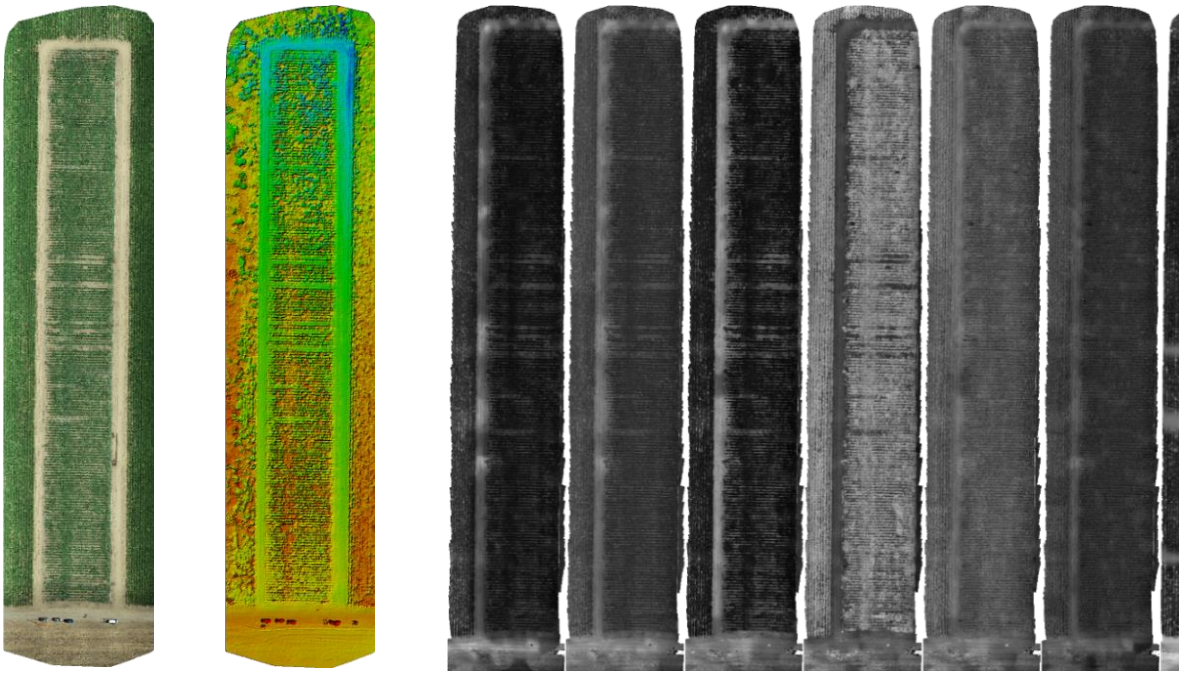


Figure 7. Orthomosaic of aerial RGB and Multispectral imagery

Using aerial RGB imagery, we successfully identified flagged target plants. With ArcGIS Pro, shapefiles were drawn to delineate these plants. Figure 10 presents an aerial view of the farm, where white polygons indicate healthy plants and red polygons represent those infected by broomrape.

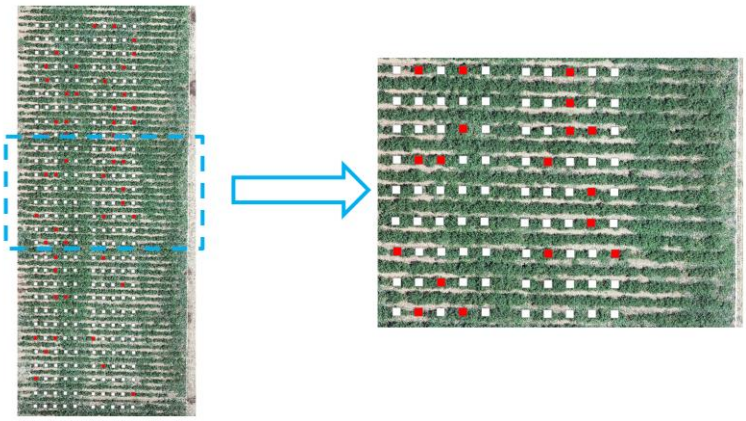


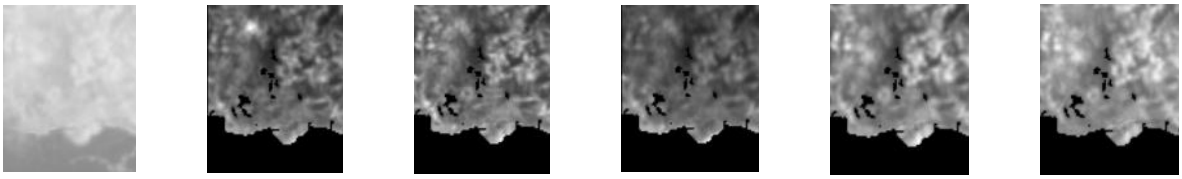
Figure 8. Aerial view of the farm with polygons indicating healthy class with white and broomrape-infected plants class with red color

Subsequently, these shapefiles allowed us to extract the flagged plants from our multispectral images, which include Blue, Green, Red, Red Edge, and NIR bands. Due to the presence of mixed pixels with soil and shaded areas in the canopy, we employed the SAVI index to focus on sunlit pixels, which is crucial for our analysis. The SAVI formula is:

$$SAVI = \frac{NIR - RED}{NIR + RED + L} \times (1 + L)$$

- NIR: Pixel values from the near-infrared band
- Red: Pixel values from the red band
- L: Factor accounting for the amount of green vegetation cover

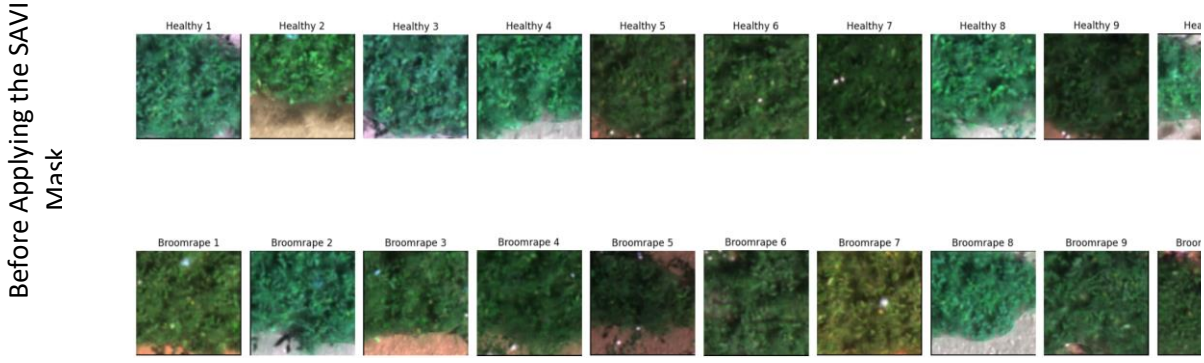
Figure 9 illustrates the SAVI mask applied to a sample plant, masking each band (Blue, Green, Red, Red Edge, and NIR).



SAVI Blue Green Red Red Edge NIR

Figure 9. SAVI mask applied to a sample plant

To highlight the effectiveness of the SAVI index in excluding shaded and soil pixels, we created RGB composite images before and after applying the SAVI mask, as shown in Figure 12.



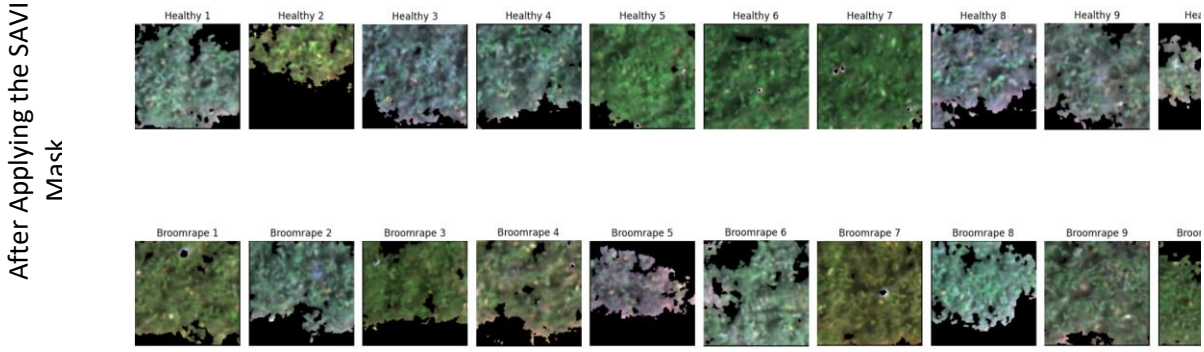


Figure 10. Comparison of images before and after applying the SAVI mask.

Figure 11 compares pixel values' mean and standard deviation for broomrape-infected and healthy classes across all bands. At the early stage (585 GDD), the patterns in all bands were similar except for the NIR band. This aligns with our leaf-level analysis, where differences were more pronounced in the NIR and shortwave spectrum. However, due to factors like environmental variability and mixed pixels, conclusions derived from leaf-level analysis might be more robust than those from the canopy level.

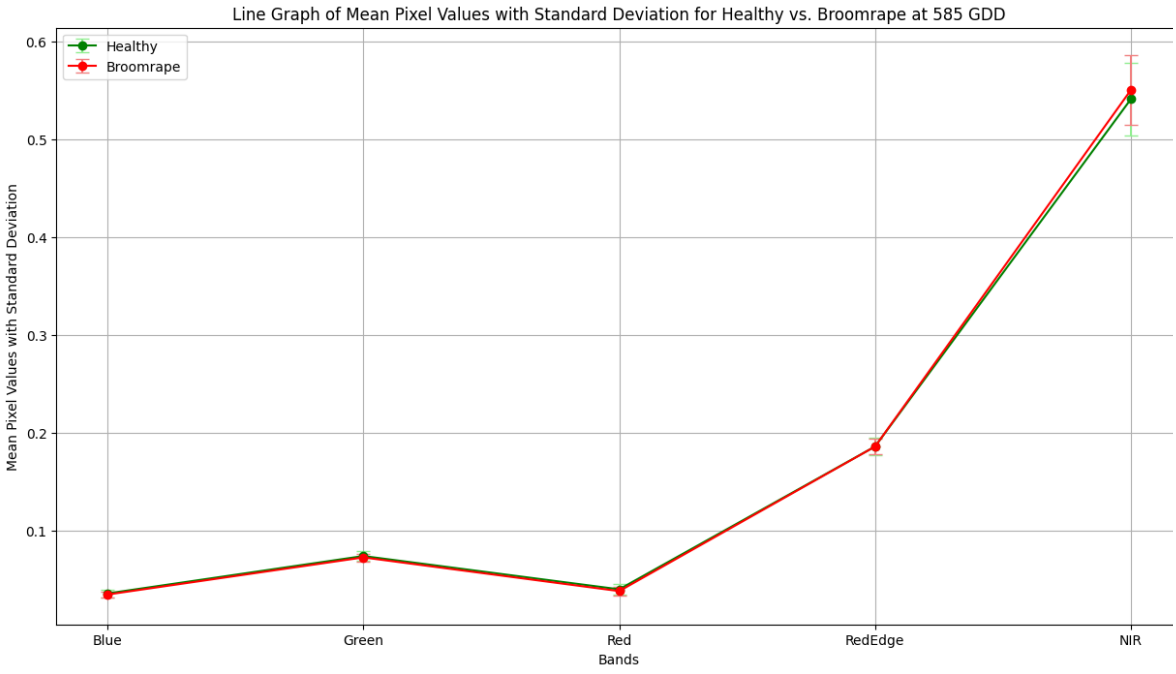


Figure 12. Comparison of the mean pixel values for broomrape-infected and healthy classes across all bands.

4.3.2. 3D Radiative Transfer Modeling and Hyperspectral Imagery in Canopy-Level:

For 3D radiative transfer modeling, we created 3D models of tomato plants at different growth stages using HELIOS software (Bailey, 2019). Figure 13 illustrates these 3D tomato plant models at key stages: flowering, fruit formation, and mature fruiting.

Flowering Fruit Formation Mature Fruiting

Figure 13. Generated 3D models of tomato plants at three different phenological stages

We integrated the 3D tomato plant objects, generated using HELIOS, into LESS (Qi et al., 2019), a 3D Radiative Transfer Modeling software. The inputs for LESS included:

- Sensor information from our hyperspectral sensor (PIKA L).
- Sun zenith and azimuth angles were calculated based on the time and location of data collection.
- Imported 3D objects from HELIOS.
- Spectral characteristics of healthy and broomrape-infested leaves derived from our leaf-level spectral data.

Figure 14 showcases the 3D scene generated in LESS.

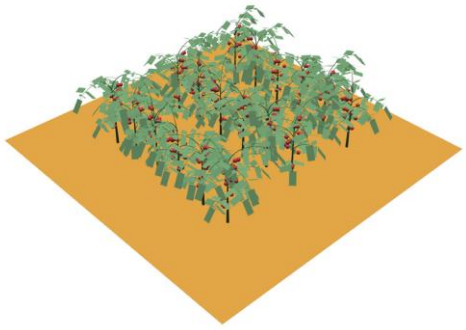


Figure 14. 3D scene generated in LESS

Our hyperspectral sensor, PIKA L, captures the 400nm to 900nm spectral range. We aimed to align the spectral reflectance in the real hyperspectral imagery with that from our LESS-generated model. Figure 15 compares these two, illustrating the correlation between actual hyperspectral imagery and our 3D Radiative Transfer model simulations.

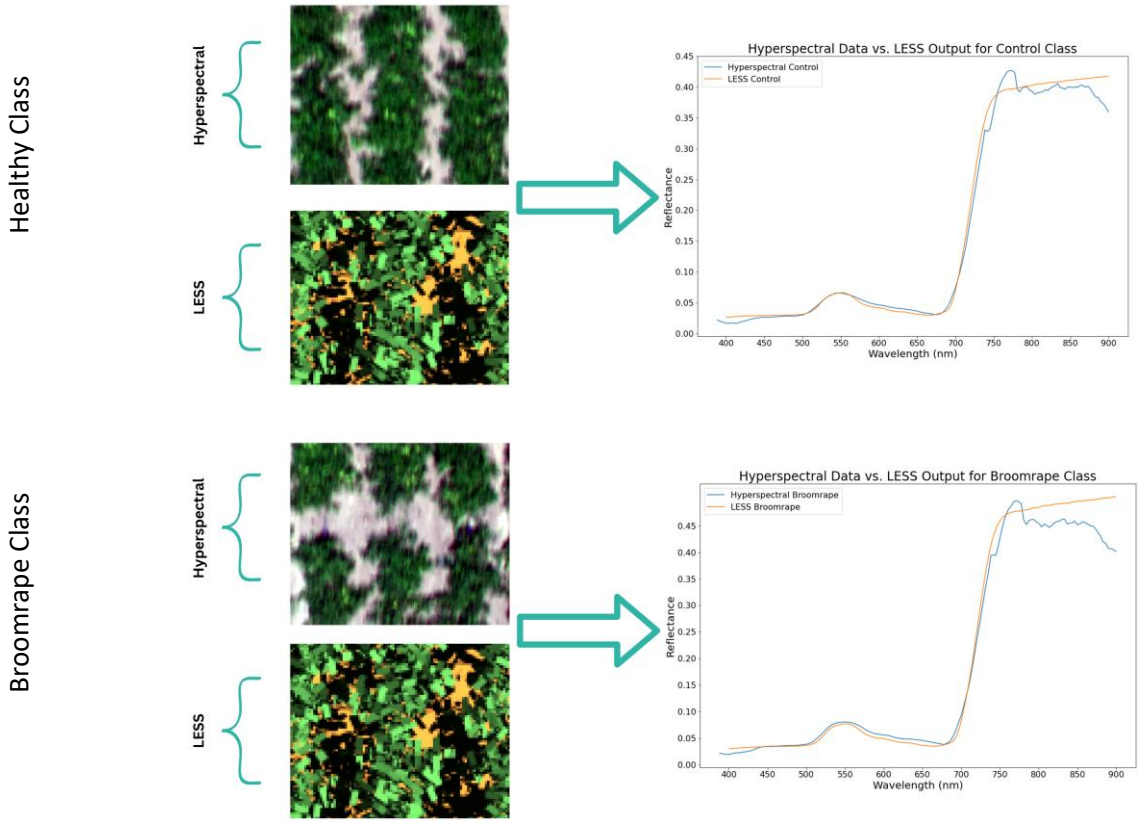


Figure 15. Comparison of aerial hyperspectral data with 3D Radiative Transfer Model

4.3.3. LiDAR Data Analysis:

Using the Ranger-UAV LiDAR system from Phoenix LiDAR Systems on DJI Matrice 600 Drone, we acquired point cloud data of our tomato farm. Figure 16 illustrates how this technology enabled us to assess the height and volume of the tomato plants.

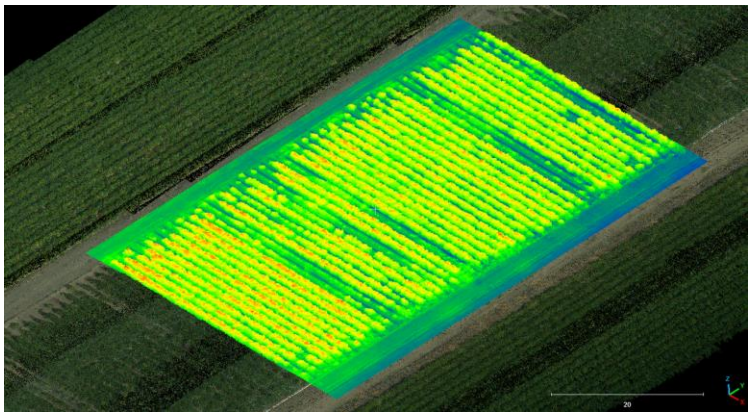


Figure 16. Visualizing the height of tomato plants from point cloud data

To differentiate soil, weeds, and tomato plants, we employed the CSF filter (Zhang et al., 2016) in CloudCompare, a point cloud post-processing software. This step ensured that our plant height and volume analysis was focused solely on the tomato plants. Figure 18 displays each stage of this process.

Tomato plants, soil, and weeds | Tomato Plants | Soil and weeds

Figure 17. Differentiate soil, weeds, and tomato plants

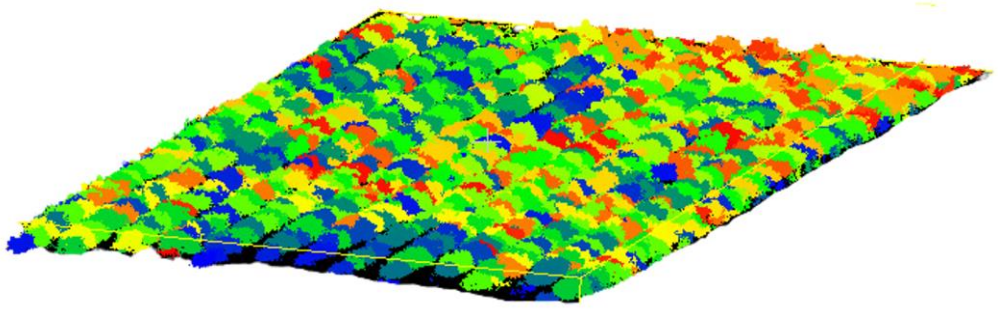


Figure 19. Isolate individual tomato plants

Furthermore, we utilized the TreeISO plugin (Xi and Hopkinson, 2022) in CloudCompare, which leverages K-means clustering algorithms to isolate individual tomato plants, as demonstrated in Figure 18. This allowed for the measurement of each plant's average height and volume. However, the resulting height and volume data did not yield significant conclusions due to the intermingling of tomato plants with weeds and their non-uniform growth patterns.

5. Discussion

Revisiting the challenge outlined in our introduction, branched broomrape remains a significant threat to California's tomato industry, necessitating urgent and effective management strategies. Our findings show promise for developing an extensive remote sensing approach for early detection of broomrape infestation at both the leaf and canopy levels in tomato plants. Results highlighted the effectiveness of various remote sensing techniques and machine learning models in identifying broomrape infestations. The Adaptive Boosting model showed exceptional accuracy at the leaf level in distinguishing between healthy and infested leaves, especially in the early growth stages. However, this study must be repeated to obtain the temporally independent data required for model validation.

At the canopy level, integrating 3D radiative transfer modeling with hyperspectral and multispectral imagery provided valuable insights, despite environmental challenges and mixed pixels. However, the leaf-level analysis yielded more robust conclusions due to controlled variables. LiDAR technology enhanced our understanding of the physical structure of tomato plants. Still, the non-uniform growth patterns and the presence of weeds at this level added complexity to definitive conclusions.

A significant outcome of our research is identifying the shortwave part of the spectrum as the most informative for distinguishing broomrape-infected tomato plants from healthy ones at the leaf level, as revealed by our machine learning models' feature importance analysis. While NIR reflectance values at the canopy level from multispectral imagery indicated some differences, they lacked the accuracy observed in the leaf-level analysis.

Looking forward, we plan to incorporate satellite imagery into our analysis to replicate the precision of leaf-level results at the canopy scale. Satellites like Sentinel-2, which capture images in the shortwave part of the spectrum, can be useful for this classification task. However, their coarse spatial resolution presents a challenge. We aim to employ novel Deep Learning methods, such as super-resolution techniques, to merge drone imagery with satellite data, enhancing spatial resolution in the shortwave spectrum. This integration promises to be a powerful tool in precision agriculture, offering more effective and sustainable management solutions for broomrape infestation in tomato fields.

Future research should focus on refining these methodologies, especially at the canopy level, to address the challenges posed by environmental variables and mixed pixels. Further, integrating these techniques into existing agricultural practices could pave the way for more sustainable and efficient management of broomrape and other agricultural pests.

6. Acknowledgements:

7. This project as leverage for other dollars: While we did not secure additional funding from other sources during this project, we recently submitted a collaborative proposal in September 2023. Working alongside our colleagues from Israel, we applied to the US-Israel Binational Agricultural Research and Development Fund (BARD), focusing on the early detection of broomrape. The proposal outlines a three-year project with a requested budget of $310,000 and substantially builds upon the results and methodologies developed in the CTRI-funded project.

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SCREENING OF A VOC SENSOR TO IDENTIFY BROOMRAPE INFESTATIONS

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Year of Project Initiation: 2023

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 will 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 for non-destructive screening.

In this first project year, we conducted a proof-of-concept greenhouse study by adapting protocols previously developed for other agricultural crops (citrus, strawberries, walnuts) to sample tomato and broomrape plants. In the greenhouse, plants were purposely infected with broomrape, and samples were taken alongside non-infected control plants. We assessed two potential mechanisms to screen for broomrape: 1) to see if broomrape, after emergence, emits a detectable profile, and 2) to see if the tomato plant VOC profile is altered after infection with broomrape.

While our findings suggest that detection of broomrape from its own VOC emissions is not likely, we did find promising results through measuring tomato plants. A machine learning algorithm correctly identified broomrape-infected tomato plants from non-infected controls with an accuracy of nearly 80% (79.2%). Given the limited sample sizes in this pilot work, these results are quite promising. Our models identified 9 specific tomato metabolites that are altered by broomrape and used by our models for screening. These metabolites may elucidate biochemical processes impacted by broomrape towards development of potential treatments or cures.

Our work continues in our current Year 2 project, which continues our proof-of-concept greenhouse study in addition to vetting our technology in a real-world field setting. Through our collaborator Brad Hanson, we will take a custom sensor technology into a broomrape-infested tomato field to determine the feasibility of our device, and to gain insights for required improvements. In further years, we will work with tomato growers to finalize a use case and protocol to translate our work into 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 volatile organic compounds (VOCs), which are small chemicals that readily evaporate into the air. 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. It has been extensively documented that the volatile emissions coming from tomato plants undergo changes when responding to stressors like drought and herbivore attack [2]. This allows for chemical sensors to utilize these gaseous plant emissions to monitor agricultural health or to detect agricultural pests.

The main *Goal* and the *Objectives* under that goal: In this first project year, our goal was to conduct a proof-of-concept greenhouse study to determine whether the broomrape parasite induces a chemical change in the tomato VOC profile, which a sensor could utilize to screen for broomrape infestations. We also assessed whether broomrape plants, upon emerging from the soil, emit a chemical profile that could be utilized for detection. Gold standard analytical approaches were used to measure emissions from tomato plants with and without broomrape attachment, as well as measurements of the broomrape plant VOCs.

In this Year 1 project, we had three major objectives:

- Objective 1: Adapt our previously developed methods to measure plant VOCs [3-8] for broomrape and tomato plant emissions
- Objective 2: Determine if broomrape emit a volatile profile that can be measured above ground
- Objective 3: Determine the exact timepoint tomato plants exhibit a shift in volatile profile upon infection by broomrape

Methodology and Results:

Objective 1: Adapt our previously developed methods to measure plant VOCs to measure tomato and broomrape plant emissions (**Completed**)

We successfully adopted our method 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

Tomato plants (18 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, 9 of 18 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. 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 1 and 2**) 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 vacuum pump. Air flow was passed through a hydrocarbon trap (Part 21991, Restek Corp., Bellefonte, PA) to scrub the air of background greenhouse VOCs. Precision-adjust air flow control valves controlled the flow to four individual purge lines, and the lines were monitored and recalibrated to each to maintain a flow of 40 sccm.

A single tomato plant or branch 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. Branches were chosen to have approximately the same number of leaves of the same age to avoid experimental biases in this methods optimization study and were marked with flagging tape to allow resampling the same branch over long duration experiments. We also were careful to ensure there was enough leaf mass placed into the enclosures to generate adequate VOC signals.

Figure 1*: With limited space in the CRF, we created a flow manifold that allows simultaneous VOC collection from four plants.*

Figure 2*: 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 magnets*

O1.3 Sampling of tomato and broomrape volatile emissions

To collect volatile emissions from tomato plants, we used an analytical chemistry approach that captured odor profiles onto a VOC sponge, commercially called “Twisters”, a small tool that plant VOCs stick to. Twisters are placed inside the branch enclosure (**Figure 2**) 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.

Every plant measurement was conducted in triplicate (ie, 3 Twisters placed inside one plant enclosure). 18 plants were followed over a course of several weeks, including broomape-infected and non-infected plants (9 plants per group). In our laboratory, plant samples underwent analysis by gas chromatography-mass spectrometry (GC-MS), the gold standard instrumentation to measure volatile chemicals. **Figure 3** shows a typical GC-MS sample of tomato plants.

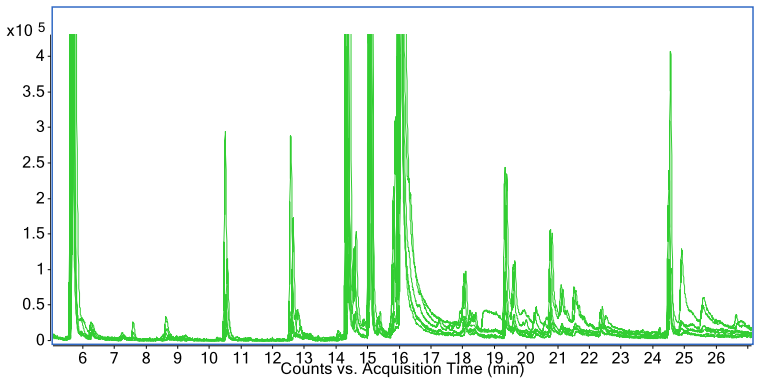

***Figure 3**: Example of a typical VOC profile of 6 tomato plants as measured by GC-MS. Shown is a portion of the complete profile. Each peak represents a chemical emitted by the plant, with the peak height related to the concentration of that chemical.*

Objective 2: Determine if broomrape emit a volatile profile that can be measured above ground (**Completed**)

VOC measurements were made from broomrape plants after emerging from the soil. **Figure 4** compares the typical VOC profile of tomato plants versus broomrape. Our results indicate that broomrape VOCs might be in such low concentration, relative to the tomato signature, that it would likely not be a reliable way to screen for broomrape. Rather, these results indicate we should focus on the tomato plant's response to broomrape parasite, discussed in Objective 3 results, as a screening mechanism.

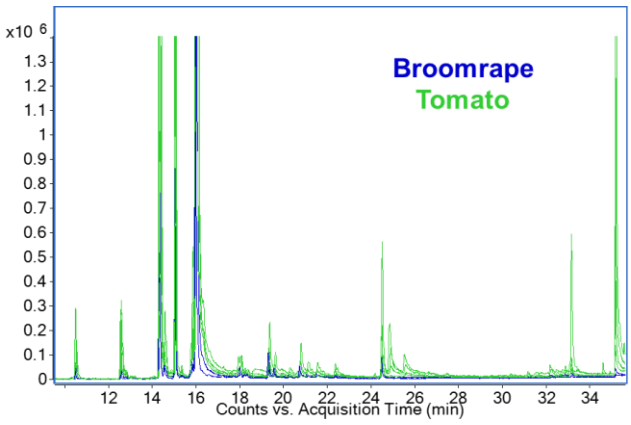

***Figure 4: (left)** Comparison of a broomrape VOC profile (blue) against tomato plants (green). Broomrape did not seem to especially emit a chemical profile that could be used for screening. Rather, screening for broomrape was successful by using tomato plant emissions (see Objective 3). **(right)** One of the broomrape plants after emergence.*

Objective 3: Determine the exact timepoint tomato plants exhibit a shift in volatile profile upon infection by broomrape (**Ongoing into Year 2**)

Year 1 results suggest broomrape attachment alters the tomato plant metabolism, and that these changes are reflected in the tomato plant VOC emissions. A developed machine learning algorithm identified broomrape-infested plants with an accuracy of 79.2%, promising results for a pilot study with limited sample sizes. We are working to confirm these findings in our current Year 2 project.

Figure 5 shows an example of how broomrape alters tomato metabolites. In this example, two compounds, alpha-pinene and m-cymene, appear to be down-regulated in infected tomato plants. This suggests that broomrape attachment alters the biochemical processes responsible for tomato plants to produce these compounds, and that a mobile chemical sensor could use these unique changes to screen tomato plants for the parasite.

Figure 6 shows a principal components analysis (PCA), a statistical method that simplifies complex datasets by highlighting the most important patterns within it. While there is some overlap between tomato samples with broomrape and without, we see a tendency of these two treatment groups to separate, indicating a difference in the VOC profile caused by broomrape.

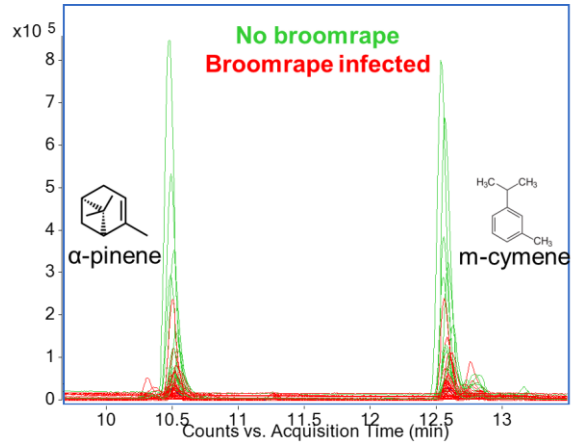

Figure 5: *Example of two volatile compounds, a-pinene and m-cymene, whose concentration decreases as broomrape attaches to the tomato plants. GC-MS data.*

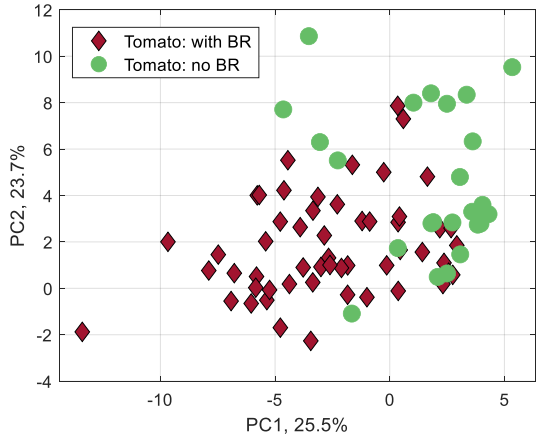

Figure 6*: VOC measurements of tomatoes with broomrape attached (red) and without broomrape (green). While there was some overlap between these two groups, the tomato profile appears to shift with broomrape infection, providing an opportunity for VOC-based screening.*

Then, partial least squares-discriminant analysis (PLS-DA) was applied, which is a machine learning method. We train the PLS-DA model on samples from tomato plants with and without broomrape. PLS-DA determines the exact chemicals needed for the model to distinguish these plants. In total, the PLS-DA model identified a constellation of 9 individual tomato metabolites (**Table 1**) to predict infection status. Seven of these compounds were identified by comparing our obtained data to the NIST 2023 database of VOCs; two compounds were not yet identified.

Our model had an accuracy of 79.2% to correctly identify broomrape-infected plants from non-infected. These results are especially promising, given the limited number of samples available to train the model. Typically, a higher number of samples to train the model yields a more accurate result. Samples collected in Year 1 will be combined with the ongoing work in Year 2 to enhance these statistical models.

Table 1. List of tomato metabolites used by the PLS-DA model to distinguish non-broomrape from broomrape-infected tomato samples. VIP scores indicate how important the compound was in the model to distinguish broomrape (higher scores = more important). The CAS number and chemical formula are provided for identified compounds.

Compound name	VIP Score	CAS Number	Chemical formula
alpha-Phellandrene	1.66	99-83-2	C10H16
Unidentified chemical #1	1.25		
Bicyclo[2.2.1]heptan-2-one, 4,7,7-trimethyl-, (1S)-	1.20	10292-98-5	C10H16O
Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	1.13	353-31-3	C10H16
Unidentified chemical #2	1.08		
2,3-Dihydro-1H-pyrazol-3-one	1.07	1000540-07-6	C7H8N2O3
2-(2-Acetoxy-1-propyl)furan, (S)	1.05	60830-70-8	C9H12O3
gamma-Terpinene	1.03	99-85-4	C10H16
Pentane, 2,3,3-trimethyl-	1.01	560-21-4	C8H18

Discussion: The objective of Year 1 was to conduct a proof-of-concept experiment to see if a chemical signature is emitted by plants that indicate broomrape infection, so that long term, mobile chemical sensors could be deployed in tomato fields to screen for broomrape infestations.

While we do not believe that broomrape plants, after emergence, emit a unique chemical signature that could be used for detection, we had promising results that the tomato plant emissions are shifted after broomrape attachment, and that this broomrape “signature” in tomato plants can be used by machine learning algorithms to identify broomrape-infected plants with an accuracy of nearly 80% (79.2%). Our model identified a constellation of 9 tomato metabolites (**Table 1**) that are altered when broomrape attaches to the tomato plant.

We have not yet been able to determine the exact timepoint by which broomrape alters the tomato plant VOC profile. This work will be completed in Year 2. Results from Year 1 and Year 2 will be combined into one manuscript that we will submit for publication at a peer-reviewed journal.

Information from Year 1 will be used to determine design choices and develop methods to construct a custom chemical sensor for broomrape detection. Our team has engineered various systems for mobile chemical sensing applications. Most relevant is our patented VOC micro-preconcentrator chip, “µPC” (US Patent #10,940,948) [9]. It contains microfabricated channels that allow intake of an air sample, which passes through a sorbent-packed cavity, trapping VOCs inside. To deploy our µPC chips, we have engineered a custom, mobile sampler), which includes a micro-controller, temperature/humidity sensors, and GPS for location data. For large scale sweeping across fields, the sampler is carried by tractor or as a payload onto a drone. Multiple µPC chips could be deployed, so that samples are taken from different portions of the field to identify areas of threat and reduce areas required to quarantine. A mobile base station with a suitcase-sized platform could be deployed in fields to analyze samples on site and provide results in near real time. These devices analyze µPC samples from the field and include machine learning

algorithms for automated data interpretation, providing a simple “pathogen detected / not detected” output to users.

These preliminary results will be confirmed in our current Year 2 study, where we will repeat the greenhouse experiment. Furthermore, we will also trial a custom chemical sensor in a tomato field to test the feasibility of our technology to screen tomato fields. Our current Year 2 study has two objectives:

1. Continue our proof-of-concept greenhouse study to assess whether broomrape or infected tomato plants emit a detectable chemical signature. Our preliminary data shows young tomato plants emit a volatile signature that shifts upon parasitization with broomrape, affording diagnostics. We will examine how quickly after infection is this shift detectable and compare against mature tomato plants. Meanwhile, we are determining whether broomrape emits a unique volatile profile after emergence that can be used to screen for infested fields via tractor or drone. We will continue this work at the Contained Research Facility with collaborator Prof Brad Hanson.
2. Test a custom, mobile chemical sensor platform in a broomrape-infested tomato field in nearby Woodland, CA. We will assemble a prototype sensor to deploy in real-world conditions in a commercial tomato field infested with broomrape for roughly 8 years. Working with collaborator Prof Brad Hanson, we will push the sensor on a bicycle-wheeled cart through tomato fields with a known infestation and determine the feasibility to detect broomrape.

Long term, our objective is to develop an inexpensive platform for rapid detection of broomrape for high-level screening of tomato fields throughout California. Our technology could direct growers as to fields that require further inspection. Furthermore, we will have identified metabolites tomato plants related to infection by broomrape, which may provide other scientists with leads for potential treatments/cures as these biomarkers can elucidate biochemical pathways up- or down-regulated by parasitization. We will continue to work with CTRI to ensure our experimental objectives match the needs of tomato growers to combat broomrape.

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: Data from this work was included in our pending National Science Foundation (NSF) Convergence Accelerator program. The proposal seeks to develop chemical sensors to reduce food waste, such as preventing tomato crops from going unharvested due to broomrape. We are thankful that CTRI has agreed to be a participant in that work, should we receive funding. Meanwhile, we continue to seek additional funding opportunities for chemical sensors to be used in agricultural settings.

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EVALUATION OF MATERIALS TO MITIGATE NEGATIVE EFFECTS OF SALINITY AND HIGH TEMPERATURES ON YIELDS OF PROCESSING TOMATOES

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Year of Project Initiation: 2023

Executive Summary: Processing tomato yields can be reduced by stress due to drought, high temperatures and/or high salinity levels. Plant activators applied to either foliage or roots have demonstrated ability to trigger physiological processes resulting in tolerance to stress conditions represent on approach that may, in the short term, alleviate yield reductions due to environmental stresses. Sub-surface drip irrigation is used to irrigate the vast majority of processing tomatoes in California. Yet, the understanding of the distribution of water and nutrients in the soil profile under SDI is incomplete. Potential benefits of altering water movement in the soil profile include improved water use efficiency and decreases in nitrogen leaching. There is the potential of favorably altering water distribution in the soil profile through the use of drip-injected soil surfactants. In two separate studies, under high temperature and drought conditions, effect of Skeepon and of Proliferate soil surfactant on yield and quality of fruit were examined under field conditions at University of California West Side Research and Extension Center. Under the high stress conditions of these studies, there were very few treatment differences. Yields and quality were poor and the materials evaluated neither improved the yields nor the fruit quality. Under the conditions of the 2023 study, which included combinations of salinity, heat and drought stress, Skeepon did not apparently improve production, but there could be benefit under less extreme stress conditions. In addition, Proliferate, the soil surfactant did not apparently increase yields nor improve quality, when applied three times at three- to four-week intervals beginning at eight weeks after planting, it may be interesting to evaluate this material when applied at very early stages of plant development. A second season will provide an opportunity to evaluate materials under conditions in which there are greater differences between drought stress treatments and full irrigation

treatments as well as a sense of the impact of alterations in some of the application timings of the test material on efficacy.

Introduction:

The vast majority of processed tomato product consumed in the US and approximately 30% of the global supply are produced in California (FAO 2021; USDA NASS 2021). Prolonged drought events and regulatory actions limit availability of water for agricultural purposes, 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^{o} – 75^{o}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.

Over 90% of processing tomatoes in California are irrigated by sub-surface drip irrigation (SDI) which helps to reduce evaporative losses, increases irrigation uniformity and greater production per unit of irrigation water as compared with furrow irrigation. However, in this sytem, water and fertilizer distribution in soil profile is poorly understood. 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.

Relative susceptibility of tomatoes to saline soil conditions is moderately sensitive with the salinity threshold at which yield impacts may be expected is 2.2 ds/m^2 with a 9% decline in yield per unit increase in salinity (Tanjiand & Kielen, 2003). Although, the potential of managing salinity with buried drip irrigation exists (Hansen & May, 2003), the salt applied with the water will accumulate in the soil, and potentially increase the levels in the root zone in the absence of leaching events to carry the salts below the root zone. The best leaching efforts will never reduce soil salinity levels below that of the irrigation water used for leaching.

The experiment evaluating mitigation potential of a plant activator applied to transplants will focus on quantification of impacts of these materials under deficit irrigation or salinity on processing tomato yield and quality. Skeepon, stimulates the acetate synthesis, which influences the priming of the jasmonic acid pathway resulting in stress tolerance (Kim et al., 2017). This material is currently being field tested internationally, and this trial is the first evaluation under Central Valley field conditions in California processing tomato production.

In a separate experiment to evaluate the utility of a drip irrigation-applied surfactant to alter the distribution of water in SDI system under field conditions will be documented and the impacts of a soil-surfactant on the yield and quality of processing tomatoes will be assessed. This project has the potential to mitigate negative environmental impacts of production through increased utilization of water and

nutrients and reduce nitrogen leaching as well as increase economic feasibility of processing tomato production that remain challenging despite recent increases in price for the commodity, which is attributable to disproportionate increases in the input costs. The 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 the *Objectives* under that goal:

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. Soil salinity levels were moderate in both trials (EC of 1.39 and 1.09 dS/m in the plant activator and surfactant studies, respectively). On 6 June, the plant activator study was transplanted and the soil surfactant study was planted on 8 June. 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 13 Jun to reduce risk of beet curly top virus. Matrix SG 4.0 oz/a was broadcast on 23 Jun and 0.5 inches of water were applied through sprinklers on 26 Jun for incorporation. Pre-plant soil nitrogen levels were moderate (25- and 19-ppm Nitrate-N at plant activator and surfactant study sites respectively). From five to thirteen weeks after planting, 160 lbs N/acre as UN-32 injected into the drip weekly. On 28 Aug, Agri-Mek SC 3.5 fl oz, Radiant SC 6 fl oz, Warior II 1.9 fl oz and Quadris Top 8 fl oz applied on 28 Aug was applied in a 30 gal/acre mixture for russet mites, worms, stink bugs and powdery mildew. In both studies, we used an irrigation manifold with 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 24 and 25 October, the plant activator study was harvested, and the soil surfactant study was harvested on 24 October. In both studies, 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. Deficit irrigation from 70 to 130 days post-transplant with Westlands Irrigation districtDistrict water (0.4 dSm)
2. Saline shallow well water (9.0 dSm) beginning 1 Aug 2023.
3. Standard irrigation

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 intention was to impose a greater deficit in treatment 1 and no deficit in treatments 2 and 3. 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. The pressurization of the water from the reservoir with saline water was low from 1 to 25 Aug resulted in under irrigation, which caused visible plant stress throughout the trial area.

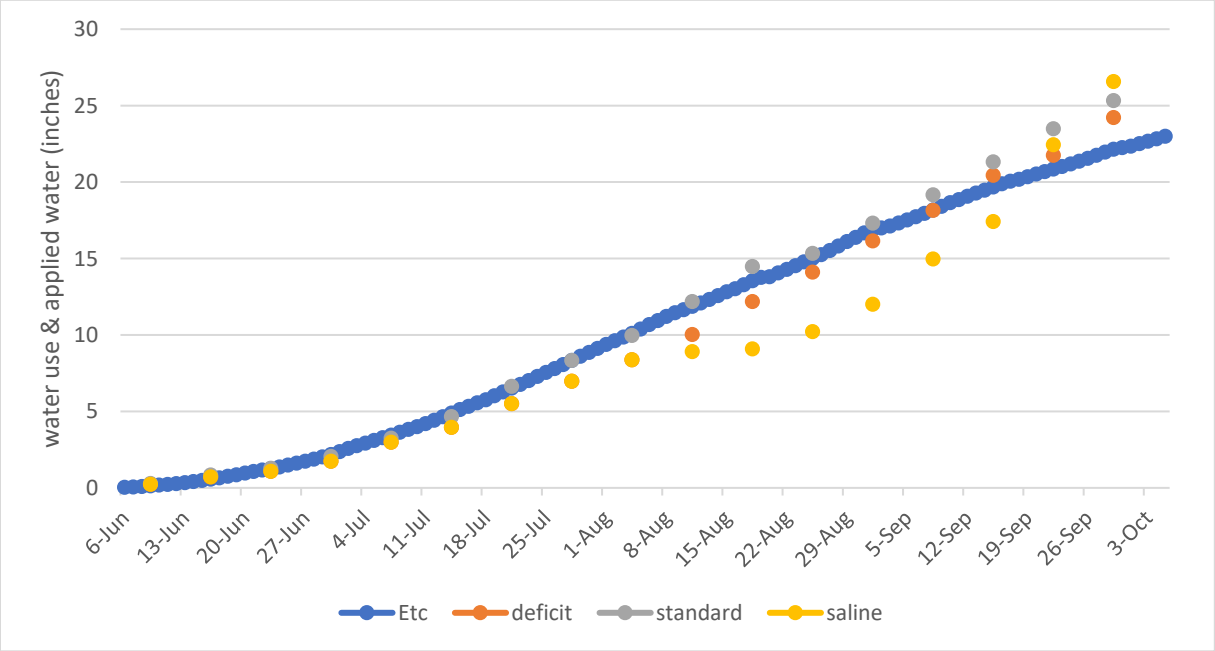

Figure 1. Cumulative calculated water use (evapotranspiration rates CIMIS station #2 Five Points x crop

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 and rots were high attributable to high population densities of Consperse stink bug, which were treated for but are difficult to control under late season conditions (Table 1). Although H1293 variety plants treated with Skeepon had fruit with significantly lower color ratings than fruit of the same variety without the treatment, no significant differences apparently attributable to Skeepon were observed (Table 2). No interactions among irrigation schedule/water quality and variety/Skeepon treatment were observed.

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**		
		(lbs/6 row ft)	red (%)	grn (%)	rot (%)
Deficit irrigation		28.36	62.16	15.93	16.72
Standard irrigation		26.44	63.31	14.24	20.34
Saline irrigation (9.0 dSm)[w]		28.82	63.34	19.09	14.34
Main plot P[v]		0.9141	0.9284	0.1200	0.1475
	H5608 - Skeepon [u]	33.69	67.07	14.08	13.99
	H5608 - No Skeepon	28.08	64.23	17.23	16.32
	H1293 - Skeepon	27.53	62.49	14.13	19.50
	H1293 - No Skeepon	22.19	57.96	20.26	18.72
Sub plot P		0.4343	0.1657	0.0821	0.3892
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] Saline water was used for all irrigations in that main plot treatment after 30 July.

[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 6 Jun.

Table 2. Influence of Skeepon plant activator on fruit quality character in Fresno County, 2023.

main plot[z]	sub plot[y]	wt/ 40 fruit (lbs)	PTAB[x]		
			color	solids	pH
Deficit irrigation		2.98	20.3750	5.3500	4.5681
Standard conditions:		2.93	20.5625	5.5938	4.5563
Saline irrigation (9.0 dSm)[w]		2.90	19.9063	5.6938	4.6000
Main plot P[w]		0.9553	0.2259	0.2254	0.3927
	H5608 - Skeepon[v]	3.10	19.5417 b[u]	5.2750 a	4.5058 b
	H5608 - No Skeepon	2.80	20.0000 b	5.3250 a	4.5667 ab
	H1293 - Skeepon	3.07	20.1667 b	5.7250 a	4.6233 a
	H1293 - No Skeepon	2.77	21.4167 a	5.8583 a	4.6033 ab
Sub plot P		0.55	0.0012	0.0375	0.0193
Main x sub plot P		0.66	0.5701	0.7436	0.5306

[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] Saline water was used for all irrigations in that main plot treatment after 30 July.

[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 6 Jun.

[t] Means within a row that are followed by a different letter are statistically different at P=0.05 according to Tukey's HSD.

<u>Soil Surfactant Evaluation</u>

The performance Proliferate under three irrigation schedules, on two varieties was evaluated in a four-replication randomized complete block experimental design. Treatments are as follows:

1. Proliferate - based on Sony soil sensors.
2. Proliferate - schedule a
3. Proliferate - schedule b
4. No Proliferate - based on Sony soil sensors
5. No Proliferate - schedule a
6. No Proliferate - schedule b

Proliferate, a penetrating humectant soil surfactant, was injected through the drip irrigation system at the equivalent of **2 quarts/per acre** into treated rows over 30 minutes, followed by 2 hours of irrigation on 28 Jul, 18 Aug, and 18 Sep.

Differences in applied water within the three irrigation schedules were subtle and average applied water over the season exceeded the evapotranspiration rates x crop coefficient (Fig 2). Sony sensors, loggers and equipment for remote access functioned well. Software was easy to access and relatively easy to get to accumulate information. However, to make the data available to other programs, such as Microsoft Excel requires coding, so there is room for improvement.

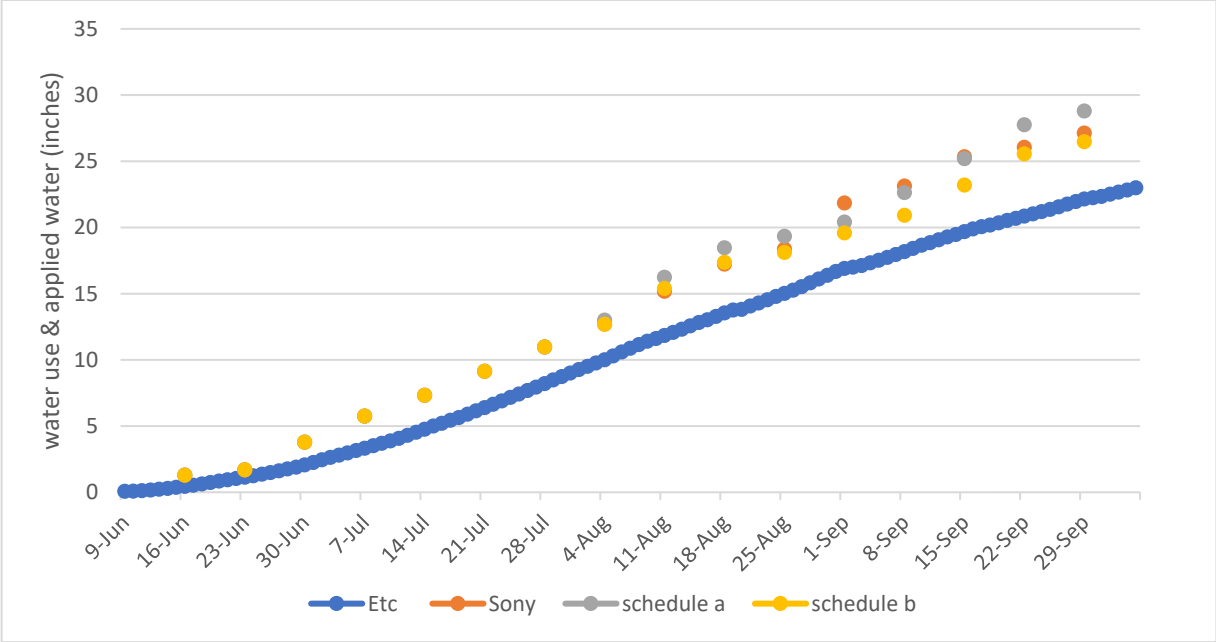

Figure 2. Cumulative calculated water use (evapotranspiration rates CIMIS station #2 Five Points x crop coefficient based on canopy coverage) and water applied in each main plot irrigation treatment in the soil surfactant evaluation.

There were no significant differences in yield or quality among treatments (Table 3). Overall, yields were low, and rots and greens were high. However, there were periods of several consecutive days during the season during which no water was available at this field due to station infrastructure issues. In addition, high temperatures during critical bloom periods in July and August contributed to extremely low yields across all treatments. As with the plant activator trial, stink bug population densities reached damaging levels and contributed to the high levels of rot observed.

Table 3. Influence of Proliferate soil surfactant on processing tomato (cv. H5608) yield and quality in Fresno County, 2023.

Surfactant treatment[z] – irrigation schedule	Total fruit (lbs/6 row ft)	Hand sort of 15 to 25 lbs fruit[y]			wt/40 fruit (lbs)	PTAB[x]		
		reds (%)	greens (%)	rot (%)		color	solids	pH
Proliferate - Sony	26.43	48.78	10.36	31.07	3.22	19.250	4.400	4.6875
Proliferate - schedule a	33.83	56.77	11.96	23.23	3.59	20.000	4.475	4.6425
Proliferate - schedule b	31.87	53.69	13.38	24.91	3.49	20.000	4.475	4.6675
No Surfactant-Sony	33.52	55.04	13.27	30.71	3.29	19.375	4.775	4.5725
No Surfactant-schedule a	29.96	50.62	9.36	24.29	3.41	20.625	4.650	4.6525
No Surfactant-schedule b	33.38	56.46	12.37	23.48	3.58	20.125	4.350	4.6925
P[w]	0.8337	0.6510	0.1889	0.7310	0.8865	0.2233	0.5145	0.5341

[z] Proliferate, a penetrating humectant soil surfactant, was injected through the drip irrigation system at the equivalent of 2 quarts/per acre into treated rows over 30 minutes, followed by 2 hours of irrigation on 28 Jul, 18 Aug and 18 Sep.

[y] 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.

[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.

Discussion:

Production of late season tomatoes is consistently challenging in Central California. However, the conditions present in the June-planted were particularly unfavorable. Very high temperatures were present at critical stages of flowering and early fruit development: High temperatures from the high 97^{o} to 105^{o}F occurred from 13 Jul to 1 Aug (Fig. 3). While the plant activator tested has provided tolerance of heat and drought stress in tomato in greenhouse studies, we did not see evidence of that performance in the Fresno County Study.

In the studies conducted in 2023, performance under extreme conditions were evaluated and these products did not show promise. 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.

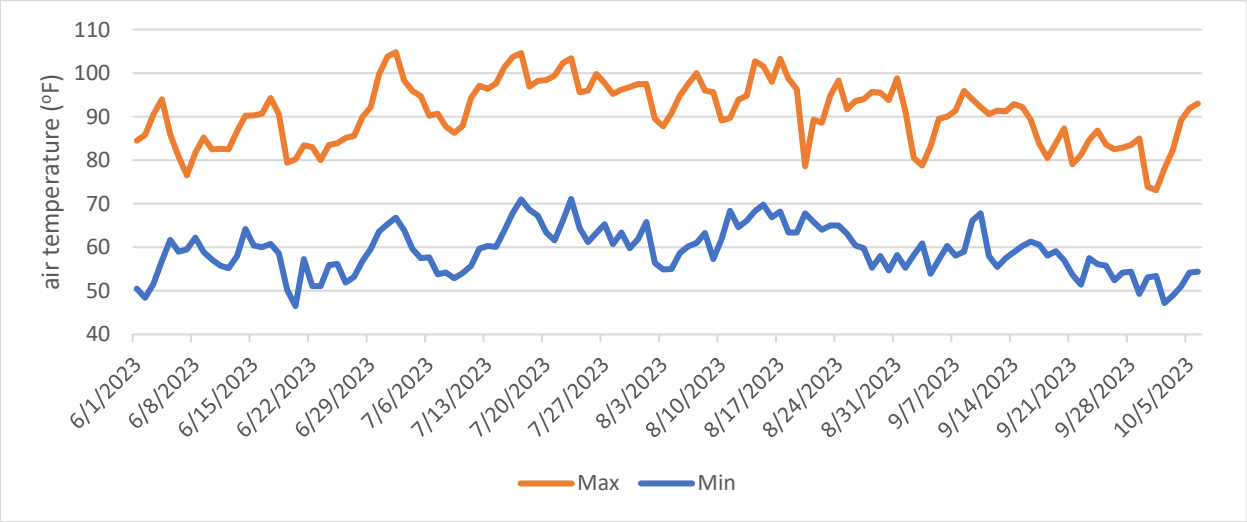

Figure 3. Daily high and low temperatures at Five Points from June to early October, 2023.

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Isaya Kisekka received funding from Sony and from AC-Planta, which was used to support travel to site by Felix Ogunmokun and additional plant and soil analysis, which is currently in progress.

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CLIMATE SMART MANAGEMENT INNOVATIONS FOR IMPROVED SOIL QUALITY, AND PRODUCTIVITY OF CALIFORNIA PROCESSING TOMATOES

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Year of Project Initiation: 2023

Executive Summary:

California processing tomato growers face unprecedented climate pressures from increased annual average temperatures and drought conditions. 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 create new growth opportunities. There is however a critical gap and need to capture and categorize the diversity of CS 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, yields and input use. The unique partnership we created in year 1 between private industry and academic researchers allowed us to start quantifying the effect of these practices on soil quality indicators, nutrient use efficiency, and yields across 19 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 an outreach publication in year 3 for processing tomato growers located in the Sacramento Valley; soil quality, 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 resources and 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:

The California processing tomato industry is under intense pressure from increased annual temperatures and the prolonged decades-long megadrought in the Central Valley (CV), with these trends set to continue[1]. Over the past decade effects have been hard-felt via major water restrictions[2] and direct impacts on reduced state-wide processing tomato production for 2022[3]. The winter rains in 2023 provided some reprieve from the drought, with a 13% increase in tomato acreage in 2023 compared to the year prior, but long-term trends still indicate intense drought pressure. California tomato growers contribute 30% of the world's processing tomatoes, a critical contribution to the global food system with a nutritious fruit product that can be stored for long periods[4]. The market opportunity for processing tomatoes remains strong, but future economic viability for California growers will require the identification of feasible Climate Smart (CS) management practices that buffer against and provide resilience against these environmental pressures.

There is growing interest in supporting markets and incentives for CS 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, water use efficiency, input use/efficiency, and soil quality 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[5,6]. 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[7]. 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[8]. 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[9]. Yields in the organic treatment were similar to conventional after 24 years, with increased resistance to losses in bad years, an important climate consideration.[10] Similar soil carbon impacts were realized after 10 years of cotton-tomato rotations at the UC WSREC experiment station in the reduced tillage and cover crop treatment, with a 9.5% increase in yield over the conventional tillage and no cover crop treatment[11]. Some studies also suggest fruit nutritional content can be higher in organically managed systems, related to flavonoids at the UCDCE[12] and sugars and vitamin C in studies from other parts of the world[13–15], a potential additional yield outcome consideration. Although long-term trials provide important insights, there are limitations with highly controlled field station experiments. Furthermore, these 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 quality 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, 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 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 and could help elucidate some earlier outcomes as well as long term management legacies.

Previous o n-farm field trials at 13 organically managed farms in Yolo county that produce processing tomatoes provide insights on mechanisms of importance to soil quality 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)[16]. These benefits are realized across various soil types and operations, highlighting the potential for growers across the CV to reap these benefits[17]. With regard to nutrient management, farms which experienced optimized C and N cycling and performance were those that included an additional N input (guano, pellets, or Chilean nitrate)[16]. 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[9]. These surprisingly different effects of two popular practices for building soil quality further indicate the importance of stacking practices and avoiding a one-size-fits-all approach.

Climate Smart strategies that build soil quality 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 an additional 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.[18,19] In California, the most popular CS practices implemented on organic and conventional farms include crop rotations, compost and/or manure applications and other organic amendments, and a growing interest in cover crops. 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 can 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,[20] 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 which set of practices, on a gradient from none/few to multiple stacked CS practices, yield the most complementary approach for building soil quality, improving water use efficiencies, improving nutrient use efficiency, 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 quality, sustainability, and yield outcomes of CS practices and identify pertinent soil indicators
2. **Objective 2 (year 1&2):** 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: Progress has been made in organizing the UCD CE dataset and in conducting a preliminary analysis and draft of a manuscript. This analysis leverages a one-year data consolidation effort by PhD student Peter Geoghan and the building of a yield, input, and soil indicator database now poised for analysis. Efforts will be 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 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 + compost and cover crops), and an organic treatment. Work on both the literature review and data analyses will continue and be prioritized in year 2 (2024).

Objective 2: We created and disseminated a comprehensive online intake survey to capture grower management practices and field characteristics for fields that growers were interested in including in our study. Fields were required to be under the same management practices for 5+ years for us to quantify the legacy effects of those practices on soil health, input use and yield and fruit quality outcomes. Recruitment for the study began in late February 2023 and concluded in mid-June . Most of respondents were from the Sacramento Valley, which dictated our geographic focus on counties west and north of Davis. Nine grower cooperators were included in the study, with a total of 18 fields. Climate smart practices included in this study were crop rotations, cover crops, conservation tillage, compost application, grazing, integrated pest management and presence of natural habitat around fields. Six fields include the implementation of multiple stacked CS practices, 5 fields with the adoption of 2 practices, and 7 fields with none or one practice were selected. This represents the low end of our goal to sample 18-24 fields, but reflects the difficulty in receiving responses from growers, follow-up clarifying questions on their practices, and the hardships everyone faced in 2023 with weather related transplanting delays and replanting fields. The intake survey can be accessed from the link below: https://ucdavis.co1.qualtrics.com/jfe/form/SV_5sQT85omwhh66yO.

Soil samples were collected (June-July, Figure 1), during full bloom/early-fruit development which is when we expect plant-soil interactions to peak as the plants are concentrating energy into flower and fruit production. During this phenological phase, we expect that microbial activity would be highest in the soil, and provide an approximation of how well the soil ecosystem is supporting tomato production. Sample collection occurred on sections of fields with the same soil type/texture and on single varieties, to minimize potential confounding factors. Soil samples were taken from three randomized locations (plot) per field. A single sample was taken from six beds in each plot along a diagonal transect across the field, at 0-15cm and 15-30cm depths. Soils from the six beds, at each depth were then composited into a single

sample per plot, per depth. We started sample analyzes in fall 2023 and will continue through spring 2024. Early results were shared at the 2023 CTRI winter meeting. Table 1 indicates the functions that are of interest to cropping systems, the indicators we are measuring for that are associated with those functions, and the important insights those will provide growers.

Figure 1. *Photos from field season: (From upper left hand corner). Photo (1): fields were sampled at full bloom - early fruit development. (2-3): auger used to sample soil at two depths (0-15cm, 15-30 cm). (4): ruler used to ensure consistent sampling depth. (5) bulk density sample, (6) Team members sampling along one of the diagonal transects, (7) 1x1m harvest sampling transect, (8) fruit sample processing in the Gaudin lab.*

Soil Ecosystem Function	**Indicators**	**Insights for Growers**
Soil fertility	**-Cation Exchange Capacity** **-pH** **-Macro/micro nutrient availability** **-Soil electrical conductivity** -Potentially mineralizable nitrogen (PMN) -Organic/Inorganic N pools	Ability of soils to store/retain nutrients, nutrient use efficiencies, crop available nutrient pools, salinity.
Soil water/water use efficiency	**-Water holding capacity** -Infiltration	Water use efficiency, cycling, and availability to 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** -Total soil organic carbon (SOC) -Minerally associated organic carbon (MAOC) -Active carbon (PoxC) -Carbon mineralization potential	Energy and nutrient flow potential for crop productivity and carbon storage potential.
Soil microbial and ecosystem communities	-**Phospholipid Fatty Acids (PLFAs)** -**Microbial biomass** -Respiration -C&N cycling enzymes	Ability to build soil quality, optimize and ensure function related to crop productivity is supported.

*Table 1. Indicators **bolded on the table** are those for which we currently have raw data for and will have some preliminary data analyses for in the coming months.*

Early results indicate lower nitrate levels in systems with stacked applications of climate smart practices and mixed managed fields (15-30cm, Figure 2). The larger pool of phospholipid fatty acid biomass (PLFA) (15-30cm) in these fields, an indicator of overall microbial biomass, may suggest that N in these fields is being retained more efficiently by the soil ecosystem. Increased nitrates in the conventional system, if not taken up by the plants or soil organisms, is subject to potential loss through leaching or off gassing during nitrification and denitrification as dinitrogen gas (N_2) or nitrous oxide (N_2O). Forthcoming data on potentially mineralizable nitrogen and enzymes associated with the nitrogen cycle, coupled with input data will provide further insights into these dynamics and nitrogen use efficiency. Some additional data will be shared at the 2024 Campbells Sustainability Summit (February 21-22). We are happy to share the slide deck if that is of interest to the board. Additionally, we will share the slide deck with all growers in the cohort, along with any field specific data we have available for them at that time.

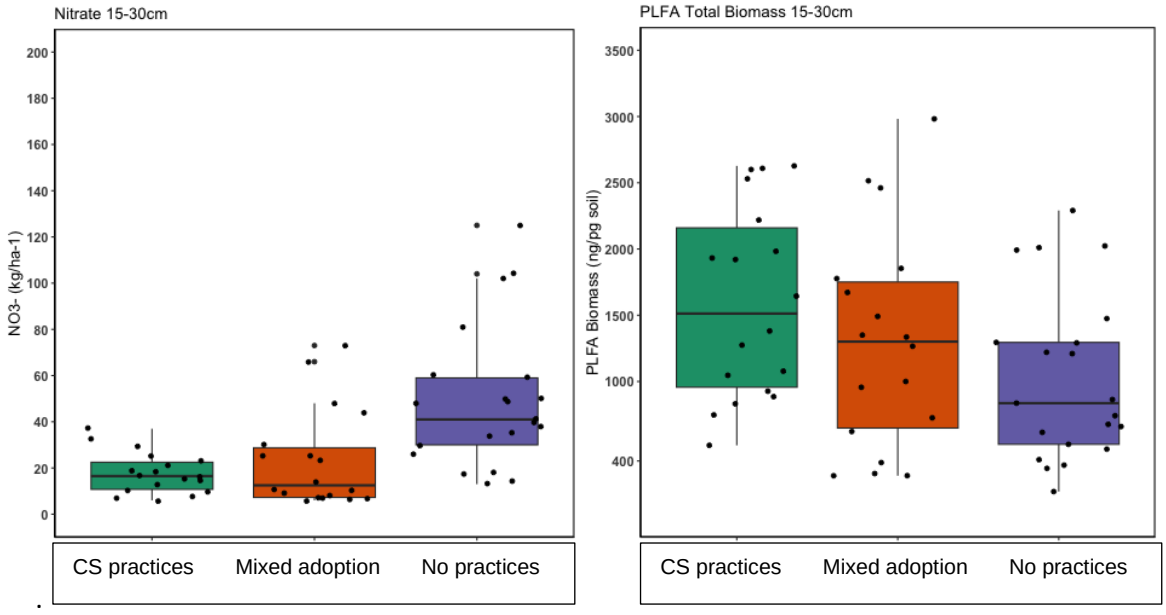

Figure 2: Nitrate and PLFA total microbial biomass in the 18 fields arranged in 3 categories. Six fields include the implementation of multiple stacked CS practices, 5 fields with the adoption of 2 practices (mixed adoption), and 7 fields with none or one practice.

Discussion

The diversity of practices in our study reflects various strategies prominent growers use to maintain the leadership of the California processing tomato industry in the Sacramento Valley. Additionally, in conversation with growers it is evident that there is interest in understanding how their management impacts soil health and metrics associated with input reduction and ecological regulation. Some have shared barriers to adoption, and changes in management due to operational shifts and market fluctuations. Cover crops, which are a popular practice in regenerative agriculture and provide benefits to the soil, can be difficult to implement due to constraints faced by processor contracts (i.e. the additional operations can complicate spring transplanting). Sub-surface drip, while creating opportunities for water-use efficiency, can limit other practices like alfalfa in rotation and the integration of livestock during this phase of the rotation. Finally, prices have not made sense for the organic certification for some growers in recent years.

During winter 2024 we are conducting follow-up interviews with growers to verify their management practices provided to us in the intake survey, to gather more nuanced management data which will help us create a more fine-tuned management gradient for data analyses, and to capture yields from the 2023 growing season on fields we sampled. We do not have any recommendations ready for the CTRI board nor the larger processing tomato community at this time; we are still in the process of gathering data, analyzing samples in the lab, and conducting initial data analyses. Visualizations of some of the available data do however provide some potential insights on where some of the results may lead to. We caution against these without further statistical analyses, in the absence of more nuanced management data, and prior to having the full data set we set out to capture from Table 1.

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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 ($56K) 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.

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QUANTIFYING THE EFFECTS OF K-PAM ON SOIL-BORNE DISEASE AND YIELDS IN LOWER SACRAMENTO VALLEY PROCESSING TOMATO FIELDS

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Year of Project Initiation: 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

Two field trials were established in Yolo and Solano counties. Both fields have a history of FRD pressure. At each site, twenty adjacent beds were chosen in areas of the field where FRD was historically high. 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 two 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. At the Yolo site only, samples were submitted to the Processing Tomato Advisory Board for quality analysis.

Results

At the Yolo site, pathogens detected included all three identified strains of the *F. falciforme* complex, Fusarium wilt (*F. oxysporum fsp. lycopersici*) and southern blight (*Sclerotium rolfsii*). Disease pressure was relatively high and none of the plant health metrics differed significantly between fumigated and nonfumigated plots at any time during the season. Disease pressure was both high and variable across the site, with the proportion of severely declined or dead plants averaging 55%, ranging from 26% in the lowest pressure plot to 84% in the highest. Yields were on average 4.6 t/acre higher in the fumigated than the nonfumigated plots, but due to high variability the confidence of a statistically significant difference between the two treatments was below 95% (p=0.055).

At the Solano site, all three strains of the *F. falciforme* complex were detected, while other pathogens were not. Symptom onset was later and symptoms were less severe than at the Yolo site. The proportion of severely declined or dead plants averaged 16%, ranging from 3% in the lowest pressure plot to 46% in the highest. Fumigation significantly reduced the proportion of dead and declined plants (p=0.008 or >99% confidence). Yields averaged 3.5 t/acre higher in the fumigated than the non-fumigated rows.

Neither site showed significant differences in the proportion of fruit culls, including sun damage or rots. Processing quality was only measured at the Yolo site, for which no significant differences were observed in fruit pH, Brix or color.

Discussion and Recommendations

Fumigation with K-Pam at 30 gal/acre costs an estimated $300/acre. Thus, at the 2023 price of $138/ton the mean yield boost from fumigation was sufficient to offset the cost of the fumigant at both sites. The average profit from K-pam, given the observed average yield increases, was $368/acre at the Yolo site and $181/acre at the Solano site. However, given the relatively narrow margin and the high variability in yields and disease pressure (particularly at the Yolo site) our results suggest that K-Pam's profitability is not a certainty. This is 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.

The high variability in disease pressure and yields at the Yolo site was likely in part due to the presence of southern blight, which has very similar symptoms to FRD but is not controlled by fumigation through subsurface drip. This pathogen is considered to be common in soil, but needs hot, wet conditions to infect tomatoes and is generally only problematic in California in hot weather when there is free soil moisture near the soil surface. The observation plots were located near the tail end of a long field that had issues with irrigation leaks, which likely led to greater soil moisture near the surface. The relatively low yield difference between fumigated and unfumigated plots at the Solano site may have been in part due to the choice of variety. The field location was chosen for its high previous disease pressure but even non-fumigated rows were generally healthy. The field variety, HM 8237, is a vigorous, large-vined plant that has consistently shown low vine decline in trials testing varietal tolerance to FRD in historically affected sites.

Taken with results from previous chemical trials, our results suggest that while fumigation can be profitable, the effects are variable. More research is needed to better understand the conditions under which fumigation is likely to be most effective. Our results from 2023 suggest that factors which may compromise K-Pam's effectiveness include the presence of other pathogens which are poorly controlled by fumigation and poor irrigation uniformity. The results from the Solano County site suggest that K-Pam

fumigation may confer some additional yield benefits even when a tolerant variety is used, but that this benefit may be marginal. Given an estimated cost of $300-$400 per acre for fumigating with K-Pam at 30-40 gal/acre, the 3.5 t/acre yield gain observed at the Solano site would break even at tomato prices of $85-$114/ton.

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 four 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 (Fig. 1).

Figure 1. Summary of chemical trials in Yolo and San Joaquin counties. Taken from Aegerter et al. (2023), used by permission

Summary of seven field trials including fungicides and/or fumigants

year	2019	2019	2019	2019	2019	2020	2021
location	UC Davis	UC Davis	Yolo Co	San Joaquin Co	San Joaquin Co	San Joaquin Co	San Joaquin Co
disease(s)	Fol	Ff	Ff	Fol	Ff	Fol & Ff	Fol & Ff
Product							
K-Pam ~30 gal	++	NT	NT	++	+ 7.2 t/a	+	+ 26 t/a
K-Pam ~15 gal	-	NT	+ 11.9 t/a	NT	NT	+	+ 13.6 t/a
Miravis	++	+	NT	++	NT	+	+ 9.2 t/a
Rhyme	-	NT	NT	-	NT	+	+ 10 t/a
Velum	-	+	NT	-	NT	-	NT
Disease level in non-treated control	68% vine decline	47% rot	73% rot	37% vine decline	20% vine decline	31% vine decline	30% vine decline
Disease *P value*	P < 0.05	NS	NS	0.01	not tested	0.06	0.0004
Yield *P value*	NS	NS	0.01	NS	0.016	NS	0.015

NT = not tested " + " = weak (statistically speaking) positive effect " ++ " and green shading = statistically significant positive effect, NS = not significant

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 f sp 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*
- *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 with a history of decline due to soilborne disease. 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 5/21 and 5/31 (24 and 40 d following fumigation) at the Yolo and Solano sites, respectively. The variety BP 74 was used at the Yolo site, and HM 8237 was used at the Solano site.

Prior to fumigation I took soil samples to determine initial nutrient status. The Solano field had a light-textured soil and the Yolo field was coming out of alfalfa, both of which are predisposing factors for potassium deficiency. However, the results of the analysis suggested both Solano and Yolo sites were in a sufficient range for processing tomato production (202 ppm K and 232 ppm K, respectively). Initial soil samples were also taken for nematode analysis, as the Solano site had a history of root knot nematode (RKN) pressure, to provide baseline values for comparison should nematode damage start to manifest during the season. However, although samples did suggest high, relatively uniform RKN populations at the Solano site (on average 500 RKN per 200 ml of soil, in comparison to an average of 10 RKN per 200 ml soil at the Yolo site), little to no galling was observed during the season on either fumigated or unfumigated rows. 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 1). Early in the season I counted all symptomatic plants, for each making a provisional diagnosis of disease and a severity rating (Table 2). I gave each a qualitative rating to each row based on overall appearance, and took a measurement of average NDVI using a Trimble Greenseeker. A difficulty at both sites (particularly Solano) was the presence of tomato spotted wilt virus (TSWV). TSWV can have very similar symptoms to FRD, including deformation and necrosis of new leaves and shoot yellowing and dieback. At the Solano site in particular, the variety used presented with symptoms very similar to those of FRD, especially for plants infected early in their development. Since TSWV is not soil-borne and is not expected to be affected by the fumigation treatment, its presence in a field can compromise disease assessment accuracy. I therefore also estimated rate of TSWV infection using AgDia Immunostrips, and when counting affected plants excluded those with clear TSWV symptoms. Twice during the season, 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.

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 1. Disease and NDVI assessment dates at the Yolo and Solano field sites. Assessments started at the onset of first symptoms and were performed approximately bi-weekly until harvest. WAT=Weeks after transplanting. The Yolo site was planted on 5/22 and harvested on 10/16, and the Solano site was planted on 5/31 and harvested on 10/27.

Yolo		Solano	
Stage	**WAT**	**Stage**	**WAT**
Early green fruit	8	Early green fruit	8
Late green fruit	10	Late green fruit	10
Early red fruit	12	Early red fruit	12
50% red fruit	14	50 % red fruit	14
75% red fruit	16	75% red fruit	16
Red fruit	19	Red fruit	18
Just before harvest	20	Red fruit	19
		Just before harvest	20

Table 2. 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 for either site (Fig. 2a). At the Solano site this may have partly been due to a high incidence of TSWV symptoms, which began early in the season, spread, and closely resembled those of FRD in severely affected plants. When TSWV counts at the early red fruit stage were added as a covariate to the model, the slight NDVI difference between K-Pam and control plots at harvest became moderately significant (p=0.10).

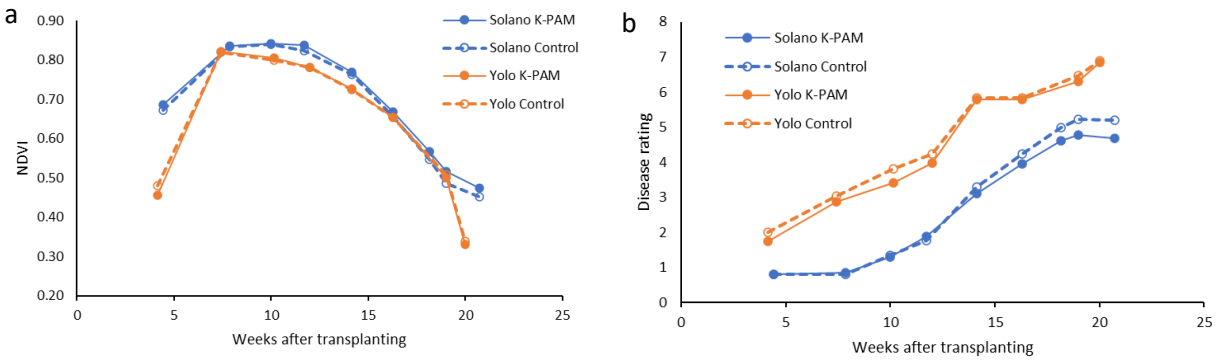

Figure 2. Average a) NDVI readings and b) soilborne disease visual rating for fumigated (K-Pam) and non-fumigated (Control) plots (n=10). Ethrel was applied at the Yolo site at 18 WAT.

Visual disease ratings did not differ between treatments at the Yolo site, although there was a slight tendency throughout in the early season for the control plots to have slightly higher average ratings than the K-Pam plots (Fig. 2b). Disease ratings became more difficult towards the end of the season at the Yolo site, as Ethrel was sprayed at 18 weeks after planting to help aid ripening. The Solano site had larger vines, and less disease pressure throughout the season. Average disease ratings started to be higher in the control plots at around 50% ripe fruit stage, and by harvest were significantly (p=0.007) higher than the average from the K-Pam rows (Fig. 2b).

According to samples submitted to the fungal pathology lab from plants with characteristic symptoms, diseases present at the Yolo site included FRD (*F. martii*, *F. noneumartii*), foot rot (*F. falciforme sensu stricto*), fusarium wilt (*F. oxysporum fsp. lycopersici*), and southern blight (*Agroathelia rolfsii*). Only FRD (*F. martii*, *F. noneumartii*) and foot rot (*F. falciforme sensu stricto*) were identified at the Solano site.

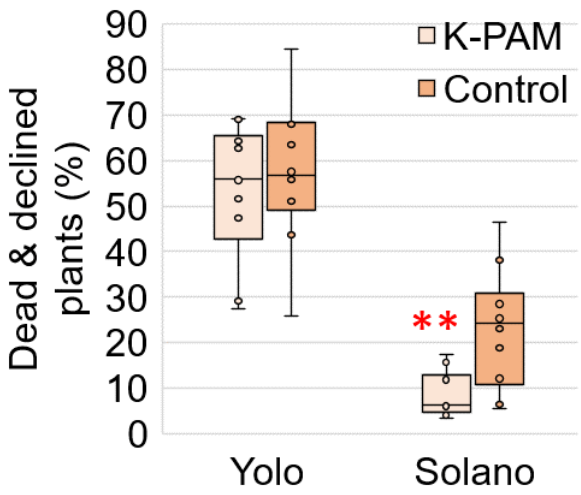

Figure 3. Percentage of dead and severely declined plants just before harvest (n=10)

The proportion of declined and dead plants were counted just before harvest. If fruits on the declined plant showed symptoms of TSWV, the plant was uprooted in order to determine if crown rot was also present, and if not the plant was not counted. At the Yolo site, the proportion of dead and severely declined plants ranged between 25% and 85% and did not differ between treatments (Fig. 3). At the Solano site, the proportion of dead and severely declined plants ranged between 4-46%, and was significantly lower in the K-Pam than control plots.

Disease pressure was not uniform at either site. In both sites, both K-Pam and fumigated rows manifested disease symptoms in patches, such that some rows under both treatments had patches of declined plants, and others did not (Fig. 4). At the Solano site, where the K-Pam reduced the proportion of dead and declined plants, vine decline was consistently less severe in the fumigated rows. In the Yolo site, this was not consistently the case.

Figure 4. Vine decline in alternating fumigated and non-fumigated rows in a diseased patch.

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

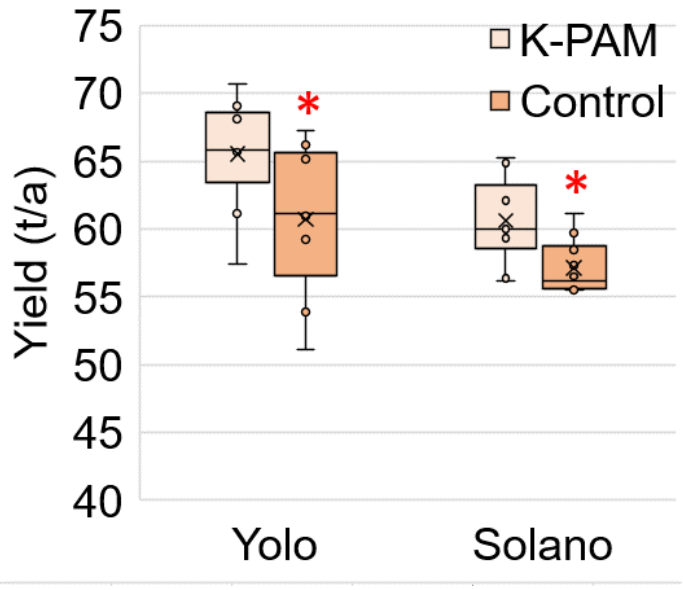

Figure 5. Total yields at Yolo (n=8) and Solano (n=10) sites. *Indicates significant difference between treatments within a site at p<0.05.

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. At the Yolo site only, 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.

The Yolo site had consistent irrigation leaks throughout the season. One non-fumigated row was discarded as an outlier, due to an early persistent irrigation leak which caused stunting along the whole row. One K-Pam row was discarded as an outlier, as equipment going through soil wet from a large irrigation leak cast large clods of soil on the bed surface all along the plot, interfering with the harvester. With both these rows excluded, the average yield for the treated rows was 65.5 t/acre and untreated rows was 60.7 t/acre), a difference of 4.8 t/acre (Fig. 5). The difference was significant (p=0.02). However, when only the stunted row was excluded, the difference was 3 t/acre and was no longer significant (p=0.09). The average yield for the treated plots was 60.6 t/acre while the untreated plot yield was 57.1 t/acre, with a difference of 3.5 t/acre (p=0.015).

No differences were measured at either site for the proportion of marketable fruit. At the Yolo site, treatment had no significant effect on fruit pH, color or Brix.

Objective 3: Share results

Results have been or will be shared with the CTRI, other researchers, and the processing tomato community through meetings including an annual California Tomato Conference in Napa, the 2023 CTRI research meeting, the 2023 University of California Cooperative Extension Vegetable Crops Program Team Meeting, and regional processing tomato production meetings for the southern Sacramento Valley and the northern San Joaquin Valley. I will also share summaries of the results through my email newsletter.

Discussion:

Previous trials with K-Pam have shown a variable effect, with yield differences ranging from slightly negative to 26 t/acre. The average yield gain from K-Pam reported from 15 trials conducted from 2017-2021 by Aegerter et al. (2022) was 9.3 t/acre. The results of the 2023 trials showed a significant effect of K-Pam at both sites; however, the average increases were lower than the average from former trials. Both sites were both relatively high yielding (above the statewide projected average of 50.8 t/acre) even in the non-fumigated rows and despite the high rate of vine decline at the Yolo site. Additionally, both sites were

planted late May, were still green and had full canopy coverage during the high July heat waves, and ripened in fairly mild weather. Since one of the mechanisms by which vine decline leads to decreased yields is through increases in sunburn and mold damage, this planting window may have also contributed to the low yield differences and the fact that there were no differences in the percent marketable fruit between treatments. The good performance of the non-fumigated plots at the Solano site could also have been partly due to the choice of variety. The field variety, HM 8237, is a vigorous, large-vined plant that has consistently performed well in trials testing varietal tolerance to FRD in historically affected sites. The Yolo variety, BP 74, has shown below-average vine decline in some trials but not in others.

Although pre-season soil testing showed soil available K to be in the published sufficiency range for processing tomato at both sites, there is a possibility that the addition of potassium (K) from K-Pam contributed towards the observed yield increases. In particular, the Solano site had a sandy soil and exchangeable K levels near the lower thresholds identified through research in the 1990s as adequate for processing tomato (Hartz et al., 1999). Some symptoms resembling K deficiency were noted (Fig. 6), but as they occurred with low consistency and severity and manifested late in the season, I did not take samples for testing. However, the fact that the yield differences at the Solano site were more consistent than were the differences in disease severity (Figs. 3,5) suggests they may have been partly due to some other factor which was more uniform across the site. Interestingly, experiments have shown *F. oxysporum fsp. lycopersici* to affect tomato plants more severely under low-K conditions (Foster and Walker, 1946), and in general K sufficiency is associated with improved disease resistance across many crops (Goss, 1968). In future experiments, especially if conducted on sandy soil, leaf K monitoring should be conducted.

Figure 6: Yellow shoulder and marginal leaf necrosis and curling observed at the Solano site, suggesting potential K deficiency

The high variability in disease pressure and yields at the Yolo site was likely in part due to the presence of southern blight, which has very similar symptoms to FRD but is not controlled by fumigation through subsurface drip. This pathogen is considered to be ubiquitous in soil and is generally only problematic in

California under unusually wet, hot conditions such as characterized 2023. The observation plots were located near the tail end of a long field that had issues with irrigation leaks, which likely led to greater soil moisture near the surface, facilitating southern blight infection. Fusarium wilt was also present at relatively high rates, considering that an F3 variety was used. However, earlier studies suggest K-Pam can be effective at reducing vine decline in Fusarium-wilt infected sites, even at relatively high disease pressure (Fig. 1). Thus, it's less likely that Fusarium wilt was a major factor reducing the difference between the two treatments.

At the 2023 tomato price of $138/ton and assuming fumigation with 30 gal/acre of K-Pam costs $300/acre, these yield differences represent an average profit of $367/acre at the Yolo site and $181/acre at the Solano site. Given an estimated cost of $300-$400 per acre for fumigating with K-Pam at 30-40 gal/acre, the 3.5 t/acre yield gain observed at the Solano site would break even at tomato prices of $85-$114/ton. However, due to the wide variability in the disease pressure the certainty on these estimates is not very high. Taken with results from previous chemical trials, our results suggest that while fumigation can be profitable, the effects are variable. Factors which may compromise K-Pam's effectiveness include the presence of other pathogens which are poorly controlled by fumigation, and poor irrigation uniformity. The results from the Solano County site suggest that K-Pam fumigation may confer some additional yield benefits even when a tolerant variety is used, but that this benefit may be marginal.

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, 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.

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T G R C

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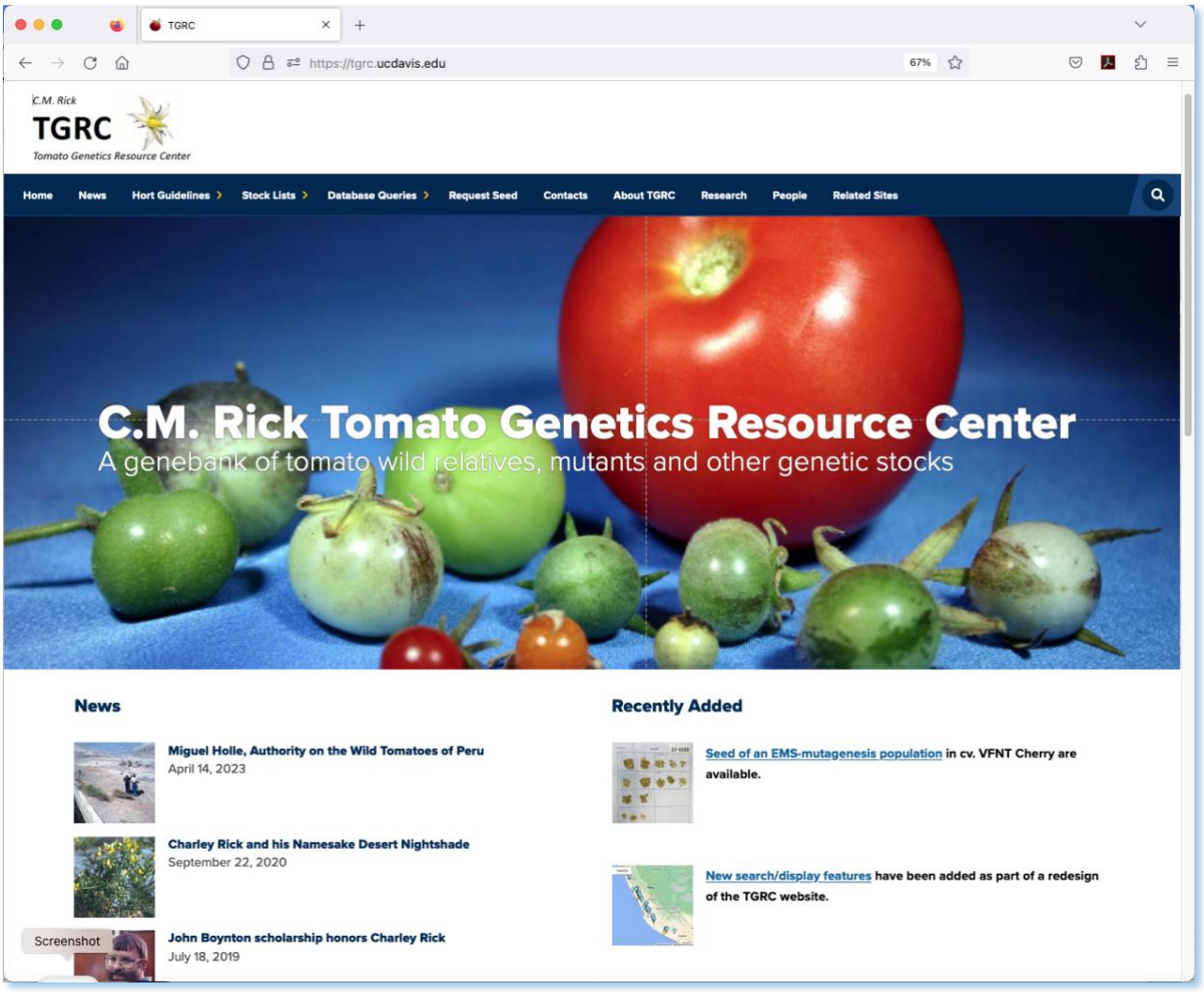
ANNUAL PROGRESS REPORT

2023



The TGRC website was redesigned and rewritten. The new site is more stable, faster, and provides additional search features and fresh content across the site.

SUMMARY

Acquisitions. We acquired 57 new accessions in 2023, most of which were a set of backcross inbred lines, donated by Dani Zamir, representing the genome of *S. pennellii* LA5240 in the background of the processing variety 'LEA'. These BILs are part of a larger set of 1400 lines that can be used study the interactions between genes controlling complex traits (epistasis). We also acquired two new stocks of cv. Rutgers: one from the USDA to replace our previous source of this variety because it had the wrong phenotype, and the other a Rutgers line with the *Ve* gene, which we obtained from Dina St. Clair at UC Davis. Several previously inactive wild species accessions that had not been multiplied before were 'rescued' from our long-term seed storage and are now available. The total of number of accessions maintained by the TGRC is now 4,535.

Maintenance and Evaluation. Over 1760 cultures were grown for various purposes, of which 433 were for seed increase, including 78 wild species accessions. Germination tests were run on 648 seed lots. Progeny tests were performed on 43 stocks of male-steriles and other segregating genes, or to check accessions with unexpected phenotypes. GMO tests were performed on 61 recently acquired stocks, all of which were negative. *S. sitiens* introgression lines were grown for marker assisted selection or for heat tolerance testing. All plants were monitored throughout development for evidence of disease. An outbreak of ToMV was detected in our fall greenhouse plantings but was eradicated. 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. 171 samples were sent up to the USDA's seed storage facility in Ft. Collins for off-site security backup.

Distribution and Utilization. A total of 4,763 seed samples representing 1,786 different accessions were distributed in response to 210 requests from 161 researchers and breeders in 21 countries. The overall utilization rate (# samples distributed / # active accessions) was 105%. Over 20 purely informational requests were also answered. Information provided by requestors indicates our stocks continue to be used to support a wide variety of research and breeding projects. Our annual literature search uncovered 110 publications that mention use of TGRC stocks.

Documentation. Our website was completely revised to improve stability and to provide a user interface consistent with other campus websites. The new website has many advantages: it is faster and more stable, and data tables are reloaded automatically from our production database on a daily basis; edits/additions to the site are easy to implement using the SmartSite web design system; webpages are scaled automatically for display on mobile devices; new query functions and new content were added. 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 continue to work towards identifying QTLs/genes contributing to seed vigor/dormancy, and seed/fruit set under heat stress conditions. We are mapping QTLs for these traits using our *S. sitiens* introgression lines, each of which contains a defined chromosomal segment from the wild nightshade in the genetic background of a modern fresh market variety. This work is 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 acquired 57 new stocks this year. Dani Zamir at the Hebrew University of Jerusalem donated 54 backcross inbred lines representing the genome of *S. pennellii* LA5240 in cultivated tomato. Also known as the "LOST" accession, LA5240 was discovered as an apparent seed contaminant in a seed sample from the IPK, Gatersleben genebank in Germany. However, we are confident LA5240 is really a derivative of *S. pennellii* LA2963, with which it shares several morphological features, including self-compatibility and various seed and whole plant traits that together make this accession relatively easy to recognize among other populations of the species. The 54 BILs – with the background genotype control variety "LEA" – were chosen by Shai Torgeman, Dani Zamir and colleagues (*PNAS* 120(14) e2205787119 and *The Plant Journal* in press) to capture nearly an entire *S. pennellii* genome in as few lines as possible. They are only a fraction of the entire BIL collection of ca. 1400 lines. The BILs were generated by backcrossing the F1 LEA x LOST hybrid to LEA for two generations, then selfing via single seed descent for several generations to allow the wild species introgressions to recombine and to become homozygous (or be eliminated). The background genotype LEA is an inbred extracted from a Heinz processing tomato hybrid cultivar. The full BIL set is expected to be useful for studying epistasis for quantitative traits such as yield. They offer the advantage of providing relatively high resolution for trait mapping due to their high level of recombination, which should make it possible to identify the specific gene(s) underlying traits of interest in most cases. Also, each line contains multiple introgressions, which makes it possible to study the effects of multiple loci interacting. The TGRC does not have the resources to maintain the full set of lines, however they can be obtained from Zamir's group.

***S. habrochaites* LA2322 growing in the greenhouse.** Originally collected in 1980, this accession had never been multiplied at Davis. [photo Matt Valle]

We also added two new stocks of cv. Rutgers after we determined that our existing accession (LA1090) is phenotypically incorrect, possibly due to an outcross in an earlier generation. Our new stock of Rutgers, LA5412, was obtained from the USDA's Plant Genetic Resources Unit at Geneva, NY as PI 647196. We also acquired a stock of cv. Rutgers *Ve* with the Verticillium resistance gene from Dina St. Clair's tomato breeding germplasm collection at UC Davis. This provides another nearly isogenic line for *Ve* (we also have it in the Ailsa Craig and Moneymaker backgrounds) and adds to our collection of Rutgers NILs (the others are mostly fruit ripening or fruit color genes).

We generated F_1 interspecific hybrids of *S. lycopersicum* cv. NC 84173 x *S. habrochaites* LA0407 and plan to maintain it in the future for use as a rootstock hybrid in grafting experiments.

We rescued a previously inactive accession of *S. habrochaites* – LA2322 from Chancleta, Amazonas, Peru – that had never been grown at Davis before. This adds one more accession from the Amazonas area, a relatively underrepresented region for tomato germplasm in general, and for *S. habrochaites* in

particular. A few obsolete or redundant accessions were dropped. The current total of number of accessions maintained by the TGRC is 4,535.

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	3,117	*S. corneliomulleri*	58
S. lycopersicum var. *ceras.*	421	*S. chilense*	115
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*	7
S. arcanum	45	*S. ochranthum*	7
S. peruvianum	71	Other	4
S. huaylasense	16	**Total**	**4,535**

MAINTENANCE AND EVALUATION

The TGRC grew over 1761 families for various purposes: 433 were for seed increases, of which 78 were wild species accessions, and 43 were for progeny tests to verify the presence of segregating genes (e.g. male-sterility loci) or to confirm phenotypes. 156 cultures were grown for introgression and analysis of the *S. sitiens* genome or to study interspecific reproductive barriers. Testing for the presence of GMOs was performed on 61 recently acquired accessions – all were negative.

Identifying accessions in need of regeneration begins with seed germination testing. We start testing seed lots after 10 years of storage. Seed samples that do not meet our minimum of 80% germination after two weeks are normally regenerated in the same 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, 648 germ tests were run on seed lots from 2013 or earlier. Average germination rates were satisfactory overall, except for *S. corneliomulleri* and *S. galapagense* for which a large share of seed lots did not meet our 80% minimum viability (Table 2).

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. Germination tests were performed by sowing 25-50 seed on ½ MS media, except for *S. lycopersicum*, *S. galapagense*, and *S. cheesmaniae*, which were sown on blotter paper.

Solanum Species	**Tested Seed Years**	**# Tested**	**Avg %Germ**	**# Low Germ**
S. arcanum	1994 - 2011	9	85.1	2
S. cheesmaniae	2003 - 2013	23	80.61	7
S. chilense	1990 - 2013	43	81.9	13
S. chmielewskii	2004 - 2010	5	89.4	1

Solanum Species	Tested Seed Years	# Tested	Avg %Germ	# Low Germ
S. corneliomulleri	1997 - 2013	15	76.9	6
S. galapagense	2003 - 2013	6	61.6	1
S. habrochaites	1992 - 2013	20	87.3	3
S. huaylasense	2005 - 2011	4	83.0	1
S. lycopersicum	2003 - 2013	410	84.7	98
S. neorickii	2005 - 2013	26	93.5	2
S. pennellii	2003 - 2013	17	92.7	1
S. peruvianum	1994 - 2013	19	84.2	4
S. pimpinellifolium	2000 - 2013	51	92.1	4
Total		**648**		**143**

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 approx. 2 acres. As usual, sequential plantings were made to spread the workload, with the first transplanting on April 24. 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.

Wilty phenotype of the *fri* (*far red light insensitive*) mutant, LA4356, which lacks phytochrome A.

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. For the mutant stocks, we sow the weakest lines first, and finish with lines of normal vigor. 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. Optimal planting dates and other growing recommendations for each species are listed on our website. 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.

We completed seed multiplications started in 2022 of a previously inactive *S. habrochaites* accession that had never been grown at Davis. This accession was ‘rescued’ from the original, and very old seed collections, which fortunately had not lost all seed viability, by using various tricks to coax a few seeds to germinate. The newly active accession, LA2322, was collected from

Chancleta, Amazonas, Peru, and is one of only a handful of collections of that species from the Amazonas region. Given the near impossibility of getting permission to make new collections in the native region, mining the existing collections for these sorts of inactive wild accessions is one way to increase diversity of the available germplasm.

Preventing the spread of seed borne pathogens is an important aspect of any 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. In the greenhouse we had an outbreak of ToMV in several groups. In response, we discarded all infected plants as well as plants in close proximity that might have been exposed in order to eliminate the virus from our greenhouse plantings. We don't know where the ToMV originated, however we think seed transmission is unlikely because all seed lots were treated with 2.75% hypochlorite for 30 mins prior to sowing to inactivate any virus in the seed coat. Seed lots received from external sources are also heat treated (3 days at 65°C) to inactivate any virus particles inside the seed. Mechanical transmission during plant growth seems the most likely source, and we have taken steps to prevent or reduce spread in the future. All our harvested seed are treated with acid and bleach as part of the seed extraction process, which should greatly reduce the likelihood of transmission via seed that we distribute.

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.

Plants of the *double dwarf* (*dd*) mutant in the field compared to wild type (+). This is one of several mutants we grew for progeny testing to verify the presence of the desired gene.

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 reestablish true breeding lines with the correct traits. This year we progeny tested 43 seed lots of male-steriles, other segregating mutants, and stocks with questionable phenotypes, including the mutants *ms-31*, *ms-38*^{40}, *gh*, *dd*, *ga*, *syv*, *alc*, *Lpg*, *Tm-2*^{a}, *w-4* and *Me*. We also grew stocks of Latin American cultivars LA0762 and LA1540 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 quantities stored at 4°C for filling seed requests. Following our standard

practice, samples of seed were treated with acid and bleach to prevent transmission of seed borne pathogens and to meet import 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. This year 171 seed samples were backed up to NLGRP.

DISTRIBUTION AND UTILIZATION

A total of 4,763 seed packets of 1,786 different accessions were distributed in response to 210 seed requests from 161 scientists, breeders, and educators in 21 countries. Relative to the size of the TGRC collection (4,535 accessions), the number of seed samples distributed represents a utilization rate of 105%. Approx. 40% of our accessions were requested at least once in 2023, demonstrating that a large share of the collection is utilized. We also answered at least 20 purely informational requests regarding our stocks, growing recommendations, and related questions.

We continue to receive many requests for introgression lines (ILs), recombinant inbred lines (RILs), and backcross inbred lines (BILs). A total of 297 seed samples of the *S. pennellii* ILs were distributed, 109 samples of the *S. habrochaites* ILs, 26 samples for the *S. lycopersicoides* ILs, and 24 samples of the *S. sitiens* ILs. We also sent out 177 samples of *S. lycopersicum* x *S. pimpinellifolium* RILs and BC-RILs, and 39 samples of *S. pennellii* BILs. Exotic germplasm libraries such as these require considerable time and expense to develop, but the investment is clearly justified by their continued long-term use in 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, which we must keep up to date with. 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. By far the most requests were for screening against ToBRFV, a major threat to production in many areas. Research and breeding for resistance to *Tuta absoluta* leaf miner continues to be emphasized, reflecting the spread of this insect pest. There continues to be strong interest in abiotic stress responses, especially drought, high temperatures and salinity. Many other requests mentioned fruit traits (quality, carotenoids, etc), or breeding-related uses, notably grafting, marker development and increasing diversity of breeding germplasm. Our stocks continue to be used for a broad array of genetic, physiological, or developmental studies, with some emphasis this year on evolutionary studies, metabolomics/secondary metabolites, and stomatal control.

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	PepMV	1	ToMV	1
Viruses:	ToBRFV	10	TYLCV	1

Unspecified viruses	4	Salinity	7	Micro RNAs	1
Bacteria:		Unspec. abiotic stresses	13	Polyploidy	1
Bacterial spot	1	**Fruit Traits**		Recombination	1
Bacterial wilt	3	Alkaloids		Transformation	1
Candidatus Liberibacter	1	Anthocyanins	1	Transcription, RNAseq	1
Phytoplasma	1	Blossom end rot	1	Unspecified genetics	5
Fungi:		Carotenoids, color	6	**Physiology / Develop.**	
Alternaria alternata	1	Flavonoids	1	ABA responses	1
Botrytis cinerea	1	Flavor, volatiles	1	Acylsugars	1
Cladosporium leaf mold	1	Fruit develop/ripening	4	Edema	2
Fusarium wilt	1	Fruit quality	4	Leaf anatomy	1
Late blight	2	Fruit sugars	1	Leaf volatiles	1
Powdery mildew	2	**Other Breeding**		Metabolomics	5
Verticillium	1	Grafting, rootstocks	6	Microbiomes	2
Nematodes	3	Germplasm diversity	4	Plastomes	1
Unspecified diseases	16	Heterosis	1	Proteomics	1
Insect pests:		Horticultural traits	1	Pollen biology	1
Corn earworm	1	Marker development	8	Roots	3
Leaf miners	1	Male sterility	1	Secondary metabolites	3
Tuta absoluta	3	Molecular breeding	1	Stomatal responses	4
Whiteflies	2	Prebreeding, wide cross	3	Trichomes	2
Unspecified insects	1	Plant architecture	2	Wounding, herbivory	4
Unspec. biotic stresses	2	Tissue culture	1	Unspecified physiol/dev	1
Abiotic Stresses		Unspecified breeding	11		
Drought	6	**Genetic Studies**		**Miscellaneous**	
Flooding	1	Association studies	2	Backup seed storage	2
High temperatures	6	Allele mining	2	Germplasm exchange	1
Low temperatures	3	Evolution, domestication	4	Instructional uses	5
Oxygen	1	Genome sequencing	5	Unspecified research	21

Our survey of the 2023 literature and unreviewed papers of previous years uncovered 110 journal articles, abstracts, theses, patents, and other publications that mention use of TGRC stocks (see Bibliography below). Many additional papers were undoubtedly missed, and cases of utilization by the private sector are generally not publicized. These publications, including many in high impact journals, demonstrate the positive impact of TGRC germplasm on basic and applied research and tomato breeding.

DOCUMENTATION

Our website was thoroughly redesigned and rewritten this year to improve stability and to conform to campus-wide website branding standards. James Cubbage in the Plant Sciences IT group rewrote the query pages, while RTC reformatted and revised the other parts of the website and added content. Overall, the new website is not only more stable but also faster and easier for users to navigate. The display format now automatically adjusts for the device type, providing a much-improved experience on mobile devices. We added a simplified accession query with a single field that requires no knowledge of how the data are structured. It uses a “fuzzy” search algorithm to find accessions even when the accession number is not a perfect match. Besides the accession number, the query also searches other key identifiers, including gene symbols for mutants or collection sites of wild species accessions. The advanced query page offers more power to find accessions based on multiple criteria. It’s similar to our previous accession query form, but

we've added the ability to search for specific gene variants (alleles) or to find accessions donated by specific individuals. Our display maps for wild species accessions now display all active accessions of the species in addition to the selected accession, which provides a quick way to find neighboring collections for stocks that are not currently available. Our geographic mapping page now has the ability to search for and plot accessions by elevation of collection. For example, one could search for collections made above 3000m, or below 500m elevation to target accessions with low temperature or high temperature tolerance respectively. We also added several biographical pages that describe the contributions of some key collaborators from Peru, Ecuador and Chile who were instrumental in finding and collecting wild tomato populations. Future website changes will be easier to implement now because most of the site is designed using the SmartSite web design environment. We expect that the programming behind the search pages will also be easier to modify in the future, with James Cubbage's help. 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 seed distribution records and the numbers of requests from different organizational categories (i.e. domestic or foreign, public, or commercial, etc.).

RESEARCH

One of our research projects is to map genetic factors (QTLs) controlling seed vigor/dormancy, seed size, and seed and fruit set under heat stress. We are using a 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 could affect seed germination. We generated CRISPR mutants in these ABA genes in the corresponding IL background (e.g. *abi4* mutants were generated in the background of the IL containing the *ABI4* locus). We plan to evaluate their germination traits once seeds become available next year. This project is funded by a grant from the Foundation for Food and Agriculture Research.

A plant of *S. sitiens* growing in the Atacama Desert at Cerro Quimal, Chile. The TGRC developed a set of prebred lines that capture the *S. sitiens* genome in the background of cultivated tomato.

Our other research focus is the study of the mechanisms of pollen rejection in tomato wide crosses. Previously we showed that pollen rejection in crosses onto *S. pennellii* pistils result in part from high level expression of an ornithine decarboxylase (*ODC2*) gene in the pistils. This acts as a barrier to pollen tube growth when pollen lack sufficient expression of a farnesyl pyrophosphate synthase (*FPS2*) gene which is required for compatibility on *S. pennellii*. We continue to explore how this pollen rejection mechanism has evolved in related wild species, particularly *S. habrochaites*, and how the ODC2-based barrier interacts with other pollen rejection mechanisms.

PUBLICATIONS

Chetelat, R.T. and X. Qin (2023) Ornithine decarboxylase gene expression mediates pollen rejection in *Solanum*. Plant Polyamine Research Workshop, Oct. 12-13, Budapest, Hungary.

Chetelat, R.T., X. Qin, and M. Valle (2023) Genetic control of unilateral incompatibility: overlapping pollen rejection mechanisms and opportunities for wide hybridization. Tomato Breeders Roundtable, Oct. 9-10, Monterey.

Chetelat, R.T., X. Qin, and M. Valle (2023) Update from the TGRC: new germplasm resources, website changes, and future prospects. Tomato Breeders Roundtable, Oct. 9-10, Monterey.

SERVICE AND OUTREACH

RTC gave presentations on the TGRC, research projects, and related topics to PLS 222 (a UCD graduate course in plant breeding), HRT 200B (graduate class in horticulture), the Tomato Breeders Roundtable, the UCD Plant Breeding Retreat, the Seed Central networking event, the Foundation for Food and Agriculture Research, and the Plant Polyamine Workshop. RTC, MV and/or XQ gave tours to and/or consulted with scientists from East West Seeds, Kagome, Takii Seeds, Syngenta, Meiogenix, and the UCD Plant Biology and Plant Pathology Departments.

PERSONNEL

Matthew Valle, Assistant Curator, supervised undergraduate students Naomi Lavin, Sabrina Colación and Jessica Carver in the greenhouse, field and seed lab. Sabrina and Naomi both graduated and were replaced by Mercury Komjak and Kallan Arimura. Jessica took over as our seed request specialist from Jay Francisco who graduated last year. Dr. Xiaoqiong Qin continues to lead our research on pollen thermotolerance and seed vigor using *S. sitiens* introgression lines, and the mechanisms of pollen-pistil incompatibility. She was assisted in the lab by undergraduate students Sarah Ng (now a graduate student at UCD) and Elizabeth Paul. Qin and her students also provided DNA marker services to the TGRC.

TESTIMONIALS

“On behalf of KeyGene Inc, I wanted to express our gratitude for the service you provide to the tomato research community. The stocks you maintain and provide are invaluable to our research as we strive to support clients that feed the world. Your support enables us to shorten our research timelines and dig deeper into tomato biology, diversity, and genetic potential. Beyond these sentiments, our recent donation to TGRC is a small token of our appreciation for service provided to date and we hope it will support your work going forward.” -- Alan Chambers

“On behalf of East West Seed breeders and researchers, I wish to thank you and the TGRC personnel who took effort in processing our seed orders and its shipment to the Philippines. Thank you so much. We look forward to your generosity in sharing TGRC’s germplasm collection this year.” -- Marilyn Belarmino

“Thanks for your wonderful services to the tomato scientific community.” -- Jianhua Zhu

“Thanks for all the TGRC does!” -- Christopher Muir

"Thanks so much! I really appreciate your help and efforts. I am so excited to receive these seeds." -- Changtian Pan

"I am writing this email to thank all the Davis team for their patient cooperation!" -- Susanna Cialli

"I appreciate the help and organization of TGRC. The contribution of TGRC will be appreciated in the future publication." -- Zhaojun Liu

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MARKER-ASSISTED BREEDING FOR POLYGENIC TOMATO SPOTTED WILT RESISTANCE IN TOMATOES

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Year of Project Initiation: 2023

Executive Summary:

Tomato spotted wilt virus (TSWV) causes tomato spotted wilt (TSW) disease in tomatoes. The viral pathogen spreads by thrips causing a widespread and destructive disease worldwide. TSW is controlled in commercial tomato production by the use of hybrids with the Sw-5b resistance gene. However, the resistant-breaking (RB) variants of the virus not controlled by the Sw-5b gene have become prevalent in some regions including processing tomato production areas in California. To mitigate the damage caused by RB strains of the tomato spotted wilt virus (TSWV), a polygenic host resistance incorporated into processing and fresh market tomatoes is critical. In this research, we aimed to: (1) utilize DNA marker-assisted breeding to introgress and stack the Sw-5b gene with other TSWV resistance genes (Sw-1 and Sw-7) in two tomato breeding lines compatible with processing tomatoes, (2) identify the best genotypic combinations of Sw-R genes with and without transgenic anti-TSWV hairpin construct conferring resistance to common and RB strains of the virus from California, and (3) evaluate and confirm the resistance and horticultural performance in growers' fields in California.

We have utilized DNA marker-assisted breeding (spring greenhouse season, 2023) to develop parental breeding lines homozygous for combinations of at least two tomato spotted wilt (TSW) resistant genes (Sw-1, Sw-5, Sw-7, and a new source of resistance derived from TSW-07). Utilizing those parental lines, twenty new F_1 hybrids (heterozygous for Sw-R genes) were created and tested for disease resistance (RB-TSWV) in California in the summer of 2023 (Table 1). Based on the data from the first field trial in California, only two F_1 hybrids derived from the TSW-07 resistant line showed promising disease resistance with no TSW infection by the end of the growing season. Sixteen F_1 hybrids carrying different combinations of the known resistant genes (Sw-1, Sw-5b, and Sw-7) as well as two other hybrids derived from TSW-07 showed different degrees of TSWV infection.

The new source of TSWV resistance (TSW-07) continues to show promising results in the research fields and with artificial inoculation of the plants with RB-TSWV (CA variant) in the greenhouse. Heterozygous F_1 hybrids derived from TSW-07 show the same level of resistance in field experiments with moderate disease pressure. However, the resistance in some of the F_1 hybrids seems to be less effective under heavy disease pressure when plants are inoculated artificially in the greenhouse or infected naturally in field trials. Thus, it is possible that the TSWV resistance associated with the TSW-07 line is a recessive trait or has an additive effect that makes heterozygous resistance less effective under heavy disease pressure. To test this hypothesis, it is important to develop F_1 hybrids homozygous for the resistance derived from TSW-07 and evaluate the resistance under heavy disease pressure. Developing DNA

markers for marker-assisted backcrossing is a crucial step to achieving this goal. Starting in 2024, we have initiated new research activities to develop DNA markers associated with the resistance in the TSW-07 line to facilitate an expedited introgression of the resistance allele/s (homozygous) into elite tomato breeding lines with promising horticultural performances.

We have utilized whole-genome sequencing data in our program to perform a genome-wide association study (GWAS) to explore possible marker-trait associations and developed two test DNA markers with potential associations with the TSWV resistance in the TSW-07 line. Disease screening by artificial inoculation with RB-TSWV (CA variant) will be performed in the greenhouse to validate marker-trait associations in spring, 2024.

Additional marker-assisted breeding activities to develop parental breeding lines and F_1 hybrids continued in the fall greenhouse season, 2023, and will be completed in early February 2024. We plan to replicate the field trial with additional test hybrids in California in the summer of 2024.

Introduction:

Figure 1. TSW symptoms on tomato fruits of mature plants with heterozygous (right) and without (left) Sw-5 gene.

Rowan County, NC summer 2019

Tomato spotted wilt (TSW) disease, caused by tomato spotted wilt virus (TSWV) and spread by thrips, is a widespread and destructive disease worldwide. TSW has been occurring with increasing regularity in more temperate regions indecent years [1]. The first incidence of tomato spotted wilt in the U.S. was reported on tomatoes in Hawaii in 1920. Since that time, TSWV has spread worldwide and epidemics of TSW have occurred in tomato-growing areas of the southeastern U.S. and California since the 1990s, concurrent with the spread and buildup of western flower thrips. Moreover, TSWV has a very wide host range and infects more than 1000 plant species, including many important vegetables, legumes, ornamental crops, and weeds, causing as much as $100 million in damage annually in the past years [2]. Because of the thrips' small size, rapid developmental time, high reproductive rate, insecticide resistance, and broad host ranges of thrips, controlling TSWV can be very challenging [3]. Proper management strategies can help slow the spread of the disease. The most effective management strategy, however, has been the use of tomato cultivars with resistance to TSWV [3]. Resistance to TSWV was found in wild relatives of domestic tomatoes, and some of these resistance genes have been moved into commercial tomato lines [4]. Commercial tomato cultivars with the Sw-1a and Sw-1b resistance genes were developed in Hawaii; however, the resistance was overcome by new TSWV strains a few years after they were deployed. A partial TSW resistance can be achieved by Sw-6 and Sw-7 to a narrow range of TSWV isolates, but this is not well characterized and not widely used in commercial tomato lines. Only the Sw-5b gene in a cluster of Sw-5 genes, Sw-5a through Sw-5e, conveys effective resistance to TSWV in tomatoes. The Sw-5b gene is the most widely deployed resistance gene for TSWV in tomatoes, interestingly, conferring resistance to several related tospoviruses including tomato chlorotic spot virus (TCSV) and Impatiens necrotic spot virus (INSV) as well, which is unusual for a virus resistance gene [5-9]. In recent years, the resistant-breaking (RB) strains of the virus not controlled by the Sw-5b gene have become prevalent in some regions including main tomato production areas in North Carolina and California (Figure 1).

The TSWV resistance conferred by the Sw-5b gene remains the only viable tool against the common race of the virus. Expedited by marker-assisted breeding approaches, the Sw-5b gene was incorporated into

multiple NCSU elite tomato breeding lines in the last three years. To make TSWV resistance more durable, the Sw-5b gene is stacked with two moderate TSWV resistance genes, Sw-1 and Sw-7, from the University of Hawaii and the University of Florida tomato breeding programs respectively. Development of advanced breeding materials homozygous for different combinations of TSW-resistant genes started three years ago and continued during this project.

Figure 2. Sw-R lines (TSW-07) showing no infection (left) after inoculation with the resistance breaking strain of TSWV, and a different Sw-R line with severe symptoms (stunting, leaf curl, leaf mosaic) that tested positive for virus accumulation with immunological assays (right).

To identify a new source of resistance or a combination of known resistant genes effective against RB-TSWV variants of the virus, a collection of breeding lines and their F_1 derivatives were challenged by the disease in on-farm trials in North Carolina and a CA resistance-breaking variant of TSWV in the greenhouse. Interestingly, one resistance source (TSW-07) outperformed all other lines and F_1 hybrids regarding resistance to a CA variant of TSWV (Figure 2). Identification of the genetics behind the new source of resistance and combining them with known Sw-R genes will be a crucial contribution to combat RB-TSWV outbreaks in California and nationwide.

At NC State University, we developed new tomato breeding lines with polygenic resistance to common and RB strains of the TSWV from California. These new lines will be compatible with processing tomato cultivars (plant growth and fruit type). They can be used by public and private processing tomato breeding programs as sources of polygenic resistance to TSWV.

Our preliminary data from the on-farm trials and greenhouse disease screening suggests that combining strong and moderate Sw-R genes reduces the risk of infection of tomato lines with RB-TSWV strains from different locations including those from California and North Carolina. We have also identified new sources of RB-TSWV resistance that to the best of our knowledge have not been utilized in any modern processing or fresh market tomato cultivars.

In this project, we utilized markers-assisted breeding approaches to combine those Sw-R genes and produced more than 20 different F_1 hybrid tomato lines along with some breading lines compatible with processing tomatoes. The first round of field trials with those 20 F1 hybrids was performed in California with some promising outcomes. We are slightly behind and in the process of testing the resistance of the different genotypes in the greenhouse with two strains of TSWV, one common and one RB strain from California.

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

Objective 1. Utilize DNA marker-assisted breeding to introgress and stack the Sw-5b gene with other TSWV resistance genes (Sw-1 and Sw-7) in two tomato breeding lines compatible with processing tomatoes

Objective 2. Identify the best genotypic combinations of Sw-R with and without transgenic anti-TSWV hairpin genes conferring resistance to common and RB strains of the virus from California

Objective 3. Evaluate and confirm the resistance and horticultural performance in the grower's field in California

Methodology and Results:

Objective 1. Utilize DNA marker-assisted breeding to introgress and stack the Sw-5b gene with other TSWV resistance genes (Sw-1 and Sw-7) in two tomato breeding lines compatible with processing tomatoes

We have utilized DNA marker-assisted breeding (spring greenhouse season, 2023) to develop parental breeding lines homozygous for combinations of at least two tomato spotted wilt (TSW) resistant genes (Sw-1, Sw-5, Sw-7, and a new source of resistance derived from TSW-07). Utilizing those parental lines, twenty new F_1 hybrids (heterozygous) were created and tested for disease resistance (RB-TSWV) in California in the summer of 2023 (Table 1).

Table 1. List of F_1 tomato hybrids with different genetic combinations

ID	Line Number (UC-CTRI)	Generation	Sw-1	Sw-5	Sw-7	New R gene
1	UC-01	F_1	-	/	-	-
2	UC-02	F_1	-	/	-	-
3	UC-03	F_1	/	-	/	-
4	UC-04	F_1	-	/	/	-
5	UC-05	F_1	-	/	/	-
6	UC-06	F_1	-	-	/	-
7	UC-07	F_1	/	/	-	-
8	UC-08	F_1	/	/	-	-
9	UC-09	F_1	/	/	-	-
10	UC-10	F_1	-	-	/	-
11	UC-11	F_1	/	-	/	-
12	UC-12	F_1	/	-	/	-
13	UC-13	F_1	-	/	-	/
14	UC-14	F_1	/	/	-	-
15	UC-15	F_1	/	-	/	-
16	UC-16	F_1	-	-	-	/
17	UC-17	F_1	-	-	/	/
18	UC-18	F_1	-	-	/	/
19	UC-19	F_1	-	-	/	-
20	UC-20	F_1	-	-	/	-

"/"= heterozygous
"-" = homozygous susceptible allele

We have also utilized whole-genome sequencing data in our program to explore possible marker-trait associations and developed two test DNA markers with potential association with the TSWV resistance in the TSW-07 line. Disease screening by artificial inoculation with RB-TSWV (CA variant) will be performed in the greenhouse to validate marker-trait associations in spring 2024.

Additional marker-assisted breeding activities to develop parental breeding lines and F_1 hybrids continued in the fall greenhouse season 2023 and will be completed in early February 2024. We plan to replicate the field trial with additional test hybrids in California in the summer of 2024.

Objective 2. Identify the best genotypic combinations of Sw-R with and without transgenic anti-TSWV hairpin genes conferring resistance to common and RB strains of the virus from California

Twenty F_1 hybrids with genotypic combinations of the three known Sw-R genes and a new source of resistance derived from the TSW-07 line have been developed in the greenhouse in the spring of 2023 (objective 1). The field experiment was conducted in the UC extension station in Fresno County, CA (see objective 3 for details). Based on the data from the first field trial in California, only two F1 hybrids derived from the TSW-07 resistant line showed promising disease resistance with no TSW infection by the end of

the growing season. Sixteen F_1 hybrids carrying different combinations of the known resistant genes (Sw-1, Sw-5b, and Sw-7) as well as two other hybrids derived from TSW-07 showed different degrees of TSWV infection.

The first round of replicated experiments with artificial inoculation was conducted in the fall of 2023; however, due to short daytime and mostly overcast day conditions, the experiment was not successful. The same collection of genotypes representing the possible genotypic combinations (proposed in this project) along with susceptible and resistant parental lines will be screened for TSWV disease resistance by artificial inoculation in the greenhouse in the spring of 2024 for a second time. This objective will help us to identify the best-performing genotypic combination of the Sw-R genes conferring resistance against common and RB strains of the virus from California.

In 2022, we cross-pollinated two NCSU Elite breeding lines (NC-109 and NC-EBR6) with our 4 best performing transgenic lines. We introgressed the transgene for several generations by marker-assisted backcross schemes to create BC_3F_2s. We are now examining these backcrosses to determine if our transgenes can still provide effective levels of resistance as hybrids with only one transgenic parent. Quantitative PCR suggests two of our lines had multiple transgene insertion events in the genome and expression of the TSWV hairpin may not be equal for each insertion. We are now screening individuals among the segregating populations for insertions that are highly expressed and provide TSWV resistance for continued breeding efforts.

Objective 3. Evaluate and confirm the resistance and horticultural performance in the grower's field in California

All commercial tomato cultivars are F_1 hybrids and it is important to examine the efficacy of resistance genes at the heterozygous levels for potential hybrid development. To evaluate the TSW disease resistance, 650 tomato plants from 19 F_1 hybrids were tested using a randomized complete block design (RCBD) in a research field with a history of RB-TSWV incidents in Fresno County, CA in the summer of 2023 (Figure 3). A Vegetable Crops Advisor collaborator from the University of California (Thomas Turini) performed the research field trial and collected the disease screening data. Twenty F_1 hybrids were included in the trial (Table 1) of which 19 hybrids produced enough seedlings and were included in the trial. One of the lines (UC-03) had a very low germination rate and was excluded from the trial. Two F_1 hybrids (UC-16 and UC-17) derived from TSW-07 showed promising resistance against RB-TSWV (CA variant) in this field experiment; however, two other hybrids (UC-13 and UC-18) derived from the TSW-07 showed TSWV incidences of 12% and 22%, respectively (Figure 3B).

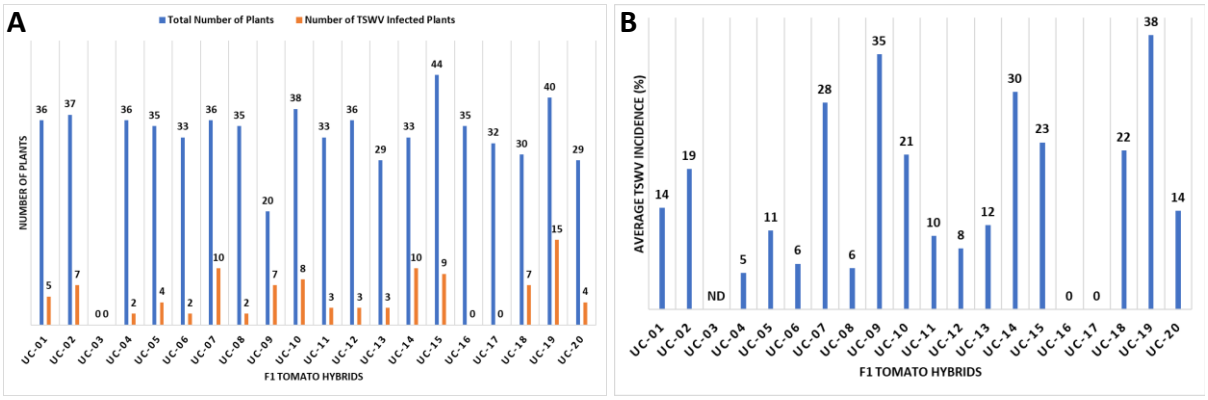

Figure 3. Incidence of TSWV disease in the replicated field trial in a research field, Fresno County, CA. **(A)** The total number of plants transplanted in the field (blue bars) and the number of plants that showed different degrees of TSWV infection (orange bars). **(B)** Average incidence of TSWV. (ND= No Data)

Discussion:

The new source of TSWV resistance (TSW-07) continues to show promising results in research fields and with artificial inoculation of plants with RB-TSWV (CA variant) in the greenhouse. Heterozygous F_1 hybrids derived from TSW-07 show the same level of resistance in field experiments with moderate disease pressure. However, the resistance in some of the F_1 hybrids seems to be less effective under heavy disease pressure when plants are inoculated artificially in the greenhouse or infected naturally in field trials. Thus, it is possible that the TSWV resistance associated with the TSW-07 line is a recessive trait or has an additive effect that makes heterozygous resistance less effective under heavy disease pressure. To test this hypothesis, it is important to develop F_1 hybrids homozygous for the resistance derived from TSW-07 and test them for RB-TSWV resistance under heavy disease pressure. DNA marker development for marker-assisted backcrossing is crucial for expediting and achieving the goals. Starting in 2024, we have initiated new research activities to develop DNA markers associated with the resistance in the TSW-07 line to facilitate an expedited introgression of the resistance allele/s (homozygous) into elite tomato breeding lines with promising horticultural performances.

We have also utilized whole-genome sequencing data in our program to perform a genome-wide association study (GWAS) to explore possible marker-trait associations and developed two test DNA markers with potential associations with the TSWV resistance in the TSW-07 line. Disease screening by artificial inoculation with RB-TSWV (CA variant) will be performed in the greenhouse to validate marker-trait associations in the spring of 2024.

Acknowledgments:

The field experiment in California was designed and conducted by our collaborator, Thomas Turini, Farm Advisor at UC Cooperative Extension Fresno County. He collected the disease incident data as well. Field trials in California are the most important aspect of this research and we are grateful for the significant contribution from Thomas Turini to this project.

This project as leverage for other dollars:

Shekasteband, Rotenberg, Whitfield, and Panthee secured new funding from the NC Specialty Block Grant Program ($185,534.10, over two and half years, 2024-2026) for developing polygenic TSWV resistance in fresh market tomatoes. We previously reported that a M.S. student (Holly McInnes) started to work on this project in 2022. Now, Holly is in the process of changing her degree level to a Ph.D. program to work on fine-mapping the resistance loci in the TSW-07 line.

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EVER A MOVING TARGET: DISEASE DIAGNOSIS, NEW PATHOGEN MONITORING, AND OUTREACH SUPPORT TO THE CALIFORNIA PROCESSING TOMATO INDUSTRY

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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.

Statewide support for tomato disease diagnostics

- Diagnosed diseases for 120 tomato samples, representing statewide distribution.
- Detected two putatively new fungal pathogens, F. solani sensu stricto and Geotrichum candidum, both from crown rots, as well as a second year of Rhizoctonia solani detection from root and crown rot (8 samples)
- Detected two putatively new bacterial diseases—bacterial pith necrosis (2 counties) and a putative wilt disease (1 county)
- Putative Fusarium crown and root rot was again very common this year--4 times more common in 2021 and 2022 than all previous years. In phenotype-based analysis of 2022 putative Forl, 16% of samples were clearly correctly diagnosed as Forl; the remainder were non-pathogenic isolates and Forl may not have been present.
- Fusarium wilt (~45 diagnoses) and Fusarium stem rot and decline (38 samples) were the next most-common diagnoses.
- Southern blight was also commonly detected this year, and I provided follow up decision support to multiple growers on how to best manage this disease.
- Saved pure cultures for over 60 isolates from diagnostic samples, for downstream pathogen characterization (new/emerging diseases), phenotyping and diagnostic tool development.
- Trained nine lab members on diagnostics this year.

***Fusarium* pathogen resistance breaking monitoring**

- Based on phenotyping of Fol isolates from F3 fields in 2022, isolates were either Fol race 3 or non-pathogenic—consistent with previous years. In total we have diagnosed Fol race 3 in 20 F3 cultivar fields state-wide.
- Processed 40 F3 cultivar fields with potential Fusarium wilt symptoms; recovered Fol from 12 F3 fields.
- Determined that 2022 putative Forl detection in an FR cultivar was not resistance breaking (the isolate was non pathogenic); however, detected putative Forl in 3 Fr cultivar fields in 2023, and these are being tested for resistance breaking.

Outreach

- Prepared a summary of five years of Fol race 4 monitoring and chronology of resistance breaking as part of a manuscript published in Plant Pathology, and this is being adapted to a UC8000 series article (in prep)

- Coordinated and presented at an in-service Vegetable Disease field day highlighting research and diagnostic updates
- Coordinated an in-service 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 Fol race 4, which has now been detected in Florida and will likely be detected in California in the near future—of concern, this year we detected Fol in 13 tomato fields, a 4-fold increase from last year.
- Characterize resistance breaking abilities in Forl isolates from Fr fields detected in 2023, and also continue resistance breaking monitoring for these pathogens in 2024.
- To improve abilities to provide accurate diagnoses that are *faster*, advance a new Fol diagnostic tool which can generate results in 3-5 days,
- Take the first steps to establish a similar rapid tool for the "Falciforme stem rot and decline" pathogen F. noneumartii.
- 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 ~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. In 2022 we also detected 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.

2023 Objectives:
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
1.2 Monitoring pathogen movement and new pathogen emergence
1.3 Curating and maintaining isolate cultures
1.4 Beta testing of new Forl diagnostics tools on diagnostic samples**.**
1.5 Diagnostics training to the next generation of diagnosticians

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
2.2 Resistance-breaking Fusarium crown and root rot—a potential new detection.

Objective 3. Provide outreach support to enable growers to utilize diagnoses for decision making.
3.1 In-service trainings including a field day and roundtable meeting coordinated by our lab
3.2 Grower-targeted outreach, including new grower-targeted outreach meeting focusing on tomato diseases and parasitic plants.
3.3 Publication of a new UC IPM field guide for tomato disease diagnosis

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 120 tomato samples were processed and diagnosed. More than half of these samples had multiple diseases. The most frequently diagnosed diseases in 2023 processing tomatoes were: putative Fusarium Crown and Root Rot (60 samples), Fusarium Wilt (40 samples), and Fusarium Rot and Decline (34 samples). Most samples came from Fresno, Yolo, and San Joaquin.

In furthering identification of Forl isolates from 2022, we phenotyped a total of 24 isolates. Of these 4 isolates (17%) 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.

Updated reports were sent out following completion of phenotype-based analysis, although information came in late 2023 and was not useful for pre-planting decision support.

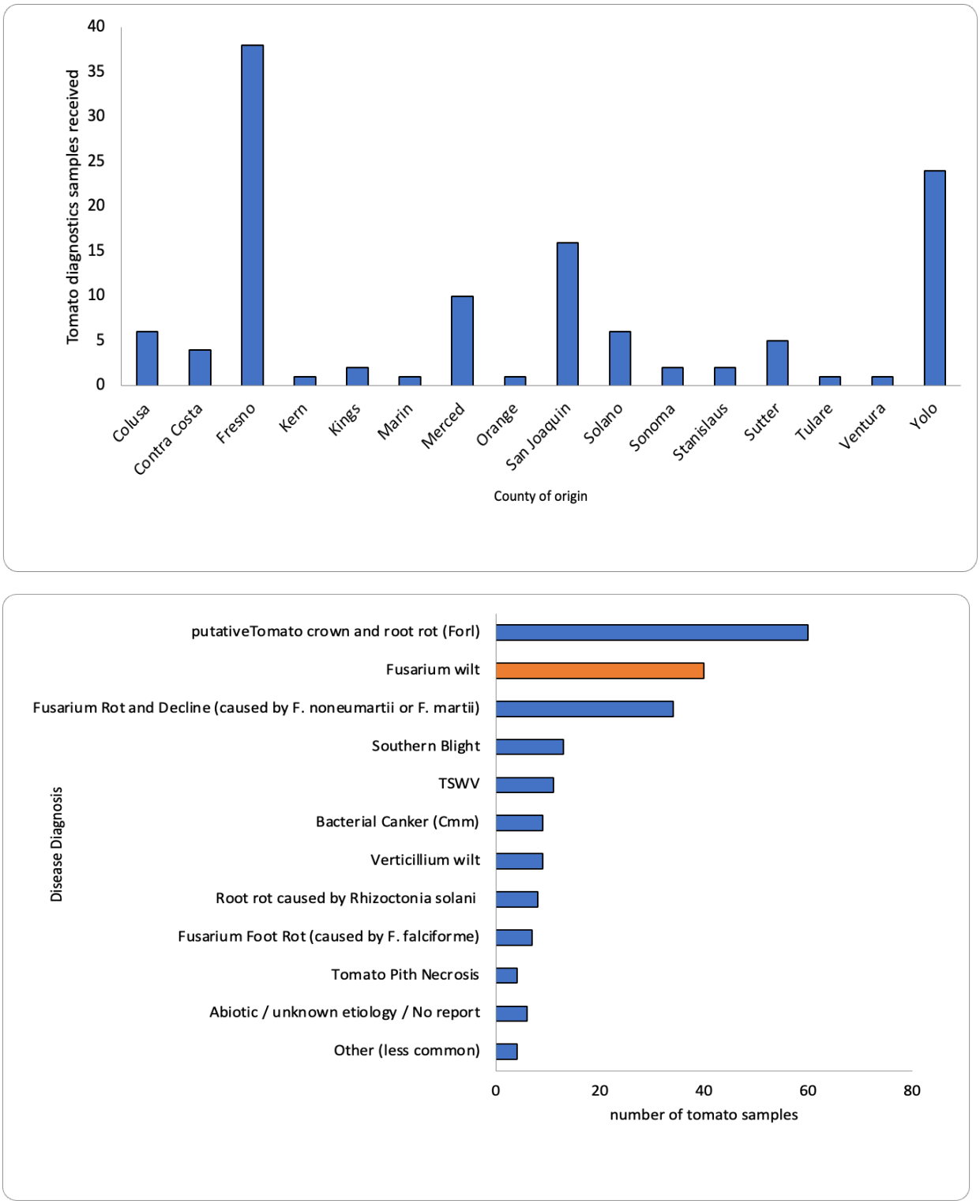

Figure 1. Number of tomato samples diagnosed in 2023 by county (top) and by disease (bottom).

1.2 Monitoring pathogen movement and new pathogen emergence

New diseases detected this year include bacterial pith necrosis and a putative bacterial wilt problem. We detected *Geotrichum candidum* in two tomato fields with crown rot, as the primary microbe associated with symptoms. We also detected F. solani sensu stricto in two tomato fields with crown rot, as the primary microbe associated with symptoms. In both cases, isolate curation is underway, for potential downstream pathogenicity tests. Beyond this, we recovered Rhizoctonia solani from tomatoes with crown and root rot for a second year—this year from eight submissions, which may indicate that this is an actual pathogen.

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

2023 tomato disease diagnoses - putative new pathogens	**No. Samples**
Tomato Pith Necrosis (caused by *Pseudomonas corrugata)*	*4*
Rhizoctonia Crown rot (Causes by *Rhizoctonia solani)*	*8*
putative foot rot caused by *F. solani sensu stricto*	*2*
Tentative crown and root rot caused by *Geotrichum candidum*	*2*

1.3 Curating and maintaining isolate cultures

We have thus far pure cultured isolates from 63 distinct samples in triplicate, saving one pure cultured isolate per sample. This included one Forl isolate from every sample where this pathogen was tentatively diagnosed, as well as every Fol from a F3 cultivar, for phenotyping. We curated 10 F. martii isolates (which currently have poor representation our collection), for diagnostic tool development downstream. In addition, we saved 3 Rhizoctonia solani isolates, for possible virulence studies if this putative pathogen appears to be impacting production downstream. We also preserved 10 single culture isolates of two bacteria species, recovered from two distinct diseases, pith necrosis and a putative wilt-like disease. Pure culturing for isolates from 2023 diagnoses continue into 2024, with an estimated 40 additional cultures to save.

1.4 Beta testing of new Forl diagnostics tools on diagnostic samples.

In total, we have conducted beta testing for 31 isolates from 16 different samples. Beta testing for the remaining 30+ samples are in progress, testing isolates in duplicate where possible.

Based on this initial beta testing effort, this tool identified 20% of isolates as Forl, 3% as non pathogens, and 45% as likely Forl. We are working on confirming this haplotype-based diagnosis via isolate phenotyping, to complete the beta test.

Table 2. Results from beta testing the new Forl diagnostic tool in 2023 diagnostic samples

			Molecular diagnosis				Haplotpye #	Lineage
Date	Sample number	Isolate number	Confirmed Forl	Confirmed Nonpath	Forl or Nonpath	Ambiguous		
7/6/2023	162023	0162023-6			X		Five_191	3D
7/6/2023	162023	0162023-8			X		Five_191	3D
7/6/2023	172023	0172023-1	X				Five_242	3D
7/14/2023	422023	0422023-1-p1				X	Five_191 (FORL or Nonpath) Five_58 (FOL)	3D 3D
7/14/2023	422023	0422023-2-p3				X	Five_191 (FORL or Nonpath) Five_58 (FOL) Five_63 (Nonpath) Five_2 (Nonpath)	3D 3D 3D 3D
7/14/2023	482023	0482023-2		X			Five_200	3G
7/14/2023	482023	0482023-5			X		Five_128	3G
9/14/23	902023	0902023-5		X			Five_141	3G
9/14/23	902023	0902023-9						
8/22/2023	902023	0902023-3			X		Five_170	3G
8/22/2023	1002023	1002023-2			X		Five_191	3D
8/22/2023	1002023	1002023-5			X		Five_191	3D
8/22/2023	1002023	1002023-8			X		Five_191	3D
9/14/23	692023	0692923-6						
9/14/23	692023	0692923-7						
9/14/23	712023	712023-2			X		Five_191	3D
9/14/23	712023	712023-6						
9/14/23	712023	712023-9						
9/14/23	922023	0922023-3		X			Five_141	3G

9/14/23	922023	0922023-4				X		Five_128 (FORL or Nonpath) Five_61 (Nonpath)	3D 3G
9/14/23	1042023	1042023-4			X			Five_128	3G
9/14/23	1042023	1042023-7			X			Five_128	3G
12/13/23	772023	0772023-5		X				Five_141	3G
12/13/23	942023	0942023-3		X				Five_141	3G
12/13/23	942023	0942023-4		X				Five_57	3G
12/14/23	992023	0992023-7			X			Five_128	3D
12/14/23	972023	0972023-8							
12/15/23	1052023	1052023-5			X			Five_191	3D
12/15/23	1092023	1092023-6			X			Five_191	3D
12/22/23	1092023	1092023-8				X		Five_191	3D

1.5 Diagnostics training to the next generation of diagnosticians.

This year I trained five new diagnostic interns (two undergraduates, two technicians, one incoming MS student) on the process of diagnosis, and provided more advanced training to four additional members of my team (two graduate students, two technicians). Of these trainees, two are currently including diagnostics positions in ongoing job searches, and five will be undergoing advanced training in 2024.

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. Consistent with this, Fol diagnoses in 2022 (2/3 fields) were Fol race 3; in addition, the isolate from one field was non-pathogenic. In total, Fol race 3 has been diagnosed in 20 F3 cultivar fields across the state. In 2023, we diagnosed diseases in 40 F3 cultivar fields this year. From these we detected Fol in 12 F3 fields. Isolates are currently be pure cultured, identity as Fol confirmed for the pure culture (using diagnostic PCR) and will be phenotyped in 2024.

Table 3. 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	12	12	TBD	TBD	TBD	TBD	TBD	TBD
Total	32	32	0	0	20	0	0	0

2.2 Resistance-breaking Fusarium crown and root rot—a potential new detection.

In 2022, we recovered putative Forl in one FR cultivar field; this isolate was non pathogenic and of note, it co-occurred with one of the FRD pathogens (F. noneumartii), which was likely contributing to symptoms. In 2023, we again diagnosed putative Forl in three FR cultivar fields. These isolates are being saved, and will be phenotyped in 2024.

Objective 3. Provide outreach support to enable growers to utilize diagnoses for decision making

3.1 In-service trainings including a field day and roundtable meeting coordinated by our lab.

We offered this dual field day and roundtable researchers lunch meeting August 28, 2023 (lunch provided by CTRI—thank you!). There were 32 participants at the field day, including 10 farm advisors, the CTRI director, 6 representatives from seed companies (AgSeeds, TS&L), and 13 students. As a new part of this field day, we spend the first part of the day doing a roundtable discussion of emerging patterns across the state, where advisors shared their observations. We then went to several field trials to discuss new topics. There were the same ~30 participants at the roundtable lunch, plus 10 additional researchers; in total, 13 researchers shared their work.

3.2 Grower-targeted outreach, including new grower-targeted outreach meeting focusing on tomato diseases and parasitic plants.

Offered 1:1 consultations on broomrape management to several growers. To develop a clearer vision on what this might involve, I had a meeting with Zach and Dave Viguie on how to better engage growers and plan to meet with CTRI board members to further this goal in 2024.

3.3 Publication of a new UC IPM field guide for tomato disease diagnosis

The finalized version of this field guide was submitted to UC IPM in November 2023.

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; early detection will enable a rapid response program aimed at preventing spread (as per outreach goal in 2024 funded work).
- 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 diseases detected in 2023 may have greater impact than previously thought, since these were likely being misdiagnosed.

- Speed of Fusarium crown and root rot diagnosis increased, improving management abilities
- Building up culture collection to develop molecular diagnosis tools for Fusarium "falciforme" stem rot and decline (FRD; as per 2024 funded work).
- 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 2024.
- The production community has increased awareness of disease issues and management options, leading to improved disease management and reduced yield losses.

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**

Developing an integrated management strategy for F. falciforme vine decline in processing tomato, including co-management with Fusarium wilt

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TS&L Seeds, Scott Picanso, 37331 State Highway 16, Woodland, CA 95695

Year of Project Initiation: 2020 for Farm advisor portion, 2018 for Swett lab portion.

Executive Summary:

Over the last four years, we have established the framework for an integrated disease management program for pathogens in the *F. falciforme* species complex (FFSC). Our work has included cultivar-based management, characterization of host range in warm and cool season crops, crop rotation guidelines, targeted weed management based on host range in weeds, and chemical-based management.

As a major foundational effort, pathosystem studies this year have allowed to us to segregate this system into two diseases, the yield loss-driving disease Fusarium Stem Rot and Decline or FRD, which is caused by *F. noneumartii* (most impactful) and *F. martii* and the non-yield-impacting Fusarium foot rot, caused by *F. falciforme* sensu stricto. This work is helping to direct research efforts on FRD, develop diagnostic tools which segregate these diseases, and segregate commercial field studies by disease system for greater informational value.

We evaluated commercial processing tomato cultivars for disease tolerance via three approaches in 2023:

1. Conducting variety evaluations in controlled trials in a FRD (*F. noneumartii*)-infested field located at UC Davis.
2. Conducting variety trials established by farm advisors in two controlled trials within pathogen-infested commercial fields, evaluating 12 cultivars and verifying the presence of FFSC and other pathogens via diagnostic efforts.
3. Conducting disease evaluations and acquiring yield data for 26 cultivars in five seed dealer variety trials when FSSC pathogen pressure is suspected, and establishing the presence of FFSC and other pathogens via diagnostic efforts.

To inform growers' decision-making for FRD management, we complied a table of conclusions from 20 variety trials conducted from 2020 through 2023, we now have a data on 47 current commercial cultivars. Studies of *F. noneumartii-F. martii* co-management indicate strong potential to use the same cultivars and chemicals to manage both species; however, limitations in cultivar studies necessitate further study to confirm these results.

In furthering warm season crop-rotation-based management of FRD (*F. noneumartii*), we established that this pathogen also causes disease in sunflower, safflower, garbanzo, and bell pepper in the field but does not appear to cause disease in melon, cotton, and corn. This work was confirmed by commercial field surveys, where *F. noneumartii* was recovered from diseased sunflower.

In rotation studies, we identified sunflower, safflower, and weedy fallow as high-risk rotations which result in tomato vine decline incidence levels similar to a tomato-tomato sequence, while cotton, corn, melon, and a weed mixture were the lowest risk. In multi-year commercial field rotation surveys, a tomato-sunflower-alfalfa-tomato rotation resulted in a 10% increase in disease development between the two tomato years.

In cool-season rotation studies, controlled greenhouse studies indicate that *F. noneumartii* can cause disease in carrot, broccoli, cabbage, and cilantro. In rotation with spinach and garlic, tomatoes developed the lowest levels of plant mortality and rotation with onion, lettuce, parsley, and alfalfa led to the highest tomato mortality levels.

In furthering FRD management via weedy host management, studies suggest that *F. noneumartii* causes disease in nightshade, tumble pigweed, cheeseweed and bindweed—all new weed hosts for this fungus. In rotation studies, fallow with warm season weed communities was high-risk, resulting in tomato vine decline levels similar to a tomato-tomato sequence.

In both cultivar and chemical studies, methods effective in managing *F. noneumartii* were also effective for other FFSC pathogens. Thus, studies focusing on management of *F. noneumartii* using chemicals and tolerant cultivars should be broadly effective against all FFSC pathogens.

INTRODUCTION

Fusarium Stem Rot and Decline (FRD) is a widespread and consistently damaging disease of processing tomatoes across the state. 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—a syndrome which we collectively refer to as premature vine decline. In certain cultivars, FRD can reduce yields by up to 60% and completely kill up to 100% of plants by harvest.

We have learned that isolates previously referred to as 'Fusarium falciforme' segregate into three different species, with diverging biological abilities to affect tomatoes. Two species, *F. noneumartii* (more virulent) and *F. martii*, cause Fusarium Stem Rot and Decline (FRD), which causes mid to late season plant

collapse and severe fruit damage, making this disease a management priority. In contrast, *F. falciforme ss* causes the minor rot disease Fusarium Foot Rot, which has no apparent effects on yield.

Farms in all major tomato-producing counties are struggling to manage this disease. Until we 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 (Lenandro et al., 2018, Weems et al. 2015). Based on this, work over the last four years has established some of the basic parameters comprising an effective IPM program for FRD in processing tomato; these include use of chemical control complemented with selection of tolerant cultivars. With completed 2023 studies, we are now able to add rotations with non-host crops to management guidelines. Independent of CTRI support, we are also working to integrate irrigation-based management into an IPM program for FRD and have been working with breeding programs to develop rapid resistance screening protocols to improve the genetic materials being released to growers.

THE MAIN GOAL AND OBJECTIVES:

Main goal: Develop an effective integrated management toolkit for Fusarium stem rot and decline in processing tomato.

Objective 1. Co-author a Fusarium Stem Rot and Decline diagnosis and management UC IPM Pest Note, which can provide cohesive pre-planting and in-season management recommendations.

Deliverables: Cohesive reference for both field diagnosis and management options for FRD

Objective 2. Developing *F. noneumartii*-tolerant cultivar recommendations and evaluating cultivars for co-management potential with Fusarium wilt

2.1 UC Davis Cultivar Trial

2.2 UCCE and Seed retailer trials, including diagnostics support from Swett lab

Deliverables: Cultivar recommendations for use in both FRD (specifically *F. noneumartii*) management and co-management with Fusarium wilt to reduce yield losses, disseminated at meetings and online

Objective 3. Developing crop rotations to minimize *F. noneumartii* inoculum load build up and subsequent losses in tomato

3.1 Developing summer crop rotations to minimize *F. noneumartii*-driven losses in tomato: tomato disease evaluations in 2021 UC Davis replicated field trial and expansion of summer rotation treatment evaluations

3.2. Assessing host status of *F. noneumartii* in winter rotation crops in the field (complete 2022/23 study; initiate 2023/24 study)

3.3 Assess winter crop rotation and fallow effects on disease development in tomato (2023 tomato planting)

3.4. Multiyear crop rotation assessments in commercial fields.

Deliverables: Crop rotation recommendations (both single and multi-year) for use in FRD management, disseminated at meetings and online in fall to reduce yield losses; additionally, identification of other crops economically impacted by F. falciforme

Objective 4. Conduct Fusarium falciforme Species Complex population studies to determine whether management practices will be broadly effective against diverse genetic groups and develop diagnostic tools

4.1 Characterize non-pathogens within the *F. falciforme* species complex, present in diseased tomatoes—include in pathogenomic analysis.

4.2 Determine whether the F. falciforme species complex species *F. falciforme* sensu-stricto contains isolates which impact tomato production.

4.3 Determine whether cultivar susceptibility profiles are similar across the three F. falciforme species complex species.

4.4 Determine whether the different F. falciforme species have similar host range and thus can be managed with the same crop rotation strategies.

4.5 Determine whether chemicals effective against F. noneumartii have similar efficacy against other species.

Deliverables: Information on broad-spectrum efficacy of cultivar-based, crop rotation and chemical-based management, understanding of management targets, and improved diagnostic abilities.

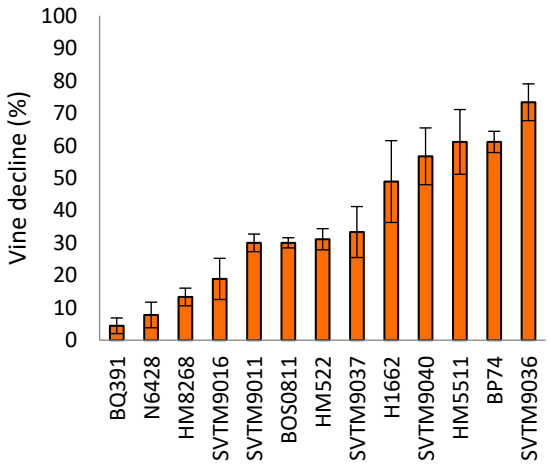
METHODOLOGY AND RESULTS

Objective 1. Co-author a Fusarium stem rot and decline diagnosis and management UC IPM Pest Note, which can provide cohesive pre-planting and in-season management recommendations

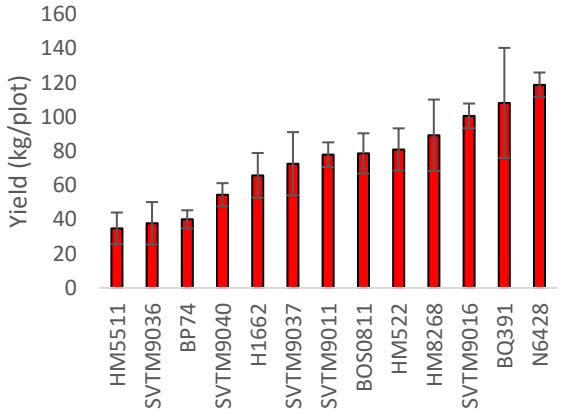
We have completed a draft of the cultivar table, summarizing results from five years of field trials (Table 5) and are currently drafting the fungicide table. A rough draft has been completed detailing diagnostic traits (with plates), contrasting to look alike diseases, describing the disease development process in the field, and detailing the distribution and impacts of the disease across the state. We are working with UC IPM to submit for review in early 2024.

Objective 2. Developing F. noneumartii-tolerant cultivar recommendations and evaluating cultivars for co-management potential with Fusarium wilt

2.1 UC Davis Cultivar Trial



At the UC Davis Plant Pathology research farm, we screened 13 commercial cultivars for resistance by inoculating transplants at 10^6 spores/ml with *F. noneumartii* (CS 109) and planting in an infested field (same isolate). The trial was arranged in a randomized complete block design, with three blocks; each treatment was allocated to one 100-ft plot, and 30-plant subplots were established for data collection. Vine decline data was collected at two timepoints (6 weeks and 3 weeks preharvest), and yield data collected at 135 days after planting.



The top performers in this trial were BQ391 and the tolerant check N6428, with the lowest vine decline and fruit damage, and the highest yields. HM8268 and HM9016 also had low decline and high yields but fruit damage was intermediate for HM8268 (43%) and high for HM9016 (60%). Intermediate performers with intermediate vine decline and yields, but lower fruit damage (less than 40%) included SVTM9011, BOS8011, and HM5522. Poor performers were HM5511, BP74, SVTM9040, and H1662, with the highest vine decline (50-73% six weeks pre-harvest), lowest yield, and intermediate to high fruit damage levels (40-80%).

2.2 UCCE and AgSeeds trials, including diagnostics support from Swett lab

In 2023, two trials were established by UC farm advisors (Fresno and Stanislaus counties) and another four trials were established by AgSeeds in close collaboration with the UC team (Sutter and San Joaquin counties). In addition to these six replicated yield trials, we also evaluated another two trials in San Joaquin County where VRD was observed and subsequently confirmed. From two of the trials, we could not confirm the presence of any FRD pathogens. At one site, the vine decline was confirmed to be due to potassium deficiency and Fus wilt (in the susceptible cultivars). At the second site, we suspect FRD, but we do not have laboratory confirmation due to inadequate plant sampling.

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 for machine-harvested yield, plot length was 75 to 100 ft. 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 some locations, we additionally have a sort out of a 5-gallon fruit subsample to determine cull rates and a PTAB analysis of fruit quality from a sample of good red fruit.

Table 2 combines the results from three trials from Sutter and San Joaquin counties with a common entry list. Varieties exhibiting the fewest plants with advanced vine decline include HM6268, HM8237, SVTM9016, SVTM1082, HMC8512, SVTM9037, N6428, HM58841, and SVTM9034. Some of these were also among the highest yielders including SVTM9016, SVTM9037, HM8237, HM58841, and N6428. Some other varieties yielded well despite moderate levels of vine decline including SVTM9041, SVTM9036, H2016, and SVTM9019. Varieties with the highest levels of vine decline included H1996, SVTM9021, SVTM9032, SVTM9023, HMC0371, SVTM9013, H1662, and HM5522. Among these, two are notable for yielding well despite high disease – HM5522 and SVTM9023.

Vine decline levels in the Fresno County trial didn’t align well with results in the above trials, perhaps because root knot nematode was a factor at the Fresno site. BP74, SVTM9040, N6428 had the lowest levels of advanced vine decline out of 12 varieties (Table 3). At the Stanislaus County site (Table 4), HM58841 had the lowest disease and highest yield. Other high yields were HM8268, SVTM9040, SVTM9037, HM5511, and BOS0811. Most of these also had relatively low vine decline, although two had high disease levels and high yield (SVTM9040 and HM5511). At the two extra sites in San Joaquin County (no yield data), varieties that stood out in terms of low rates of vine decline included HM8237, HM8268, SVTM9016, SVTM9041 and HMC8512.

Table 1. Details on variety trials evaluated in 2023.

County	planting date	evaluation date(s)		harvest date	#cvs	#rep	Yield/culls/fruit quality data?	FRD pathogens lab confirmed	other diseases
Sutter	17-Apr	2-Aug	15-Aug	15-Aug	25	3	yield, AgSeeds	*F. noneumartii*	Southern blight
Sutter	20-Apr	2-Aug	15-Aug	1-Sep	25	3	yield, AgSeeds	*F. noneumartii* and *F. martii*	Southern blight
San Joaquin	3-May	3-Aug	15-Sep	16-Sep	11	3	no	*F. noneumartii* and *F. martii*	
Sutter	5-May		6-Sep		25	3	yield, AgSeeds	None	Fus wilt, K deficiency
Stan.	19-May	28-Jul	25-Sep	26-Sep	15	4	Yield, sort out and fruit quality, UCCE	None confirmed	
San Joaquin	31-May	20-Sep	12-Oct	18-Oct	25	3	Yield, AgSeeds; sort out, PTAB, UCCE	*F. noneumartii* and *F. martii*	Fusarium wilt
Fresno	8-Jun		20-Oct	20-Oct	12	4	no, unable to harvest	*F. noneumartii*	Root knot nematode
San Joaquin	8-Jun		19-Oct	19-Oct	27	1	no, not a yield trial	*F. noneumartii*	

Table 2. Evaluation of AgSeeds variety trials; three locations in Sutter and San Joaquin counties. For disease rankings 1 = least vine decline; for yield 1 = highest yield.

	Vine decline (%)								Yield (tons/acre)				
Variety	field 1		field 2		field 3		Disease rank		field 1	field 2	field 3	mean	Yield rank
HM8268	0.0	b	5.8	d	9.6	a	1		71.33	47.50	43.59	54.14	14
HM8237	1.6	ab	10.9	cd	8.1	a	2		72.49	48.64	58.32	59.82	3
SVTM9016	0.8	b	9.4	cd	11.6	a	3		69.93	58.56	58.52	62.34	1
SVTM1082	0.4	b	4.9	d	19.2	a	4		63.53	49.73	43.56	52.27	19
HMC8512	2.5	ab	14.7	bcd	7.6	a	5		66.94	43.72	44.64	51.77	22
SVTM9037	1.5	b	3.6	d	20.2	a	6		71.56	56.93	51.93	60.14	2
N6428	1.3	b	4.1	d	22.2	a	7		71.18	51.34	47.96	56.83	7
HM58841	1.7	ab	3.8	d	22.2	a	8		71.43	53.41	48.53	57.79	6
SVTM9034	4.2	ab	6.3	d	17.7	a	9		68.36	53.76	41.94	54.69	13
SVTM9011	1.7	ab	10.8	cd	18.7	a	10		61.21	46.62	42.20	50.01	25
N6475	2.0	ab	3.0	d	29.8	a	11		66.42	50.18	36.29	50.96	24
BP74	5.4	ab	9.5	cd	22.7	a	12		66.09	48.70	46.48	53.76	15
SVTM9019	2.3	ab	7.5	d	28.8	a	13		71.35	56.29	39.99	55.88	10
SVTM9036	13.4	ab	22.4	bcd	13.1	a	14		75.28	47.09	51.78	58.05	5
H2016	2.6	ab	29.6	bcd	19.2	a	15		70.56	43.08	54.60	56.08	8
SVTM9041	3.6	ab	37.5	abcd	11.1	a	16		75.50	49.35	52.79	59.21	4
HM5511	4.8	ab	36.8	abcd	11.1	a	17		68.53	47.13	40.66	52.11	20
SVTM9040	13.9	ab	26.8	bcd	14.1	a	18		65.46	43.48	52.17	53.70	16
HM5522	16.6	a	6.4	d	44.9	a	19		70.16	49.15	48.51	55.94	9
H1662	1.3	b	15.3	bcd	52.5	a	20		64.35	43.17	46.19	51.24	23
SVTM9013	8.5	ab	49.5	abcd	11.6	a	21		64.79	40.84	51.26	52.30	18
HMC0371	5.3	ab	31.7	bcd	34.3	a	22		66.89	51.55	46.14	54.86	12
SVTM9023	6.0	ab	58.6	abc	19.7	a	23		75.00	42.50	49.26	55.59	11
SVTM9032	3.8	ab	62.0	ab	25.8	a	24		65.74	38.29	40.99	48.34	26
SVTM9021	5.7	ab	61.1	ab	26.3	a	25		67.94	42.65	47.91	52.83	17
H1996	12.3	ab	86.4	a	48.5	a	26		69.67	36.11	50.21	52.00	21
trial mean	4.7		23.8		22.0				68.91	47.68	47.56		
			<0.000		*0.012*								
P value	*0.001*		*1*		*5*				*<0.05*	*<0.05*	*0.0011*		

Table 3. UCCE-established variety trial in a commercial field, Fresno County.

Variety	Advanced vine decline (%)	
BP74	7.0	d
SVTM9040	8.5	cd
N6428	11.3	bcd
HM5511	13.0	abcd
SVTM9036	13.3	abcd
SVTM9037	14.3	abcd
H1662	19.5	abcd
SVTM9016	20.8	abcd
HM8268	26.8	abcd
SVTM9011	28.5	abc
HM5522	29.8	ab
BOS0811	32.5	a
mean	18.75	
P value	*0.0005*	
MSD 5%	21.03	

Table 4. UCCE-established variety trial in a commercial field, Stanislaus County. Note that at this site FRD pathogens are suspected but are not confirmed. Other diseases may be contributing.

Variety	Advanced decline %	Yield (ta/ac)	Fruit damage (%)	color	Soluble solids	pH
HM58841	3.8	54.8	4.5	18.6	6.0	4.52
H1996 grafted	6.3	66.9	3.1	18.8	4.7	4.46
SVTM9037	10.0	45.6	4.7	19.4	5.5	4.37
BP74	10.0	38.4	6.5	18.4	5.9	4.50
HM8268	10.0	46.8	6.4	17.4	5.9	4.49
N6428	11.3	35.4	4.7	19.3	5.2	4.52
SVTM9011	11.3	38.1	5.9	18.3	6.4	4.44
SVTM9036	12.5	40.7	5.4	19.5	5.7	4.49
BOS0811	12.5	41.4	3.9	18.8	5.5	4.41
SVTM9040	18.8	45.8	3.6	19.5	5.7	4.41
HM5511	30.0	41.5	5.5	18.5	6.0	4.52
SVTM9016	38.8	32.2	4.4	19.4	5.6	4.33
BP101	38.8	34.4	4.8	19.1	5.1	4.48
HM5522	45.0	39.3	3.1	18.3	6.2	4.37
H1662	53.8	38.3	3.9	19.5	5.2	4.46
Mean		42.6	4.7	18.8	5.6	4.45
P value		*<0.0001*	*0.1393*	*<0.0001*	*<0.0001*	*<0.0001*
HSD0.05		*19.9*	*4.4*	*1.3*	*0.7*	*0.1*

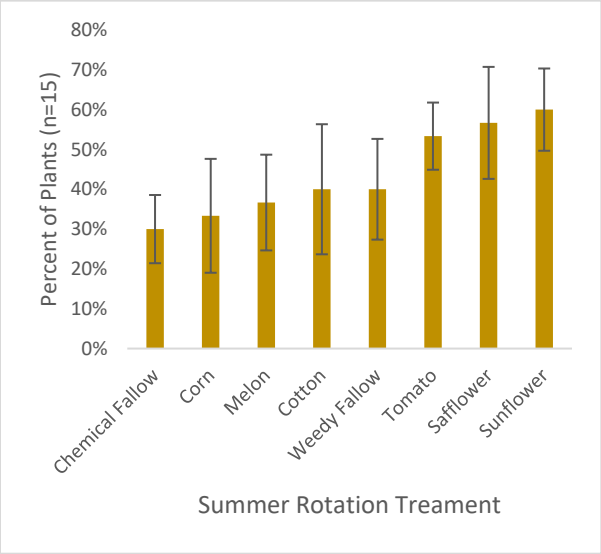
Table 5. Yield performance in 20 variety trials from 2019 to 2023; five sites on UCD campus, all others commercial fields with confirmed Fusarium stem rot and decline. Summary excludes varieties that are no longer in the top 50.

Variety	Trials	Summary of performance	Normalized yield	2023 loads rank	Traits of note
HM8237	9	Tolerant, EFH	1.17	1	Fr, EFH
SVTM9025	5	Moderately tolerant with EFH	1.15	23	Fr, EFH
HM58841	13	Tolerant of FRD at most sites, EFH, susceptible to Fus wilt race 3	1.10	4	EFH, F2
SVTM9016	17	Tolerant, EFH	1.09	2	EFH
N6428	19	Tolerant, EFH	1.08	8	EFH
SVTM9041	5	low to moderate rates of vine decline, EFH, need additional data	1.08	new	EFH
SVTM9037	11	low to moderate vine decline, EFH	1.07	28	EFH
SVTM9019	10	Tolerant, EFH	1.06	13	EFH
H5608	5	did well at most locations, moderately tolerant	1.05	19	
N6434	3	did well at 2 of 3 sites	1.05	28	
BOS0811	3	moderate tolerance? EFH, need more data	1.02	18	EFH
HM5522	9	susceptible to FRD and Fus wilt race 3, although yields decently despite decline	1.02	7	Fr, F2
HMC0371	4	moderately susceptible	1.01	new	early, Fr
SVTM9023	4	susceptible to vine decline, but has EFH and yields decently	1.01	3	EFH
SVTM9036	12	high yields under low disease pressure and has EFH, but very susceptible to vine decline	1.01	new	EFH
HM8268	9	low vine decline at 7 locations, yields decent	1.00	12	
SVTM9034	4	low to moderate vine decline	1.00	new	early
H2016	6	moderately susceptible, but manages to yield decent in problem fields	0.99	10	
SVTM1082	4	low decline at 3 of 4 sites	0.96	17	
SVTM9013	6	variable vine decline rates	0.96	5	
SVTM9021	4	moderately susceptible	0.96	20	Fr
SVTM9040	8	variable rates of vine decline	0.96	new	
H1996	6	susceptible to vine decline, but has EFS trait	0.95	6	EFS
SVTM9038	3	susceptible to vine decline, EFH	0.95		EFH
HMC8512	6	low to moderate vine decline	0.92	new	
N6475	4	low vine decline at 3 of 4 sites, EFH	0.92	24	EFH
H1662	9	variable performance, did well at 1 location, medium to poor at others	0.91	16	
SVTM9032	6	susceptible to vine decline	0.91	21	early, Fr
BP74	9	variable performance, did well at 3 locations, medium to poor at others, need more data	0.90	22	
HM5235	5	moderately susceptible	0.90	37	
SVTM9011	10	moderately susceptible	0.90		
HM5511	7	variable vine decline rates	0.85	35	Fr
HM4909	6	variable performance	0.82	45	Fr
N6416	2	susceptible	Only 2 sites	34	early
BQ273	2	medium performance, 2 sites	Only 2 sites	30	early
BQ403	2	medium performance, 2 sites	Only 2 sites	31	early

Objective 3. Developing crop rotations to minimize F. noneumartii inoculum load build up and subsequent losses in tomato.

3.1 Developing summer crop rotations to minimize *F. noneumartii*-driven losses in tomato: tomato disease evaluations in 2022 UC Davis replicated field trial and expansion of summer rotation treatment evaluations.

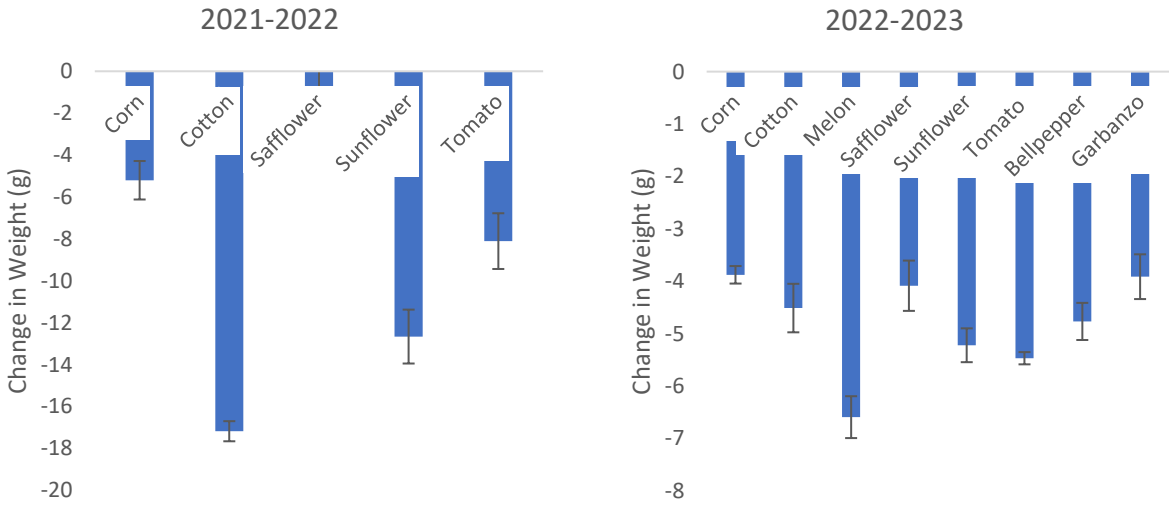
This summer we finished the multiyear summer crop rotation studies initiated in 2021. For the summer of 2022, an infested field was planted to safflower, sunflower, melon, cotton, corn, pepper, garbanzo, and tomato (as a known host control) in semi-randomized complete block design with three blocks. We included a weedy fallow and chemical fallow plot for comparison. At the end of the summer, these plots were incorporated, taking care to make sure that each crop remained in its respective plot. In May 2023, this field was planted to tomato so we could evaluate the effect of planting these different summer crops on disease development in tomato. The tomatoes were monitored for symptoms throughout the summer and evaluated for disease 2 weeks pre-harvest, and at harvest. Data was collected on canopy decline, plant death, and rot incidence in above and below ground tissues. Symptomatic plants were collected to confirm causal agent of disease. Once data on disease in tomato was collected, we were able to compare and combine with data from the 2021-2022 summer rotation trial to get a robust idea of what rotation crops potentially lead to higher or lower levels of disease in subsequent tomato plantings. We utilized the chemical fallow plots as a baseline for a 'good rotation' treatment as there are no potential hosts to increase inoculum. The tomato plots act as the baseline for a 'poor rotation' choice as tomato is a known host that would be increasing inoculum and thus leading to higher levels of disease in future years.



In both rotation trials we saw that rotating with safflower and sunflower led to similar or higher levels of disease as rotating with tomato and are thus poor rotation choices. This is reflected in the combined data that shows the safflower and sunflower treatments grouping with tomato. Corn, melon, and cotton appeared to be better rotation choices, with a corn, melon, and cotton rotation lead to similar levels of disease as the chemical fallow treatment. From this data, we can tentatively recommend not rotating with safflower or sunflower if F. noneumartii is present and instead rotating with corn, melon, or cotton. Further analysis of additional disease data (rot incidence, canopy decline severity) is underway to further characterize effects of rotations on tomato disease outcomes.

In addition to evaluating disease in tomato, we are also assessing effects of these crops on inoculum loads based on both pathogen DNA loads (soil collected, qPCR assay under development) as well as by assessing the rate of decomposition of each summer rotation crop over the winter to better understand how each crop may be contributing to soil inoculum and subsequent disease. The slower the infested crop residues decompose the longer that tissue may be contributing to soil borne inoculum loads. At the end of the summer multi crop trial, we collected plant materials from each crop and dried them down at room temperature for 3-4 weeks. The litter was then inserted into mesh bags, weighed, and buried in the field. These bags were then dug up in the spring and reweighed to get an idea of how the material had

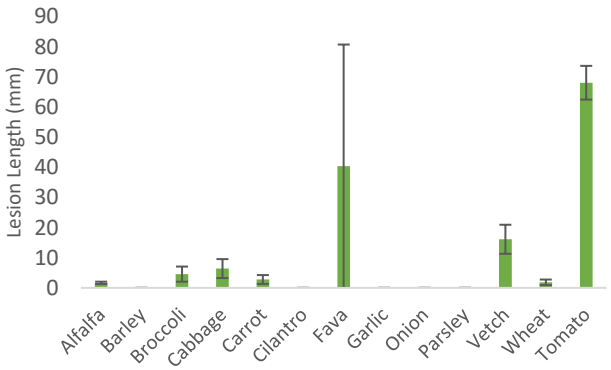
decomposed. Based on this study, the known host safflower decomposed very slowly compared to the known hosts sunflower and tomato, indicating greater potential for infested safflower to continue contributing to inoculum over time.



Change in weight of crop litter buried over the winter. Melon tissues become moldy during dry down and so could not be used for the 21-22 study.

3.2. Assessing host status of F. noneumartii in winter rotation crops in the field (complete 2022/23 study; initiate 2023/24 study)

Thus far we have primarily focused on the host range of F. noneumartii in warm-season crops. This year we initiated host range studies of F. noneumartii in cool-season crops in both greenhouse and field. The greenhouse trials allowed us to study a wider range of cool season crops in a short amount of time while the field studies allow us to confirm whether the environment under which the crops are produced is conducive to disease development.



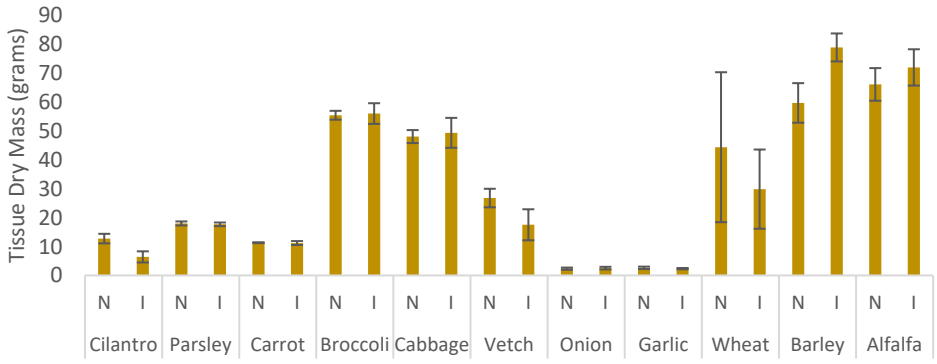
Lesion length in cool season crops inoculated with F. noneumartii.

Greenhouse studies. Greenhouse studies were initiated in the late winter/early spring of 2023 and the field trial was planted in the fall of 2022 and evaluated in the late spring/ early summer of 2023. Trials were laid out in a randomized complete block design with 3 blocks, 3 plants per crop per block per treatment (inoculated or non-inoculated). The study was divided into 3 trials, each containing an assortment of cool season crops plus tomato as a known host control. We tested cilantro, parsley, carrots, cabbage, broccoli, alfalfa, vetch, fava bean, onion, garlic, wheat, and barley. Crops were rated for canopy symptoms and evaluated for presence of rot in the roots, foot, crown, and stem, and stem lesion length. In addition, we also evaluated the effect on plant health by evaluating impacts on total canopy biomass (evaluated as dry weight).

Non-hosts. No rot developed in inoculated parsley, onion, garlic, or barley indicating these are not hosts to F. noneumartii. Although rot developed in fava, this was observed in both inoculated and non-inoculated plants and F. noneumartii could not be recovered, indicating fava is also not likely a host.

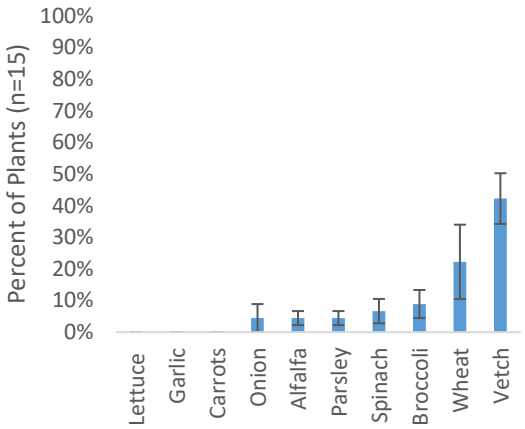
Hosts. Disease symptoms developed in carrot, broccoli, cabbage and cilantro, and putatively in vetch. F. noneumartii was recovered from all crops, indicating these may be hosts. Specifically, we observed distinctive rot symptoms in carrot, broccoli, and cabbage, although canopy biomass was not affected; F. noneumartii-like isolates were recovered from all crops (further identification underway). In addition, cilantro plants rapidly developed decline (rot could not be evaluated); reflecting this, canopy biomass was reduced by 50% in the inoculated treatment (isolations could not be conducted). No non-inoculated carrot, broccoli, cabbage or cilantro developed rot symptoms. Vetch developed rot in both the inoculated and non-inoculated plants and canopy biomass was 35% lower in the inoculated treatment, indicating a potential pathogen effect of *F. noneumartii*. *F. noneumartii*-like isolates were recovered from only inoculated plants; analysis of identity is underway.

Unclear host status. The host status of wheat and alfalfa is unclear. Rot developed in inoculated and non-inoculated plants; there was no significant effect of *F. noneumartii* on canopy biomass in either crop, although on average biomass in wheat was 30% lower in the inoculated treatment. F. noneumartii-like fungi were recovered from both crops, and analysis of identity is underway.



Effect of inoculation with F. noneumartii on crop biomass production in cool season crops. 'N' stands for non-inoculated; 'I' stands for inoculated.

Field studies-crop hosts. To determine whether *F. noneumartii* causes disease in cool season crops under winter growing conditions, we stablished a field trial in fall of 2022 in a field containing F. noneumartii-infested tomato debris, evaluating lettuce, garlic, carrot, onion, alfalfa, parsley, spinach, broccoli, wheat and vetch. All crops were direct seeded except parsley and onions which were transplanted. The field was laid out in a randomized complete block design with 3 blocks, 12 70ft plots per block. Crops were monitored through the winter and then evaluated for disease in early April. For each crop, 15 random plants were pulled per plot and rated for percent canopy in symptomatic and presence of rot in the roots, foot, crown, and stem. Any symptomatic plants were collected to determine whether F. noneumartii was present in diseased tissue.



Lettuce, garlic, and carrots did not develop any rot symptoms. Vetch had the highest incidence of rot (42% of plants), followed by wheat (22%) but we were unable to recover F. noneumartii from the symptomatic plants. All other crops developed rot in 4-9% of plants; F. noneumartii similarly was not recovered from any symptomatic plants. As this host range trial was part of the winter rotation study, we had to plant the field to tomatoes in late April. This meant many of the crops (eg. garlic, carrots, onion, alfalfa) did not complete their full growing season; running for the full crop cycle can provide a more complete picture of the ability for F. noneumartii to cause disease in these crops.

Family	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
Brassicaceae	*Sisymbrium irio*	London rocket
Montiaceae	*Calandrinia menziesii*	Redmaids

Field study-winter weed hosts. In addition to cool season crops, we also evaluated the host range of *F. noneumartii* in winter weed communities. This included six weed species from five different plant families. Symptoms were not detected in any winter weeds and we were not able to recover the pathogen from any plants.

3.3 Assess winter crop rotation and fallow effects on disease development in tomato (2023 tomato planting)

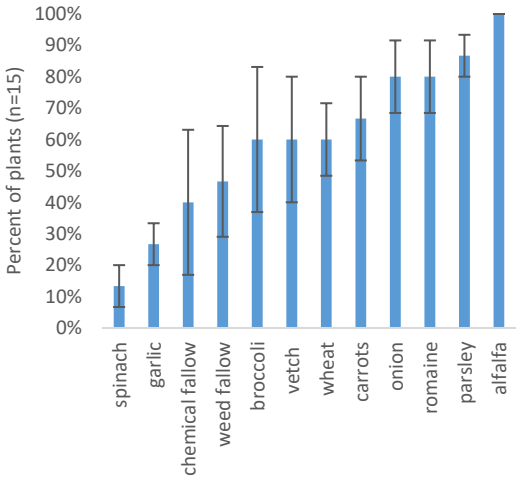
This trial was laid out in a randomized complete block design with 3 blocks, each containing 12 70-ft treatment plots. Each treatment plot was planted to a different winter rotation/cover crop. These included vetch, wheat, broccoli, spinach, parsley, alfalfa, onion, garlic, carrot, and lettuce. In addition, we had a chemical fallow and weedy fallow treatment. The trial was machine transplanted in early May. Within each 70-ft plot, we established a 15-plant monitoring plot to evaluate canopy symptoms. Rot symptoms were collected destructively from plants outside the monitoring plots. Disease symptoms were monitored through the summer with two evaluations at the end of the season, one 2 weeks preharvest, and one at harvest. As with the summer rotation trials, we also evaluated litter decomposition rate between the different crops to better understand how these crops may be contributing to soil inoculum and overall soil organic matter. In April, plant material from all rotation treatments was collected and dried down. This material was then put in mesh bags and weighed before being buried in the field in their respective plots. These bags were then dug up and weighed at three time points throughout the summer.

We observed a wide range in disease development between the rotation treatments, from as low as 10% of plants in decline all the way to 100% of plants in decline at harvest. Plots planted to spinach and garlic resulted in tomato decline incidence which was lower than chemical fallow (13-26% of plants dead at harvest). Garlic did not develop any symptoms in the field or greenhouse trial, pointing to some

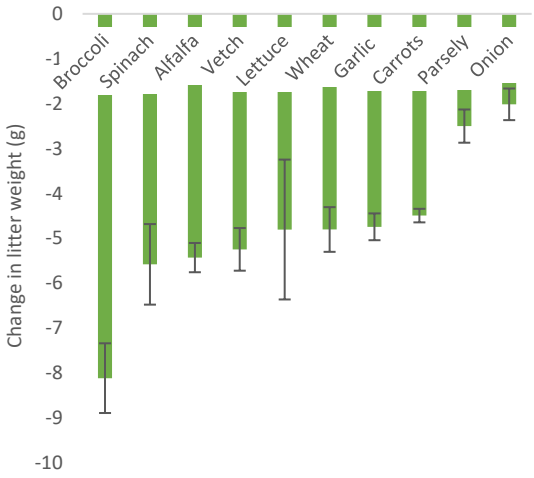
correspondence between host status and inoculum load contribution (greenhouse data lacking for spinach).

Broccoli, vetch, wheat and carrots resulted in tomato decline incidence (60-67% of plants dead at harvest) similar to chemical fallow (40% of plants dead at harvest). While carrot developed symptoms in greenhouse trials, symptoms did not develop in the field, providing some correspondence between host status and disease risk. However, both vetch and broccoli developed symptoms in field and greenhouse trials, suggesting that in some cases, host crops may still be low risk. Both of these crops have reported pathogen-suppression abilities which may be at work here. In the case of broccoli, activity requires breakdown of tissue to release the biofumigant product, and in our trial we were able to show high rates of broccoli tissue breakdown. Overall, more rapid tissue breakdown may also reduce inoculum loads by removing the food source for the pathogen.

The onion, lettuce, parsley, and alfalfa treatments had higher levels of disease (80%-100% of plants dead at harvest), which were greater than the chemical fallow based on standard error. This indicates these crops pose a greater disease risk than chemical fallow. None of these crops appear to be hosts based on either greenhouse study and develop little or no rot in the field. It is therefore unclear why disease levels were so much higher in these treatments. It is noteworthy that two of these crops, parsley and onion, had the slowest decomposition rates; if this tissue was providing a food source for the pathogen, this might account for higher disease pressure. However, this does not account for lettuce and alfalfa contributions.



Percent of plants dead or severely declined at harvest in the different winter rotation/cover crop treatments. The chemical fallow plot acted as out baseline for disease development.



Cool season crop residue decomposition over the summer.

3.4. Multiyear crop rotation assessments in commercial fields.

To complement single year trials, we are conducting multi-year rotation studies in four commercial grower fields (thus far). In these trials, we first established base-line pathogen loads in the field using transect-based quantification of vine decline incidence (three 100-ft transects with random row and paces into row) in F3 cultivars (to exclude Fusarium wilt, which has similar symptoms) and conducted fungal isolations, to confirm association of FRD (either *F. noneumartii* or *F. martii*). From there, we are conducting annual evaluations of FRD-associated disease incidence. When the field returns to tomato, we assess disease incidence as above to determine whether different multi-year rotations have the potential to alter

losses in tomato (either increase or decrease). We also assessed weeds as hosts in all four fields; weeds included nightshade, nutsedge, and Amaranth species.

Yolo site 1 was rotated through sunflower and two years of alfalfa. This rotation resulted in a 1.5-fold *increase* in disease, from 17.5% plants in decline in 2019 to 27.71% in 2023. Data from our controlled rotation trials supports this as we saw both sunflower and alfalfa lead to higher levels of disease in tomato.

SJ site 2 was rotated in into cucumber for one year, after which disease was 55% lower in tomato, although it is noteworthy that the cultivar replanted was HM58841, which has resistance to FRD. A subsequent rotation to tomato (again), using HM8237 (R unknown) resulted in a 1.5-fold increase. The relative role of cultivar vs crop rotation here is unclear.

In 2023, were also able to evaluate a tomato field which has been rotated to safflower. Safflower is a host to *F. noneumartii* based on our greenhouse and field host range trials and we did observe a low incidence of mild symptoms and were able to isolate the pathogen from a few symptomatic plants in this field.

Table 6. Grower rotation survey showing the percent of premature vine decline (PVD) across years for tomatoes (listed as cultivars) before and after rotations; decline levels for rotation crops also given if known.

Site	2019	%PVD	2020	%PVD	2021	%PVD	2022	%PVD	2023	%PVD
Yolo 1	SV1082 (F3)	17.5% ± 5%	Sunflower	0%	Alfalfa	NA	Alfalfa	NA	HM8237 (F3, Fr)	27.7% ± 6.8%
Yolo 2	BQ413	59.7% ± 8%	Wheat	ND	Sunflower	ND	Tomato	-%	Survey ended	
SJ 1	SV1082 (F3)	16.4% ± 0.3%	Cucumber	0%	Cucumber	ND	fallow		Almond survey ended	
SJ 2	-	-	SV9013 (F3)	25.9% ± 5.6%	Cucumber	0%	HM58841 (F2)	11.5% ± 4.8%	HM8237 (F3, Fr)	17.6% ± 6.2%
SJ 3	-	-	SV9013 (F3)	38.8% ± 2.3%	SV9011 (F3)	15%	wheat fb teff	0%	Safflower	0%
SJ4							Tomato	8.2% ± 1.7%	N6428 (F3)	4.0% ± 1.6%

Objective 4. 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 4.1-4.2, we expanded upon last summer's multi-isolate field trial. This year we included a total of 8 isolates (plus a non-inoculated control) across all three species (including modern and historical *F. noneumartii* isolates) 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.

Table 7: Treatment description with CS isolate number, species designation, and virulence designation

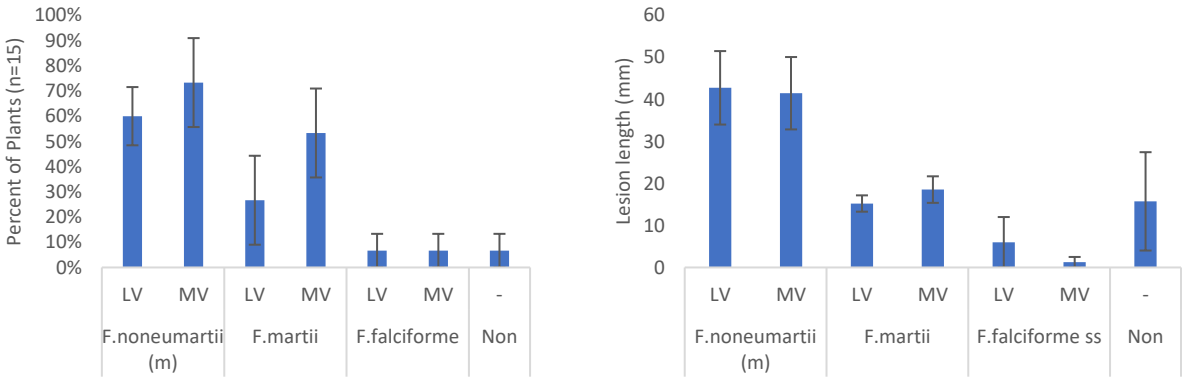
Treatment	Virulence	Isolate	Species	Cultivar
T1	Low	CS870	*F. noneumartii* (m)	SVTM9032
T2	High	CS109	*F. noneumartii* (m)	SVTM9032
T3	Low	CS410	*F. noneumartii* (h)	SVTM9032
T4	High	CS1276	*F. noneumartii* (h)	SVTM9032
T5	Low	CS162	*F. martii*	SVTM9032
T6	High	CS91	*F. martii*	SVTM9032
T7	Low	CS918	*F. falciforme*	SVTM9032
T8	High	CS966	*F. falciforme*	SVTM9032
T9	na	na	Non	SVTM9032

The trial was designed as a randomized complete block design with three blocks, nine 70-ft treatment plots per row. 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 every 2 weeks from 17-Jul to 11-Sep. On 13-Sep, the monitoring plots were harvested to quantify overall yield impact and a sample of the fruit was sorted to quantify fruit quality impact.

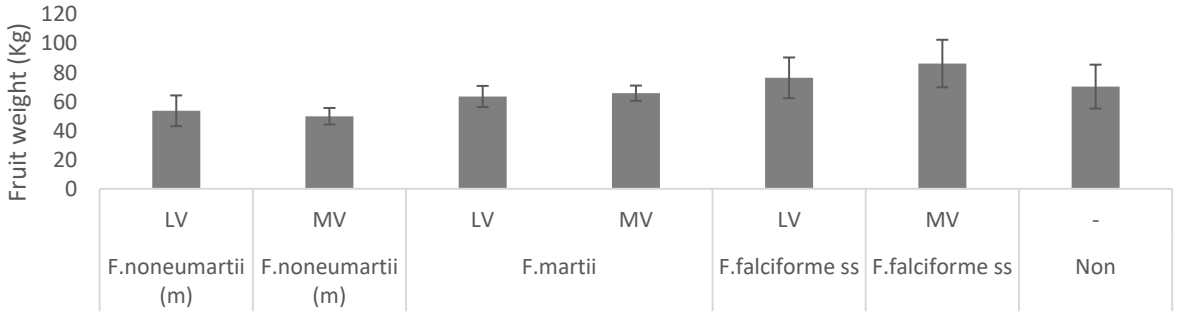
4.1 Characterize non-pathogens within the F. falciforme species complex, present in diseased tomatoes—include in pathogenomic analysis.

As previously mentioned, we choose a less virulent and more virulent isolate for each species to characterize both the between species and within species diversity. These designations appeared to line up in the field trial with more virulent isolates causing more sever disease and less virulent isolates causing less virulent disease.

We also further characterized the between species diversity of this disease. The *F. falciforme sensu-stricto* isolates appeared to be non-aggressive (as discussed further in the next objective) and the *F. martii* isolates appeared to be as virulent as the *F. noneumartii* isolates. We did not observe a strong economic impact from the different species. Interestingly, *F. falciforme ss* had a higher yield than the non-inoculated plots. As expected, *F. noneumartii* had the largest reduction in yield.

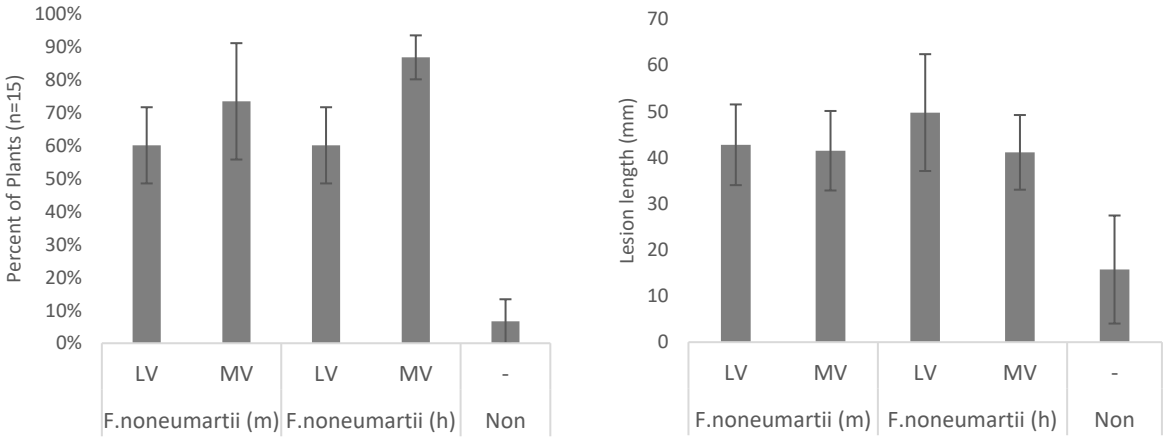


Incidence of tomatoes dead or declined (left) and internal lesion size (right) at harvest. LV = less virulent isolate, MV = more virulent isolate

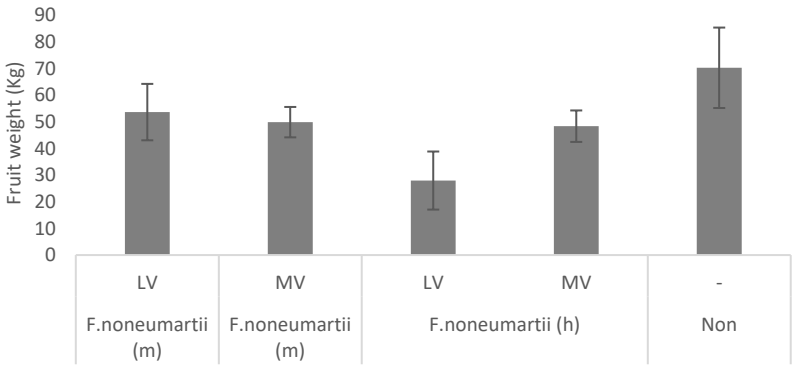


Total plot yield for all three species. LV = less virulent isolate, MV = more virulent isolate

As part of characterizing species diversity, we also included historical isolates to further investigate the cause of the observed symptom shift. We observed a similar incidence of plant death at harvest as well as internal lesion length in the modern and historical isolates of *F. noneumartii*. This further supports the hypothesis that the pathogen has not changed or become more aggressive but instead there is another factor (such as environmental or cultural) that has changed how this disease progresses.

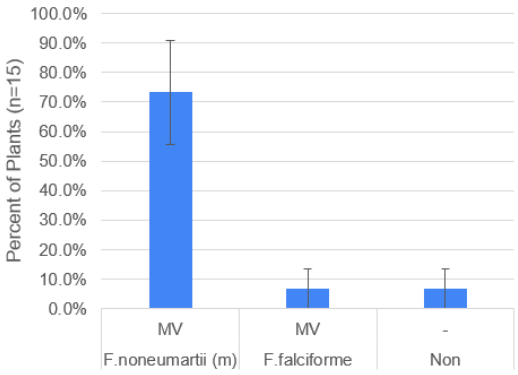


Incidence of tomato vine death (left) and internal lesion length (right) at harvest. The (m) or (h) denotes modern or historical isolates respectively. LV = less virulent isolate, MV = more virulent isolate

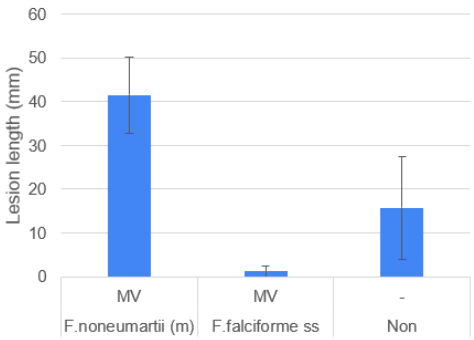


We observed a slight economic impact caused by both the modern and historical *F. noneumartii* isolates, though, interestingly, the less virulent historical isolate had the largest reduction in yield.

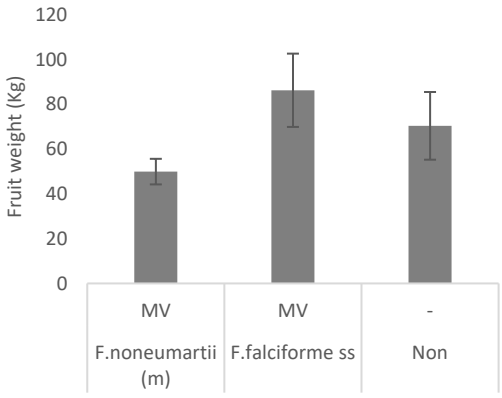
4.2 Determine whether the F. falciforme species complex species *F. falciforme sensu-stricto* contains isolates which impact tomato production.



As we saw in preliminary greenhouse studies, the *F. falciforme sensu-stricto* species appears to be mostly nonpathogenic. At harvest, there were less than 10% of plants dead or declined in the *F. falciforme sensu-stricto* treatments while there was over 70% of plants in the *F. noneumartii* treatment plots. (see left)



We also observed very minor rot in the *F. falciforme* ss plots while the *F. noneumartii* plots had lesions over 40mm in length. (see left)



The lack of pathogenicity of the *F. falciforme sensu-stricto* species was also reflected in the yield data. The *F. falciforme ss* plots had greater yield then the *F. noneumartii* plots and did not appear to have an economic effect.

4.3 Determine whether cultivar susceptibility profiles are similar across the three F. falciforme species complex species.

Assessment of the cultivar susceptibility profiles was undertaken with a high throughput greenhouse assay. Cultivars were chosen based on variety evaluation trials. Using this preliminary data, we grouped cultivars into three groups, most resistant to *F. noneumartii*, moderately resistant to *F. noneumartii*, and least resistant to *F. noneumartii.* And then used the assay to determine if cultivars fell into these groups when inoculated with the other species.

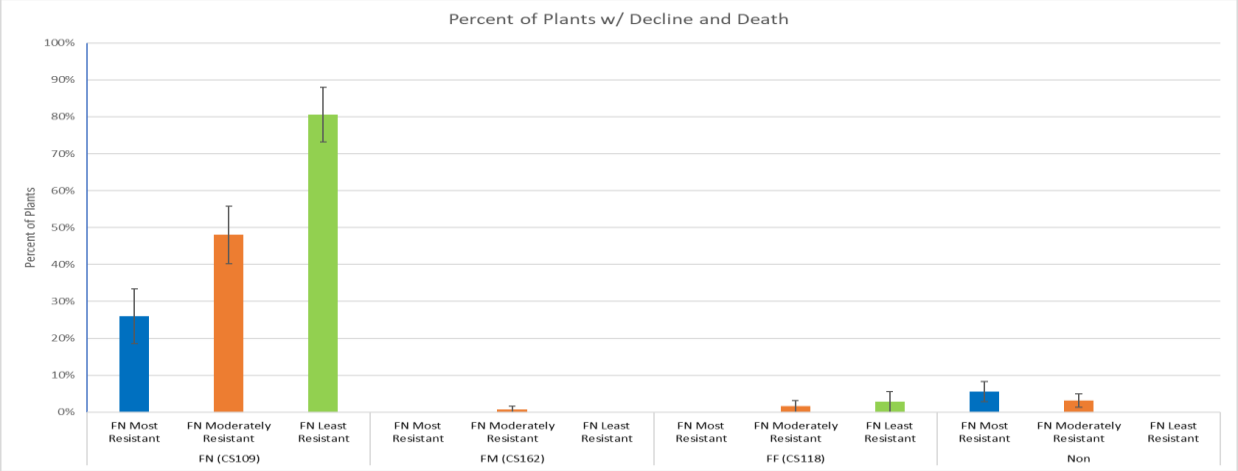
Table 8. Cultivars used in the trial and their FRD resistance level based on field trials.

Cultivar[y]	Normalized[z] yield	Normalized[z] fruit damage levels	Normalized[z] vine decline at harvest	FRD disease resistance level
SV9016	1.16	0.52	0.82	high
N6428	1.13	0.65	0.87	high
H5608	1.10	0.77	0.44	high
HM58841	1.05	0.86	1.04	high
HM5235	1.00	1.39	0.90	medium
HM4909	0.92	0.97	1.13	medium
H1310	0.89	1.07	1.08	medium
HM3887	0.88	1.35	1.33	low
SV8011	0.86	1.07	1.37	low
AB311	0.82	1.07	1.28	low

[x] Data compiled from work by Swett and Aegerter (unpublished).
[y] Cultivars SVTM9016 and SVTM9032 were chosen for comparison purposes because they were used in previous studies with FRD lineages (Collinson and Swett, unpublished).
[z] For normalized data, numbers below one represent low cultivar performance. Numbers equal to one represent average cultivar performance. Numbers over one represent high cultivar performance.

The assay was arranged in a complete block design done in 72-cell plug trays with three replicates and four treatments (*F. noneumartii*, *F. martii*, *F, falciforme*, and non-inoculated). Each 72-cell plug tray was one replicate:treatment. Cultivars were sown in the plug trays on 23-Sept-2022, and then inoculated four weeks later. For inoculations, each plant was wounded just below the soil line and then the whole tray was submerged in a spore suspension of the appropriate species (for non-inoculated, the trays were dipped in plain 0.1% water agar). Trays were then top dressed with soil mixed with extra inoculum to make sure the wound was below the soil line. Great care was taken to not cross contaminate treatments. Plants were monitored for disease development and rated for canopy over 4 time points. At the final time point, 17 weeks after seeding, plants were removed from the trays and rated for presence of above and below ground rot. Once plants were rated for death and decline, they were sorted into the aforementioned categories based on each cultivar's response to *F. noneumartii.* We then compared these categories across the different species expressing the same pattern.

Overall, cultivars that were the most resistant to the *F. noneumartii* species were also most resistant to the other species and did not develop no death or decline when inoculated with the *F. martii* or *F. falciforme* species. The cultivars that were least resistant to the *F. noneumartii* species were also the least resistant to the other species. This suggests that cultivar susceptibility profiles line up across species and that we can use cultivar-based management tools designed around the *F. noneumartii* species for all the species. However, this study was conducted under non-optimal (winter) conditions and overall, symptoms were minor across treatments; further studies under optimal conditions are needed to confirm these patterns.



Percent of tomatoes of each cultivar with decline and death sorted into F. noneumartii *resistance profiles.*

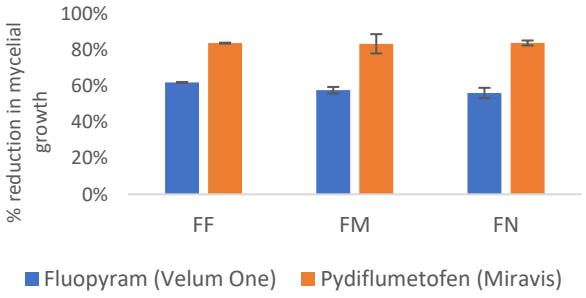
4.4 Determine whether the different F. falciforme species have similar host range and thus can be managed with the same crop rotation strategies.

So far, we have worked to confirm the biological host status of a selection of warm and cool season crops to *F. noneumartii* in greenhouse trials. We used this information to determine whether different *F. falciforme* species have similar or different host ranges. We selected three hosts and three non-hosts from both warm- and cool-season crops and inoculated these with the three species in the FFSC. This trial was laid out as a randomized complete block design with three blocks, each containing three crops per treatment. Due to the late start of the 2023 field season we were not able to start this trial until later than planned. For this reason, it is still in progress and we do not have the final data on the multi-species host range.

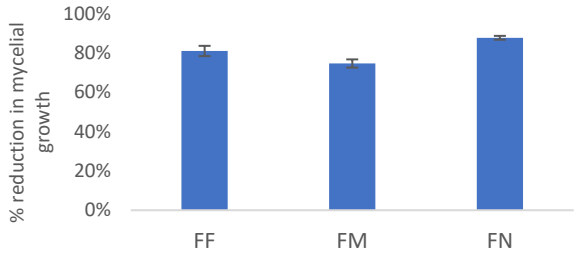
4.5 Determine whether chemicals effective against *F. noneumartii* have similar efficacy against other species

Two isolates from each species were chosen for this study: CS687 and CS966 which are *F. falciforme sensu stricto* (FF), CS162 and CS951 which are *F. martii* (FM), and CS109 and CS411 which are *F. noneumartii* (FN). Fungicides tested include Velum One (fluopyram) and Miravis (pydiflumetofen). Fungicides were applied to agar plates using the Eddy Jet spiral platter. Cellophane strips colonized by each isolate were then placed onto plates with fungicide. Plates were then incubated at 25 ˚C. Controls consisted of plates with isolate strips but no fungicide. The first trial used 1000 ppm of the active ingredient of both fungicides. Data was collected five days after strips were applied. EC50 values from the plates were inconclusive due to low fungicide concentration, so percent reduction at the highest fungicide concentration (1000 ppm) was instead calculated from one representative plate from each isolate. Each plate contained two strips of the same isolate, and data was collected from both the left and right sides of each strip. Percent reduction was determined by comparing the percent change in growth of plates with fungicide compared to the control plates. The second trial used 5000 ppm of pydiflumetofen. Data was collected six days after strips were applied. Percent reduction was calculated in the same way as trial one but with five plates per isolate. Neither fungicide at 1000 ppm was enough to completely kill the fungal isolates at any dose. Both fungicides had a similar effect on all three species at the lower dose. At the higher rate, pydiflumetofen appeared to have slightly greater efficacy against F. noneumartii (88% grower reduction) compared to *F. martii* (75% reduction) and *F. falciforme sensu stricto* (81% reduction.

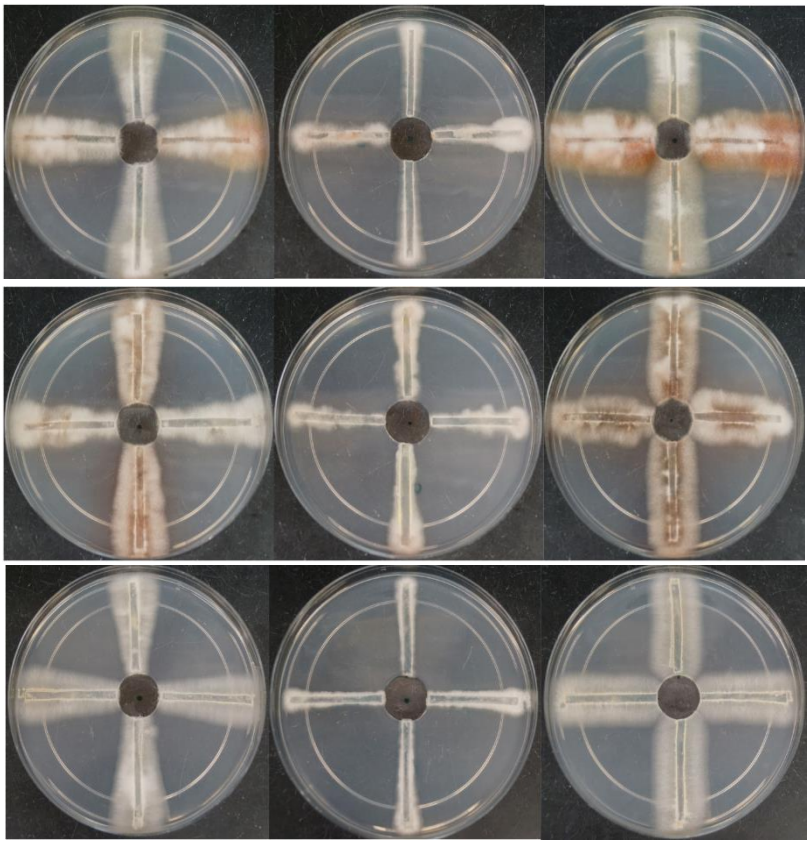
Overall results indicate that fungicides are similarly effective against the different species. Of note, between the two fungicides, pydiflumetofen had a stronger effect at slowing down mycelial growth than fluopyram.



Effect of 1000 ppm of fluopyram and pydflumetofen on reducing hyphal growth of F. falciforme sensu stricto (FF), F. martii (FM) and F. noneumartii (FN)



Effect of 5000 ppm of pydflumetofen on reducing hyphal growth of F. falciforme sensu stricto (FF), F. martii (FM) and F. noneumartii (FN)



Fluopyram, pydflumetofen, and control plates from trial 1 (1000 ppm).

Top row: *F. falciforme* isolates, with CS687 on the left/right sides and CS966 on the top/bottom. The first plate is fluopyram, the next pydflumetofen, the final the control.

Middle row: *F. martii* isolates, with CS162 on the left/right sides and CS951 on the top/bottom. The first plate is fluopyram, the next pydflumetofen, the final the control.

Bottom row: *F. noneumartii* isolates, with CS109 on the left/right sides and CS411 on the top/bottom. The first plate is fluopyram, the next pydflumetofen, the final the control

DISCUSSION

To assist growers in selecting varieties for FRD management, we complied a table based on combined results from 20 variety trials conducted from 2019 through 2023, and this table is being incorporated into the pest note, together with a fungicide trial table in preparation, as well as basic information on the disease (symptoms, distribution, pathogen identity).

This year we confirmed several newer FRD-tolerant cultivars including HM8268, HM8237, BOS0811, and SVTM9041. We also identified poorer performing varieties (to avoid using) including BP74, HM 5511, SVTM9032, SVTM9040, and H1662

Host range studies in 2023 have identified several new putative hosts for this pathogen, including carrots, broccoli, cabbage and cilantro. Economic impacts may also occur in these other crops, in rotation with tomato. For example, cilantro plants appeared highly susceptible, and would likely be a poor tomato rotation. In most cases, crops that developed symptoms in the greenhouse rarely developed symptoms in the field. We believe this lack of symptoms was caused by the cool winter temperatures and the short length of the trial. As the field had to be planted to tomatoes for the winter crop rotation study, the some of the cool season crops were not able to complete their full growing season, which likely resulted in underrepresentation of the ability for this fungus to cause disease. To better understand field host range, downstream studies are needed in which the crops are allowed to grow over their full crop cycle.

Our work has also identified several disease-enhancing rotations, including sunflower and safflower. In some cases, putative non-host crops such as alfalfa appear to be increasing disease risk; it is possible that the fungus can build up inoculum as a saprophyte on incorporated tissue. Understanding the saprophytic stage of this fungus and the role of crop organic matter in driving inoculum load build up can help to interpret and manage this effect. Interestingly, although both broccoli and vetch are putative hosts, both resulted in relatively low disease levels in tomatoes; both of these crops are reported as Fusarium suppressive, which may account for lower disease levels—an effect that merits further study. Overall, further work is needed in cool season crops to develop diverse rotation options for tomato growers.

While single year trials can provide some insight into disease risk, in most cases multi-year rotations between tomato crops are common. Commercial field surveys over time have allowed us to start to understand impacts of common multi-year rotations on tomato disease, and also understand the effects of repeat tomato plantings on disease development. We established that a 1-year cucumber rotation reduced disease in tomato, but subsequent planting of tomato (again) increased disease. Additionally, a multi-year commercial field rotation surveys, a tomato-sunflower-alfalfa-tomato rotation resulted in a 10% increase in disease development between the two tomato years.

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 tested fava, vetch, and alfalfa, all of which did not develop symptoms. We will be repeating this work to confirm these results. Other families, including the grass (corn), mallow (cotton) and cucurbit (melon) families appear to include species which thus far are not good hosts. We will further examine these relationships with additional grasses (barley).

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 Falciforme Species Complex (FFSC). 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*. Last year, we characterized a third virulent species: *F. martii*. This year we determined that *F. falciforme sensu stricto* is a weakly pathogenic species. From our controlled field trials, we have seen that *F. falciforme* sensu stricto may be so mild that it is not a management target as it does not appear to cause economic losses, but more work is needed to confirm this.

In evaluating co-management options for FFSC pathogens, cultivar trials broadly indicate that cultivars that are more resistant to *F. noneumartii* are also more resistant to the other species, although these trials need to be repeated under conditions more optimal for disease development. Similarly, chemicals (Miravis and Velum One) effective against *F. noneumartii* also appear effective against the other species. Thus, studies focusing on management of *F. noneumartii* using chemicals and resistant cultivars should be broadly effective against all FFSC pathogens.

Next steps:

As new cultivars are becoming available, it is important we repeat cultivar trials each year to provide up to date recommendations. For 2024 we would like to repeat the UC Davis controlled cultivar trial to evaluate 12-14 cultivars in a *F. noneumartii* infested field. In addition, we would provide diagnostics support to multiple commercial cultivar trials across the state.

Moving into next year we would like to complete our work with winter rotation crops, further characterizing the host range of *F. noneumartii* in winter crops and weeds and repeating the winter rotation study. To better evaluate the winter crops for symptoms, we propose to expand the field host range study and allow a section to grow into the summer so that the crops can fully mature and develop disease. We have seen some promising results from the first year of the winter rotation study, but this needs to be repeated to confirm these results are consistent across years. We also have promising results from multi-year rotation studies, and several ongoing sites that we would like to continue to collect data from. These collaborative commercial trials provide a valuable corroboration of single-year studies and enable us to evaluate multi-year rotations, which would not otherwise be feasible.

Over the last few years, we have done extensive surveys evaluating weeds for symptoms in FRD infested fields (controlled and grower fields) and evaluated the effect of weedy fallow on disease in tomato. We have found numerous common summer weeds to be hosts to *F. noneumartii* (nightshade, amaranths, horse weed, bindweed) which could increase soil inoculum. To further study this relationship and understand the overall risk of different weed species, we need to characterize host range in a controlled replicated manner. This summer we did a controlled pilot weed host range study where we had individual weed species level plots replicated across a field. This allowed us to not just determine which weeds are hosts, but also how often individual plants of a species are infected and develop symptoms. We would like to repeat this study in summer weeds and expand into cool season weeds.

Thus far we have furthered our understanding of the pathogen make up of this disease and the overall species complex to which it resides. We have shown *F. noneumartii* and *F. martii* to be highly virulent, economic species in need of management tools and *F. falciforme* ss to be a weak pathogen and potentially not an economic target for management. We need to further study *F. falciforme* ss to confirm its virulence and effect on yield before we remove it as a target. We also need to continue to characterize the within-species diversity to determine if all isolates of a particular species behave similarly. This will allow us to recommend isolates for further study and resistance breeding.

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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. 9/15/2023-12/31/2025. Aim: this project is looking at *F. falciforme* species complex pathogens across all California crops; it is not tomato specific (no support for crop rotation studies focused on tomato) but includes diagnostic and host range assessment efforts for pathogens of tomatoes.

NIFA-AFRI "Pathogenomics-Based Development of Crop-Specific Diagnostics Tools For Emerging And Expanding Fungal Diseases In The U.S." $1 mil. June 1, 2020-May 30, 2024. Of note, due to collaborator inactivity during covid, the Swett portion of this grant was directed to additional technician costs and there are no longer any dollars remaining. However, there is still support for other Co-PIs on the grant working to support California tomato producers.

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INVESTIGATION OF UNUSUAL OUTBREAKS OF CURLY TOP IN THE NORTHERN COUNTIES, RESPONSE OF TOMATO VARIETIES TO NEW BCTV STRAINS AND CONTINUED STATEWIDE DIAGNOSTICS FOR STRAINS OF BCTV AND TSWV

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Executive Summary:

In 2023, we continued our virus surveillance efforts in processing tomatoes in California. Because of the unusual outbreak of curly top disease (CTD) in the northern production area in 2021 and 2022, we performed regular field monitoring for CTD and beet leafhoppers (BLHs) on yellow sticky cards (YSCs) in Yolo and Colusa counties in 2023. We also focused on spotted wilt in the central and northern production areas through conducing field monitoring and field visits as well as receiving spotted wilt samples from our surveillance network of Farm Advisors, PCAs, growers, industry personnel and other stakeholders. **Curly top 2023.** The 2021 and 2022 outbreaks of CTD were associated with unusually hot dry windy weather in March and April and proximity to foothills, suggesting altered flights of beet BLHs carrying the rare BCTV-SpCT strain. Thus, the cool wet spring of 2023 provided the perfect conditions to determine if there was a correlation between these weather conditions and the unusual CTD outbreaks. The finding of little or no CTD in monitored fields in 2023 and very few BLHs captured around fields supported the idea that the extreme weather conditions facilitated these outbreaks. There is probably greater BLH mortality when conditions are cool and wet (BLHs like desert conditions). Thus, spring weather conditions may be a useful CTD predictor, at least in the northern production area. **Spotted wilt 2023.** During the above mentioned fields surveys, we began observing

spotted wilt symptoms in fields planted with resistant varieties in Yolo and Colusa counties, though at relatively low incidences. Initially, we detected the YPT RB strain in spotted wilt samples, as expected based on 2022 results. However, as the season progressed, many samples tested negative for the YPT strain, especially those from Colusa and Sutter counties. The reason for this was the emergence of a 'new' CPN RB TSWV strain! This was totally unexpected because this strain has only been reported from Spain. Most fields had spotted wilt by the end of the season, but still at low incidences, whereas fields in some hot-spot areas had high levels of spotted wilt. In addition, an unusual spotted wilt symptom phenotype was observed in some fields in the northern and central production areas, and this was attributed to the genetics of the underlying hybrid once Sw-5b resistance is broken rather than the 'new' CPN strain. In 2023, we did not detect RB TSWV strains in thrips emerging from the soil. We again ran the thrips DD modal that allows for targeting the critical 2nd and 3rd generations, thereby slowing spotted wilt spread. In 2023, we collected or received 317 processing tomatoes, the majority of which were spot wilt from resistant varieties. We continued to be very active in outreach and conducted field visits, gave presentations at grower meetings, and provided written materials, including many diagnostic reports.

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 viruluferous 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 it 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.

Main goals:

- Monitoring of processing tomato fields in the Northern Counties for beet leafhoppers and CTD and determining virus levels in BLHs and major strains involved in the 2023 growing season.
- Continued statewide surveillance and diagnostics for virus diseases, with an emphasis on curly top (BCTV) and spotted wilt (TSWV) and the strains that overcome the Sw-5b resistance, i.e., resistance breaking (RB) TSWV and running the DD model for thrips and TSWV.
- Maintain a strong outreach effort to provide timely reporting of results and recommendations to Farm Advisors, PCAs, growers and ag industry professionals. In this regard, we have worked in close collaboration with the new Farm Advisor Patricia Lazicki on this project.

Methodology and Results:

Objective 1. Surveillance for appearance and prevalence of viruses affecting processing tomatoes in 2023 with emphasis on BCTV and RB-TSWV.

The virus surveillance system brings together our 1) long-time experience and diagnostic tests that allow rapid and precise diagnostics and 2) our network (Farm Advisors, growers, PCAs and industry personnel). In 2023, this involved the diagnosis and testing of 317 for processing tomato samples from 8 counties (Table 1). In 2023, we detected four viruses that represented three levels of concern for the industry. This included **1) diseases/viruses of low incidence and no economic loss,** tomato necrotic spot (caused by ToNSV); **2) diseases/viruses of regulatory concern,** tomato brown rugose fruit virus (ToBRFV) and associated tobamoviruses (ToMMV and ToMV); and 3) **diseases/viruses of current economic importance**, i.e., spotted wilt caused by TSWV and curly top caused by BCTV. In terms of TSWV and BCTV, we continue working on outbreaks of each virus in the Northern counties, i.e., 1) a severe and unusual outbreak of curly top and
2) the first widespread detection of RB-TSWV. **In handling the virus disease issues facing tomato production in California in 2023, the surveillance system functioned as intended in providing a central location for processing, diagnosis and rapid reporting of test results and associated information for samples with virus-like symptoms.**

Table 1. Total tomato samples collected or received from processing tomato fields and results of tests for different viruses

County	Total Samples	Number of samples tested for each pathogen:			
		TSWV	BCTV	ToNSV	Tobamovirus
Colusa*	53	49	4	0	0
Sutter*	33	12	21	0	0
Yolo*	149	143	4	0	2
San Joaquin	6	6	0	0	0
Solano	3	3	0	0	0
Madera	2	0	0	0	2
Fresno	69	43	21	5	0
San Diego	2	2	0	0	0
Total	317	258	50	5	4

*Northern Counties

ToNSV was only detected in Fresno. In 2023, we did not detect ToNSV in northern counties as we observed in 2022. As previously observed, infected plants recovered from these symptoms and little plant to plant spread by this virus, which is transmitted by pollen-mediated thrips transmission. Thus, although incidences in some fields in Fresno County were high, we still do not believe that this virus is a threat to tomato production.

BCTV situation in 2023-Central production area.

The incidence of curly top was relatively low in Fresno County, also possibly a benefit of the cool and wet weather. Symptoms of curly top and spotted wilt were observed in tomato fields beginning in May. PCR tests of representative symptomatic leaves indicated the BCTV strains associated with curly top symptoms in the Central Valley were mostly BCTV-CO and less BCTV-Wor, (Tables 2).

Table 2. Strains of beet curly top virus (BCTV) detected in samples of tomato plants with symptoms of curly top collected or received in 2023.

County	No. of samples	Multiplex PCR for mild and severe type BCTV strains				PCR with BCTV strain-specific primers					
		mild-type	severe-type	Mixed**	Negative	BCTV-SpCT	BCTV-CO	BCTV-Wor	B CTV-LH71	CO+Wor	Other mixed
Colusa*	4	1	0	0	3	0	1	0	0	0	0
Sutter*	21	12	6	0	3	6	5	3	0	4	0
Yolo*	4	2	1	0	1	1	1	0	0	1	0
San Joaquin	---	---	---	---	---	---	---	---	---	---	---
Solano	---	---	---	---	---	---	---	---	---	---	---
Madera	---	---	---	---	---	---	---	---	---	---	---
Fresno	21	14	1	4	2	1	10	2	0	2	4
San Diego	---	---	---	---	---	---	---	---	---	---	---
Total	50	29	8	4	7	8	17	5	0	7	4

*, northern California counties.

**, mixed infection of a mild-type and a severe-type BCTV strain.

BCTV situation in 2023-Northern production area .

Typically, CTD is most prevalent in Fresno, Kings and Kern Counties and is also observed in Merced, Stanislaus, and San Joaquin Counties. However, in 2021 and 2022, there was a widespread outbreak of curly top in the Northern Counties that stretched from Yolo to Glenn. In 2023, eight processing tomato fields in the northern production area (4 in Colusa and 4 in Yolo counties) were selected for monitoring incidence of CTD, populations of BLHs with YSCs and detection of BCTV in the BLHs captured on YSC. These fields were in locations were CTD was severe in 2021 and 2022.

Results of field surveys conducted from April to July revealed trace amounts of CTD symptoms in the eight monitored fields, even in those planted next to foothills areas where incidences of CTD were high in 2021 and 2022 (Table 2). The low incidence of CTD in the monitored fields in 2023 was also observed in other commercial fields in the northern production area by growers, PCAs and Farm Advisors. This also was reflected in the small number of CTD samples received in 2023, i.e., 50 samples only and most were not CTD. There also were no requests for field visits to investigate possible CTD outbreaks in 2023. Notably, the unusual BCTV-SpCT strain, which was involved with the early season (April-May) curly top outbreaks in northern counties in 2021 and 2022, was only detected in Sutter County in 2023 (Table 2).

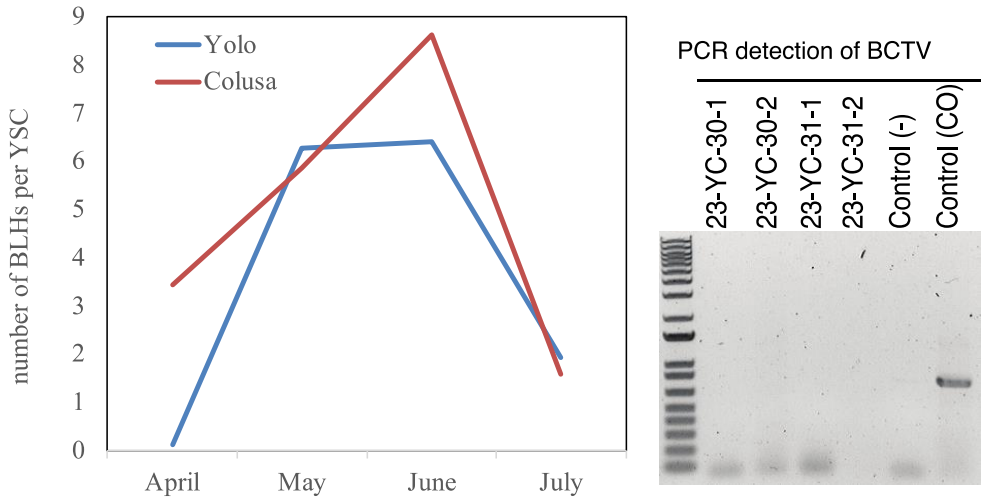

Figure. 1. Number of beet leafhoppers (BLH) detected on yellow sticky cards yellow (YSC) from eight processing tomato fields in Yolo and Colusa counties in 2023 and the detection of beet curly top virus (BCTV) in BLH from four representative cards..

RB-TSWV situation: 2023.

The surveillance system helped recognize and confirm outbreaks of spotted wilt disease in fields with resistant processing tomatoes in the northern production area in 2023. Furthermore, we established that two RB TSWV strains were causing spotted wilt in resistant varieties in the

northern production area in 2023: YPT and the new CPN strain. The YPT strain was presumably introduced in this area in 2021, whereas the new CPN strain apparently emerged in the northern production area in 2023 (Table 3).

We developed a specific primer pair to detect this 'new' CPN strain and validated a RT-PCR test specific for this RB strain. We used RT-PCR tests and pathogenicity tests to confirm that the RB-TSWV CPN strain was widely distributed in the northern production area in 2023, but was not detected in samples from the central production area (e.g., Fresno County) (Table 3). Furthermore, isolates of the new CPN strain broke Sw5b resistance and were highly virulent in pathogenicity tests, more so than isolates of the YPT strain tested at the same time. In Northern Counties, the new CPN variant was predominant in Sutter and Colusa counties, whereas in Yolo County the YPT and CPN strains were detected at similar levels (Table 3).

Table 3. Number of samples per county were the RB-TSWV variants were detected in samples collected in 2023.

County	RB-TSWV variants				
	CPT	YPT	CPN	mix	Total
Colusa	0	10	37	2	49
Sutter	0	4	8	0	12
Yolo	0	62	75	3	140
San Joaquin	0	6	0	0	6
Madera	0	0	0	0	0
Fresno	0	43	0	0	43
San Diego (Oceanside)	0	2	0	0	2

Through mechanical inoculations in two TSWV-resistant tomato varieties, it was confirmed that the new variant of RB-TSWV can overcome the resistance conferred by the Sw5 gene (Table 4).

Table 4. Pathogenicity test of selected RB-TSWV isolates in resistant tomato varieties.

ID Sample	County	RB-TSWV variant	Resistant tomato varieties (Sw-5 gene)*		Susceptible tomato variety (no Sw-5)*
			N6415	HM3888	Glamour
23-26	Yolo	CPN	9/9 (3+)	9/9(3)	9/9 (3)
23-38	Colusa	CPN	9/9 (3)	9/9 (3)	9/9 (3)
23-49	Sutter	CPN	9/9 (3)	9/9 (3)	9/9 (3)
23-51	Sutter	YPT	9/9 (2)	9/9 (2)	9/9 (2)
23-24	Oceanside	YPT	9/9 (3)	9/9 (3)	8/9 (3)

Finally, we did not detect ToBRFV in processing tomato samples from California in 2023, consistent with results of previous years. We did detect ToBRFV in blotchy tomato fruit purchased at a Trader Joe's store in Davis, CA (Yolo County) and that was most likely imported from Mexico. We confirmed ToBRFV infection with our LAMP and RT-PCR tests. This shows the virus continues to come into California in such fruits.

Objective 2. Predict thrips generations with the DD model and detect TSWV in thrips emerging in the spring.

RB TSWV was not detected in adult thrips recovered from yellow sticky cards put out early in the season in Fresno. Table 5 presents the months, number of YSC, number of thrips on YSCs and the number of these thrips in which RB-TSWV was tested with our RT-PCR test.

Predictive degree day (DD) model for thrips.
We continued to run the thrips degree day (DD) model through the ANR website: http://ucanr.edu/sites/TSWVfieldriskindex/Thrips_Population_Projections/. This model predicts the appearance of thrips generations, but does not predict actual numbers of thrips. Based on our knowledge of the biology of the virus-thrips interactions, and it is recommended to target 2-3rd generation adults for insecticide treatment, in order to slow the spread of the virus by virus-carrying adults, with the urgency to spray increased if TSWV has also been detected in the field.
The DD model was run in 2023 and provided updates on thrips generations that can inform growers of the most effective times to target thrips (period of 2-3 generations) as well as appearance of spotted wilt symptoms. In general, the DD model predicted delayed thrips generations, consistent with the cool wet weather in the spring.

Table 5. Detection of TSWV in early season thrips emerging from soils using yellow sticky cards (YSC) in Fresno.

Date of sampling	# of YSC	# of thrips/ YSC	# of RT-PCR positive for RB-, WT-, NSm-	Observation
March, 2023	4	85	0	---
April, 2023	23	1066	0	---
May, 2023	7	>2123	0	---

Objective 3. Maintain a strong outreach effort to provide timely reporting of results and recommendations to Farm Advisors, growers, PCAs, and industry personnel.

During 2023, we continued our outreach efforts, as indicated below.

- 2023 UCCE Northern San Joaquin Valley Processing Tomato Meeting, Wednesday, February 8th, 2023, 8:30 – 12:00 pm at Robert J. Cabral Agricultural Center – Assembly Room 2, 2101 E Earhart Ave, Stockton, CA 95206. (talk delivered by Dr. Tomas Melgarejo)
- Beet Curly Top Virus Informational Meeting, Friday, March 24, 2023, 1:30-3:00 pm at 100 Sunrise Blvd., Colusa, CA 95932.
- Tomato spotted wilt virus—incidence, symptoms, and management Meeting, July 7th, 2023, 1:30 -2:30 pm at Norton Hall, 70 Cottonwood Drive, Woodland, CA 95695.

- Field visits were made to Yolo County (with Patricia Lazicki) and Colusa County (with Gerry Hernandez) to evaluate curly top and view other disease. This field visits were carried out mainly in the tomato fields that we monitored BLH populations but also, we visited several other tomato fields in Yolo and Colusa counties.
- Presentation at Napa Processing Tomato Meeting November 13, 2023.

Discussion:

The wet and rainy weather conditions in 2023 were a welcome relief for the processing tomato industry and also provided the contrasting weather conditions to test the hypothesis that it was the hot dry windy weather in springs of 2021 and 2022 that facilitated the unusual outbreaks of CTD occurred in the northern production area. Thus, the finding of little curly top in monitored field and in other commercial fields, as well as the low population of BLH supported this idea and may useful for trying to predict CTD, at least in the northern production area. Thus, with what is predicted to be another wet year in California, we would predict low CTD incidence in 2024. For the central production area, the detection of low levels of BCTV in BLH from YSC in Fresno was consistent of relatively low CTD incidences in most fields. Conversely, when weather conditions are hot dry and windy in the spring, growers may want to use a systemic insecticide treatment of transplants, especially for fields near foothills areas or other risk factors.

It was no surprise to observe spotted wilt in processing tomatoes field in the northern production area in 2023 because: i) the YPT RB strain was introduced in 2021 and ii) most fields are planted with resistant varieties. However, the incidence in most field remained low (<5%) in most fields, suggesting that most thrips did not have TSWV at this time and that growers were spraying for thrips. Higher incidences were observed in fields in hot-spot areas, e.g., Knight's Landing and Colusa (near the levee) and some were associated with late planting when more virus-carrying thrips are present. We also identified the emergence of the 'new' CPN RB strain that seems to have emerged in Colusa and Sutter counties in 2023 based on it being the predominant strain in samples from these counties. The YPT strain was still detected in many samples, especially from Yolo County. However, it was quite remarkable was remarkably rapid and the CPN strain spread and competed effectively with the YPT strains. The pathogenicity test results showing that isolates of the CPN strain were highly virulent, consistent with the capacity to spread. We failed to detect the CPN strain in spotted wilt samples from the central production area and all were infected with the YPT strain. There results indicate continuing and changing pressure on the Sw-5b resistance gene, and potential for higher incidences under favorable conditions. Thus, growers and PCAs should i) plant clean transplants, ii) avoid risk factors, iii) monitor for thrips and TSWV iv) target the 2nd and 3rd generations as predicated by our DD model, v) prompt sanitation, and vi) avoid bridge crops (lettuce, radicchio and fava beans. It still makes sense to plant Sw-5b varieties, but most importantly we need to identify sources of resistance to these emerging RB TSWV strains.

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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. We also would like to thank our dedicated and hard-working undergraduate students Yige Chen and Eric Hickerson. Special thanks to Emeritus Farm Advisor Gene Miyao who is tirelessly walking tomato fields and is our eyes and ears in the field!

EVALUATION OF NEW AUTOMATED WEEDERS IN PROCESSING TOMATOES

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Year of Project Initiation: 2023 (similar projects were funded in 2020 – 2022. See Vinchesi and Stoddard, 2020)

Executive Summary:
Current automated weeders use machine learning to recognize crops and weeds. These automated weeders have been widely adopted in vegetable crops like lettuce because they improve hand weeding efficiency. The objective of this project was to determine if new recent generation automated weeders can provide similar benefits to the processing tomato industry. A Kult robotic cultivator, Carbon Robotics laser weeder, a 2015 Robovator, and Steketee finger weeder were evaluated in five different trial locations and planting dates in 2023. Not all equipment was evaluated at each location. The finger weeder provided good weed control (average 61% control) with minimal cost, and was found safe to use on processing tomatoes. It was probably the best option of the equipment tested. The use of intelligent weeders/automatic weeders/robotic weeders evaluated in this project significantly reduced weed pressure and reduced hand weeding time (vs the UTC) 30% to 64%, but were slow as compared to the less expensive but fairly effective finger weeders. Cost savings ranged from $14 - $96 per acre. Both the Kult and the Robovator caused some crop injury – plant stand was reduced 16 - 20% as compared to the other treatments. The most effective cultivators in lettuce are the Stout Smart Weeder and the Farmwise Titan weeders, which use AI technology. These cultivators need to be tested in processing tomatoes as we suspect they would work well. Those companies were not ready to work in commercial processing tomatoes in 2023. Additional research is planned in 2024.

Introduction

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 planting/transplanting often moves the soil and herbicides, resulting in little weed control in the plant row. The result is that hand weeding is often still required.

Automated weeders, or robotic weeders, offer the ability to remove weeds within the crop row. Research has shown that automated weeders reduced labor use in lettuce production by an average of 38-45%

without reducing yield, though this was dependent on the unit used, field efficiency, production conditions, and weed pressure (Fennimore and Tourte, 2019). Potential problems with this equipment include reliability, accuracy, and risk of crop loss from the machines.

Several new automated weeders have been introduced to the market during the past two years. These "smart" machines are more capable at differentiating the crop and weeds than the first automated weeders, such as the Robovator, which have been evaluated for 3 seasons in processing tomatoes in Merced and Colusa Counties. In research trials conducted in the Salinas area on various crops, these new weeders were very effective at removing weeds in the plant line without damage to the crop, and this resulted in a significant reduction in hand weeding time. Current automated weeders such as the Stout Smart Weeder and Kult use machine learning to recognize crops and weeds. These automated weeders have been widely adopted in vegetable crops like lettuce because they improve hand weeding efficiency. The objective of this project was to determine if new recent generation automated weeders can provide similar benefits to the processing tomato industry as they have in leafy vegetables.

Objectives:

- **1:** Evaluate the performance of auto weeders for controlling weeds in the plant row in processing tomatoes.
- **2**: Conduct a simple economic analysis on cost savings from auto weeders

Methods

Objective 1: Evaluate the performance of auto weeders in processing tomatoes.

While the original proposal was written with the intent of doing a thorough evaluation of the newest automated weeders commercially available, lack of company interest and/or difficulty accessing the equipment during the cultivation window after transplanting severely limited the machines tested. In the end, we tested the Kult robotic cultivator and the Carbon Robotics laser weeder. These were compared to a 2015 Robovator robotic cultivator, and finger weeder, and a standard system utilizing Matrix (rimsulfuron) herbicide applied in a band about 2 weeks after transplanting (Figure 1). These machines are automated weeders and both are capable of weeding around crop plants to control weeds in the row. Trials were done in four commercial fields (Soledad, Gilroy, and Dos Palos) and at the UC Davis field research lab in Davis. Not all treatments were evaluated at each location (Table 1).

For each location, treatments were imposed 2 to 3 weeks after transplanting and compared to the grower's normal program in commercial fields. Weed counts and tomato stand counts were taken before and after cultivation. Hand weeding times were measured to determine the effect of cultivator type on labor inputs by timing 1 person to manually remove emerged weeds growing in the plant row with a hula-hoe for the entire plot. Yields were estimated at the Teixiera Farms location by hand harvesting 10 ft from the center of each plot. Tractor speeds were measured to estimate cultivation time per acre. Treatments were replicated a minimum of four times and arranged in a RCBD at each location, mean separation was conducted using Fisher's protected LSD.

Figure 1. Clockwise from upper left: Kult, Robovator, finger weeder, and laser weeder.

Table 1. Location and equipment evaluation summary:

	LOCATION and DATE				
	Gilroy 25-May	**UC Davis 20-June**	**Soledad 23-May**	**Dos Palos 1 25-May**	**Dos Palos 2 28-June**
TREATMENT	Paul Mirassou	Veg crops farm	Josh Roberts	Dan Burns	Kirk Texiera
1.. Untreated control (UTC)[1]	X	X	X	X	X
2. Kult robotic weeder	X	X		X	
3. Carbon Robotics laser weeder			X		X
4. Robovator (2015)					X
5. Steketee finger weeder		X			X
6. Matrix herbicide banded 4 oz/A		X			X

[1]*UTC treatments were normal grower weed management.*

Results

Objective 1: Evaluate the performance of auto weeders in processing tomatoes.

Gilroy. The Kult autoweeder was evaluated at the Paul Mirassou farm near Gilroy, CA May 25, 2023. Field set up was on single row 60" beds; plant spacing was 14". Weed counts were made before and after cultivation and percent weed control was calculated. Main weeds at this location were nightshades. Following cultivation, hand weeding was conducted and timed. The Kult cultivator removed 91% of the weeds. Hand weeding times were 9.1 hours per acre in the control plot and 3.3 hours per acre following cultivation with Kult, a reduction of 64%.

Davis. This report presents findings from a study conducted June 20, 2023, at the UC Davis's Vegetable Crop Field Station. The objective of the study was to assess weed control efficacy and crop injury of three treatments, Kult, Steketee finger weeder, and Matrix herbicide at 4 oz/A, compared to an untreated control. Field set up was SSDI single row 60" beds with 12" plant spacing. Treatment design was a randomized complete block with 4 replications. Plot lengths were 200 ft for the cultivator treatments, and 100 feet for the Matrix herbicide and untreated control. Weed counts were recorded before and after treatment, and the main weeds were pigweeds, purslane, bindweed, nutsedge, puncture vine, lambsquarters, and malva.

The Kult cultivator removed 87% of the weeds which was significantly more than the Finger Weeder which removed 60% of the weeds. Hand weeding times per acre were similar with both cultivators Kult 1 hour/A and Finger weeder 1.2 hour/A. Both cultivators significantly reduced hand weeding times compared to the control of 52% and 44%, respectively (Table 2).

This project was part of the UC Davis Weed Day tour on June 21, 2023, and the equipment was demonstrated. About 80 people were in attendance.

Table 2. Weed control results, UC Davis field site.

Treatment	PRE Weed Count	POST Weed Count	Hand weeding	Weed Control[1]
	number per 1000ft^2		hours/A	%
1. Control	37.5	37.5	2.06 a	0 c
2. Finger Weeder	28.3	10.8	1.16 b	60.47 b
3. Kult	23.3	3.0	0.98 b	87.15 a
LSD (p = 0.05).			0.442	15.6
Treatment Prob (F)			0.001	0.001

[1] *Percent control rating based on comparisons to the non-treated control.*

Soledad. A trial was conducted at Soledad, CA in an organic processing tomato field with Josh Roberts at JV farms May 23, 2023. The treatments were the Carbon Robotics laser weeder and an untreated control. Weed densities measured before and after laser weeding were collected in 100 ft long sample areas. Each treatment was replicated 4 times.

The laser weeder removed 79% of the weeds and reduced hand weeding times from 6.35 hours in the control to 2.8 hours in the laser weeded plots, a reduction of 56%. However, the laser weeder operated very slowly, less than 1 mph.

Dos Palos 1. A trial was conducted near Dos Palos, CA, with San Juan Ranch on May 25, 2023, in a conventional processing tomato field with the Kult robotic weeder and compared to adjacent areas. Field set up was SSDI single row 60" beds with 12" plant spacing and was about 3 weeks old at the time of treatment. Grower's standard herbicide program was Treflan + Dual PPI; no Matrix had been applied at the time of cultivation. The Kult robotic weeder occasionally errored and removed tomato plants while weeding, which resulted in a significant (p 0.07) 20% reduction in plant stand as compared to adjacent rows: 4367 compared to 5500 plants per acre. Weed pressure was very low at this location and remained that way for the duration of the trial, and therefore no weed control data were collected.

Dos Palos 2. Due to lack of weed pressure at the first location, the trial was repeated in a late season commercial field with Teixiera Farms, near Dos Palos, CA, beginning June 28, 2023. Field set up was SSDI double-row 80" beds with 24" plant spacing and was 2 to 3 weeks old at the time of treatments. Carbon Robotics laser weeder was evaluated on June 28, while the Robovator and finger weeder treatments were done on July 10. A banded application of Matrix at 4 oz/A was applied on July 12 using a backpack sprayer at 40 psi and T-Jet 8002 nozzles in a 12" band over the top of the tomatoes. Plots were 1 bed x 175 ft long and replicated 5 times. Weed and plant injury ratings were made from a 15' section within each plot before and after each treatment. Main weeds included lambsquarters, pigweed, purslane, puncturevine, and nightshade. In general, both the Robovator and the finger weeder had better weed control than the laser weeder, however the laser weeder was evaluated very early after transplanting and there was a secondary flush of weeds that emerged after treatment. As a result, the laser weeder had only 12% weed control as compared to the UTC plots, but still had a 30% drop in hand weeding time (Table 3). In-row weed control was 62% to 85% for the finger weeder and Robovator, respectively. Hand weeding times were significantly reduced from 3 hours/A to 1 hour/A, a reduction of 67% (Figure 2). The Matrix plots were weeded by a hoeing crew on July 19 before an evaluation could be made.

Yield results are shown in Table 3. This was a very late field, and yields are low, average about 25 tons/A, but there were no significant differences in yield between treatments.

Table 3. Pre and post weed counts, % control, and hand weeding times for each treatment, Teixeira Farms 2023.

Post Plant Treatment	PRE plants/A	PRE weeds/A	POST plants/A	POST weeds/A	crop injury, %	control, %	hand weeding hours/A	$ 17.49 $/A	yield T/A
1. UTC	6531	5225	6444	8708	---	---	3.0	$ 52.35	24.053
2. Carbon Robotics laser weeder on June 2	6270	4267	6270	4354	0.0%	12.4%	2.1	$ 37.08	23.615
3. Robovator on July 10	6444	7053	5486	1045	14.7%	85.4%	1.0	$ 18.06	22.838
4. Steketee Finger Weeder on July 10	6792	7227	6705	1742	1.3%	61.6%	1.1	$ 18.42	27.181
5. Matrix herbicide on July 12*	6182	6705	6182	---	0.0%	---	---	---	26.430
Average	6444	6095	6217	3962	4.0%	53.1%	1.80	$ 31.48	24.823
LSD 0.05	ns	ns	642	6074	---	32.2	1.2	$ 20.93	ns
CV, %	6.4	96.3	9.4	57.6	---	41.6	48.3	48.3	15.7

All weed counts from 6" band in center of bed.

Matrix herbicide plots were hand weeded before weed counts were performed.

LSD 0.05 = Least significant difference at the 95% confidence level. Post weed statistical analysis performed on square root corrected data; arithmetic means shown.

--- = no data, or analysis could not be performed

CV = coefficient of variation

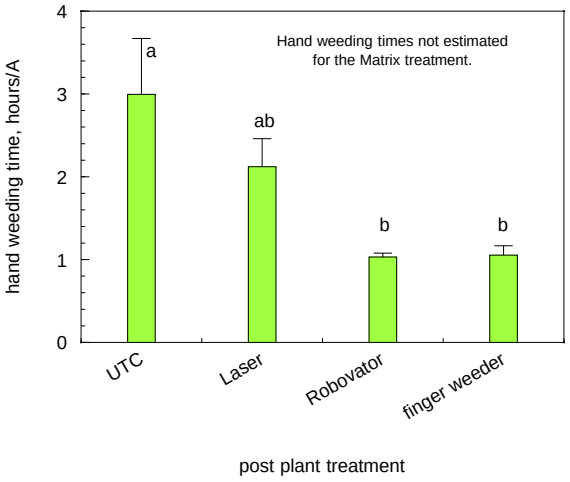


Figure 2. Hand weeding times for the Teixeira Farms location.

Objective 2: Conduct a simple economic analysis on cost savings from auto weeders.

A simple economic analysis was performed using hand weeding times and equipment speed. Hand weeding estimates were made by measuring the amount of time for one person to hand weed the center of the plots immediately after the cultivation or laser weeding treatments. Equipment estimates were based on measured working speed for each implement and assumed a 20 ft working width. Hand weeding costs were estimated based on an hourly rate of $17.49 per hour ($16.50 + 6% overhead); tractor drivers at $22.50 per hour. Measured speeds were 0.8 mph for the laser weeder, 1.8 mph for the Robovator and Kult, and 3.8 mph for the finger weeder. The economic analysis does not consider additional expenses such as equipment cost, tractor fuel, or maintenance. No hand weeding times were made for the Matrix herbicide treatments in Dos Palos or in Davis. The following chart shows estimates used for this analysis:

Implement	Speed	A/hr[1]	Labor[2] $/A
Kult robotic weeder	1.8 mph	4.4	$5.11
Carbon Robotics laser weeder	0.8 mph	1.9	$11.84
Robovator robotic weeder	1.8 mph	4.4	$5.11
Steketee finger weeder	3.8 mph	9.2	$2.45

[1] *Number of acres per hour based on 20-ft tool bar.*

[2] *Tractor driver labor rate calculated at $22.50/hr.*

At all locations except Dos Palos 1, hand weeding times were significantly reduced, and therefore weeding costs were also reduced as compared to the untreated control even with the inclusion of the equipment labor charge (Table 4). Cost savings ranged from $14 to $96 per acre, depending on location. The cost of Matrix herbicide, $45/acre, was relatively high, however, Matrix provides much longer weed control that eliminates the need for a second hand weeding, which was not measured in these trials. According to the newest cost study on processing tomatoes (2023), typical hand weeding costs are $230 per acre (Aegerter, et al., 2023).

Table 4. Simple economic analysis based on hand weeding costs and equipment speed.

TREATMENT	Gilroy 25-May	UC Davis 20-June	Soledad 23-May	Dos Palos 1 25-May	Dos Palos 2 28-June
	Paul Mirassou	Veg crops farm	Josh Roberts	Dan Burns	Kirk Texiera
		$ per acre per location			
1.. Untreated control (UTC)[1]	$159.16	$36.03	$111.06	$0.00	$52.35
2. Kult robotic weeder	$62.83	$22.25	---	$5.11	---
3. Carbon Robotics laser weeder	---	---	$60.81	---	$48.92
4. Robovator (2015)	---	---	---	---	$23.17
5. Steketee finger weeder	---	$22.74	---	---	$20.87
6. Matrix herbicide banded 4 oz/A[2]	---	$45.00+	---	---	$45.00+

[1] *UTC treatments were normal grower weed management.*

[2] *Matrix herbicide costs shown are for herbicide application only, assuming 1 application made at 3.8 mph. Since hand weeding times were not measured for this treatment, values shown are less than would have been estimated.*

Acknowledgements: Many thanks to the grower cooperators who made this test possible: Paul Mirassou, Josh Roberts, Dan Burns, and Kirk Terixiera. Additional thanks to the equipment manufactures/retailers for their participation: Joe Sutton, Sutton Ag; Christian Kirchoff, K.U.L.T.; and Edgar Perez with Carbon Robotics.

References

Aegerter, B., Lazicki, P., Miyao, G., Stewart, D., and Goodrich, B. 2023. Sample costs to produce processing tomatoes in the Sacramento Valley and northern Delta – 2023. UC Cooperative Extension, Department of Agriculture and Resource Economics, Davis, CA. https://coststudies.ucdavis.edu/en/current/

Fennimore, S. A. and L. Tourte. 2019. "Regulatory burdens on development of automated weeding machines and herbicides are different". Outlooks on Pest Management. August 2019: p 147-152.

Vinchesi-Vahl and Stoddard, 2020. Cost-benefit analysis of automated planters and cultivators in processing tomatoes. FINAL REPORT TO CTRI. https://files.constantcontact.com/11ae1167201/9936bead-8f5b-46e3-bf54-7383768cc199.pdf

2024 BOARD APPROVED RESEARCH - PROJECT LIST

CTRI ANNUAL RESEARCH FUNDING: FINAL BOARD DECISIONS (NOVEMBER 30, 2023)				
2024 TOTAL OF ALL PROPOSALS RECEIVED: $923,843				
2024 TOTAL AFTER FINAL BOARD DECISIONS: $677,118				
Broomrape Containment, Control and Management		**Research Lead**	**Institution**	**$353,420**
2020 Start	Broomrape: Devt. of Long Term Mgmt. Options: CA Commercial Field Conditions & Contained Research Facility Ongoing Work	Brad Hanson	UC Davis	$ 54,347
2020 Start	Broomrape: Devt. of Long Term Mgmt. Options: Chilean Commercial Field Conditions *	Juan Carlos	UC Davis - Chile	$ -
2021 Start	Developing best equipment sanitation practices for broomrape and other high-profile soil borne pathogens; to mitigate field-to-field spread*	Cassandra Swett	UC Davis	$ 54,158
2022 Start	Determining the population structure of Phelipanche ramosa and Orobanche aegyptiaca field detections in California	Adam Schneider	UW-LaCrosse	$ 36,876
2022 Start	Developing Tomato Lines Resistant to Branched Broomrape, a Critical California Pest*	Neelima Sinha	UC Davis	$ 15,000
2022 Start	Inducible Suberin for Tomato Drought Tolerance (root architecture)	Siobhan Brady	UC Davis	$ 87,866
2023 Start	Detection of Broomrape Infestations with Remote Sensing*	Alireza Pourezza	UC Davis	$ 36,345
2023 Start	Screening of a VOC Sensor to Identify Broomrape Infestations*	Cristina Davis	UC Davis	$ 68,828
Agronomic/Water/Nutrient Management				**$ 54,339**
2022 Start	Adapting the 'CropManage' weather-based irrigation decision-support tool – Further Investigation of Pulse Irrigation *	Zheng Wang	UC Extension	$ -
2023 Start	Evaluation of materials to mitigate negative effects of salinity and high temperatures on yields of processing tomatoes	Tom Turini	UC Extension	$ 21,312
2023 Start	Climate Smart Mgmt. Innovations for Improved Soil Quality, and Productivity of CA Processing Tomatoes	Amelie Gaudin	UC Davis / UC Extension	$ 18,997
2023 Start	KPAM in Soils with RKN AND Fungal Challenges - Impacts on Yield and Disease Severity	Patricia Lazicki	UC Extension	$ 4,725
2024 New	Assessment of Novel Transplanter Performance and Economics	Patricia Lazicki	UC Extension	$ 9,305
Germplasm and Variety Development				**$ 48,475**
1991 Start	C. M. Rick Tomato Genetic Resource Center	Roger Chetelat	UC Davis	$ 25,000
2024 New	Leveraging germplasm resources for genetic discovery and deployment of salt stress resilience	Greg Vogel	Cornell	$ 23,475
2021 Start	Marker-assisted breeding for polygenic tomato spotted wilt resistance in tomatoes*	Reza Shekasteband	NCSU	$ -
Insect & Invertebrate Management				**$ 33,422**
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	$ 33,422
2011 Start	Evaluation of Alternative Nematicides for the Control of Root-Knot Nematodes of Processing Tomatoes - with addition of novel biocarriers *	Jaspreet Sidhu	UC Extension	$ -
Pathogen Management				**$176,587**
2017 Start	Disease diagnosis, pathogen movement / emergence monitoring, new pathogen ID and F4 monitoring for the CA processing tomato industry	Cassandra Swett	UC Davis	$ 42,282
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	$ 53,323
2020 Start	Developing an integrated mgmt. strategy for F. falciforme vine decline in processing tomato, including co-management with Fusarium wilt	Brenna Aegerter	UC Extension	$ 6,267
2024 New	Evaluation of Insecticide Programs in Processing Tomatoes for the MGMT of BCTV and TSWV Vectors and Viruses	Tom Turini	UC Extension	$ 7,715
2017 Start	Viral Diagnostics*	Robert Gilbertson	UC Davis / UC Extension	$ 67,000
Weed Control and Management				**$ 10,875**
2023 Start	Evaluation of new automated weeders in processing tomatoes	Scott Stoddard	UC Extension	$ 3,950
2024 New	Controlling in-row weeds with post plant applications of pre-emergent herbicides	Scott Stoddard	UC Extension	$ 6,925

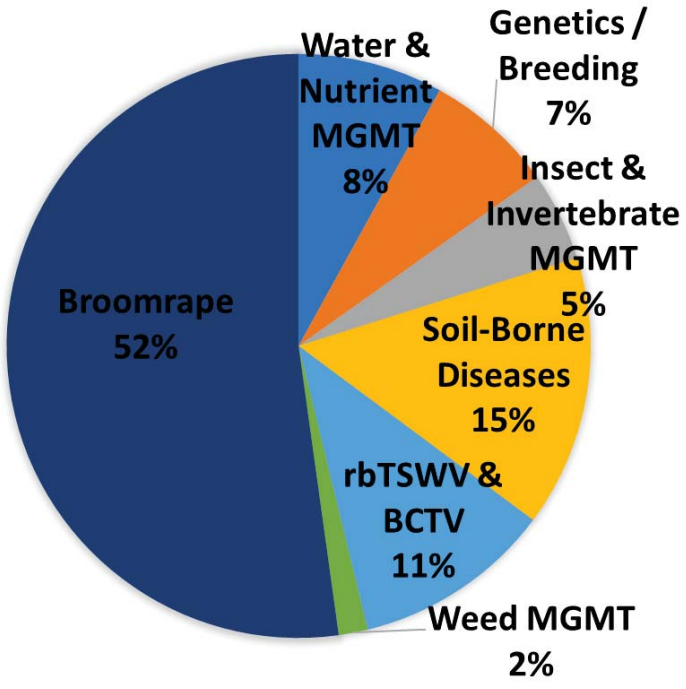
* Denotes a project which is ongoing with the review and confidence of the CTRI, but with 100% outside funding

*High Potential but TBD cost-sharing support from CLFP and CDFA. Total potential savings of $122,666.

2024 RESEARCH - DOLLAR ALLOCATION

2024 Approved Project Funding			
Category	**Funding**		**%**
Broomrape	**$**	**353,420**	**52%**
Field Management	$	54,347	
Sanitation*	$	54,158	
Population Genetics	$	36,876	
Genetic Resistance*	$	102,866	
Detection*	$	105,173	
Agronomic	**$**	**45,034**	**7%**
Genetics	**$**	**48,475**	**7%**
University Breeding Projects	$	23,475	
TGRC-Rick Center	$	25,000	
Pest Management	**$**	**210,009**	**31%**
Diagnostics Support	$	42,282	
Fusarium et al.	$	59,590	
Viral (BCTV & TSWV)*	$	74,715	
Root-Knot Nematode	$	33,422	
Product & Process	**$**	**-**	**0%**
Automation	**$**	**13,255**	**2%**
Transplanter Technology	$	9,305	
Weeding Technology	$	3,950	
Weed Management	**$**	**6,925**	**1%**
TOTALS	**$677,118**		**100%**

*High Potential but TBD cost-sharing support from CLFP and CDFA. Total potential savings of $122,666.



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2023

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