ANNUAL RESEARCH REPORT

for

2020

FULL VERSION

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

TABLE OF CONTENTS

INTRODUCTION

Welcome, this report marks the 52nd 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 ($0.07/paid ton in 2020) 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 600 research projects have been supported. These projects have primarily focused on improving field production, particularly in the areas of: pest management (250+ projects); variety development, pre-breeding and variety evaluation (150+ projects); agronomics (100+ projects); market development and process quality (75+ projects); and automation (25+ projects). Figure 1 charts our long running research categories over time.

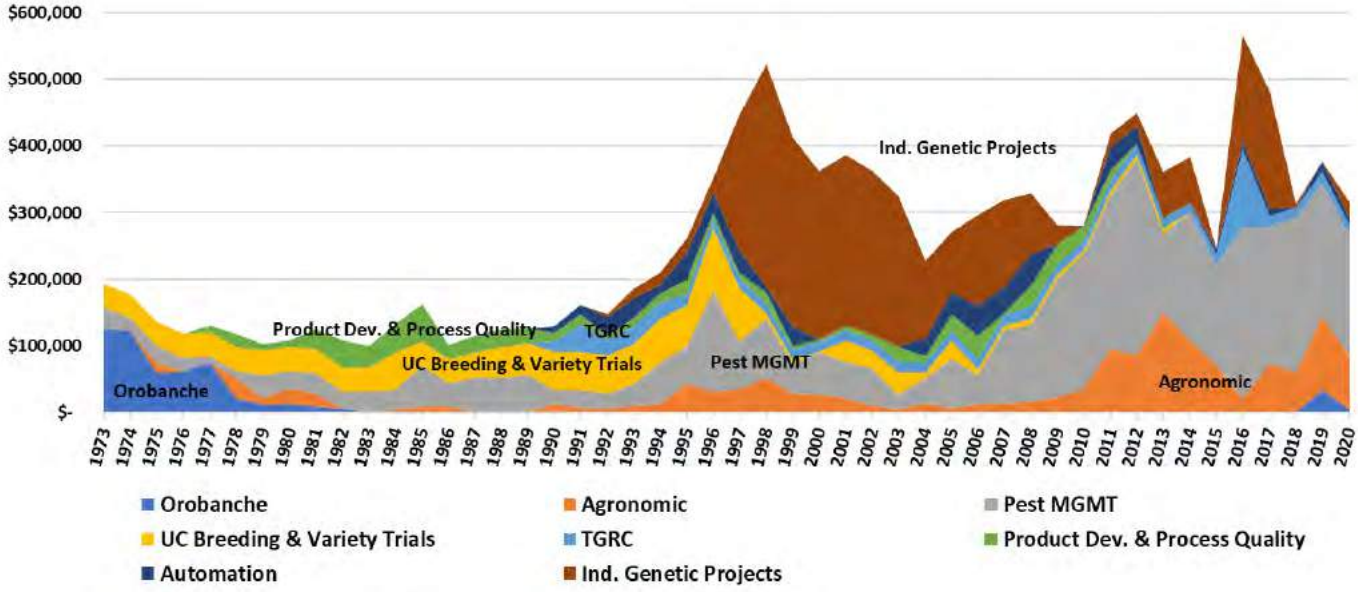


Figure 1. Expenditure by category through time (1973-2020)

As evidenced by a membership which represents over two thirds of the paid tons in 2020 (see Figure 2) and 52 years of historical expenditures, the CTRI has invested significantly (over 12 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 2020.

Additional resources for growers and allied industry can be found on the pages of www.tomatonet.org and by joining the industry email alert system also found on the home page of www.tomatonet.org.

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

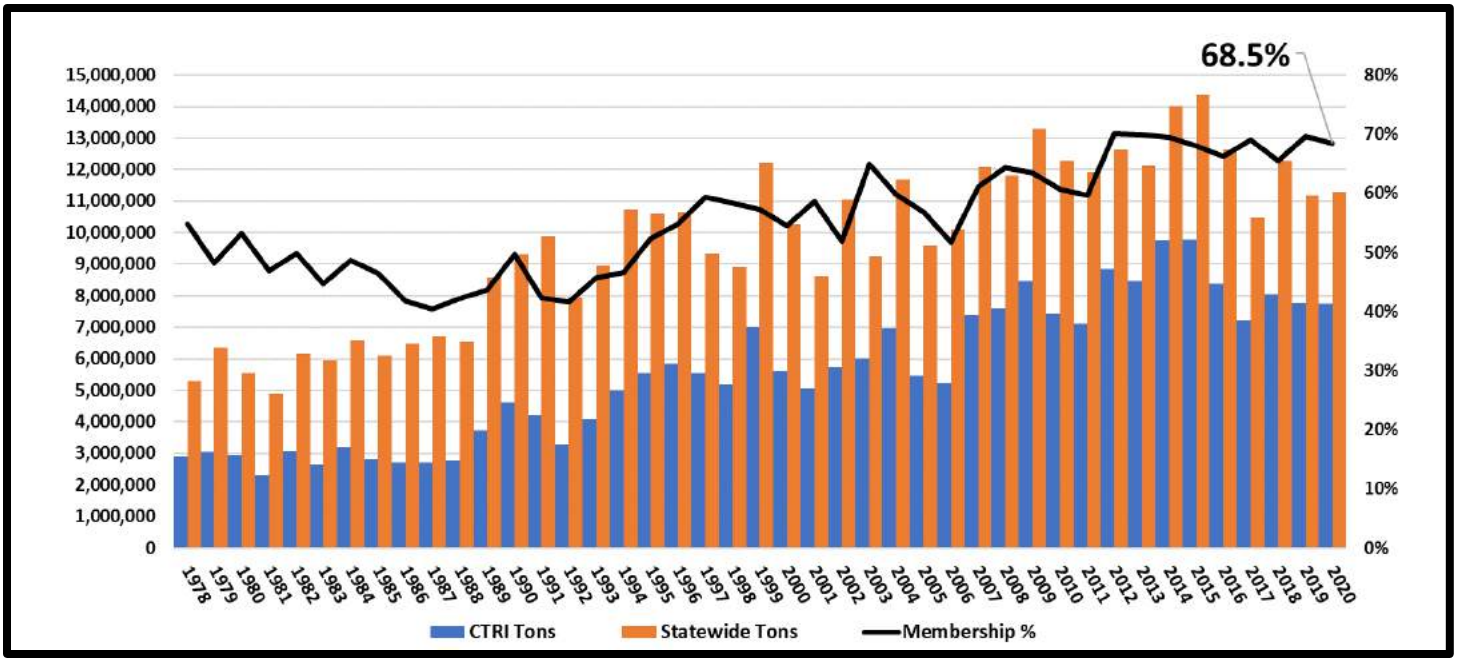
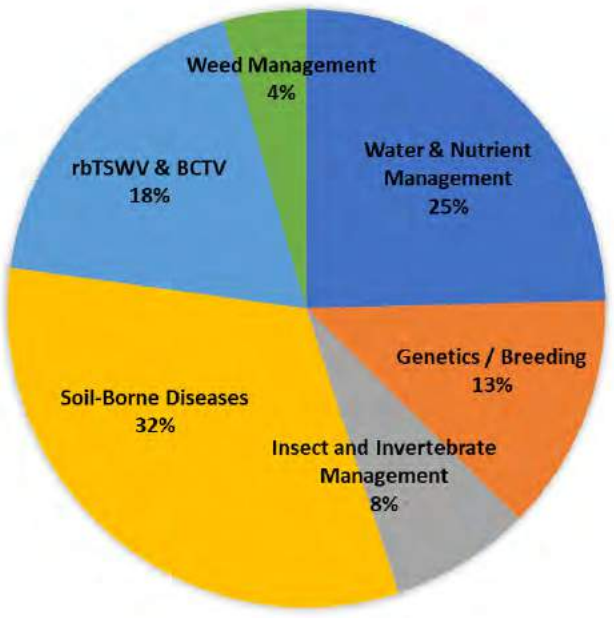


Figure 2. Membership & Assessment through time (1978-2020)

2020 RESEARCH - DOLLAR ALLOCATION

2020 Actual Allocations			
Category	**Funding**		**%**
Agronomic	$	81,655	26%
Genetics	$	39,937	13%
Bacterial	$	-	0%
Southern Blight	$	4,875	2%
RKN	$	12,000	4%
Fusarium et.al.	$	95,449	30%
BCTV	$	5,270	2%
TSWV	$	51,882	16%
Weed MGMT	$	9,100	3%
Broomrape	$	4,879	2%
Other Insect Pests	$	11,770	4%
TOTALS	**$**	**316,817**	**100%**



California Tomato Research Institute

2020 CTRI FUNDED RESEARCH PROJECTS				
2020 TOTAL FUNDING: $316,817				
Agronomic/Water/Nutrient Management			**$**	**81,655**
2020 New	How do different soil types impact physiology, yield, and quality under late-season deficit irrigation?	Mallika Nocco	$	24,268
2019 Start	Optimizing Potassium Fertilizer Uptake Efficiency while Minimizing Costs in Processing Tomato	Nicole Tautges	$	20,371
2019 Start	Effects of soil management on processing tomato associations with mycorrhizal fungi	Rachel Vannette	$	23,000
2019 Start	Influence of Compost Application Rates and Timing on Nitrogen Management and Processing Tomato Productivity and Quality	Zheng Wang / A. Fulford	$	9,016
2020 New	Securing the future of highly productive annual cropping systems in California	Jeff Mitchell	$	5,000
Germplasm and Variety Development			**$**	**39,937**
1991 Start	C. M. Rick Tomato Genetics Resource Center	Roger Chetelat	$	15,000
2020 New	Completion of Insect Resistance Source Line for Transfer Resistance to Insects and Insect Transmitted Virus Processing Tomato	Martha Mutschler	$	15,000
2020 New	Breeding for Water Stress Tolerance by Combining Two Wild Species in Tomato	Dina St. Clair	$	9,937
Insect and Invertebrate Management			**$**	**23,770**
2011 Start	Evaluation of Alternative Nematicides for the Control of Root-Knot Nematodes of Processing Tomatoes	Jaspreet Sidhu	$	12,000
2019 Restart	Consperse Stink Bug IPM Update	Tom Turini	$	11,770
Pathogen Management			**$**	**157,476**
2020 New	Evaluation of Streptomyces isolates as biocontrol agents for Southern Blight of Tomato	Isolde Francis	$	4,875
2017 Start	The resistance breaking strain of TSWV in CA processing tomatoes: Monitoring, improved detection and screening for resistance	Robert Gilbertson	$	51,882
2020 New	Beet leafhopper efficacy comparison	Tom Turini	$	5,270
2020 New	Control strategies for F. falciforme, a newly recognized and widespread cause of premature vine decline	Brenna Aegerter	$	6,649
2017 Start	Developing accurate, rapid and cost effective tools for diagnosis and predictive monitoring of Fusarium pathogens of tomato	Cassandra Swett	$	25,000
2017 Start	Developing effective crop rotation strategies for Fusarium wilt management	Cassandra Swett	$	26,000
2018 Start	Control strategies for F. falciforme, a newly recognized and widespread cause of premature vine decline	Cassandra Swett	$	19,800
2017 Start	Disease diagnosis, pathogen movement / emergence monitoring, new pathogen identification and Fusarium wilt race 4 monitoring in support of the processing tomato industry	Cassandra Swett	$	18,000
Weed Control and Management			**$**	**13,979**
2019 Start	Imaging Technology for Rapid Identification of Broomrape Parasitized Tomato Plants	Mohsen Mesgaran	$	4,879
2019 Start	Weed control and cost-benefit analysis of automated cultivators to control within-row weeds in processing tomatoes	Amber Vinchesi-Vahl	$	9,100
2020 TOTAL OF ALL FUNDED PROJECTS			**$**	**316,817**

HOW DO DIFFERENT SOIL TYPES IMPACT PHYSIOLOGY, YIELD, AND QUALITY UNDER LATE-SEASON DEFICIT IRRIGATION?

MALLIKA NOCCO

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Key Takeaway(s) of this project:

- Commercially available imaging tools (e.g., Ceres Imaging) can aid in timing and sensing in-season crop responses to late-season deficit irrigation at the farm scale
- Up to 3.14" of water savings were achieved in the 2020 season with minimal impact to yields and improved °Brix
- The most promising strategy for attaining over 4" of savings is to go "slow and low" with deficit irrigation—start early (immediately post-flowering or ~day 60 initiation) and initiate low to moderate stress
- Study sample yields had high variability within treatments in the 2020 season; we plan to increase yield transect size and number in 2021

Introduction:

Processing tomatoes are one of the most valuable annual crops in California. Late season deficit irrigation has been shown to increase fruit quality and nutrient concentration with minimal impact on yield (Mitchell et al., 1991; Amor and Amor, 2007; Jensen et al., 2010; Ripoll et al., 2016; Lu et al., 2019). The processing tomato industry is in a unique position to demonstrate leadership across California via widespread adoption of late season deficit irrigation into commercial practice, as it is one of the few crops that may have demonstrable, marketable fruit quality and nutrition co-benefits with controlled, late season water deficits. However, the industry needs decision support to determine when to initiate deficits and how much water stress to apply.

Recent work has used canopy cover as an accessible and reliable tool (monitored as the nondifferentiated vegetation index or NDVI) for managing deficit irrigation in tomato (Li et al., in prep). NDVI could be used at the leaf or canopy scale to predict the moment that a deficit strategy should be used to maximize °Brix and yield. Before recommending this as the best and simplest way to understand when to initiate deficit irrigation, it is important to confirm how the lowering of NDVI is related to plant water stress; especially in soils with different physical properties. Contrasting work in Spanish processing tomato has shown that NDVI may potentially lag behind the moment that physiology switches to ripening and that soil moisture or water potential may be the

better estimate (Campillo et al., 2016). Additionally, it is important to tease out the benefits of thermal mapping, actual ET, and soil water potential for informing how much water stress to apply to processing tomato and identifying stress responses. In order to provide the most specific recommendations to maximize brix and fruit quality, we need to understand which indicator is the best tool for deficit irrigation management in different types of soils. The goal of this work is to (1) develop a clear monitoring guideline for late season deficit irrigation using commercially available decision support tools (e.g., Ceres NDVI/Thermal imaging, Tule ET sensors) in processing tomato in order to maximize nutrition, quality, and yield under increasing groundwater salinity, (2) provide processing tomato growers a "menu" of commercially available decision support tools with costs, benefits, and tradeoffs associated with implementation.

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

The goal of this work is to quantify tradeoffs and benefits from late season deficit irrigation and assess options for in-season decision support for deficit irrigation in processing tomatoes. The objectives of this work (after grower input to in December 2019 in and COVID modifications in April 2020) are:

- Develop a clear monitoring guideline for deficit irrigation in tomato using NDVI, crop water stress, and ET indicators from commercially available services (e.g., Ceres Imaging, Tule). The commercially available services component is the COVID-19 modification of this project, as these services were deemed essential when university travel and field operations were not.
- Assess relationship between irrigation, yield, quality, and nutrition to inform guidelines
- Ground truth previous deficit irrigation findings in commercial fields, include different soil types and cultivars

Methodology and Results:

We are conducting an on-farm late season deficit irrigation study in partnership with Scott Schmidt of Farming 'D' Ranch in Five Points, CA. We initiated this experiment in 2020 and will continue in 2021 and 2022 for a total of three field seasons. Decision support systems (e.g., Ceres Imaging, Tule technologies) were used in the 2020 season to inform water management and provide both spatially-explicit data related to phenology and stress as well as continuous monitoring of actual evapotranspiration. In addition to water use analyses, we sampled for yield, quality (and nutrients (Vitamin A, Vitamin C, lycopene) to better understand their tradeoffs. We chose these particular nutrients based on the literature suggesting that they may increase with late season deficit irrigation and consultation with the Tomato Wellness team as to which nutrients are prioritized for marketing the nutritional benefits of processed tomato products.

Because there was some uncertainty in what 2020 research looked like as well as the need to relocate instrumentation to a field with enough acreage in one tomato cultivar to manipulate irrigation treatments, we chose a field with a later growing season. Details about the field experiment are below:

Variety: 5508 (149 acres)
Transplanting date: 5/21/20**; Harvest date**: 10/08/20
Experimental Treatment (Figure 1): We initiated 4 treatments including a control, managed per Scott Schmidt's usual protocol (described below). Based on Li et al., (in prep), we decided there is no longer a need to test a 25% reference ET (ET_o) treatment—it was too far removed from having a reasonable return on investment. Here, we focused on manipulating timing as well as applying stress. The irrigation experiment was replicated on the commercial field using three blocks (needed for statistical analyses) to account for variation in tomato growth patterns that was observable in the CERES imagery and using Scott Schmidt's existing knowledge of the variability of irrigation on the field.

Control (14 beds per block): Grey areas with a "C" marked. This was Scott Schmidt's usual protocol. The only reason that we demarcated the control as a treatment is because we sampled tomatoes from these plots at harvest for yield, quality, and nutrition analyses.

- **Day 85 (8/19/20):** ~75% ET_o =36 hours or ~1.43" over 6 days
- **Day 105 (9/2/20):** ~50% ET_o = 26 hours or ~1.03" over 6 days
- **Day 120 (9/17/20): no irrigation until harvest**

Low stress (12 beds per block): Fig. 1 blue areas with "L" marked. This treatment initiated deficit earlier and eliminates the ~75% ET component.

- **Day 85 (8/19/20):** ~50% ET_o = 26 hours or ~1.03" over 6 days
- **Day 120 (9/17/20): no irrigation until harvest**

Moderate stress (12 beds per block): Fig. 1 orange areas with "M" marked. This treatment started early and eased into 50% earlier than the control.

- **Day 85 (8/14/2020):** ~50% ET_o= 26 hours or ~1.03" over 6 days
- **Day 105 (9/2/20):** ~37% ET_o= 18 hours or ~0.72" over 6 days
- **Day 120 (9/17/2020): no irrigation until harvest**

High stress (12 beds per block): Fig. 1 red areas with "H" marked. This was the highest stress treatment that may yet have some ROI. Previous studies took this down to 25%, but there was no ROI justification to keep going that low.

Day 85 (8/14/20): initiate ~37% ET_o= 18 hours or ~0.72" over 6 days
Day 120 (9/17/2020): no irrigation until harvest

Figure 1. Experimental field location SW of Five Points (left) and experimental treatments (right). Tule ET sensor and sensor monitoring area outlined by green rectangle in SE corner of the field

Actual ET and stress monitoring: A Tule surface renewal sensor (Figure 1; right) was installed in the SE portion of the field with its area of measurement outlined. This sensor measured actual evapotranspiration in the control portion of the field. Additionally, we initiated biweekly-weekly CERES imaging flights to collect multispectral and thermal imagery. An exciting result is that CERES thermal imagery shows in-season crop responses to the deficit irrigation (Figure 2). This means that (1) deficit treatments "worked" and caused measurable plant water stress and (2) CERES thermal imaging has potential to serve as a decision support tool for deficit irrigation in processing tomato with further development and groundtruthing.

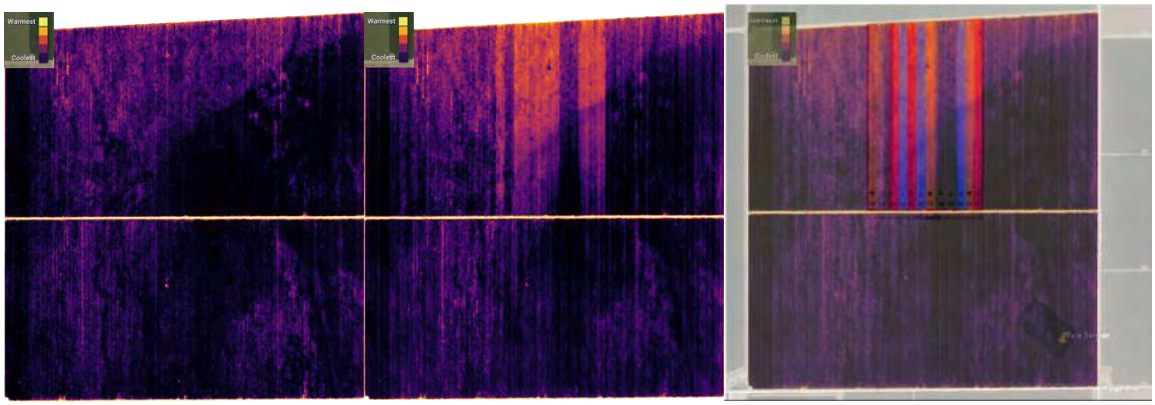

Figure 2. CERES thermal imagery from 8/9/20 (left, prior to deficit treatments) and 8/31 (center, ~2 weeks after deficit initiated). The right image has the treatments overlaid on top of the thermal imagery to display the crop water stress. Higher temperatures indicate more stress (low ET) and lower temperatures indicate less stress (higher ET).

Yield and co-benefits: We hand-harvested yields on 10/7-10/8 (immediately prior to and during machine harvest) from transects and scaled up to field-based estimates (n=2-3 transects per treatment). We sampled for quality and nutrition by collecting a gallon-size bag from three different locations in each treatment block (9 locations per treatment). Harvested quality tomato samples were transported to the PTAB facility in Huron, CA on the day of sampling and analyzed for quality (°Brix, color, acidity). Harvested nutrition samples were immediately frozen to preserve nutrient concentrations. Samples were transported frozen to Davis, CA and shipped frozen to Medallion Labs (General Mills subsidiary, Minneapolis, MN) for certified analysis of Vitamin A, Vitamin C, and lycopene.

We present average yield and quality data by treatment in Figures 4-6 and are currently working through additional analyses to examine the statistical differences between yield, quality, and nutrition among irrigation treatments. Though the control treatment had the highest yields, there are small numerical differences in yields between treatments (Figure 4) with high variability in yield estimates. In order to reduce variability between treatments, we plan to sample from larger and more transects next year. There were also small or no numerical differences between the rotten or green tomato yields. However, Figures 4-6 provide the average and standard deviation of quality parameters by treatment. We also note that hand-harvested yields are unusually high relative to statewide reported yields, which is a common issue in plot-scale field studies of processing tomato. We plan to calibrate our hand-harvest yield estimates using control treatment harvest information if possible. The high-stress treatment had the highest °Brix, while the control treatment had the highest PTAB color score. The moderate stress treatment has the highest acidity (lowest pH) and the lowest yields. For 2020, the relative water savings were 1.33”, 2.109”, and 3.142” for the low, moderate, and high stress treatments compared to the control.

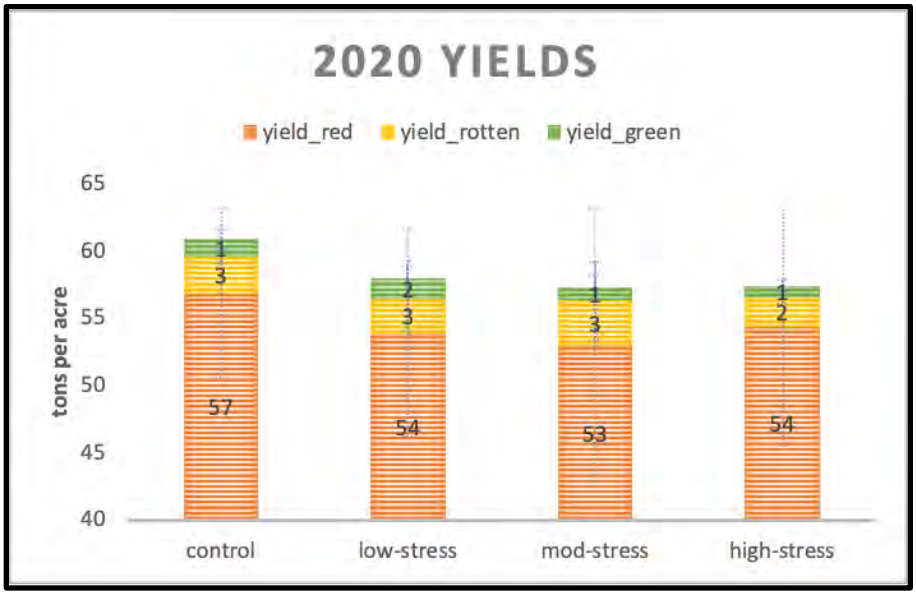

Figure 3. Marketable (red or orange tomatoes), rotten (no structural integrity), and green tomato yields by irrigation treatment for 2020 season

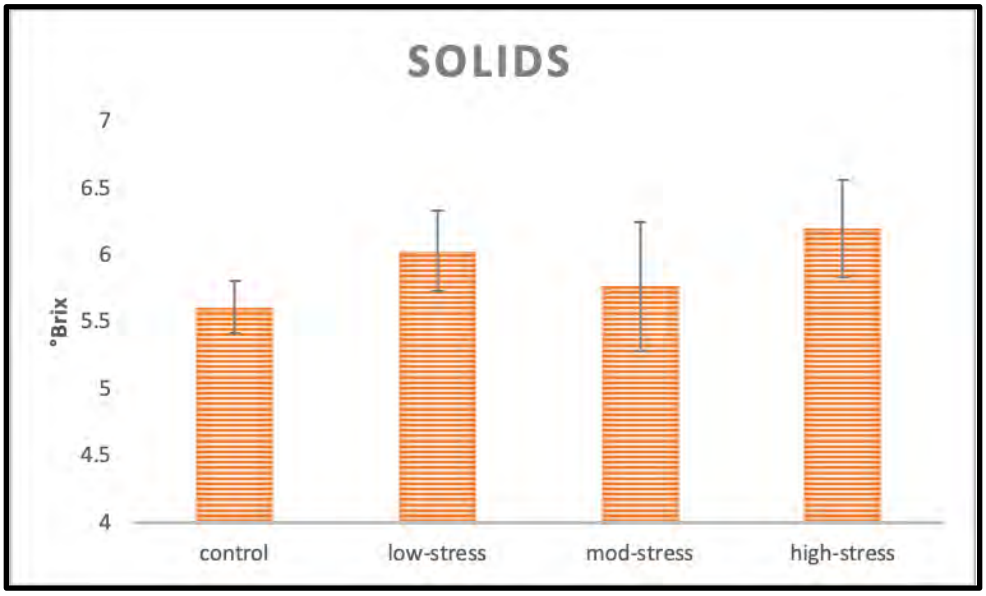

Figure 4. Solids content (°Brix) measured by irrigation treatment for the 2020 season

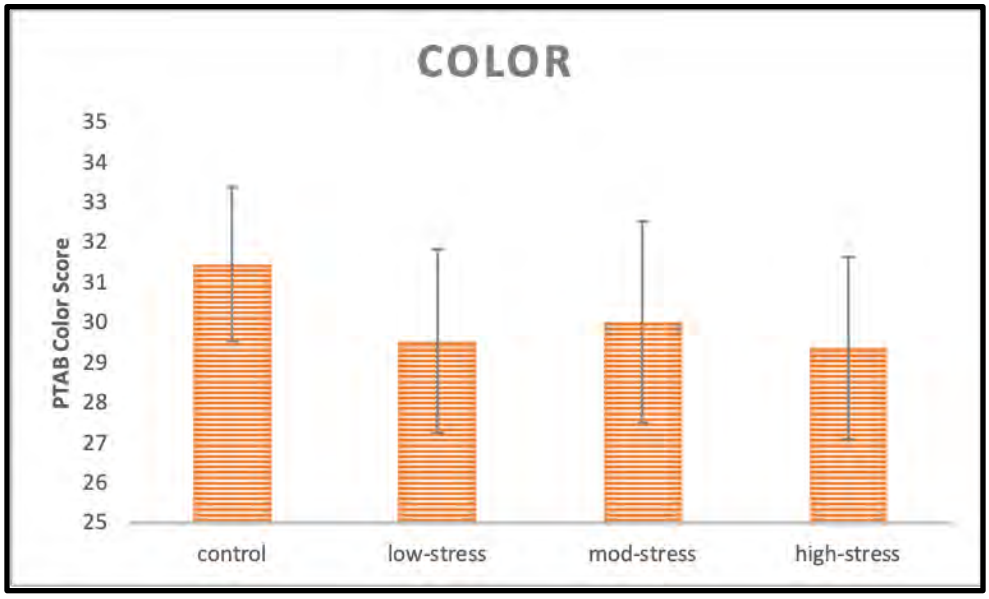

Figure 5. Color measured as PTAB Color Score for each irrigation treatment in the 2020 season

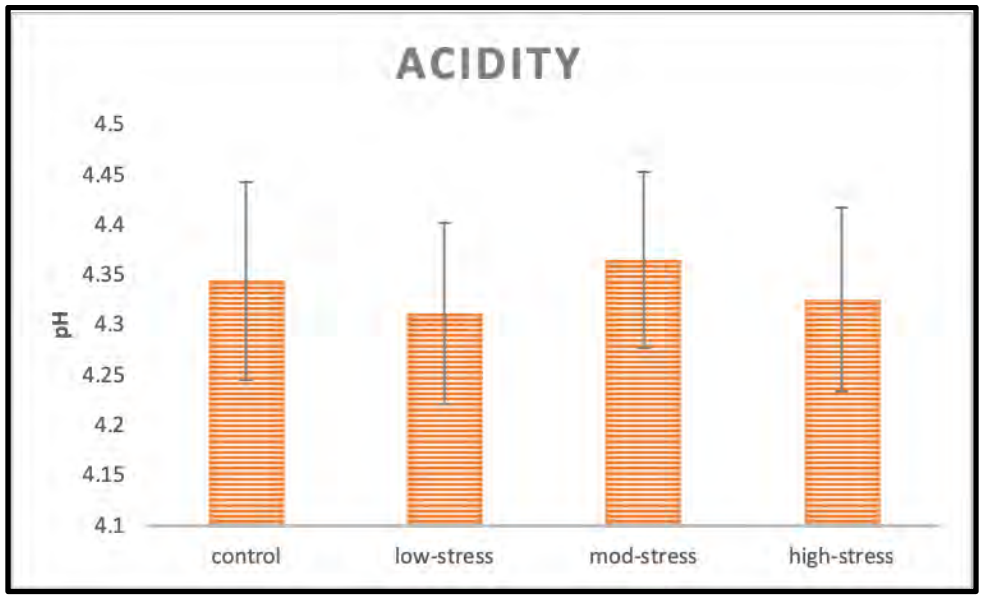

Figure 6. Acidity measured as pH for each irrigation treatment in the 2020 season

Discussion:

In order to reap benefits and cope with continued water scarcity, our target is to reliably reduce the water requirement for processing tomatoes by 4" without impacting yields (e.g., from 30" to 26" in the Five Points region). These recommendations need to be "variety-agnostic" in order to grow with the constant introduction of new cultivars of processing tomato. In year 1 of this study, we were able to demonstrate savings of up to 3.142" accompanied by an average yield reduction of 3 tons/acre and an average increase in °Brix of 0.59. The most promising and logistical approach for improving savings without compromising yield is to further manipulate timing and initiate earlier mild-to-moderate stress or take a "slow and low" approach to deficit irrigation. In years 2 and 3 of this study, we will be further testing the benefits and tradeoffs of different decision support tools to inform the timing and magnitude of irrigation (Figure 7). We want to expressly acknowledge the limitations of drawing conclusions based on one year of data—especially with significant variability in yields that were observed. Ideally, at least three years of data are needed in order to develop a full picture of benefits, tradeoffs, and quantitative relationships among irrigation treatments, decision support tools, yield, quality, and nutrition.

Figure 7. Benefits and tradeoffs of different decision support tools for deficit irrigation schematic

Acknowledgements: We would like to acknowledge Scott Schmidt of Farming 'D' Ranch for partnering and supporting this project in a very challenging and uncertain year. We greatly appreciate the logistical and operations assistance from Farming 'D' Ranch as well as the on-field perspectives and discussions related to this work. Additionally, we would like to thank Bob Huttmacher for providing freezer space to immediately freeze tomatoes for nutrition analyses and his insights related to this project. Finally, we are thankful to Tom Turini for his expertise, insight, and support as the sole UCCE Advisor co-investigator on this project.

This project as leverage for other dollars:

We leveraged CTRI funds from this project to secure a CDFA Specialty Crop Block Grant titled, "Optimizing irrigation innovation, soil health, and salinity management in California processing tomato systems". We are thankful for CTRI's funding of the deficit irrigation component of the larger proposed project and have leveraged these funds as match for the larger grant application. The value of that grant is $394,497 spread out over 30 months starting on 11/1/20 and as the title suggests, would add additional objectives related to soil health and salinity management to the deficit irrigation study underway including a field trial at the Westside REC. We also leveraged these funds (equipment, field site, infrastructure) as part of a new CDFA Specialty Crop Block Grant proposal led by Cassandra Swett focusing on deficit irrigation and plant health. Finally, we submitted a proposal to the CLFP Tomato Processing Research program ($24,801) to fund additional nutrition analyses for both the on-farm work and the CDFA trial at the Westside REC as there is an additional relationship between salinity and nutrition that needs quantification. Our long-term goal is to build a comprehensive understanding of how deficit irrigation impacts yield, quality, human nutrition, soil health, and plant health (Figure 8) as well as how existing decision support systems can be used to co-manage water, diseases, and soils.

Figure 1. Long-term plan for matching funds

References:

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Lu, J., G. Shao, J. Cui, X. Wang, and L. Keabetswe. 2019. Yield, fruit quality and water use efficiency of tomato for processing under regulated deficit irrigation: A meta-analysis. Agric. Water Manag. 222: 301–312.

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Ripoll, J., L. Urban, B. Brunel, and N. Bertin. 2016. Water deficit effects on tomato quality depend on fruit developmental stage and genotype. 190: 26–35.

OPTIMIZING POTASSIUM FERTILIZER UPTAKE EFFICIENCY WHILE MINIMIZING COSTS IN PROCESSING TOMATO

NICOLE TAUTGES

Project Leader and any Co-PIs: Radomir Schmidt, Project Scientist, Russell Ranch Sustainable Agriculture Facility, University of California, Davis.

Cooperating Personnel:

Israel Herrera, Farm Manager, Russell Ranch Sustainable Agriculture Facility

Key Takeaway(s) of this project:

- Although yield measurement variability meant differences between the control and K application treatments were not significant, the trends in yields showed 1-4 tons/acre higher yields with supplemental 50 lb K/acre application.
- Profit calculations based on mean yield differences and fertilizer costs ranged from $27/acre loss to $203/acre profit depending on treatment and fertilizer choice.
- There were strong positive correlations between yield and tomato vine K concentration; and between tomato vine K concentration and tomato fruit pH.
- There were no correlations between yield and K application rate, soil K, fruit K or incidence of tomato yellow shoulder disorder.

Introduction:

Tomato plants are relatively high users of soil K, with total plant uptake rates of 200 to 350 lb K per acre (Hartz et al. 2002). Harvest of a tomato crop can result in the removal of 180 lb K per acre in a lower-yielding field to 270 lb K per acre in a high-yielding field (4 to 6 lb K per ton of fruit removed; Hartz et al. 2002). Availability of K in the soil decreases with depth and K diffusion in soil is extremely limited, suggesting that sufficient levels of exchangeable K throughout the soil volume, and/or targeted application of K fertilizer to the tomato root zone via fertigation, may be necessary to meet crop needs. In subsurface drip-irrigated systems, the tomato root zone is concentrated around the drip tape. Therefore, over time, the root zone can become depleted of nutrients, particularly P and K (Hartz 2008). While much research on processing tomato K requirements and optimal fertigation application in California has been conducted by T. Hartz and E.M. Miyao in the past two decades, K management remains "a complicated issue" in drip-irrigated processing tomato (Hartz 2009). Previous work has identified yield benefits from applied K fertilizer in drip-irrigated processing tomato fields in California with less than 200 ppm soil K and, in the case of soils with inherently high Mg to Ca ratios (like many soils in the Sacramento Valley), with K making up less than 2 percent of a soil's cation exchange (Hartz 2009). Hartz (2009) also identified early fruit set (starting around 30 days after transplanting; Figure 1) as the period of rapid K uptake by tomato plants from the soil, suggesting that whatever the method of K application, soil K must be present at levels sufficient to satisfy rapid uptake at this time, and that this soil K must be in the soil exchangeable pool.

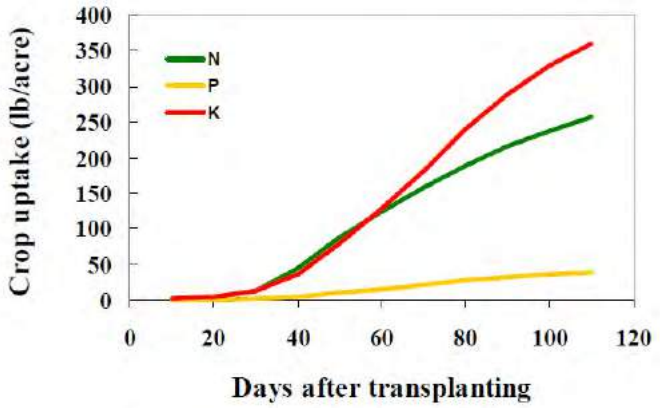

Figure 1. Pattern of macronutrient uptake in high-yield processing tomato (from Hartz 2009).

Fall spreading: Very little information exists regarding timing of availability from broadcast or banded applications of dry forms of K fertilizer in California soils in the fall. While Sullivan et al. (2017) state that most of the K applied in broadcast applications of KCl are immediately available, they do not discuss any factors of K exchange in soil, including the effect of soil texture and organic matter on K adsorption, retention, and release from broadcast applications. For application rates, while full replacement of K removed via tomato fruit would require from 180 to 270 lb K per acre to be applied each year to tomatoes, Hartz (2009) found that 100 to 150 lb K per acre is likely the rate that maximizes economic returns on the K fertilizer investment.

Fertigation: Subsurface drip irrigation has become a popular method of irrigation in processing tomatoes in California due to the yield advantage, and because subsurface drip enables fertigation, a tactic which is widely believed to increase the percent of applied fertilizer taken up by plants. In particular, targeted placement of K to the tomato root zone may be important, given the limited diffusion of K in soils and the concentration of tomato root growth around the drip tape. Fertigation application of K has been observed to improve marketable tomato fruit yields by 5 to 15 percent in K-deficient soils (Hartz et al. 2005; Hochmuth and Hanlon 2017). Conversely, foliar applications, though popular, have not been observed to improve tomato plant K uptake or fruit yields. Application timing could impact fertilizer uptake efficiency and fruit set, given that the rapid K uptake period is steep in magnitude and relatively long compared to N and P (Figure 1). Hartz et al. (2005) tested K fertigation treatments at rates of 225 lb K per acre initiated at early fruit set (early June) compared to late fruit set (end of June) stages and concluded that early application timing tended to increase yield by increasing the number of fruits per plant, but that the high rates of K applied in that study may not be economical given the cost of injectable K fertilizers. Clearly, availability of soil K is essential at early fruit set stages for improvement of fruit yields and suggests that investment in a K fertigation early in the season at modest rates is likely worthwhile. Moreover, whole-season availability supported by a split application injected two times per season could improve fertilizer uptake efficiency (i.e., unit K taken up per dollar investment in K fertilizer) as well as support multiple timings of flower and fruit set throughout the season, which may be increasingly important as earlier heat waves intensify and damage pollination and fruit set.

Given the clear connection between soil K availability and tomato yields, but with high rates of K application economically unviable and the most appropriate methods of K provision uncertain, we tested three methods of relatively low-levels of K application (100 lb/acre in 2019, 50 lb/acre in 2020) to determine if low K applications can significantly increase yields and which method of application provides the best cost to benefit ratio. Results from the 2019 season with Split application of 100 lb K/acre suggested that 50 lb K/acre may be sufficient to show yield increases. In order to test minimal supplemental K application, 50 lb K/acre were used for the 2020 growing season.

The main *Goal* and the *Objectives* under that goal: Our goal was to repeat and build upon the experiment conducted in year 1 by identifying the method and timing of K fertilization that results in the greatest uptake of K in tomato while minimizing costs; i.e., getting the most K uptake per dollar investment, when comparing fall broadcast to in-season fertigation. Supplemental K of 50 lb/acre was therefore applied in three treatments: i) fall shank application, ii) early season fertigation, iii) early and mid-season split fertigation (Table1).
Our specific objectives were to:

1) Measure exchangeable K in soils.
2) Measure tomato tissue-K levels during at least two time points during the season, especially at the early flower and early fruit stages.
3) Measure fruit yields among treatments and evaluate K fertilizer economic cost/benefits in terms of fruit yields, application costs, and returns.
4) Compare fruit quality outcomes of K fertilizer treatments, including color, pH, soluble solids content, and yellow shoulder disorder incidence.
5) Compare K fertilizer treatment effects on the above described indicators in tomato following winter fallow (conventional) versus following winter cover crops.

Table 1. Supplemental and typical management K application in test plots for the 2020 season.

	K application (lb/acre)				
	Supplemental			Typical management	
Treatment	**October 2019**	**6/8/20**	**7/13/20**	**7/17/21**	**7/21/20**
Control	-	-	-	15	15
Full	-	50	-	15	15
Split	-	25	25	15	15
Shank	50	-	-	15	15

Methodology and Results:

In mid-October 2019, potassium sulfate was shanked into the tops of the beds at a rate of 50 lb K/acre, in a conventional winter-fallowed tomato system ("Conv") and winter cover cropped tomato system ("Conv+WCC"). The rate was determined following baseline soil testing and results of previous season's K application. Tomatoes were transplanted around 5/1/2020, and the Full fertigation application and the first half of the Split application was injected the week of June 8, around 40 DAT. Soil and plant tissue samples were collected in the first week of September, approximately one week before tomato harvest. Tomato yields were collected via machine harvesting entire rows of tomatoes in the plots, within treatments. Subsamples of the machine-harvested fruit were collected and taken to a PTAB station to undergo analysis for color, pH, and soluble solids content, using industry standard methods. Tomato yellow shoulder incidence was assessed by manual counts of clearly visible tomato "shoulders" in photographs taken 8/28/20; three photographs were taken per test plot. Economic cost/benefits were calculated by comparing all treatments to the no-K control, by subtracting the revenue from tomato fruit sales in the control treatment from the revenue in the K fertilized treatments, to obtain the additional profit earned by using K fertilizer. We assumed a tomato price of $72/ton and a fertilizer spreading cost of $25/acre. While KCl was used in the study, costs were also calculated for the hypothetical scenario where a manager chose to use potassium thiosulfate (KTS), a more expensive liquid fertilizer product. We did not take into account the cost of cover crops in these analyses.

1) Exchangeable K in soils

In order to measure the fate of supplemental K in the treatment plots, we sampled soil and analyzed it for exchangeable K levels before harvest. While there were significant differences between K soil concentrations between the systems (P=0.0007), the differences in soil K were not significant within each system (Conv or Conv+WCC). Interestingly, soil K in the conventional system trended lower than control in all supplemental K treatments (Figure 2).

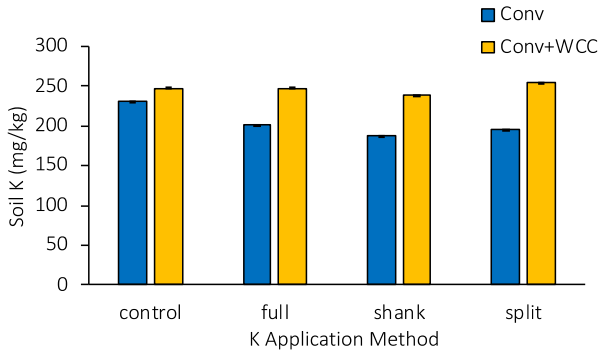

Figure 2. Exchangeable soil K at tomato harvest time. Conv = conventional system with winter fallow, Conv+WCC = conventional system with a legume+oat winter cover crop.

2) Tomato tissue K content measurement.

During the 2019 growing season, early- and mid-season leaf samples collected according to fertility monitoring recommendations (CDFA-FREP 2019, California Fertilization Guidelines) did not show significant differences between K treatments and control (Figure 3 a, b). During the 2020 growing season mid-season leaf samples were not collected due to field-work restrictions due to the ongoing Covid 19 pandemic. Instead, plant tissue samples were collected at the beginning of September, approximately one week before harvest, and both tomato fruit and tomato vine tissues were analyzed for their nutrient content (Figure 3 c,d). The tomato fruit nutrient content, including K concentration, was consistent across all treatments (Figure 3 c). The K content in the tomato vine tissue was more variable across treatments (Figure 3 d).

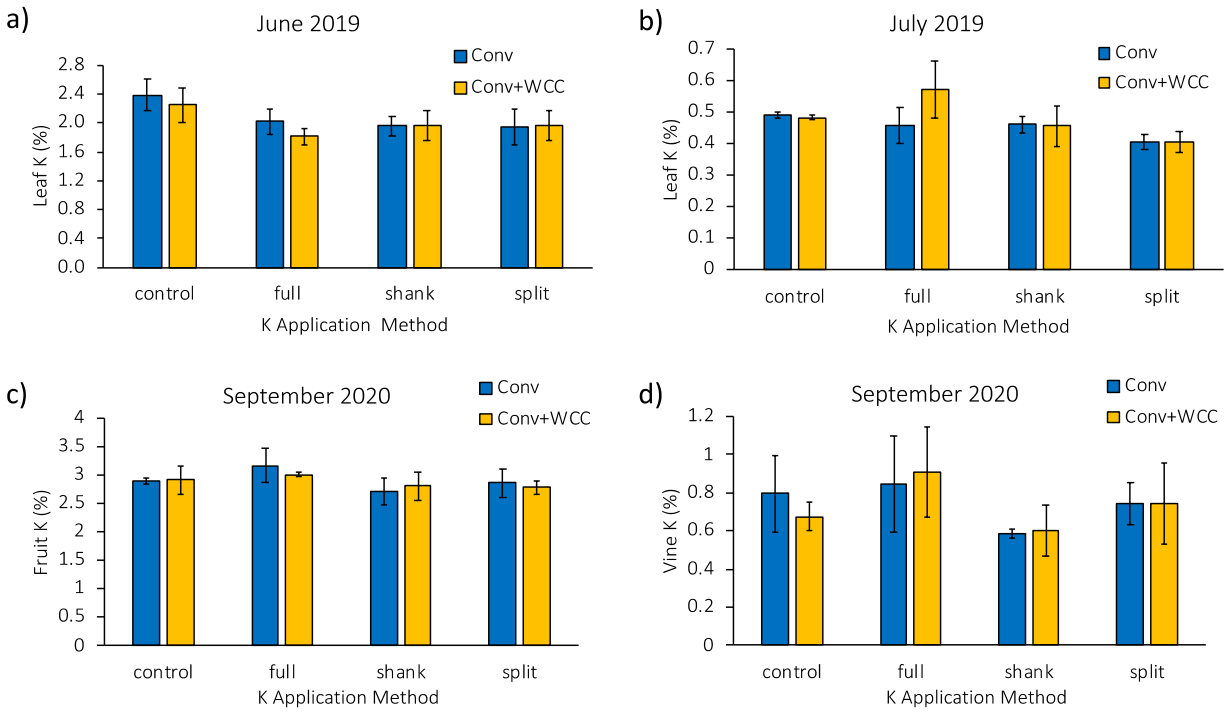

Figure 3. Tomato plant tissue K content. a) 2019 June leaf tissue K; b) 2019 July leaf tissue K; 2020 September tomato fruit tissue K ; 2020 September tomato vine tissue K. Conv = conventional system with winter fallow, Conv+WCC = conventional system with a legume+oat winter cover crop.

Although there were no significant differences in vine tissue K between treatments, correlation analysis showed significant, strong positive correlations between vine tissue K and yield, as well as vine tissue K and tomato fruit pH (Table 2, Figure 4). There was also a weak positive correlation between yield and vine tissue P content (Table 2).

Table 2. Spearman correlation analysis of tomato yields and fruit quality measures vs soil and plant tissue nutrient concentrations.

2020		Yield and fruit quality measures			
		Yield	Hue	Brix	pH
Soil	NO_N				
	NH4_N		-0.45+		-0.52++
	P				
	K			0.46+	
	S				
Tomato vine	Total N				
	P	0.43+	0.47+		0.76++++
	K	0.70+++			0.79++++
	S				
Tomato fruit	Total N				
	P				
	K				
	S				
Disease incidence	Yellow shoulder				
Fertilizer	K application		-0.51+		

Significance indicators: + P < 0.05, ++ P < 0.01, +++ P < 0.001, ++++ P < 0.0001
Blank cells indicate no significant correlation

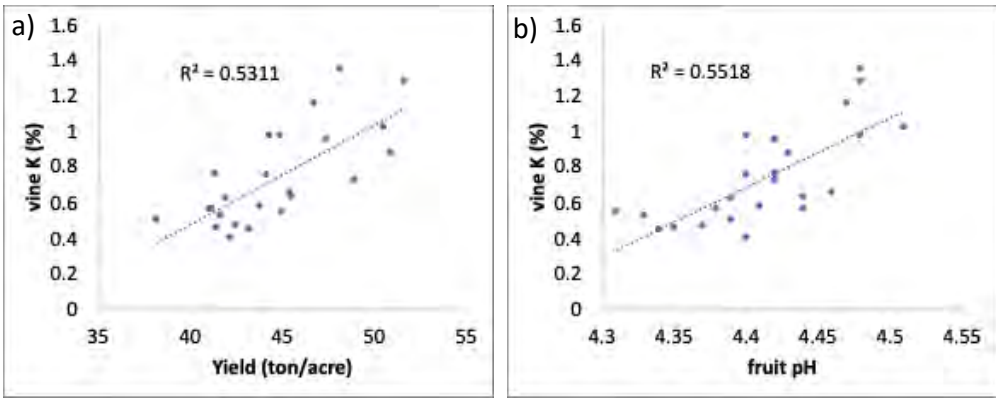

Figure 4. Relationship between tomato vine K and a) yield, or b) fruit pH. Trendline R^2 values are indicated in each figure.

3) Fruit yields and K fertilizer economic cost/benefits

Overall yields were lower in 2020 compared to 2019 for all treatments (Figure 5). While yields in the K amended treatments were 3-5 tons/acre higher than the control in 2019, in 2020, the K amended treatment yields were on average 0.5-4 tons/acre higher. However, due to relatively high variability in yield measurement in the individual test strips, ANOVA analysis did not indicate statistical differences in yields for the 2020 season. With the above caveat, economic benefits were calculated for each of the treatments in each system based on mean differences in yield compared to the respective controls, processing tomato returns, and K fertilizer costs (Table 3). For ease of comparison, 2019 costs were used for both years. In 2019 the best performing treatment was the split treatment with early- and mid-season K application. In 2020, the full treatment with a single, full K application in early season performed the best (Table 3).

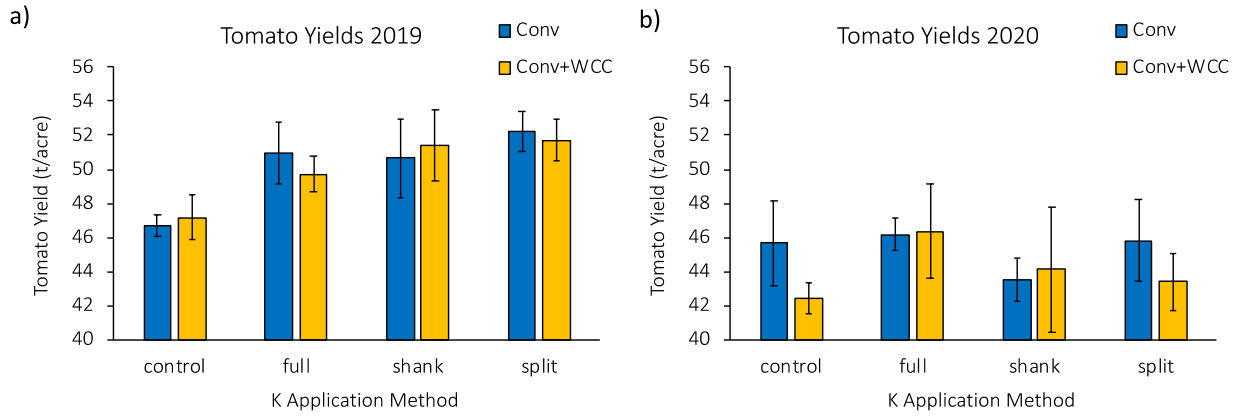

Figure 5. Tomato fruit yields, measured via machine harvest at Russell Ranch on a) 9/4/2019, and b) 9/9/2020. Conv = conventional system with winter fallow, Conv+WCC = conventional system with a legume+oat winter cover crop.

Table 3. Profits or losses associated with supplemental K application at Russell Ranch for the 2019 and 2020 tomato growing seasons. Conv = conventional system with winter fallow, Conv+WCC = conventional system with a legume+oat winter cover crop.

Year	System	Treatment	Profit/loss based on fertilizer choice		
			KCl	KTS	K_2SO4
2019 (100 lb K/ac)	Conv	full	$225.49	$119.49	
	Conv	split	$317.62	$131.62	
	Conv	shank			$190.90
	Conv + WCC	full	$101.91	-$84.09	
	Conv + WCC	split	$244.91	$58.91	
	Conv + WCC	shank			$206.67
2020 (50 lb K/ac)	Conv	full	$170.95	$118.18	
	Conv	split	$144.08	$91.30	
	Conv	shank			-$27.72
	Conv + WCC	full	$202.98	$190.21	
	Conv + WCC	split	-$10.80	-$23.57	
	Conv + WCC	shank			$74.18

Assumed returns/costs: tomato $72/ton
Dry K_2SO_4 $0.78/pound K_2O equivalent
Liquid KCl $0.66/pound K_2O equivalent
Liquid KTS $1.54/pound K_2O equivalent

4) Fruit quality outcomes of K fertilizer treatments, including color, pH, soluble solids content, and yellow shoulder disorder incidence.

There were no significant differences in color, pH or soluble solids content between control and K application treatments (Table 4). Similarly, there was no difference in yellow shoulder disorder incidence between any of the treatments (Table 5). Overall, there was weak but significant correlation between Hue and supplemental K fertilizer application, as well as soil NH_4 concentration and vine P content (Table 2). There was a weak correlation between Brix scores and soil K concentration, and strong positive correlation between tomato fruit pH and tomato vine K and P content as well as weak negative correlation between pH and soil NH_4 concentration (Table 2).

Table 4. PTAB analysis results among K fertilizer treatments in two systems at the RR location. Conv = conventional system with winter fallow, Conv+WCC = conventional system with a legume+oat winter cover crop.

System	Treatment	Hue	Brix	pH
Conv	Control	22.5 ± 0.5	5.23 ± 0.15	4.46 ± 0.06
	Full	22.0 ± 0.5	5.17 ± 0.21	4.42 ± 0.09
	Split	22.3 ± 0.3	5.00 ± 0.10	4.41 ± 0.02
	Shank	22.2 ± 0.3	5.10 ± 0.10	4.43 ± 0.02
Conv + WCC	Control	22.7 ± 0.8	5.30 ± 0.00	4.38 ± 0.05
	Full	21.7 ± 0.3	5.30 ± 0.17	4.42 ± 0.06
	Split	21.7 ± 0.3	5.20 ± 0.17	4.40 ± 0.06
	Shank	21.7 ± 0.3	5.30 ± 0.17	4.39 ± 0.05

Table 4. Tomato yellow shoulder incidence based on field photograph counts. Conv = conventional system with winter fallow, Conv+WCC = conventional system with a legume+oat winter cover crop.

System	Treatment	Yellow shoulder incidence (%)
Conv	Control	34.5 ± 12.7
	Full	31.6 ± 8.3
	Split	36.0 ± 8.0
	Shank	29.6 ± 13.1
Conv + WCC	Control	41.2 ± 18.0
	Full	33.0 ± 15.9
	Split	34.3 ± 17.1
	Shank	34.5 ± 14.3

5) K fertilizer treatment effects on tomato following winter fallow (conventional) versus following winter cover crops

Overall there was little difference between the conventional fallow and conventional winter cover crop systems in terms of yields, fruit quality or tomato tissue nutrient levels. One surprising observation was the trend for lower soil K concentrations near harvest time in the conventional fallow system with supplemental K (Figure 2). The lower soil K concentrations did not appear to result from increased plant K uptake, as they did not appear to be reflected in higher yields or higher plant tissue K content. Based on differences in mean yield values, supplemental K application was typically more profitable in the conventional fallow system than in the cover crops system, but the results were inconsistent over the 2019 and 2020 growing seasons (Table 3).

Discussion:

A number of studies have shown that K fertilizer application can improve tomato yields (Hartz et al. 2005; Hartz 2009; Hochmuth and Hanlon 2017), but that fertilizer application at rates calculated to replace K removed during harvest is likely not economically practical (Hartz 2009). The purpose of this study was to test low levels of supplemental K applied by three different methods (fall spreading and in-season single or double fertigation) on tomato yields and profitability. Tests with 100 lb/acre K application in the 2019 season demonstrated increased yields and moderate increases in profit per acre achieved in most of the treatments. For the 2020 season we applied 50 lb/acre K using the same three treatments. The calculated profit indicators were similar to the 2019 season, although three out of the 10 calculated K application scenarios showed a loss, compared to 1 out of 10 the previous season. In addition, the mean yield increases with supplemental K were lower with the 50 lb K/acre than with 100 lb K/acre and more variable, to the extent that changes in yield between control and test treatments were statistically not significantly different.

It should be noted that due to rising cost of operations at Russell Ranch fewer yield strips were measured in 2020 than in 2019. In 2019 tomatoes from all three rows in each test plot were weighed at harvest. We learnt late in the 2020 season that to repeat the same protocol would have cost approximately 2/3 of the total grant amount, and was thus not feasible. In consequence, we were only able to measure the yield from a single row per test plot. The reduced number of yield measurements contributed to the apparent yield variability and thus negatively impacted the statistical significance tests.

The results of the study over the 2019 and 2020 seasons showed consistent yield and economic benefits with the application of supplemental K at 100 lb/acre. With the addition of 100 lb K/acre the Split treatment (KCl) resulted in the greatest returns relative to the control by an additional $240-310 per acre in both systems, though returns were greater in the Conv system, due to the higher yields in that treatment and system. The Fall Shank treatment showed the most modest profits, especially when spreading costs (fuel, labor, etc.) were considered. This season, the results with 50 lb K/acre application were not consistent enough to definitively demonstrate application benefits. Nevertheless, based on calculations using the mean tomato yield from each treatment, the Full treatment (KCl) resulted in the greatest returns relative to the control by an additional $170-200 per acre. However, due to the statistical uncertainty of these results, 100 lb K/acre may be the minimum amount that provides statistically conclusive proof of benefits in the soils under study.

To find other relationships between the various parameters under study this year, and specifically to determine if any of the measured variables could have an impact on yield or fruit quality, we carried out Spearman correlation analyses. Tomato vine nutrients P and K showed a strong positive correlation with tomato yield and fruit pH. However, there was no clear relationship between tomato vine nutrient content and K application method or rate. The correlation between tomato tissue K concentration and yield is consistent with reports of increased yields in high K fertilizer application trials (Hartz et al. 2005; Hartz 2009), while the lack of clear relationship with the tested K application strategies may suggest that K uptake is a more complex interplay of nutrient availability, soil moisture, plant growth stage, and other biotic and abiotic factors. Optimizing soil nutrient application is of increasing importance as it lies at the nexus of profitability, soil health and environmental regulations. Optimal soil nutrients maximize yields and maintain soil function while minimizing wasteful nutrient leaching that can also lead to downstream deleterious effects. Soil K availability and fate, as well as the relationship to other soil nutrients, is an area deserving of further research. We plan to undertake a wider literature search on plant nutrient uptake in the coming months with the aim of designing a new experimental study focused on tomato K uptake to submit to CTRI for consideration in the 2021 proposal solicitation cycle.

Acknowledgements: This project would not have been possible without the assistance of Israel Herrera and all farm staff working on the Century Experiment plots at the Russell Ranch Sustainable Agriculture Research Facility.

This project as leverage for other dollars: We have two CDFA healthy soils program projects (HSP-1 and HSP-2) exploring compost application in tomato cropping systems. Both projects are three-year duration, and both

projects were funded to approximately $250,000 each. HSP-1, now entering its third year, is primarily concerned with comparison of GHG emissions under different compost and cover crops treatments. HSP-2 is exploring the use of coarse (grade B) compost in tomato systems.

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EFFECTS OF SOIL MANAGEMENT ON PROCESSING TOMATO ASSOCIATIONS WITH MYCORRHIZAL FUNGI

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Key Takeaway(s) of this project:

- Two years of AMF addition to field-grown processing tomato were performed in fields maintained in organic, conventional or mixed management
- Plant variables including root colonization, leaf damage, foliar nutrients, and yield were monitored
- AMF inoculum did not affect plant growth, yield, herbivore damage or root colonization but was associated with variation in nutrient concentrations (N, Al, Fe, S) in some soil types.
- One caveat is that we found very low root colonization rates and our results might not reflect outcomes in different environments and experimental conditions.

Introduction:

Mycorrhizal fungi form extensive associations with tomatoes and are typically beneficial for tomato growth, tolerance to deficit irrigation, stabilizing soil aggregates and resistance to insect pests (Cavagnaro et al. 2006, Vannette and Hunter 2009, Pineda et al. 2010). In cropping systems, soil management practices can substantially influence the amount and type of mycorrhizal fungi in soils, and the extent to which they associate with plant roots (Lekberg and Koide 2005, Lekberg et al. 2008). *The general hypothesis addressed in this project is* that farming practices, especially compost additions and cover cropping, influence soil microbial communities, including mycorrhizal fungi which in turn directly impact agronomic and physiological traits and potential outcomes of mycorrhizal inoculants on growing tomato plants. There is significant interest in microbial products but it is unclear if they increase yields under field conditions or under which management conditions they may be most effective. ***Our field experiments performed in working processing tomato fields aim to fill this gap and address if AMF inoculum offers benefits to processing tomato health—including nutrient composition and pest resistance-- and yield and how these may vary with management.***

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

- Perform a second year of mycorrhizal inoculum addition experiment at the Century Experiment at Russell Ranch on a larger scale across 3 long term soil management backgrounds.
- Evaluate reliability of lipid biomarkers to predict root colonization by mycorrhizal fungi, as a tool for growers to evaluate mycorrhizal availability in their fields.
- Complete analysis of experimental results from previous years and write up for dissemination
- Make recommendations and produce outreach and education material to inform growers about cultural practices which enhance mycorrhizal fungi abundance.

Methodology and Results:

***Objective 1:* Assess effects of mycorrhizal inoculant on tomato yield across 3 soil management backgrounds.**

In 2019 and 2020, we performed experiments at the Century Experiment at UC Davis, a long-term agricultural experimental site, where we established micro-plots within each of the long term management treatments: organic (cover crops, compost), conventional (winter fallow, chemical fertilizers), and mixed (cover crops, chemical fertilizers). During planting, the inoculum product MycoApply Endo (Mycorrhizal applications) was applied to transplants per application instructions, by dipping transplants in the inoculum solution before transplanting. Control sub-plots were also prepared as above, but without the mycorrhizal product. Whole plants were harvested at 6 weeks post transplanting for nutrient analysis, biomass, and insect damage, as well as mycorrhizal colonization and yields at harvest. In 2019, plants in all subplots were assessed for insect damage, biomass and yield. In 2020, we scaled up the experiment, treating entire rows were treated in 9 replicate plantings and individual plants were harvested from within each row as replicates. Data on plant nutrients and yield were collected in 2020. For statistical analysis, plots were considered random effects.

Plants within each subplot were processed with standard methods used at Russell Ranch and yield and canning and nutritional quality assessed. Root samples were harvested, cleared and stained to verify mycorrhizal colonization.

Results:

Effects on root colonization, plant growth and yield

In 2019, AMF inoculation increased root colonization overall, but particularly in soils managed organically or with a legume cover crop (Fig 1). Total fruit and red fruit mass was lower in organic and mixed (winter legume) soils compared to conventional (Fig 1) but was not affected by AMF treatment.

2020, neither soil management nor AMF addition affected root colonization levels assessed visually (Fig 1, $p>0.3$ all comparisons and interactions). In addition, neither soil management nor AMF treatment affected plant dry mass at 6 weeks post transplant (Fig 2, $p>0.20$ all comparisons and interactions). Yield was decreased in organic fields compared to conventional and legume fields ($p=0.02$) but we did not detect an effect of AMF inoculum on yields overall or in any management type (Fig 3, $p>0.4$ for all comparisons).

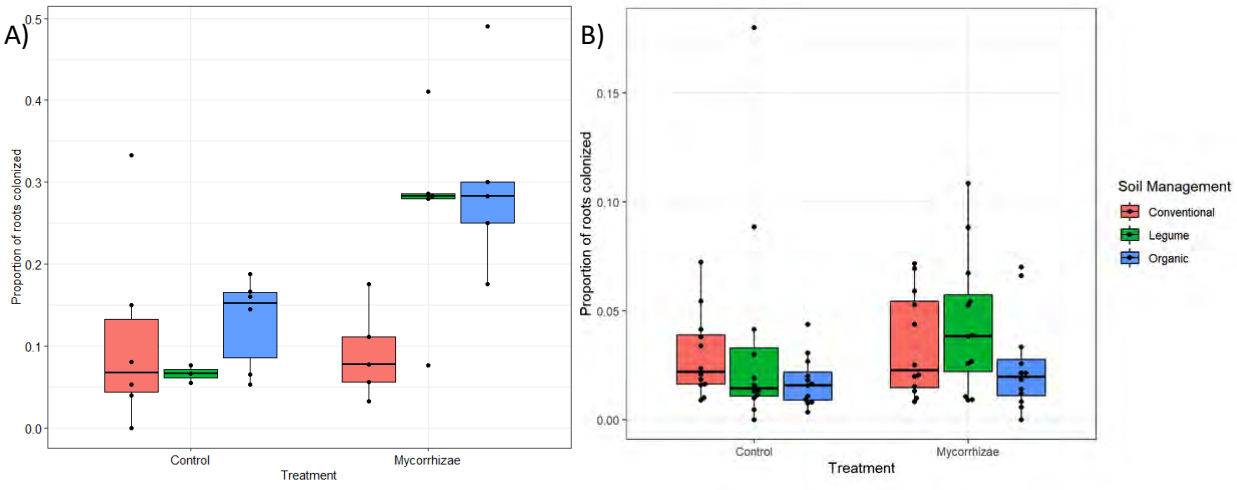


Figure 1. Effect of AMF inoculum addition on tomato root colonization in A) 2019 and B) 2020. In 2019 (panel A), AMF inoculum addition increased root colonization (p=0.02), particularly in organic and legume managed soils (p=0.07). In 2020 (panel B), AMF inoculum addition did not significantly enhance root colonization of processing tomatoes by mycorrhizal fungi (p>0.05).

A) B)

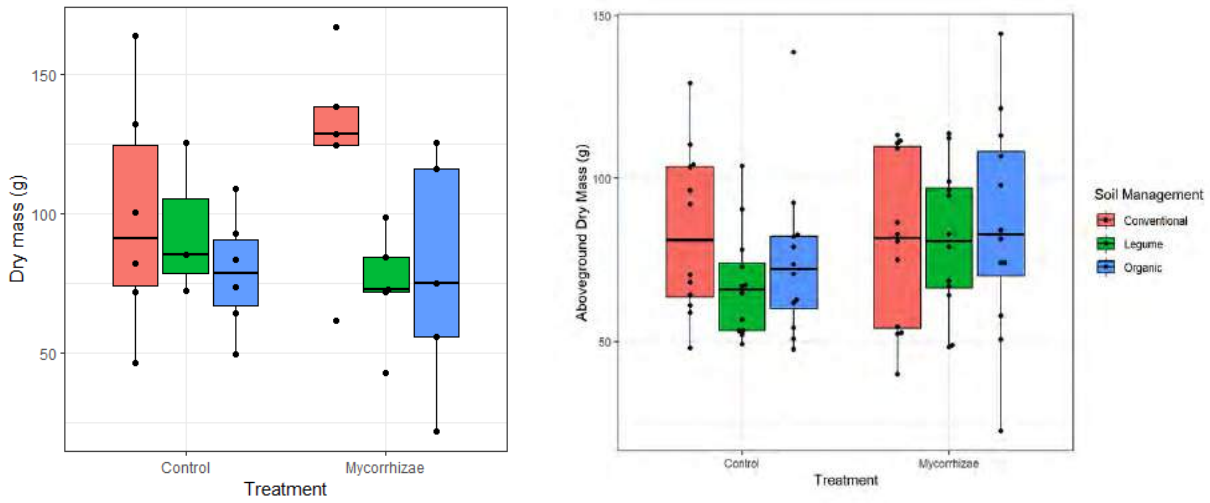
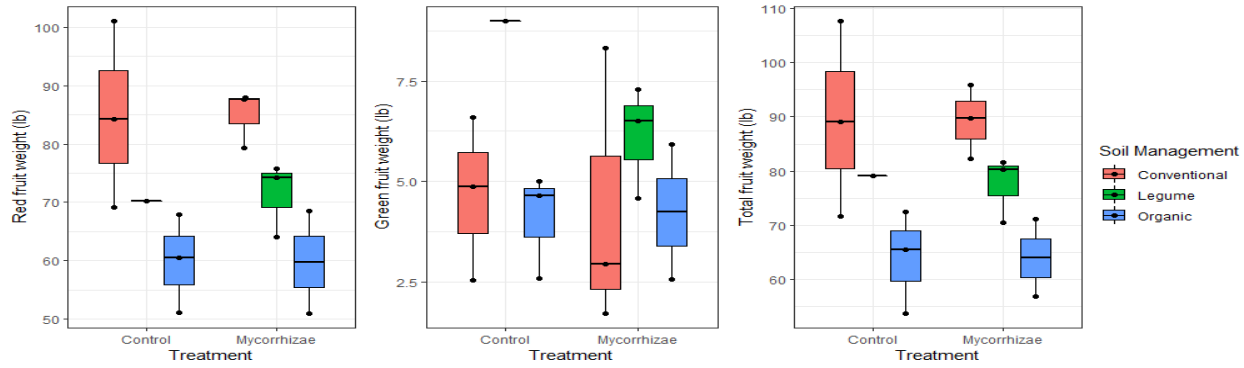


Figure 2. Effect of management and treatment on aboveground dry mass 6 weeks post transplant date in A) 2019 and B) 2020 experiments. Neither AMF addition treatment nor management affected dry mass in 2019 and 2020 (p>0.20).

A) **B)** **C)**



D)

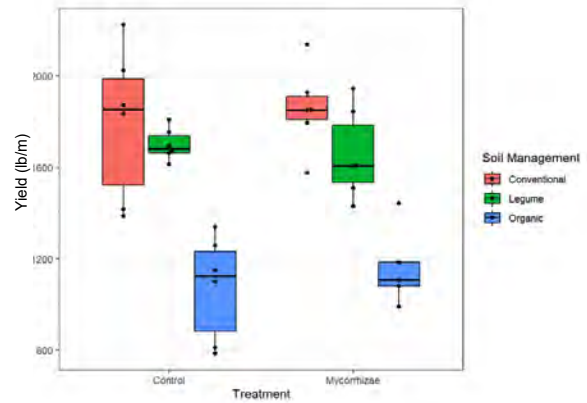


Figure 3. Yield. In 2019 (A-C), processing tomato yield was affected by soil management (p=0.004), but not AMF inoculum (p>0.40). In 2020 (D), yield in processing tomato was affected by soil management (p=0.02), but not AMF inoculum addition (p=0.48) or the interaction between soil management and yield (p=0.78).

Plant nutrients

In 2019, foliar nutrient levels were only rarely affected by soil management, and not affected by AMF treatment (Fig 4). Macronutrients, including nitrogen, phosphorus, potassium, magnesium were not significantly affected by soil type nor AMF treatment ($p>0.05$). Calcium and sulfur were significantly higher in plants grown in organic soils ($p<0.001$) but unaffected by AMF treatment ($p>0.10$).

Foliar manganese was higher in conventional soils (Fig 6, $p<0.01$) than organic soils, while copper was highest in organic soils ($p<0.01$). Sodium was greatest in legume (mixed) soils ($p<0.01$). Other micronutrients including boron, iron, aluminum and zinc did not differ among soil treatments or AMF treatment ($p>0.10$).

Plant nutrient levels were affected by soil management and AMF treatments (Fig 5). Leaf nitrogen levels were affected by inoculation, management and their interactions ($p<0.001$). Specifically, leaf N was lower in organic fields ($p<0.001$), but only in this soil type did mycorrhizal treatment increase N levels ($p=0.004$). Leaf phosphorus was higher in organically managed soils ($p<0.001$) but was not affected by AMF addition nor its interaction with soil management ($P>0.40$). Foliar potassium was higher in organic soils but was not affected by AMF addition nor its interaction with soil management ($P>0.40$). Foliar calcium was not affected by soil management, AMF addition nor its interaction with soil management ($P>0.40$). Foliar magnesium was lower in organic soils ($p=0.006$) but was not affected by AMF addition nor its interaction with soil management ($P>0.40$). Foliar sulfur was higher in organic fields ($p<0.001$), but only in this soil type did mycorrhizal treatment decrease S levels ($p=0.002$). Foliar manganese was lower in organic fields ($p<0.001$) and higher in mixed management fields ($p=0.02$), and mycorrhizal addition had a management-specific effect on Mn levels ($p=0.03$). Foliar iron concentrations did not vary by soil management ($p>0.20$), but mycorrhizal addition had a management-specific effect on Fe levels ($p=0.03$). Foliar copper concentrations were higher in organic soils ($p<0.01$) but were not affected by mycorrhizal treatments ($p<0.30$). Foliar boron levels did not differ among any treatments ($p>0.40$). Foliar aluminum did not differ significantly by management ($p>0.2$), but mycorrhizal addition had a management-specific effect on Al levels ($p=0.03$). Foliar zinc was greater in organic soils ($p=0.0087$) but no effect of mycorrhizal treatment was detected ($p>0.70$). Sodium levels were impacted by both mycorrhizal treatment ($p = 0.004$), and soil management type ($p < 0.001$). They were lower in organic soils, and overall lower in mycorrhizae-treated plants.

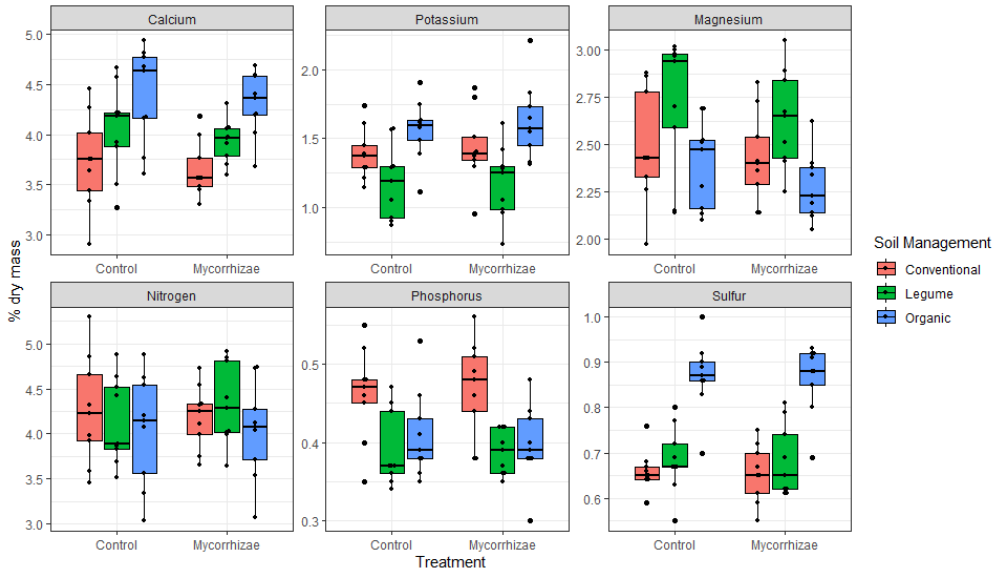


Figure 4. Effects of soil management and mycorrhizal treatment on foliar macronutrient concentrations (% dry mass) in 2019 experiment.

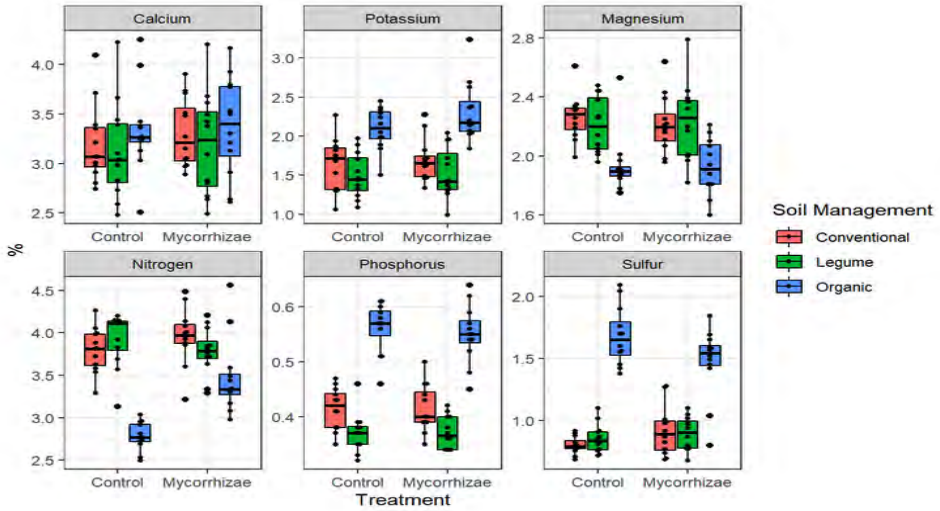


Figure 5. Effects of soil management and mycorrhizal treatment on foliar macronutrient concentrations (% dry mass) in 2020 experiment.

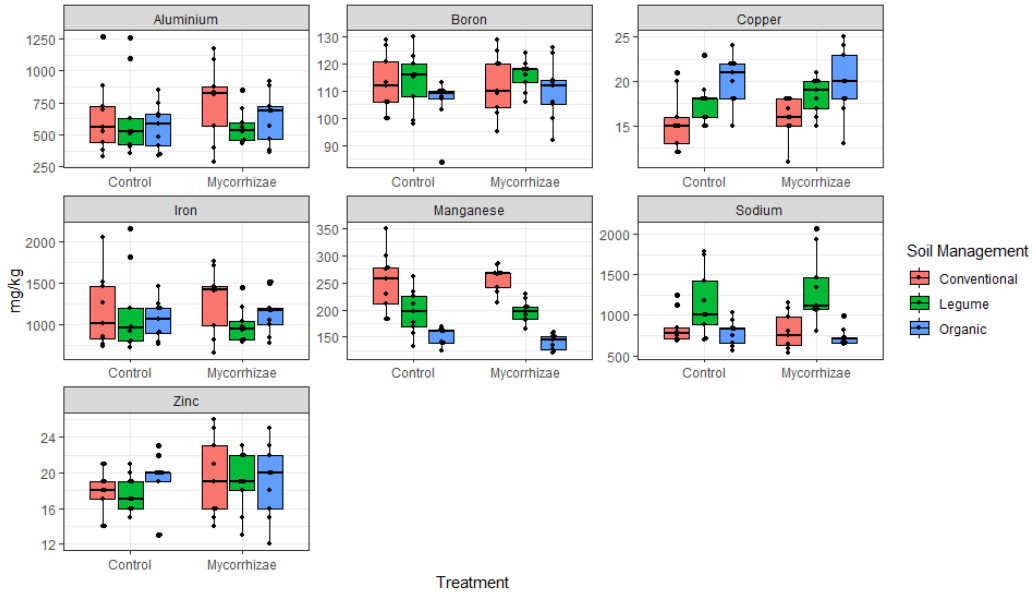


Figure 6. Effects of soil management and mycorrhizal treatment on foliar micronutrient concentrations (mg/kg) in 2019 experiment.

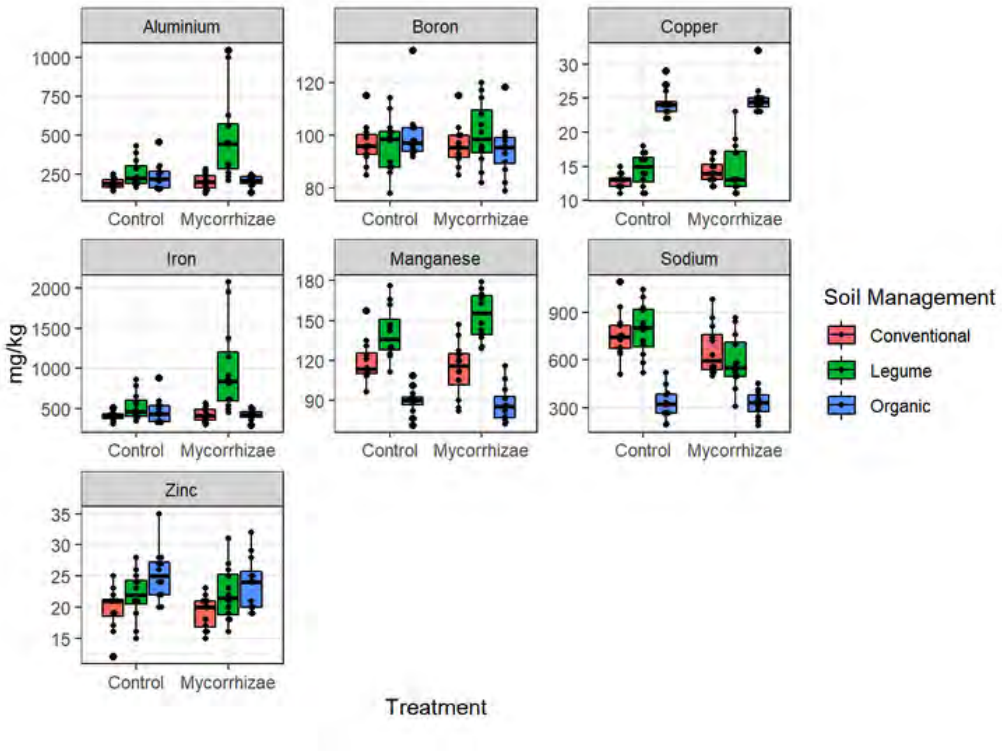


Figure 7. Effects of soil management and mycorrhizal treatment on foliar micronutrient concentrations (mg/kg) in 2020 experiment.

Objective 2: Evaluate reliability of lipid biomarkers to predict root colonization by mycorrhizal fungi

Previous studies (including the CTRI-funded tomato field soil health survey project) suggest that soil phospholipid (PLFA) biomarker analyses may be a better indicator of mycorrhizae among fields than root colonization counts. Further, characterization of lipid biomarkers in the soil rather than the tomato roots allows for better spatial comparison among parts of fields, or among different fields. We compared rhizosphere and bulk (away from plant) soil mycorrhizal numbers using PLFA methods and value as a mycorrhizal biomarkers at 30 DAT and 60 DAT to also obtain an idea of how mycorrhizal numbers change over the growing season.

Results: Soil PLFA markers for mycorrhizal fungi were not correlated with the root colonization metrics in the 2020 experiment (Fig 8). Interestingly, there was no effect of soil management or mycorrhizal addition on total PLFAs, although there was significant variation in total PLFAs among plots, suggesting variation at small scales (our samples were collected in the root zone and bulked among 3 subsamples).

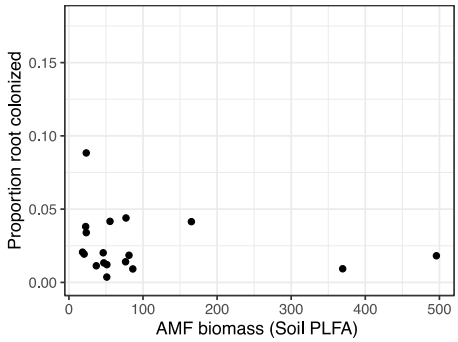


Figure 8. No significant relationship between PLFA-quantified AMF biomass in bulk soil and the proportion of root colonized by mycorrhizal fungi in cleared and stained roots. Notably, root colonization values were very low (less than 10% root colonization).

Objective 3: Complete analysis of experimental results from previous years and write up these results

Results: We have completed the analysis of results from 2019 and 2020. Results from 2019 indicate a potentially economically important effect of AMF but significant variability was found in among-plot response. Because some of the results from 2019 and 2020 are conflicting and suggest a context-specific effect of AMF, we are pursuing additional experiments. To support this work, we have secured additional funding from Valent (see below), to conduct additional experiments in 2021.

Other notable results from 2019 experiment included analysis of plant defense hormones. We found that plant defense hormone ACC (ethylene precursor) did not vary with soil treatment, AMF addition, nor their interaction ($p>0.10$). Abscisic acid (ABA), involved in bud dormancy and plant development, varied among soil types, and was greatest in the legume (mixed) treatment ($p<0.01$). Jasmonic acid (JA), a plant hormone involved in defense signaling, did not vary among soil types ($p>0.10$) but was slightly higher in plants that did not receive AMF treatment ($p=0.06$). Indole acetic acid (IAA) did not vary with soil management or AMF treatment ($p>0.30$). Interestingly, salicylic acid (SA), was decreased by AMF, but only in the legume (mixed) treatment (interaction $p=0.06$).

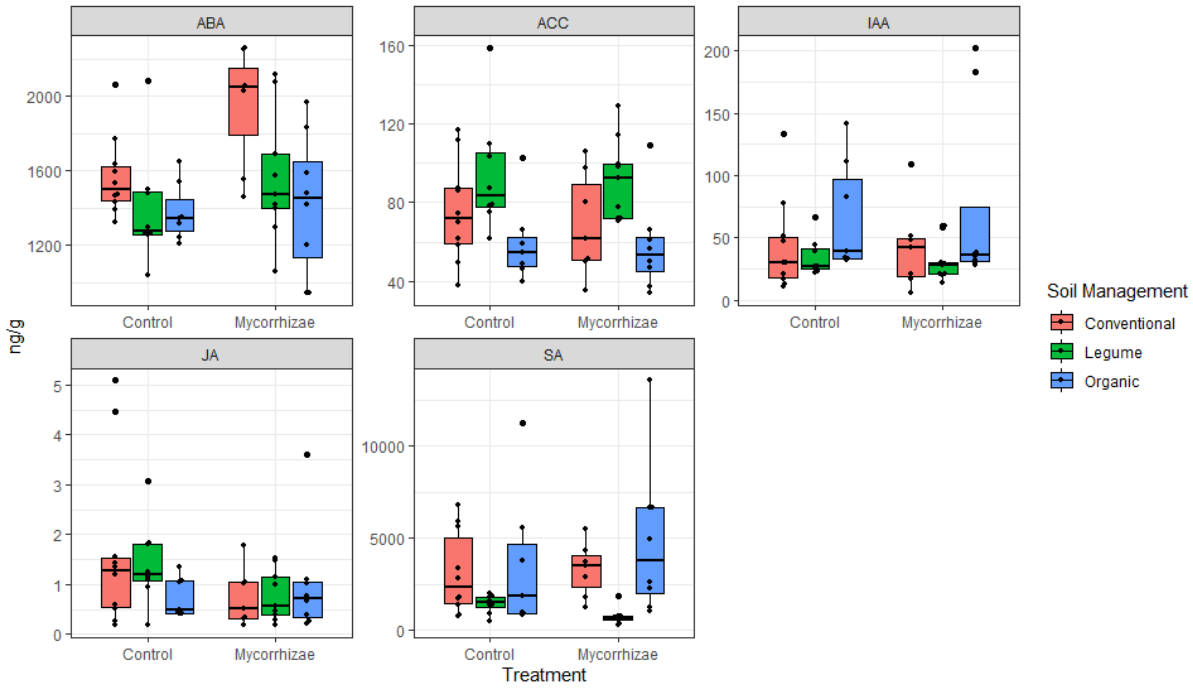


Figure 9. Effects of AMF and soil management on plant hormones in 2019 experiment measured by LC-MS/MS.

We also examined if AMF treatment affected herbivory on tomato leaves. We found no effect of AMF nor soil type on thrips damage, chewing damage, evident pathogen damage, nor aphid damage (Fig 10, $p>0.10$). In addition, using a choice experiment comparing between AMF inoculated plants and control plants within a soil treatment, we found that leafhoppers did not display a significant preference between leaf types ($p>0.20$).

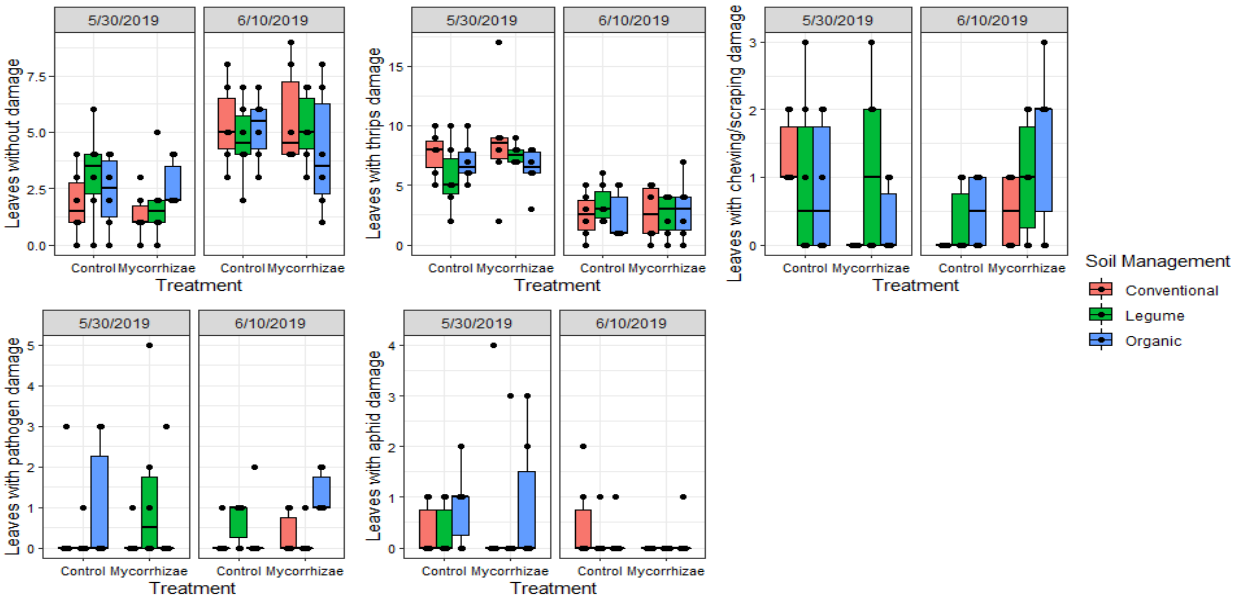


Figure 10. Effect of AMF inoculum addition and soil management on foliar damage by various sources.

Objective 4: Make recommendations and produce outreach and education material to inform growers about cultural practices which decrease attractiveness to leafhoppers.

Our results do not support the hypothesis that AMF affect insect damage. In addition, we do not find strong support for the hypothesis that mycorrhizal addition affects yields or fruit quality. As a result, we cannot, solely based on these data, recommend that tomato growers in Yolo county use mycorrhizal inoculum to improve yield or reduce insect damage. However, we note that AMF colonization levels are extremely low, even compared to previous reports of tomato. It may be that soil conditions at the Century Experiment, management practices, or other conditions are not amenable to the growth or persistence of mycorrhizal fungi in this cropping system, or that the varieties planted here are not amenable to associating with mycorrhizal fungi. In addition, our sequencing efforts including targeted primers for mycorrhizal fungi were not successful and very few sequence data were recovered that matched mycorrhizal fungi in this system, further supporting our findings that AMF are present in low abundance in our system.

Discussion: Although mycorrhizal fungi are known to form extensive associations with tomatoes and are typically beneficial for tomato growth, tolerance to deficit irrigation, stabilizing soil aggregates and resistance to insect pests (Cavagnaro et al. 2006, Vannette and Hunter 2009, Pineda et al. 2010), our results here suggest that the soils and / or plants used in our experiment did not support the growth or colonization of processing tomato by mycorrhizal fungi. In cropping systems, soil management practices can substantially influence the amount and type of mycorrhizal fungi in soils, and the extent to which they associate with plant roots (Lekberg and Koide 2005, Lekberg et al. 2008). We find evidence that mycorrhizal fungi are in general very limited in the focal fields and scant evidence that the addition of mycorrhizal inoculum can increase mycorrhizal colonization. Specifically in 2019, we found slight increases in AMF colonization with inoculum, but only in the organic soils. In 2020, we found no effect of AMF inoculum on root colonization or most measured variables except for a few nutrient-related variables. We conclude that the organisms contained in this product are not compatible with the management or plants at the Century Experiment. In conclusion, our results do not support the use of this product for improving yield or reducing herbivore pressure for processing tomatoes grown in Yolo county. If any effect is present, it is at most context-dependent—perhaps depending on the tomato variety planted or existing soil conditions. One hypothesis has been that systemic fungicides applied to conventional and mixed systems had a significant impact on AMF biomass and root colonization but we did not observed significant differences in Organic certified plots which did not receive any fungicides. Finally, our sampling 6 weeks after transplanting might have been too early and inoculum might need to be reapplied periodically and at later growth stages when nutrient uptake is higher for maximum effectiveness. While there is significant interest in microbial products, our field experiments

performed in working processing tomato fields do not support the hypothesis that AMF inoculum has significant effects on processing tomato health—including nutrient composition and pest resistance-- and yield. It may be that other products or application methods or interactions with management or plant stress conditions could reveal effects of mycorrhizal products. However, we did not detect significant growth enhancements from these treatments in either of our field experiments (2019 or 2020).

Acknowledgements: We acknowledge support from Mycorrhizal Applications (Valent) in providing AMF product for agricultural application. We thank Israel Herrera and others at Russell Ranch Century Experiment for their support. We acknowledge Ward Laboratories for PLFA analysis and Penn State lab for Nutrient analysis.

This project as leverage for other dollars: This is a metric the CTRI began collecting in 2017 to help communicate to our stakeholders the value driven into the industry by the researchers we work with AND the significance of this research, generally. If you were successful in receiving grants other than through CTRI to pursue related research questions where did those grants come from, what was their dollar value in 2020 and if they were multi-year grants, what will their dollar value be in subsequent years?

Thanks to CTRI support, we were able to forge a strong working relationship with Valent Bioscience, the company supplying the inoculum for this study. We have secured 4 more years of funding to support additional research ($386,216, 4/1/2020- 3/31/2025, PI Gaudin). Our objectives are to repeat this experiment with 1) no fungicide applications on any treatment, 2) a different tomato variety and 3) more sampling during the season so we can determine at what point in the growing season inoculation might generates the greatest mean proportion of colonization across all soil managements. Finally, we will quantify potential long-term effects of AMF inoculation on soil health metrics, and examine the interaction long-term organic and conventional management have on these outcomes. Valent Bioscience has embarked on assessments of long-term soil health outcomes of inoculation across long term experimental platforms in the US and the Century Experiment will be a flagship sites for irrigated semi-arid landscapes.

Project Leader and any Co-PIs: Zheng Wang, Vegetable Crops Farm Advisor, UCCE Stanislaus County; Anthony Fulford, CE Advisor, UCCE Stanislaus County; Costanza Zavalloni, Associate Professor, Dept. of Agricultural Program, CSU-Stanislaus.

Cooperating Personnel: T&M Farms, Inc. (Grower Cooperator).

Key Takeaway(s) of this project: Overall, we see the long-term potential of using composts in improving soil nitrogen translocation and accumulation for processing tomato production from this one-year composted study.

Specifically,
a) From November 2019 to March 2020, we did not see any difference in soil inorganic nitrogen accumulation.
b) Short of rainfall and dry soil during this bare-ground period were not beneficial for compost to help with inorganic nitrogen accumulation.
c) The combined rainfall for November 2019, January and February 2020 was less than 2 inches with 0 in February.
d) Tomato leaf petiole analysis indicated higher nitrate content at mid-season and/or early season after compost application.
e) Greater soil CO_2 respiration in April 2020 prior to transplanting tomato was detected for composted soil than the unamended control, potentially indicating greater microbial activities and nutrient releasing early in the growing season.
f) Composted plots yielded 10% higher by average than the unamended control in one field, while the other field produced comparably for all treatments.

Introduction: For processing tomato in the central valley, there is a time frame of 4 to 7 months between spreading compost and transplanting the next year's tomato plants. Understanding the potential of the compost to influence nitrogen dynamics during the subsequent growing season is critical to estimate nutrient contribution and better match nitrogen availability to processing tomato demands, leading to an improved nitrogen use efficiency, fruit yield and quality. Most research focused on the performance of compost on plant growth and development but rarely investigated the interaction of compost with soil properties during the period prior to planting. Given to the unique production pattern of processing tomato, the best time of applying compost is fall before new beds are listed. Therefore, it is necessary to understand how fall-applied compost can influence the soil nitrogen mineralization before planting and the subsequent plant growth and yield formation. This study started monitoring soil nitrogen status and total mineralization from Fall 2019 to the end of season 2020, covering an entire stage of compost "life span". On the other hand, we measured that composted plots translocated more nitrate from soil to plant vegetative tissues and eventually to fruit than the unamended controls. Although we did not measure nitrate leaching, this sufficiently indicated that composting can potentially make plants use fertilizers more efficiently and reduce nitrate loss. In other words, composted plots may contribute a larger portion of what has been fertilized to soil for plant uptake. This could be particularly welcomed by growers who are impacted by the restrictive regulation of minimizing soil and water contamination.

The main *Goal* and the *Objectives* under that goal: The major goal is to develop a better understanding of how green waste compost application rate influence processing tomato nitrogen use efficiency, fruit yield, and quality. There are three specific objectives under this main goal:

- Monitor soil physical and chemical properties and mineralized N from compost application to before tomato transplanting (November 2019 – March 2020).
- Determine the impacts of compost application rates (5, 10, and 15 tons/acre) on nitrogen mineralization, nitrogen use efficiency, and how these N-related dynamics affect plant development, yield, and fruit quality (estimated time to complete: April 2020 to September 2020).
- Synthesize data and disseminate results through newsletters and trade magazine articles (April 2020 to the end of the grant and beyond).

Methodology and Results:

Methodology

Compost application: In October 2019, we applied the compost at 5, 10, and 15 tons/acre to the designated field area. Soils were incorporated right after application for a better contact. Tomato beds were then listed to form treatment rows for each rate and a non-composted control at early November. Compost and soils were sampled before and after application to get a baseline data of the compost nutrient and soil routine characteristics. Then, buried bags were made by filling soil from each composted treatment and control plots, and then buried underneath soil at a 6-inch depth (Fig. 1). Six bags were placed at each treatment, and one bag will be taken out of soil at a monthly basis. The first set of bags were taken out at early December and sent to the UC Davis Analytical Lab for nitrogen mineralization measurement.

Planting: The fields were machine transplanted on May 18 and 19, 2020 using variety 'SVTM9008'. Plants were placed in a double row setting with a population of 9000 per acre.

Sampling: One buried bag was taken out of the soil from November 2019 to March 2020. Bags were brought to the lab and incubated to measure inorganic nitrogen mineralization for each composted and unamended treatment. Starting in April 2020, due to the shelter-in-place order, we decided to take soil samples at pre-plant, mid-season, and late-season on April 16, July 30, and September 17, 2020 and incubate them in the lab instead of going to the field each month. These samples were used to simulate the real-field situation and incubated to measure inorganic nitrogen mineralization after planting. Please note that the actual measurement/sampling dates were a few days earlier or later than the original plan not only due to the pandemic but also to the impact of wildfire and poor air quality and that soil samples incubated in the lab only included the two extremities (15 tons/acre and unamended control). These samples and measurements indicate the nitrogen mineralization over time during the growing season (May to September 2020). Plant leaf samples were taken monthly on July 3, 30, and August 27, 2020 to measure leaf nitrate and potassium concentration and monitor nitrate translocation from soil to plant tissues throughout the growth period.

Growth measurement: Stand count were made twice on June 12 and 26, 2020. NDVI leaf coverage data were measured monthly on June 26, July 22, and August 25, 2020. Leaf chlorophyll concentration was measured on the same day as leaf nitrate and potassium measurements.

Harvest: Machine harvest was conducted on October 9, 2020. Fruit weight from each plot was recorded and transferred to a tons/acre basis. Fruit from each plot was sub-sampled and sorted by grade: red, pink, green, blossom end rot, sunburn, and mold. After weighing fruit of each grade, red fruit was sampled in a 1-gal zip-lock bag for PTAB quality measurement (pH, HUE, titratable acidity, and Brix). This part combining the soil and leaf nitrate measurements will tell a whole story of nitrate translocation throughout the growth cycle.

Fig. 1. Soil samples were taken from each composted area and control plot and then sieved to get finer particles (Top 2); Filled the soil (pre-moistened) into the bag to 6 inches (Middle 2); Bags were buried 6 inches deep (Bottom 2). One bag from each treatment will be taken out of the soil each month (from November 2019 to April 2020) to measure nitrogen mineralization.

Results

Before planting (November 2019 to March 2020): Compost was sampled before applying for nutrient and physical property analysis. The average moisture, %N, and C:N are 24.8%, 1.7, and 16:1 (Fig. 2).

Fig. 2.

A & L WESTERN AGRICULTURAL LABORATORIES, INC.

Report No: 19-304-039
1311 Woodland Avenue, Suite 1 • Modesto, California 95351 • (209) 529-4080
Account No: 1213-D

Send to: UC COOP EXT-STANISLAUS COUNTY
3800 CORNUCOPIA WAY, STE. A
MODESTO, CA 95358

Submitted by: ANTHONY FULFORD

Date Received: 10/31/2019
Date Reported: 11/05/2019

ANAYSIS REPORT

Lab Number	Sample Id:	Moisture %	Nitrogen %	C:N Ratio
20632	L1 R1	22.43	1.90	15:1
20633	L1 R2	22.85	1.96	14:1
20634	L1 R3	22.56	1.67	17:1
20635	L2 R1	26.38	1.58	16:1
20636	L2 R2	27.65	1.74	15:1
20637	L2 R3	27.18	1.46	17:1

Compost property analysis performed by the A&L Lab in November 2019.

The overall soil inorganic nitrogen accumulation was not greater for composted soil vs. the control in three out five months for both fields. Similarly, both the composted soil and unamended plots exhibited mainly net N

immobilization and very limited organic N mineralization in three out of four months for both fields (Tables 1 and 2). Whereas soil CO_2 respiration measured in the spring (April 2020) prior to transplanting was numerically greater for compost (15 tons per acre) vs. the control (53.03 vs. 43.28 CO_2-ppm in field 1 and 49.03 vs. 38.18 in field 2).

Table 1. Mean accumulated inorganic N (NH_4-N + NO_3-N) during wintertime for both fields.

	Inorganic N in Field 1 (ppm)					
	November	December	January	February	March	Overall
5 tons/ac.	19.45	9.28	10.15	15.13	18.11	14.42
10 tons/ac.	17.56	11.16	11.28	13.78	17.64	14.28
15 tons/ac.	18.77	9.36	3.74	9.64	16.36	11.57
Control	15.11	8.72	14.67	9.28	21.04	13.76
P-value	0.15	0.96	0.11	0.42	0.47	0.29
$LSD_{0.05}$	4.03	10.09	8.98	9.00	6.46	3.29
	Inorganic N in Field 2 (ppm)					
5 tons/ac.	18.94	13.61	11.82	16.19	18.24	15.76
10 tons/ac.	25.14	14.71	13.33	14.35	18.9	17.28
15 tons/ac.	22.02	6.44	7.05	12.72	22.5	14.14
Control	24.34	11.74	22.62	19.23	31.23	21.83
P-value	0.63	0.66	0.20	0.62	0.15	0.04
$LSD_{0.05}$	11.14	15.24	14.93	11.06	12.72	5.39

Table 2. Mineralized N content from December 2019 to March 2020 in both fields.

	Mineralized N (ppm) in Field 1 [Month (T) – November (T_0)]				
	November (T_0)	December	January	February	March
5 tons/ac.	-	-10.17*	-9.3	-4.32	-1.34
10 tons/ac.	-	-6.4	-6.28	-3.78	0.08
15 tons/ac.	-	-9.41	-15.03	-9.13	-2.41
Control	-	-6.39	-0.44	-5.83	5.93
	Mineralized N (ppm) in Field 2 [Month (T) – November (T0)]				
5 tons/ac.	-	-5.33	-7.12	-2.75	-0.7
10 tons/ac.	-	-10.43	-11.81	-10.79	-6.24
15 tons/ac.	-	-15.58	-14.97	-9.3	0.48
Control	-	-12.6	-1.72	-5.11	6.89

*Negative number indicates nitrogen immobilization and positive number indicates nitrogen mineralization.

After planting (May to October 2020): Changes of nitrate concentrations in soil samples from pre-plant to the end of season had a reverse trend of nitrate concentrations in tomato leaf petiole (Table 3 and Fig. 3). The soil nitrate concentration is the lowest at mid-season, while leaf petiole was observed to have the highest concentration of nitrate, indicating the flow or translocation of nutrient from soil into plant tissues. In Figure 2, we noticed more active nitrate translocations from soil-vegetation-fruit for composted treatments than the unamended control. Please note dates reported in the following tables and figures have been transformed according to Julian calendar.

Table 3. Soil nitrate concentrations measured at pre-plant, mid-season, and late-season (Julian Days).

	Field 1-Soil Nitrate Concentration (ppm)		
	Pre-plant (107 day)	Mid-season (212 day)	Late-season (261 day)
0 tons/acre	32.8	9.8*	14.0
15 tons/acre	34.3	7.5	16.5
	Field 2-Soil Nitrate Concentration (ppm)		
0 tons/acre	37.8	6.5	17.0
15 tons/acre	40.8*	10.8*	20.0

*indicates a higher nitrate concentration than the other treatment at P<0.05.

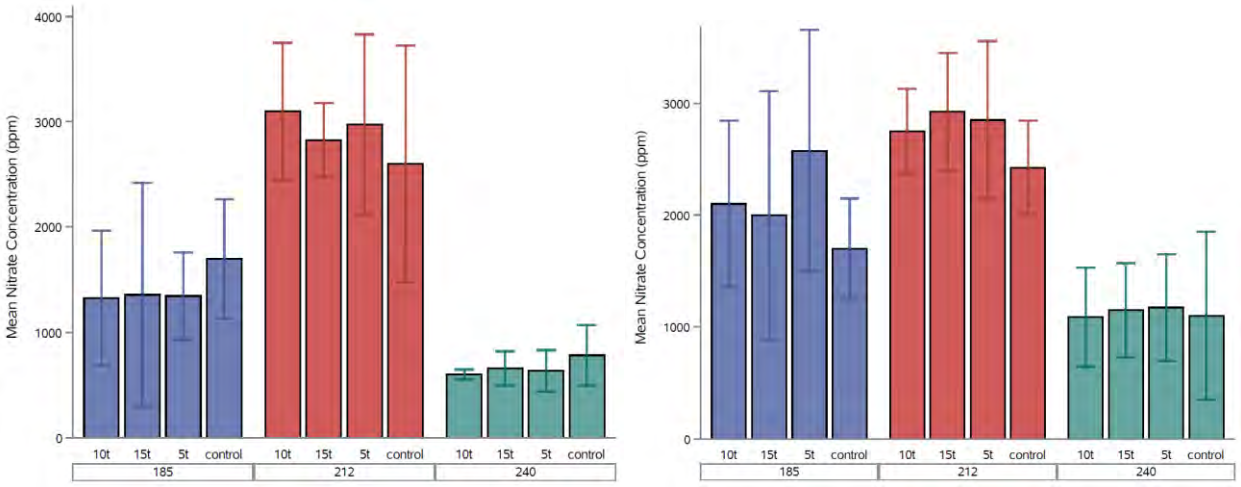


Fig. 3. Leaf petiole nitrate concentration at each date for field 1 (left) and field 2 (right).

For plant development, a higher leaf canopy coverage was observed for 15 tons/acre treatment in field 1. Overall, no difference in chlorophyll concentration was found for composted soil compared to the unamended control (Table 4).

Table 4. Mean NDVI readings and leaf chlorophyll concentration at each measuring date for both fields.

	Field 1-NDVI (Julian date)			Field 2-NDVI (Julian date)		
	178	204	238	178	204	238
5 tons/ac.	0.35	0.73	0.74	0.40	0.73	0.79
10 tons/ac.	0.35	0.73	0.73	0.39	0.74	0.80
15 tons/ac.	0.36	0.74	0.74	0.40	0.74	0.80
Control	0.35	0.71	0.72	0.40	0.71	0.78
P-value	0.99	0.13	0.07	0.98	0.42	0.29
$LSD_{0.05}$	0.10	0.02	0.02	0.09	0.04	0.02
	Field 1-Chlorophyll (umol.m^{-2})-Julian date			Field 2-Chlorophyll (umol.m^{-2})-Julian date		
	185	212	240	185	212	240
5 tons/ac.	602.53	514.85	474.45	504.88	475.43	432.50
10 tons/ac.	567.23	494.92	439.98	495.83	515.95	431.98
15 tons/ac.	589.22	486.53	446.58	513.10	485.42	441.23
Control	594.98	513.50	441.67	500.40	491.30	427.80
P-value	0.10	0.41	0.05	0.97	0.41	0.90
$LSD_{0.05}$	28.7	42.5	26.96	81.41	51.89	39.47

For yield, composted plots yielded 10% higher by average than the unamended control in one field, while the other field produced comparably for all treatments (Table 5). For fruit quality, we did not see any difference among treatments (Table 6).

Table 5. Fruit yield from each compost application treatment in both fields.

Application Rate	Yield in Field 1 (tons/acre)	Yield in Field 2 (tons/acre)
5 tons/acre	62.6 AB	69.7 A
10 tons/acre	63.6 A	68.0 A
15 tons/acre	63.2 AB	66.9 A

Unamended control	58.2 B	66.6 A
$HSD_{0.05}$	5.2	5.4

Mean fruit yields followed by the same letter are not significantly different at $P \leq 0.05$ according to Tukey's Honest Significant Difference (HSD).

Table 6. Fruit quality from each compost application treatment in both fields.

		Field 1 Fruit Quality		
Application rate	Brix	pH	TA	HUE
5 tons/acre	4.88	4.28	1.73	20.0
10 tons/acre	4.78	4.30	1.64	20.4
15 tons/acre	4.88	4.31	1.55	20.0
Control	5.00	4.30	1.67	20.2
		Field 2 Fruit Quality		
5 tons/acre	5.03	4.35	1.68	20.2
10 tons/acre	5.05	4.38	1.57	20.4
15 tons/acre	5.08	4.35	1.51	20.3
Control	5.00	4.38	1.64	20.4

Discussion: Overall, we see the long-term potential of using composts in improving soil nitrogen translocation and accumulation for processing tomato production from this one-year composted study. In the future, we need understand how composting interacts or changes the crop nutrient demand and uptake, that way growers may adjust their fertilization and irrigation schemes. We can utilize online tools (e.g., CropManage) to quantitatively monitor plant nutrient demand and uptake dynamics at a real-time and site-specific basis after spreading compost and maybe other amendments as well.

Acknowledgements: We want to thank Tom Maring and Mark Maring for allowing us to conduct this project on their commercial field and their help with fruit harvest. Also, we want to thank Recology and ALB for providing compost and spreading to the field. Lastly, thanks are delivered to Stanislaus Food Products and Morning Star for offering the service of fruit quality analysis.

ONGOING ANNUAL SUPPORT OF THE TOMATO GENETICS RESOURCE CENTER

ROGER CHETELAT

Project Leader: Roger T. Chetelat, Geneticist, Dept. of Plant Sciences, University of California, Davis

SUMMARY:

Acquisitions: No new accessions were acquired this year. A few obsolete or redundant accessions were dropped, and some previously inactive accessions were 'rescued'. The current total of number of accessions maintained by the TGRC is 4,405.

Maintenance and Evaluation: 1000 cultures were grown for various purposes, of which 307 were for seed increase, including 55 wild species accessions. Germination tests were run on 697 seed lots. Progeny tests were performed on 44 stocks of male-steriles and other segregating genes, or accessions with unexpected phenotypes. 62 stocks of *S. sitiens* introgression lines were grown for marker assisted selection or for drought tolerance testing. Other stocks were grown for research on interspecific reproductive barriers and/or for wide crosses to *S. ochranthum* and *S. juglandifolium*. All plants grown for seed distribution were monitored in all stages of growth for evidence of disease; TSWV was a big problem in our greenhouse this year. Newly regenerated seed lots were split, with one sample stored at 5° C for filling seed requests, the other stored in sealed pouches at -18° C for long term preservation. 209 seed samples were sent to the USDA for backup storage.

Distribution and Utilization: A total of 4289 seed samples representing 2035 different accessions were distributed in response to 184 requests from 155 researchers and breeders in 28 countries; many purely informational requests were also answered. The overall utilization rate (the number of samples distributed relative to the number of accessions available) was 97%, which is lower than in previous years, likely due to the effects of Covid-19 restrictions. 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 123 publications that mention use of TGRC stocks.

Documentation: Updated stock lists were published on our website. New images of mutants and wild species were uploaded. Passport data on new accessions was added. Revised guidelines for seed germination were posted on our website. Seed request records and passport information on seed samples submitted for off-site back up were provided to the USDA for uploading to their GRIN-Global database.

Research: The TGRC continued research on interspecific reproductive barriers and introgression of the *S. sitiens* genome. We isolated and characterized *ui3.1,* a gene expressed in pistils that is part of a novel mechanism of pollen rejection. We were awarded a grant from the Foundation for Food and Agriculture Research to study seed quality and pollination under heat stress.

ACQUISITIONS:

The TGRC acquired no new stocks this year. However we finished seed multiplication of BILs and other stocks added in recent years. Obsolete or redundant accessions were dropped, and inactive accessions were 'rescued' from old seed lots whenever possible. The current total of number of accessions maintained by the TGRC is 4,405.

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
S. lycopersicum	3,423
S. pimpinellifolium	332
S. cheesmaniae	42
S. galapagense	28
S. chmielewskii	16
S. neorickii	47
S. arcanum	45
S. peruvianum	69
S. huaylasense	16
S. corneliomulleri	53
S. chilense	115
S. habrochaites	120
S. pennellii	47
S. lycopersicoides	23
S. sitiens	13
S. juglandifolium	5
S. ochranthum	7
Other	4
Total	**4,405**

MAINTENANCE AND EVALUATION:

The TGRC grew ca. 1000 families for various purposes: 307 were for seed increases, including 55 wild species accessions, most grown in the greenhouse; 76 were for introgression and analysis of the *S. sitiens* genome; 44 were for progeny tests to verify the presence of segregating genes (e.g. male-sterility loci) or to confirm phenotypes.

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 threshold of 80% germination after two weeks are normally regenerated in the same year. Seed lots that meet the threshold are retested again every two 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, 697 seed lots from 2010 or earlier were tested for germination rates. Average germination values continued to be relatively high for most species (Table 2). Seed of the Galapagos endemics *S. galapagense* and *S. cheesmaniae,* as well as the tomato-like nightshades *S. lycopersicoides, S. sitiens, S. juglandifolium* and *S. ochranthum*, are always hard to germinate. In our germ tests this year, growth on ½X MS medium gave the best results. Also, seed lots stored at -18ºC often did better than samples of the same seed lots that had been stored in our working collection, at +5ºC.

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

Solanum spp.	Dates of Tested Lots	# Tested	Avg % Germ	# Low Germ
S. arcanum	1984 - 2010	23	90.0	0
S. cheesmaniae	2004 - 2010	9	77.8	3
S. chilense	1990 - 2010	46	86.0	9
S. chmielewskii	2007	1	100.0	0
S. corneliomulleri	1983 - 2010	24	90.2	0
S. galapagense	2004 - 2010	4	86.5	1
S. habrochaites	1991 - 2010	26	88.5	3
S. huaylasense	2004 - 2010	2	75.0	1
S. lycopersicum	2003 - 2010	416	80.0	137
S. neorickii	2005 - 2010	17	87.2	2
S. pennellii	2003 - 2010	19	92.2	0
S. peruvianum	1979 - 2010	33	86.0	6
S. pimpinellifolium	2000 - 2010	66	90.0	10
S. sitiens	2001 - 2003	6	76.0	1
S. lycopersicoides	2001 - 2008	4	81.5	0
S. ochranthum	2000	1	64.0	1
Total		**697**		**174**

Most stocks of *S. lycopersicum* and selfing *S. pimpinellifolium* are planted in the field, unless they require greenhouse culture, with each family represented by 8 or 9 plants. Our field plot this year occupied ca. 1.5 acres, similar to recent years. As usual, sequential plantings were made to spread the workload, with the first transplanting on April 29. Conditions were generally favorable throughout the growing season, despite the usual heat spells, and plants were mostly disease free. However, our field was infested with volunteer tomato plants from previous years' seed, necessitating careful and thorough weeding to avoid contaminating newly harvested seed.

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 to maintain heterozygosity and minimize inbreeding as each successive stock increase depletes genetic variation. This year we regenerated several accessions of *S. juglandifolium* and *S. ochranthum,* fruit of which require up to 12 months to ripen.

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. Conditions in the greenhouse were excellent, however we had a heavy outbreak of TSWV in the Fall and had to replant a large number of groups. We continue to treat samples of newly harvested seed lots with acid and bleach to prevent transmission of seed borne pathogens and to meet import requirements for certain countries.

All stocks grown for seed increase or other purposes were systematically checked to ensure that they expressed the correct phenotypes. New accessions were evaluated in greater detail, with the descriptors depending upon 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.

Many genetic stocks, including various sterilities, nutritional, and weak mutants, cannot be maintained in true-breeding condition, hence have to be transmitted from heterozygotes. Progeny tests must therefore be made to verify that individual seed lots segregate for the gene in question. Other accessions may show unexpected segregation or off-types due to outcrossing, and need to be progeny tested to reestablish true breeding lines. We progeny tested 44 seed lots of male-steriles, other segregating mutants, and other stocks with questionable phenotypes. This year's progeny tests included the mutants *ms-2, ms-16, ms-25, ms-32, ms-46,* and *ps-2*, as well as two *S. habrochaites* accessions.

Samples of newly regenerated seed lots were catalogued, with most of the seed stored or at -18°C for long term storage, and smaller samples stored at 5°C for filling seed requests. We made a change to how we dry seeds. In the past we used Zeolite beads to dry seed to ultralow moisture levels prior to sealing in foil pouches. However, we now dry seeds at 5°C / 15% RH, then seal them and freeze. This is easier and reduces the risk of over drying. As in the past, large samples 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 209 samples were sent to NLGRP.

DISTRIBUTION AND UTILIZATION:

A total of 4289 seed packets of 2035 different accessions were sent in response to 184 seed requests from 155 scientists, breeders and educators in 28 countries. Relative to the size of the TGRC collection (4,405 accessions), the number of seed samples distributed represents a utilization rate of 97%. Nearly half of our accessions were requested at least once in 2020, confirming that a large share of the collection is used each year. We also answered at least 22 purely informational requests regarding our stocks, growing recommendations, and other types of questions. Demand for our stocks was substantially less than in recent years, due no doubt to Covid-19 restrictions on research activities at many institutions.

We continue to receive many requests for introgression lines (ILs) and recombinant inbred lines (RILs). A total of 19 requests and 130 seed samples were processed for the *S. pennellii* ILs, 11 requests and 250 samples for the *S. habrochaites* ILs, and 13 requests and 322 samples for the *S. lycopersicoides* ILs. We also sent out 321 samples of *S. lycopersicum* x *S. pimpinellifolium* RILs in response to 6 requests. 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 repackaging seeds, entering the information into our database, providing cultural recommendations, obtaining phytosanitary certificates and import permits, 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 our crew must keep up to date with. For instance, this year the European Union countries began requiring that imported tomato seed be tested for Tomato Brown Rugose Fruit Virus (ToBRFV). This is not feasible for us, given the thousands of different seed lots that would need to be tested, so we request users provide a Letter of Authority granting exception to this rule.

Information provided by recipients regarding intended uses of our stocks is summarized in Table 3. As in previous years, there was a notable emphasis on biotic stresses (57 requests),
especially viral, bacterial and fungal diseases, both for breeding purposes and for more basic research. A large number of stocks were requested for screening for emerging diseases, including the new tobamovirus, ToBRFV. There continues to be much work on abiotic stress responses (28 total), particularly drought and high temperatures. Many requests mentioned research on fruit traits (10), particularly color and flavor. As usual, there were many requests for breeding uses (24), especially marker development and prebreeding. As in the past, a large number of requests (35 total) were for physiological or developmental studies, with notable interest this year in circadian and light responses, and root traits, including mycorrhizae and root microbiomes.

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

Category	
Biotic Stresses	
Viruses:	
ToBRFV	2
TSWV	1
TYLCV	2
Viroids	1
Unspecified viruses	1
Bacteria:	
Bacterial spot	2
Bacterial speck	2
Bacterial wilt	1
Unspecified bacteria	3
Fungi:	
Cladosporium	1
Botrytis	2
Early blight	2
Fusarium spp.	2
Oidium powdery mil.	1
Phytophthora spp.	2
Septoria	1
Target spot	1
Verticillium	3
Unspecified fungi	1
Nematodes	1
Unspecified diseases	17
Insect pests:	
Potato beetle	1
Spodoptera exigua	1
Unspecified insects	3
Parasitic plants	
Unspec. biotic stresses	3

Category	
Abiotic Stresses	
Drought	6
High temperatures	10
Low temperatures	2
Salinity	8
Unspecified abiotic	2
Fruit Traits	
Carotenoids, color	4
Flavor, volatiles	4
Fruit quality	2
Breeding	
Grafting, rootstocks	2
Haploid induction	1
Internode length	1
Male sterility	2
Marker development	8
Prebreeding, wide hyb.	5
Unspecified breeding	5
Genetic Studies	
Allele tests	1
Chloroplast engineer.	1
Diversity	1
Evolution	1
Gene expression	2
GWAS	2
Mapping, QTLs	4
Mutation	1
Recombination	1
Polyploidy	1
Transformation	3

Category	
Unspecified genetics	9
Physiology & Develop.	
ABA	2
Acylsugars	2
Anthocyanins	3
Auxin responses	1
Circadian responses	3
Flower morph/develop	1
Herbivore microbiome	1
Inflorescence devel.	2
Leaf anatomy	1
Leaf senescence	1
Light responses,flower.	3
Metabolites	1
Mycorrhizae, rhizosphere microbiome	4
Nutrition	1
Plastids	1
Pollen growth, fertiliz.	1
Root traits	1
Shade avoidance	1
Stomatal develop.	1
Thiamine metabolism	1
Tritrophic interactions	1
Wounding, defense	2
Miscellaneous	
Seed backup storage	1
Instructional	1
Unspecified research	15

Our survey of the 2020 literature and unreviewed papers of previous years uncovered 123 journal articles, reports, abstracts, posters, theses, and patents that mention use of TGRC stocks (see Bibliography, below). Many additional publications were undoubtedly missed, and cases of utilization by the private sector are generally not publicized. These publications, many in high impact journals, demonstrate that the TGRC germplasm collection is a vital global resource for basic and applied research and breeding involving tomato.

DOCUMENTATION:

Additional images of mutants and wild species accessions were uploaded to our database and are accessible via our website. We updated passport data on existing accessions to correct errors or incorporate new information. On our website we revised our guidelines on seed germination to reflect recent improvements we made using tissue culture media. We also created a new page describing the *S. sitiens* introgression lines developed by the TGRC. We posted updated lists of available monogenic stocks (mutants), wild species, and miscellaneous genetic stocks (cultivars, breeding lines, etc.). We added links to new publications and to relevant online resources and events. 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 user categories (domestic or foreign, public or private institution, etc.).

RESEARCH:

Research at the TGRC currently focuses on deciphering the genetic mechanisms of pollination barriers that prevent or limit crosses between cultivated tomato and related wild species. Dr. Xiaoqiong Qin in our lab isolated and characterized a gene (*ui3.1*) expressed in pistils that prevents pollen of cultivated tomato from fertilizing ovules of the wild species *S. pennellii.* We previously characterized a pollen expressed gene (*ui10.1*) from *S. pennellii* that overcomes this barrier. This newly identified pollen rejection mechanism acts independently of the well-known self-incompatibility system present in wild tomatoes and other Solanaceae.

Another project in the lab aims to develop new germplasm resources that incorporate genetic material from the wild nightshade *S. sitiens.* Last year we reported developing a set of 56 introgression lines (ILs) representing the genome of *S. sitiens* in the genetic background of cultivated tomato. Each line contains a small piece of one chromosome from the wild parent. As a whole, the lines capture 93% of the donor genome in the background of the fresh market inbred NC 84173. We continue to refine and improve this resource by isolating recombinant plants containing shorter chromosomal segments, by isolating homozygous (stable) genotypes, and by producing sufficient seed. With our UC Davis colleagues Dr. Kent Bradford (PI) and Dr. Barbara Blanco-Ulate (coPI), and Dr. Alfred Huo (coPI) at the University of Florida, we were recently awarded a grant from the FFAR (Foundation for Food and Agriculture Research) to study seed quality and pollen responses to heat stress. The TGRC will use the new *S. sitiens* ILs to map the genetic regions (QTLs) involved in seed dormancy/germination, seed size and shape, and pollen germination and growth under heat stress.

Unique among the tomato species, *S. sitiens* plants (foreground) lack anthocyanins in all stages of growth. Analysis of introgression lines show this trait is controlled by mutations on chromosomes 2 and 10. The ILs express many novel traits and can facilitate their genetic analysis.

PUBLICATIONS:

Chetelat, R. T. (2020) The tale of a wild tomato's discovery. https://sustainable-secure-food-blog.com/2020/09/22/the-tale-of-a-wild-tomatos-discovery/

SERVICE AND OUTREACH:

RTC gave lectures on the TGRC, research projects, and related topics to HRT 200B (a UCD graduate course in horticulture) and PLB 154 (a UCD undergraduate course on Plant Breeding). RTC gave an invited presentation to the California Plant and Soil Conference, hosted by the California Chapter of the American Society of Agronomy.

RTC was interviewed by High School student Jasper Zeitlow for a paper on plant breeding and hybridization.

RTC met and consulted with scientists from the University of Haifa (Israel) and the Crop Trust, and participated in a scientific advisory board meeting for the HARNESSTOM project and consulted for the Periodic Table of Food Initiative's Biobanking Strategy.

RTC wrote a blog post about Charley Rick and his discovery of *S. sitiens* in the Atacama Desert of Chile. Written for nonscientists, the blog is part of a series by the American Society of Agronomy and the Crop Science Society of America that highlights the role of crop wild relatives in food security.

PERSONNEL:

Matthew Valle continues as our Assistant Curator, overseeing undergraduate students Anastasis Matthews, Maxine Nixon, Carina Chavez and Erin Reim. Former undergraduate student assistant Grace Mackie graduated this year. Dr. Xiaoqiong Qin continues her research on reproductive barriers and *S. sitiens* introgression, while providing the TGRC with lab services (DNA marker analysis, GMO testing, phytopathology, etc.). Yoji Nakajima, a tomato grower from Japan, spent several weeks at the TGRC to learn about the wild species and help us in the greenhouse. Undergraduate student Timothy Mulgrew completed an internship in the lab.

TESTIMONIALS:

"Thank you also, for all you do to support the tomato research community." – Tom McKnight

"Thanks for the work you guys do at TGRC – I've recently received some seeds from you and it was an easy and straightforward process." – Ben Mansfeld

"As a tomato breeder, I can't say enough on the importance of germplasm collection and diversity. TGRC and your research program helps greatly in many ways in our success as one of the main seed suppliers worldwide." – Chunxiao Jiang

"Thanks for the continued great contribution of the TGRC to tomato academia and industry." – James Brusca

"Your TGRC website is a valuable source of information." – Hidde Boersma

Dense hairs on the *Wo-Ln* double mutant. Our mutant stocks include single, double or multiple marker stocks that can be used to study how genes interact to control trait expression.

COMPLETION OF INSECT RESISTANCE SOURCE LINE FOR TRANSFER RESISTANCE TO INSECTS AND INSECT TRANSMITTED VIRUS PROCESSING TOMATO

MARTHA MUTSCHLER

Project Leader:
Martha A Mutschler; Professor
Plant Breeding and Genetics Section, SIPS; Cornell University, Ithaca, NY 14850
Cooperating Personnel: Cooperating Personnel: Lynn Veenstra and Richard Ozminkowski, HeinzSeed.

Key Takeaway(s) of this project: Considerable progress was made towards the main goal and objectives of this project during year 1. Critical advances were made in modifications of the Chr2, Chr3 and Chr10U introgressions, in combining these newly modified introgressions in lines, and testing the resulting new lines for acylsugar level and plant traits as each advance was made. The work also revealed acylsugar QTL interactions that might require adjustment of breeding plans, but open new opportunities to achieve the high acylsugar levels needed for optimal control of WFT and TSWV.

Introduction: ***Rationale for need***. Western flower thrips (WFT) are major pests of tomato. Thrips feeding can stunt/kill young plants, delay crop maturity, blemish fruits and reduce yield. More critically, WFT are "supervectors" of plant viruses including *tomato spotted wilt virus* (TSWV), which severely reduces crop yield and quality. Use of tomato hybrids with the *Sw-5* TSWV resistance gene protected CA tomato production until 2016 – 2018, when *Sw-5* breaking strains emerged and rapidly spread in CA. Incorporation of other TSWV resistance genes is possible, but new resistance genes are scarce, and could also fail if used as the sole control of TSWV. Leafhopper transmitted BCTV has also been regionally important in some years and spread of whiteflies/TYLV in CA is also of concern. A new sustainable means of controlling the insect transmitted viruses is needed. Acylsugars, produced at high levels by the wild tomato *S. pennellii*, are strong insect control compounds, deterring many species of insects from feeding and/or oviposition on plants. Since acylsugars protect through strong feeding/oviposition deterrence, rather than by toxicity to insects, acylsugars mediated insect control should be sustainable, rather than leading to the development of acylsugar-resistant insect biotypes. (Mutschler and Wintermantel, 2006)

The original acylsugar benchmark line CU070126 has moderately high levels of acylsugars. Additional breeding produced a set of tomato lines in CU071026 producing acylsugars at higher levels (Leckie et al 2012) and/or of different chemical structures (Smeda et al 2016, 2017, 2018). The newer benchmark line CU17NBL have similar moderately high levels of acylsugars as CU070126 but is far superior for fruit set and seed production. Both lines have negative plant or fruit characteristics due to negative trait QTL contained in the *S. pennellii* introgressions that also contain necessary acylsugar QTL. The work in this project is creating new tomato lines that have the high levels of acylsugars needed for optimal WFT/TSWV but do not carry the negative trait QTL.
Prior work developing and testing lines built a solid basis for this project. Cooperators used CU071026 and derived acylsugar lines producing acylsugars at higher levels and/or of different chemical structures in laboratory and field insect/virus trials show that:

- Acylsugar lines control WFT, significantly deterring feeding/oviposition of WFT on acylsugar tomato lines with higher acylsugar levels (Leckie et al 2016; Ben-Mahmoud et al 2018, 2019).
- Differences in acylsugar level and/or chemistry can impact the degree of control of WFT and whiteflies. (Leckie et al 2012, 2016, Smeda et al, 2018).
- The acylsugar lines with the strongest WFT control also significantly reduce the likelihood of infection by TSWV in lab tests (Smeda et al, 2018, Ben-Mahmoud et al, 2018, 2019) and percentage of plants infected by resistance breaking TSWV (TSWV-RB) in 2018 & 2019 California field trials against Sw-5 resistance breaking TSWV (Gilbertson and Turini, CA trials 2018 to 2020, unpub).
- Multistate field trials show that, due to environment conditions and tomato production methods, acylsugar lines produce the highest acylsugar levels in CA; this trait is particularly well suited for deployment in CA tomato production.
- The additional QTL that had been transferred into CU071026 to create the lines used in CA trials were also transferred into CU17NBL, to create high acylsugar level lines with higher seed germination and fruit set for

the continuing trials in California.

The work accomplished in year 1 moved the development of tomato lines that produce high levels of acylsugars and are also free of the negative QTL present in CU071026 and CU17NBL, much closer to completion. The completed lines, with information collected during their development, and the new genetic markers created, will facilitate use of acylsugar mediated insect/virus control by seed companies for the creation of commercial processing tomato hybrids that possess this critical trait.

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

The **main goal** is to create tomato lines carrying the introgressions required for optimal acylsugar levels and that are free of QTL that result in undesirable traits, such as off type vine and small fruit. The optimal acylsugar levels are those shown to control WFT and significantly reduce TSWV infection, and control leaf hopper/BCTV, as well as other insect pests.

The objectives to meet this goal include:

1. Complete the modification / testing of the *S. pennellii* introgressions from CU17NBL so that each modified introgression retains its acylsugar QTL but has lost its negative trait QTL.
2. Combine the resulting modified introgressions together in lines and confirm that resulting lines both retain the levels of acylsugar expected and no longer retain the targeted negative traits.
3. Determine best way to achieve the increased acylsugar level optimal for WFT/TSWV control: using QTL6 or FA2QTL, FA7QTL, or FA2QTL/FA7QTL. Both of QTL raises acylsugar level 80% to 100% in prior lines; FA7QTL introgression is free of negative trait QTL, but the QTL6 introgression carries the *Sp* gene for the negative trait indeterminate vine growth. Introgressions carrying FA7 or QTL6 will be independently transferred to lines with the modified introgressions (steps 1 and 2), and the resulting sets of lines will be tested for acylsugar level and plant/fruit characteristics.
4. Provide seed of lines with modified introgressions to seed companies, with full information to facilitate the transfer of the acylsugar mediated insect/virus control into modern processing lines and hybrids. We will also release the suite of SNP markers, created in year 1, to assist the seed companies in transferring this trait to commercial lines/hybrids.

Methodology and Results:

Methodology:

1. Self and Cross Seed production. Self seed is produced in greenhouse grown plants by vibrating flowers to insure good pollination. Cross seed is produced by manually emasculating female flower buds and pollinating them with pollen from flowers of male parent plants. Fruit are harvested when ripe and deseeded, this seed is acid/base treated, thoroughly rinsed, then dried, weighed, and packaged. Mutschler's staff are routinely do this work; no problems are anticipated

2. Plant Growth: This project uses only greenhouse grown plants, which allows for the most rapid progress (2.5 to 3 generations/year). Plants are grown using standard greenhouse growing methods.

3. *Marker assisted selection.* Marker assisted selection is well supported in tomato by availability of the complete tomato genomic sequence and the creation of thousands of markers with locations spread over tomatoes 12 chromosomes. Prior breeding for acylsugar production in tomato heavily utilized in -lab PCR to distinguish tomato, heterozygous and *S. pennellii* genotypes for positive selection for the presence of the *S. pennellii* introgressions. In year 1 a set of 70 SNP markers were created and tested to allow use of service labs to handle very large populations to accelerate progress. Both methods are working well, no problems are anticipated with these techniques

4. Acylsugar assay. Acylsugar levels are routinely determined by the standard acylsugar assay, developed by Mutschler's lab, and described in prior papers (Leckie et al 2012, Smeda et al, 2016, 2017 and 2018). The assay is robust, has been used for years, and no problems are anticipated

5. *Measuring phenotypes for negative traits*: several introgressions initially had small fruit QTL which reduces fruit weight from 20% to 50%; fruit weight is typically measured to measure the phenotype for this trait. Small fruit QTL on chromosomes 2, 3, 7, and 8 have been eliminated. As the modified introgressions are combined, fruit weight will be monitored to determine if the changes made have fully eliminate this trait. Tomato off flavor (chr3L) is very noticeable by taste, but data showing the location of this the off-flavor QTL vs Acylsugar QTL in the Chr3L

region allows selection by marker in seedlings, so relatively small number of plants require fruit taste tests. Determinate/indeterminate vine (Chr6) impacts height of vine (which is easily measured). Since the Sp gene responsible for this trait has been sequenced a marker within the gene itself is available, making seedling screens simple. Excessive branching/ unusual foliage pattern (3U) is visually rated and has already been eliminated in several lines. No problems are anticipated.

6. *Process of modifying the introgressions, using all methods listed above*. First, we produce large seed lots for the desired generation of plants with recombinations in the targeted introgression. The best seed is from plants homozygous for all the introgressions except the one being targeted for modification, which should be heterozygous; this maximizes efficiency. Then we grow a large population of seedlings, which are screened with markers within the ends of the introgression to be modified. The size of the population is determined by the size of the introgression, or any prior knowledge about how closely the acylsugar and the negative QTL are positioned within the introgression. We use the resulting marker data to identify plants with a recombinations in the targeted introgression. Plants with recombinant introgressions are potted, assayed for acylsugar and for the altered trait (fruit size, etc.) that is carried by the introgression, and self-pollinated to produce seed. In the next generation, markers are used to select for homozygosity for the segregating region to select plants that are homozygous tomato for a portion of the introgression, reducing introgression size. These plants are potted, then tested for acylsugar level and whatever negative trait was associated with the introgression. Comparisons of the modified introgressions with the original introgression in the same background indicates which modified version of that introgressions allow plants to produce acylsugars at the same level but have eliminated the negative trait. This work started in year 1 and will continue in year 2.

7. *Combining modified introgressions into a single line.* It is advisable to combine all the modified introgressions into a single line, so that we can confirm desired acylsugar level and the improvement of plant/fruit type are achieved when the modified introgressions are combined. We already have the final versions of the Chr7, ModChr2, and ModChr10U introgressions combined in a line. As modifications of the other introgressions are finalized, they will be backcrossed into this line to produce the final line, possessing only modified introgressions. We would test acylsugar level and plant type as each introgression is added to the line. The resulting line would facilitate the most rapid transfer into processing tomato.

Year 1 results for the listed objectives:

1. Complete the modification / testing of the S. pennellii introgressions from CU17NBL so that each modified introgression retains its acylsugar QTL but has lost its negative trait QTL.

2. Combine the resulting modified introgressions together in lines and confirm that resulting lines both retain the levels of acylsugar expected and no longer retain the targeted negative traits.

These two objectives progress separately for different introgressions, and the objective two work starts as soon as obj 1 work on an introgression is completed. Therefore, it is easiest and clearer for the reader if the progress on these two objectives is discussed together for each introgression.

I. Improving Sub introgressions 3U and 3L

I. a) The original 3L/3U line most selections with modification of 3U or 3L lacked the Chr7 and Chr8 introgressions present in CU17NBL. Line I is an elite Chr2/3U/3L/Chr10U introgression line in which the 3U region is reduced from 10Mb to 4Mb and lacks the QTL3.1 The lower acylsugar level of Line I could have been due to the lack of the 3.1 QTL, the lack of the Chr7 and Chr8 introgressions, the lack of all three of these QTL. Adding Chr7 & Chr8 introgressions to the line I created the desired Chr2/AL3.2/3L/Chr7/Chr8/Mod10U single plant selections; the acylsugar level of these plants were increased, but lower than CU17NBL.

I. b) Creation of 3U and 3L sub-introgressions from the original Chr3 introgression also identified and mapped the three acylsugar level QTL: AL3.1 & AL3.2 (in the 3U sub-introgression) and AL3.3 (in the 3L sub-introgression). Screening additional generations identified plants with additional modifications of the 3L and 3U sub-introgressions, refining QTL locations.

I. c) Progress has been made on creating versions of chromosome 3 that possess three sub-introgressions containing the AL3.1, AL3.2 and AL3.3 QTL, respectively. BC progeny derived from Line I and a sister line homozygous for AL3.1 and AL3.3 identified plant identical to Line I, but also heterozygous for AL3.1, and which have significant higher acylsugar level than line I, indicating the value of AL3.1 QTL.

I. d) Prior work demonstrated that the off-flavor QTL associated with the 3L sub-introgression could be separated from the AL3.3QTL by recombination, and the relative locations of these two QTL in 3L. Work in 2020

identified several plants with an independent recombination in Chr3L of the type predicted to eliminate the off-flavor QTL. Work in 2021 will identify progeny plants retaining AL3.1 and free from the off-flavor QTL.

II. Combining the introgressions Mod2 and Mod10U for removal of small fruit QTL: Work in 2020 completed creation of lines that combined the Mod10U introgression and the Chr7 and Chr8 introgressions in lines homozygous for Chr2 and the 3U/3L sub-introgressions, creating a Chr2/3u/3L/Chr8/Mod10U line that produces acylsugars at levels similar to that of CU071026. Progress has been made combining the Mod2 introgression into this Chr2/3u/3L/Chr8/Mod10U line; the remaining work in this process will take 2 more generations, or about 7 or 8 months.

III. Introgression Chr8: are any modifications necessary?

Plant type associated with the Chr8 introgression is shorter in height, more compact, unlike the more open vine of most determinate fresh market tomatoes. F1 hybrids from crosses of fresh market and processing tomato have a vine type similar to that of fresh market tomato, so the compact structure of the processing tomato is a recessive trait. However, the F1 hybrids from crossing processing tomato with acylsugar lines containing Chr8 introgression retain a processing tomato-like vine, suggesting that the Chr8 introgression has an QTL for compact vine which should be retained, not removed.

3. Determine best way to achieve the increased acylsugar level optimal for WFT/TSWV control: using QTL6 or FA2QTL, FA7QTL, or FA2QTL/FA7QTL.

I. a) **Chr6 introgression.** The Chr6 introgression has a QTL that nearly doubles acylsugar level over that of CU071026 or CU17NBL, resulting in lines that best control WFT/TSWV. In 2020, screening progenies of plants with recombinations in the Chr6 introgression located an acylsugar QTL in the upper 0.7Mb region that also contains the undesired Sp (indeterminate vine) gene. We produced seed lots from plants only heterozygous for this region, to facilitate identification of plants with recombinations needed to eliminate Sp while retaining the acylsugar QTL. After the desired modified Chr6QTL introgression is identified, it will be transferred to the ModChr2/3u/3L/Chr8/Mod10U line being produced in 2. above.

I. b) **Alternative means to raise acylsugar**: In a separate USDA funded project, we serendipitously that the presence of the FA2QTL, the FA7QTL, or both introgressions increases acylsugar level substantially in the CU17NBL background, although not in CU070126. This difference is due to the presence of the Chr8 introgression only in CU17NBL, suggesting the increased acylsugar level is the result of interaction between the acylsugar QTL in Chr8 introgression and (most likely) the FA7QTL. Tests Dec 2020 confirmed that addition of FA2QTL and FA7QTL introgressions into CU17NBL doubled acylsugar level. The FA2 and FA7QTL introgressions are not associated with negative traits. This line already has both the AL7QTL and the FA7 QTL combination on chromosome 7. The FA2QTL introgression would have to be combined with Mod2 introgression, but this alteration could be done while transferring the FA2 and FA7 introgressions into the ModChr2/3u/3L/Chr8/Mod10U line described above. We have started this transfer. We are also increasing seed of the FA2/FA7/CU17NBL line, for use in collaborative WFT/TSWV trials in California.

4. *Provide seed of lines with modified introgressions to seed companies, with full information to facilitate the transfer of the acylsugar mediated insect/virus control into modern processing lines and hybrids.* Work on this objective will largely be accomplished after the other three objectives are completed. However, we are keeping interested seed companies informed of progress, and they are awaiting release of the lines. The information on the new set of markers to support transfer of the modified introgressions will be posted on a web site and provided as files to the seed companies receiving the acylsugar lines.

Summary Tables: these tables present the same information as the text, in a more concise format.

Table 1: Summary of progress to date and work to be completed for introgression from CU17NBL

Introgression	Modification needed	Status of work	Work to do
Chr2	Remove small fruit QTL	Modification completed; confirmation test complete,	Combine Mod2 introgression in final lines with other modified introgressions.
Chr3U	Bring 3.1QTL and 3.2 QTL into coupling on chromosome 3	Coupling achieved, but selected plants are heterozygous	Select plants homozygous for coupled 3.1QTL and 3.2 QTL, test plants for acylsugar level compared to CU071026 and CU17NBL. Transfer into final line with other modified introgressions
Chr3L	eliminate off flavor QTL	Relative position of negative QTL and acylsugar QTL determined, plants with modification predicted identified,	Select plants homozygous for recombination, test acylsugar level and fruit flavor to identify desired recombination
Chr7	None needed	NA	NA
Chr8	None currently needed	None currently	Once lines with modified Chr2, Chr3, and Chr10U introgressions are created, fruit size will reveal if Chr8 introgression has a small fruit QTL
Chr10U	Remove small fruit QTL	Modification completed; confirmation test completed	Combine Mod2 introgression in final lines with other modified introgressions.

Table 2: Summary of progress to date and work to be completed for introgressions that raise acylsugar level.

Introgression	modification	Status of work	Work to do
Chr6	Remove small fruit QTL	QTL for increased level of acylsugar and for Sp gene both located in upper 0.7Mb of this introgression.	Select plants with recombination in this region to separate the acylsugar QTL and Sp. After that, two more generations will be needed to identify which, if any, recombination eliminates Sp, and to transfer Mod6 to a final Mod2/Mod3U/Mod3L/7/8/Mod10U line
FA2QTL and FA7QTL introgressions	FA2QTL introgression must be in coupling with Mod2 introgression, not original Chr2 introgression	Selected BC1 plants heterozygous for FA2QTL and FA7QTL for transfer of the two introgressions into final line; selections still heterozygous for some other introgressions	Two or three generations needed to transfer FA2/FA7 QTL into a Mod2/Mod3U/Mod3L/7/8/Mod10U line, and to select for FA2QTL to be in coupling with Mod2.
	No modification needed FA7QTL introgression, which is in coupling with AS7QTL introgression		

We are pleased with the progress in year 1, despite a 2-to-3-month delay caused by Covid19 response at CU spring 2020. The possibility of using FA2/FA7QTL to double acylsugar level instead of the QTL6 introgression is critical. Since we have not yet separated the Chr6QTL from Sp, and since the FA2 and FA7 introgressions do not carry negative QTL, it might be better to use the latter approach to doubling acylsugar level. However, it would still require bringing FA2QTL and the ModChr2 introgression into coupling. We will strive to complete all the remaining work in objectives 1 to 4 in year 2, however due to the need to continue both approaches to doubling acylsugar level, as well as the 2 to 3 months delay due to Covid 19, completion of all the goals might require some year 3 work. This will become more clear late spring 2021.

The completed lines that have the optimal high acylsugar level and are free from the targeted negative traits, information collected during their development, and the new genetic markers created in this project, will facilitate use of acylsugar mediated insect/virus control trait by seed companies for the creation of commercial processing tomato hybrids that possess this critical trait. The potential benefit of commercial hybrids with acylsugar mediated WFT/TSWV control to the industry is best estimated by the value of the reduction of the direct costs of insect/virus control (reduced insecticide applications to prevent damage) added to the value of the reduction in loss/damage of crops due to insects/virus. The exact value of this total benefit would vary over years since insect / disease pressures vary year to year.

Acknowledgements: I would like to thank HeinzSeed for funding a 1:1 match of funding for this project; I would also like to thank Rich Ozminkowski and Lynn Veenstra of HeinzSeed for very useful periodic discussions of this project. I also thank Tom Turini, who has been running WFT/TSWV trials in CA, which include acylsugar tomato lines.

This project as leverage for other dollars: For the proposal submitted last year, Heinz indicated that it would provide an equal amount for support of this project for two years.

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Key Takeaway(s) at this date:

Progress has been made on sample preparation for Δ13C analysis (a measure of water use efficiency) of dried leaf samples from tomato introgression lines and their hybrids that were evaluated in the 2019 field season. More work needs to be done in the lab to finish sample preparation, followed by Δ13C analysis by the UC Davis Stable Isotope Facility (SIF) and statistical analyses. Since mid-March, the pandemic has caused work stoppages and major delays due to safety-based restrictions on campus research activities. SIF was closed down for months due to the pandemic, and is only recently is re-opening slowly.

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

Objective 1: Complete the Δ13C analysis on leaf samples collected from 2019 field experiments and complete statistical analyses of all traits to identify introgression lines (ILs) and their IL hybrids that have higher water use efficiency.

Expected Results: The identification of higher water use efficient ILs and their IL hybrids that can serve as a resource for breeding water use efficient cultivars.

***Progress* towards and *Preliminary Findings* on project objectives to date:**

Completed Activities:

The majority of dried leaf samples from the 2019 field experiment have been ground into a fine powder (1030 of 1050 samples) by graduate student Amy Groh and an undergraduate student assistant. Of these, 566 samples have been weighed and placed into tin capsules in trays, following the specifications of SIF. These 566 samples are ready for submission to SIF for Δ13C analysis. SIF has recently started accepting samples again after months of closure due to the COVID-19 pandemic. Initial statistical analyses of trait data including maturity, horticultural, and yield traits for the 2018 and 2019 field experiments are in progress by Amy.

Activities to be Completed:

Preparation of the remaining 484 leaf samples (of 1050) for SIF and submission of these samples to SIF for Δ13C analysis. Subsequent statistical analyses of all 2019 Δ13C data from SIF.

The 566 samples that were previously prepared will be submitted to SIF for Δ13C analysis in September. In order to safely restart work in our lab (an enclosed indoor space with limited airflow) while ensuring personnel safety, safe operating procedures will be designed and employed to minimize the possibility of Covid-19 transmission. Subsequently, in the lab Amy will prepare the 484 remaining leaf samples this fall, and submit these to SIF as soon as possible, depending on the SIF sample acceptance rate and queues. The timeline for receiving Δ13C data for our samples from SIF is unknown at this time. It depends on sample queues, the course of the

pandemic, and the ability of the SIF staff to work safely in the coming months. The SIF staff is doing the best they can under challenging circumstances.

Amy will complete statistical analyses and receive statistical advising once the 2019 Δ13C data for all 1050 leaf samples is available from SIF.

What's next for this project?:

Next steps for this project cannot be determined until we receive the 2019 Δ13C data from SIF and conduct statistical analyses. The expected results for this project include identifying the best performing introgression lines (IL) and IL hybrids for WUE (Δ13C), yield and horticultural traits for 2018 and 2019. All data will be made publicly available. The best performing ILs and their hybrids can be employed in breeding water stress-tolerant tomato varieties.

This project as leverage for other dollars:

In recent years the St.Clair tomato breeding research project had at least four 3-year grants (each approximately $500,000) from USDA-NIFA to work on the genetics of water stress tolerance in tomato. Currently there are no other grants supporting this work.

EVALUATION OF ALTERNATIVE NEMATICIDES FOR THE CONTROL OF ROOT-KNOT NEMATODES

JASPREET SIDHU

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Key Takeaway(s) of this project: None of the products seemed to have a long lasting effect in managing root knot nematodes in 2020 field trial. Further research is needed to evaluate these products as well as soil analysis to identify the plant parasitic nematode populations at the trial site.

Introduction: Root knot nematodes (RKN) are a major pest of processing tomatoes grown in California. Most commercially grown processing tomato varieties now incorporate the MI gene to provide a single gene resistance to RKN. However, each year incidences of moderate to severe RKN injury seem to occur more frequently. Some failure of the MI gene can be attributed as being due to resistance buildup to this single mode of resistance by RKN to this gene. Other instances of failure of processing tomatoes with the MI gene are still debatable but may be due to high soil temperatures. Other methods of RKN control is with the use of expensive pre-plant soil fumigants. This has become increasingly concerning due to regulatory and economic constraints. Alternative methods of nematode control need to be studied in field situations to quickly identify other possible control strategies. Since 2012 we have shown new non-fumigant nematicides products can significantly reduce nematode damage on processing tomatoes. Nimitz by Adama became available for use by tomato growers in 2016 in California partly due to the efforts of this CTRI funded project. A second nematicide by Bayer Crop Science, Velum, was released in 2017 with a limited list of crops on the label. A third nematicide product by DuPont, Salibro was announced in 2017 but currently is not registered for tomatoes.

Main goals: Conduct small plot field trials to evaluate the efficacy of Nimitz, Velum, Salibro, Calcium cyanamide, and possibly a developmental product

What's next for this project: Depending upon availability of new products, we would like to continue screening these already available nematicides for an additional year in order to generate more data and validation for these products.

Methodology and Results: This study was conducted as a small plot field trial on our root knot nematode infested site at the Shafter research farm, Shafter. A RKN susceptible tomato variety 'Halley' was hand transplanted onto 60- inch beds on July 1, 2020. There were four replications and six treatments in this trial arranged in a randomized block design. Rates, timings and method of application for each treatment is listed in Table 1. Each plot was 20 feet in length with a five feet buffer between plots. Treatments were applied either as a pre-plant or post-plant application as soil drench using four gallon watering cans. The plots were irrigated using a surface drip and maintained using standard agronomic practices. The trail was terminated on October 28, 2020. Tomato plants were lifted using the beet lifter and then pulled out from the soil. Roots were washed and rated for root galls on a scale of 0-10 (0 means no visible galls and 10 means extensive galling on the roots). Results are shown below in Table 2. Data on root galling was analyzed using SAS (statistical analysis software).

Table 1. Treatments, rate/ A, application schedule and timings

Trt	Application time	Rate /Acre	Date applied
Control			
Velum	At planting	6.5 Oz/ A	07/01/2020
Nimitz	At planting	5 pt/ A	07/01/2020
Salibro	At planting, 28 d after planting	30.7 fl oz/A	07/01/2020 July 28, 2020
Calcium cyanamide	At planting	200lbs/ A	07/01/2020
DP1	At planting	11.4 fl oz/ A	07/01/2020

In comparing these products side by side at harvest there was some reduction in nematode injury as compared to the non-treated control (Table 2). In the past, Nimitz and Velum have shown to be reliable and consistent performers but this year these products were relatively less effective. One of reasons could be exceptionally heavy population pressure in the nursery and extensive damage on the roots that made it hard to see any significant difference. However, the two new products Calcium cyanamide and a developmental product performed a little better than these products. Therefore, for 2021, we propose to repeat this trial to evaluate and validate more on these products in the field and may also be looking at any new nematicide products that come along. One additional aspect to the trial will be mid-season gall evaluation.

Table 2. Mean root galling on the roots in different treatment

Trt		Mean root galling
1	Non-treated control	8.68a
2	Velum	7.28ab
3	Nimitz	7.06ab
4	Salibro	7.04ab
5	Calcium cyanamide	6.05b
6	DP1	6.12b
		$F_{5,18}$=2.70 P=0.054
***means followed by same letter are not significantly different**		

Discussion:

Research on alternative control options that are highly efficacious, economically viable, and environmentally safe would be highly beneficial to the processing tomato industry. Nimitz by Adama became available for use by tomato growers in 2016 in California partly due to the efforts of this CTRI funded project. A second nematicide by Bayer Crop Science, Velum, was released in 2017 with a limited list of crops on the label. A third nematicide product by DuPont, Salibro was announced in 2017 but currently is not registered for tomatoes. In our 2020 trial, we compared four treatments with non-treated control and the standard Nimitz. None of the treatments were significantly different for root galling at harvest and the results indicate that none of the treatments had a long lasting effect on root knot nematode levels in the soil. Nimitz and Velum have been consistent performers in the past trials but were not very effective in the 2020 trial. One of the possible explanations could be exceptionally high pest pressure and some changes in the soil profile with high number of stubby root nematodes. However, Calcium Cyanamide and a developmental product showed some potential in the very first trial. Therefore, further evaluations are needed to better determine the efficacy of these products as sole treatments and in combination with other products and their potential and continued use by the processing tomato industry and sustainable management of root knot nematodes.

Pic 1. Root galling on roots across different treatments

Acknowledgements: We thank the California Tomato Research Institute for providing funding for this research and the private industry for providing us with products used in this study.

This project as leverage for other dollars: Funding from Industry collaborators ($5000)

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Key Takeaway(s) of this project:

- Under the conditions of the 2020 study, insecticides evaluated were not effective in reducing damage due to Consperse stink bug. In previous work, reductions in feeding damage were documented where Warrior II was applied and combinations of neonicotinoids and pyrethroids; however, even when differences in damage were significant, damage levels remained higher than ideal.
- Consistent with previous work, the 2020 results demonstrated increased coverage deep in the canopy with the Nutrien berm-blower air assist sprayer as compared to the conventional sprayer.

Introduction:

Consperse stink bug (*Euschistus conspersus)* can reach extremely damaging levels in some fields or sub-regions within extremely important processing tomato production areas in Fresno County. The fruit decay associated with stink bug feeding injuries is common in fields with high stink bug population densities. This fruit decay is categorized as "mold" by the state graders. Furthermore, the damage may result in complete degradation of the fruit which can cause a substantial decrease in yield.

Cullum and Zalom (2000) published a degree day model for Consperse stink bug development and had developed a system for scheduling treatments based on duration of life cycle stages. Insecticide applications targeting nymph stages of Consperse stink bug are more effective. When Consperse stink bug is initially detected in the field, the calculation of accumulated degree days begins. The assumption is that egg laying has begun at the time of detection. With the developmental threshold for Consperse stink bug set at 54°F, 150 degree days (dd) are required for egg hatch, 408 dd from 1st instar to 3rd and 386 from 4th to adult. The degree day accumulation for areas in which a CIMIS (California Irrigation Management Information System) station are located can be accessed at http://www.ipm.ucdavis.edu/calludt.cgi/DDMODEL?MODEL=CSB&CROP=tomatoes.

A CTRI-sponsored project was conducted from 2013 to 2015 in which seasonal population development and utility of trapping were documented and relative efficacy of insecticides were evaluated. The benefit of using pheromone baited traps was demonstrated. Insecticide treatments with highest levels of activity against this target included pyrethroids and neonicotinoids of insecticides currently registered. However, in consideration of damage in 2018, there was apparent need to revisit this issue and further develop an integrated management program.

Very early in 2019, substantial overwintering populations were detected under heavy leaf cover in a permanent crop in January, but a combination of factors including altered cropping practice and lower degree day accumulation contributed to generally lower Consperse stink bug population densities than in the previous season. In Dec 2019, *Chlorochroa uhleri* (identified by E. Hannon Fresno Co. Ag Commissioner Entomologist) was detected on Russian thistle in a weedy field near a processing tomato production area. While this species may inflict the same damage as Consperse, the processing tomato damage documented in Fresno County since 2013 has consistently and exclusively been associated with Consperse stink bug. Economic damage due to stink bug feeding was not observed in 2019 nor in 2020 in production areas where losses were reported in 2018.

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

- Refine baited trapping for early detection of Consperse stink bugs.
- Evaluate spray technology to increase insecticide coverage deep in canopy, increase efficacy and decrease stink bug damage.
- Compare insecticides that were not sufficiently evaluated under current production conditions.

Methodology and Results:

Trap lure evaluations although planned were not conducted in 2020. There are intentions to conduct this work in 2021.

For insecticide efficacy and for alternative spray technology components of this project, AB0311 transplants were mechanically planted on 22 May into a Panoche clay loam soil at the University of California Research and Extension Center (UC WSREC) in Fresno County. Sixty inch beds with drip irrigation tubing buried at 10 inches were used and all irrigations were through buried drip. No insecticides other than those included in the trial were applied. AlphaScent-baited traps were deployed in the field on 1 July and used to time the first application.

Chemical efficacy Materials compared included the following:

Material and rate
Assail 70WP 1.2 oz
Mustang Max 4 fl oz
Mustang Max 4 fl oz/a + Admire 2.2 fl oz+ Warrior II 1.92 fl oz
Warrior II 1.92 fl oz/A
Minecto Pro10.0 floz product/acre
Sefina 14 fl oz + Dyne-Amic 0.25%
Sefina 6 fl oz + Dyne-Amic 0.25%
Untreated Control

Applications were made with a backpack CO^2-powered sprayer at 30 psi and the volume was equivalent to 40 gallons per acre with 0.25% MSO unless another surfactant was included. The spray boom was equipped with three TeeJet DGTJ-60 8003VS at 19-inch spacing. All materials were applied on 3 September. The first Consperse stink bug detection in the pheromone baited trap in the field was 18 Aug, which was 440 dd >54°F before the first application based on data from the Five Points CIMIS Weather Station FIVE_PTS.A (CIMIS #2, Five Points/WSFS USDA)). Therefore, eggs laid on 18 Aug would be at early instar development by the time of the application.

Treatments were arranged in a four-replication randomized complete plot design. Plot size was a single 60-inch bed x 70 ft. Four row feet of canopy were shaken and lifted on the north side of the bed and the ground was examined for stink bug on 9 September. Stage of development of the stink bug was noted. In each plot, 13 ft of a single 60" bed was hand harvested and weighed on 26 and 27 Sep. Twenty to 25 lbs of fruit were hand sorted into red, green, sunburn, rot and stink bug feeding injury. Per acre yield and percentage of fruit in sorted categories was calculated. Analysis of Variance was performed on all data and Least Significant Difference (LSD) at P = 0.050 was calculated.

In the insecticide efficacy trial, there were high levels of damage. Some treatments had numerically lower levels of rot & feeding damage, but these treatments were not significantly different that the untreated control (Table 1). In addition, the insect counts were similar among treatments as well (Table 2).

Table 1. Yield and quality of processing tomatoes in Consperse stink bug insecticide efficacy comparison in Fresno County, 2020.

Insecticide trade name and rate per acre (active ingredient)[z]	yield (tons/ acre)[y]	hand sorted fruit categorization[x]				
		red (%)	green (%)	rot (%)	Stink bug feeding (%)	rot & feeding (%)
Mustang Max 4 fl oz	43.29	56.16	8.15	20.69	14.99	34.33
Warrior II 1.92 fl oz/A	35.64	48.84	6.28	31.00	13.88	35.69
Assail 70WP 1.2 oz	31.66	45.30	5.77	31.81	17.12	42.70
Sefina 6 fl oz + Dyne-Amic 0.25%	44.60	48.67	8.64	25.97	16.73	42.79
Minecto Pro10.0 floz product/acre	30.50	40.11	4.19	35.10	20.59	44.38
Mustang Max 4 fl oz/a + Admire 2.2 fl oz+ Warrior II 1.92 fl oz	38.45	50.11	7.65	26.37	15.87	44.89
Untreated Control	41.99	47.40	8.22	32.28	12.09	48.93
Sefina 14 fl oz + Dyne-Amic 0.25%	46.44	49.84	7.38	26.85	15.94	55.70
LSD $_{0.05}$	NS[w]	NS	3.69[v]	13.37	NS	18.86
CV (%)	28.44	27.48	35.68	31.78	41.77	28.79

[z] All materials were applied in the equivalent volume of 40 gallons per acre with a CO_2-pressurized sprayer on 3 Sep

[y] Thirteen row ft of a 60 in bed per plot was hand harvested and weighed on 22 and 23 Sep. Yield in tons per acre was calculated.

[x] Twenty to twenty-five lbs of fruit were hand sorted into categories; red, green, rot, stink bug feeding damaged, and both rot and feeding damaged, and percentages were calculated.

[w] Means appearing above 'NS' are not significantly different from one another at P=0.05.

[v] Means appearing above the Least Significant Difference are the statistically different at P=0.05 if their difference is greater than that number.

Table 2. Consperse stink bug densities and fruit chemistry in insecticide efficacy comparison in Fresno County, 2020.

Insecticide trade name and rate per acre (active ingredient)[z]	stink bug total[y]	PTAB laboratory[x]		
		color	solids	pH
Assail 70WP 1.2 oz	0.50	21.13	5.58	4.428
Mustang Max 4 fl oz	1.50	20.75	5.60	4.340
Mustang Max 4 fl oz/a + Admire 2.2 fl oz+ Warrior II 1.92 fl oz	1.25	20.75	5.63	4.410
Warrior II 1.92 fl oz/A	0.00	21.00	5.40	4.443
Minecto Pro10.0 floz product/acre	0.75	21.50	5.30	4.413
Sefina 14 fl oz + Dyne-Amic 0.25%	0.75	20.88	5.53	4.388
Sefina 6 fl oz + Dyne-Amic 0.25%	1.25	21.25	5.63	4.365
Untreated Control	1.25	20.63	5.60	4.410
LSD $_{0.05}$	NS[w]	NS	NS	NS

[z] All materials were applied in the equivalent volume of 40 gallons per acre with a CO_2-pressurized sprayer on 14 July.

[y] On 9 Sep, four row feet of vine was shaken and lifted. The ground was inspected and all live stink bugs were recorded.

[x] Forty red fruit per plot were submitted to the Processing Tomato Advisory Board Laboratory at the facility at the Los Gatos Cannery in Huron on 22 and 23 Sep.

[w] Means appearing above 'NS' are not significantly different from one another at P=0.05.

Alternative spray technologies: One alternative sprayer was compared to a commercial standard and an untreated control. Entries included were the Nutrien air-assisted sprayer, and a conventional spray rig (Teejet XR8003VK @ 19" spacing, 40 psi). The GenZ Technologies air assisted shielded sprayer included in the comparison in 2019 was not included in 2020 due to issues within the company and poor performance in the first season. Mustang Max 4

fl oz/a + Admire 2.2 fl oz+ Warrior II 1.92 fl oz was applied in the equivalent volume of 40 gallons per acre with 0.25% MSO on 10 September. The first Consperse stink bug detection in the pheromone baited trap in the field was 18 Aug, which was 23 days (601.6 dd >54°F based on data from the Five Points CIMIS Weather Station FIVE_PTS.A (CIMIS #2, Five Points/WSFS USDA)) prior to the application. Therefore, eggs laid on 18 Aug would be at late instar development by the time of the application.

Plots were arranged in a four-replication randomized complete block with 120 x 20 ft plots. Water-sensitive cards were deployed with water sensitive portion of the card facing up at ground level, and 6 and 12 inches above ground level. At 6 and 12 inches, the cards are doubled with a water sensitive portion of the cards exposed on both sides, which would approximate upper and lower leaf surfaces. In each plot, 13 ft of a single 60" bed was hand harvested and weighed on 23 and 24 Sep. Twenty to 25 lbs of fruit were hand sorted into red, green, sunburn, rot and stink bug feeding injury. Per acre yield and percentage of fruit in sorted categories was calculated. Data was subjected to Analysis of Variance and Least Significant Difference (LSD) at P = 0.050 is presented.

In the alternative spray technologies comparison, coverage differed among treatments (Figure 1). Coverage of the cards at the ground position and lower surface at the 12 inch elevation were significantly higher in the Nutrien berm-blower treated plots than in the plots treated with the conventional sprayer (Table 3). However, neither insect counts nor rot and stink bug feeding damage separated statistically (Tables 4 and 5).

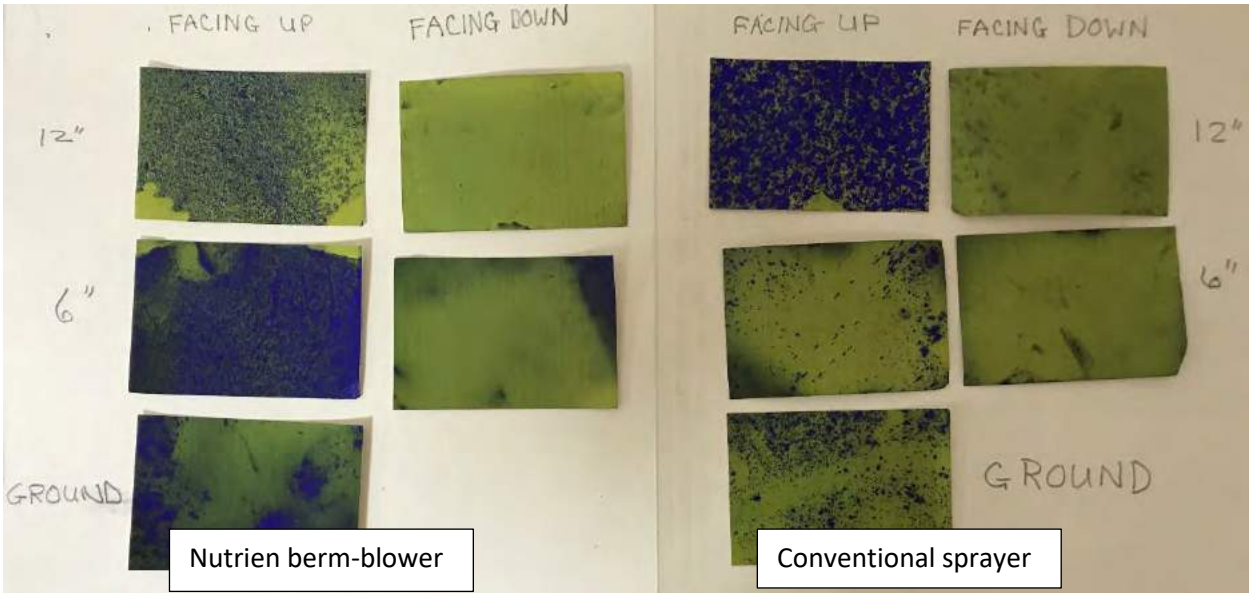

Figure 1. Photo of cards from replication 1 showing spray coverage of water sensitive cards secured in processing tomato canopy provided by sprayers in comparison, Fresno County, 2020.

Table 3. Spray coverage of water sensitive cards secured in processing tomato canopy provided by sprayers Fresno County, 2020.

Sprayer[z]	coverage (%) relative to position of water-sensitive cards[y]

	Ground, facing up	6", facing up	6", facing down	12", facing up	12", facing down
Nutrien berm-blower	33.00	49.17	8.00	77.83	4.67
Conventional	29.67	38.33	7.67	82.00	5.17
Probability[x]	0.05	0.07	0.18	0.32	0.05

[z] A air assist, Nutrien berm-blower, sprayer and a conventional sprayer were used to apply 40 gal/a on 10 Sep.

[y] Water sensitive cards were positioned face up at ground level, at 6- and 12-in above ground facing up; and at 6- and 12-in above ground facing down. Percentages of the cards covered were estimated and recorded.

[x] A probability of 0.05 or indicates a significant difference according to a paired t-test.

Table 4. Yield and quality of processing tomatoes in sprayer comparison for Consperse stink bug control in Fresno County, 2020.

		hand sorted fruit categorization[x]				
Sprayer[z]	**yield (tons/ acre)**[y]	**red (%)**	**green (%)**	**rot (%)**	**Stink bug feeding (%)**	**rot & feeding (%)**
Nutrien berm-blower	40.79	51.52	6.08	14.19	35.20	49.39
Conventional	31.65	43.09	10.42	9.74	43.82	53.56
Untreated	41.24	45.19	8.77	12.05	41.03	53.08
LSD $_{0.05}$	NS[w]	NS	NS	NS	NS	NS

[z] A air assist, Nutrien berm-blower, sprayer and a conventional sprayer were used to apply 40 gal/a on 10 Sep.

No insecticides were applied in the untreated treatment.

[y] Thirteen row ft of a 60 in bed was hand harvested and weighed on 24 Sep. Yield in tons per acre was calculated.

[x] Twenty to twenty-five lbs of fruit were hand sorted into categories; red, green, rot, stink bug feeding damaged, and percentages were calculated.

[w] Means appearing above 'NS' are not significantly different from one another at P=0.05.

Table 5. Consperse stink bug densities and fruit chemistry in sprayer comparison for Consperse stink bug control in Fresno County, 2020.

	stink bug total[y]	PTAB laboratory[x]		
Sprayer[z]		**color**	**solids**	**pH**
Nutrien berm-blower	3.00	20.17	5.67	4.42
Conventional	2.17	20.50	5.87	4.45
Untreated	2.33	20.50	5.77	4.46
LSD $_{0.05}$	NS[w]	NS	NS	NS

[z] A air assist, Nutrien berm-blower, sprayer and a conventional sprayer were used to apply 40 gal/a on 10 Sep.

No insecticides were applied in the untreated treatment.

[y] On 14 Sep, four row feet of vine was shaken and lifted. The ground was inspected and all live stink bugs were recorded.

[x] Forty red fruit were submitted to the Processing Tomato Advisory Board Laboratory at the facility at the Los Gatos Cannery in Huron on 24 Sep.

[w] Means appearing above 'NS' are not significantly different from one another at P=0.05.

Discussion:

Research was conducted in Fresno County in 2019-20 to address issues of crop loss and high mold attributable to Consperse stink bug feeding in 2018 Fresno County processing tomatoes. Projects conducted to add detail to and refine an integrated management program was needed because the current standard was not commercially acceptable in some situations. Trap optimization components of this study were not conducted in 2020 but evaluated in 2019 when two lure types were tested in a commercial site and the AlphaScent lure was consistently more effective. Chemical efficacy was evaluated in replicated studies during both seasons. In 2019, no treatment delivered superior control to pyrethroids or pyrethroids/neonicotinoid mixtures and there was substantial feeding damage in those treatments as well. In 2020, there were no significant benefits in applications of materials that had provided significant reductions in damage in the past. Alternative spray technology: Two alternative sprayers were compared to a commercial standard in 2019 and one was compared to the standard in 2020. Over both seasons, water-sensitive cards deployed at ground level, and two elevations within the canopy showed that the Nutrien berm-blower sprayer was superior to the commercial standard. It is likely that there is not one technique that alone will deliver consistent, commercially acceptable levels of control. Based on numerous observations and a known life cycle of this pest, identification of sources of Consperse insect is likely to be a critical component of management.

Since the initiation of this study in 2019 and in earlier work in the region, there has been specific documentation of overwintering sites and areas where the insect was reproducing. High Consperse stink bug densities have been noted under leaf cover of some permanent crops, which poses a risk to nearby tomatoes. Additionally, high densities have been documented in grain crops and on certain weeds including wild radish and black mustard early in the season. Sanitation and planning, where possible, can help in avoidance of economic damage when our other tools are less efficacious and less consistent than ideal.

Acknowledgements:

- Kevin Ruble and Rick Blankenship (Woolf Farms)
- Gene Hannon (Entomologist at Fresno County Agricultural Commissioner's office)
- Charles Jepson (CEO Gen Z Technology)
- Ron Avila and Jorge Saldona (Nutrien Ag Solutions)
- Merf Solorio, Eli Pimentel, Jaime Solorio, Bert Garza, Nelson Vallejo and Tracy Waltrip (UC West Side Research and Extension Center
- Herman Daniel Delgado (UC Coop. Ext. Fresno County)

This project as leverage for other dollars:

- I received an unrestricted gift of $2000 from BASF Agrochemical.
- There was support from Nutrien Ag Solutions in providing equipment and personnel for equipment adjustment and insecticide application in the sprayer comparison study in both 2019 and 2020.

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THE RESISTANCE BREAKING STRAIN OF TSWV IN CA PROCESSING TOMATOES: MONITORING, IMPROVED DETECTION AND SCREENING FOR RESISTANCE

ROBERT GILBERTSON

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Key Takeaway(s):

- Rapid and specific LAMP (loop-mediated amplification) tests have been developed for RB TSWV and *Tomato brown rugose fruit virus* (ToBRFV) that provide results in <2 hours and do not require a high-tech laboratory
- RB TSWV is prevalent in Fresno and Merced Counties, but still largely absent from the Northern Counties
- No evidence of ToBRFV infection in processing tomatoes in open fields in California in 2020

Introduction:

Spotted wilt disease of tomato caused by *Tomato spotted wilt virus* (TSWV) has emerged as a serious constraint on processing tomato production in California, with the potential to cause substantial economic losses. This was seen in the mid-2000s when outbreaks of TSWV, associated with high populations of thrips, caused millions of dollars of losses to the processing tomato production. Through the support of the CTRI, we learned about aspects of the biology of TSWV and the Western flower thrips vector (*Frankliniella occidentalis*) in Central California and utilized this information to develop an integrated pest management (IPM) program for spotted wilt in California. This program has helped growers and PCAs manage the disease and minimize economic losses. A critical component of this IPM program has been the development and widespread planting of processing tomato cultivars with the Sw-5 gene, a single dominant resistant gene that confers a high level of resistance to TSWV, i.e., inoculated plants do not develop symptoms of TSWV (although they may be infected with the virus).

The situation with TSWV in the Central Valley of California changed dramatically in 2016, when fresh market tomato varieties with the *Sw-5* gene developed spotted wilt-like symptoms in Fresno County, e.g., in Cantua Creek and Firebaugh. Using diagnostic tests, we confirmed that these plants were infected with TSWV, and we further established that this was a resistance-breaking (RB) strain of TSWV (RB-TSWV) based on results of molecular and plant inoculation tests. RB strains of TSWV have been previously reported in countries of Europe, e.g., Italy and Spain, so this was not a new or unexpected phenomenon. In 2017 and 2018, surveys of processing tomato fields in California for RB-TSWV revealed widespread infection of Sw-5 varieties with RB-TSWV in Fresno and Merced Counties. We also established that this RB-TSWV could infect and cause spotted wilt symptoms in major Sw-5 processing tomato varieties grown in California. Given the importance of resistant varieties in the IPM package for spotted wilt management in California (and other parts of the world), the emergence and establishment of the RB-TSWV strain in the major processing tomato counties of California poses a significant threat to the processing tomato industry.

Main goal: The main goal of this project is understand the potential threat posed to the processing tomato industry by the newly emerged RB-TSWV, and to develop strategies to minimize the impact of this virus. We also want to continue to provide rapid and precise diagnostics and information on other tomato-infecting viruses that are a concern to the industry, e.g., the new emergent tobamovirus, *Tomato brown rugose fruit virus* (ToBRFV), and pospiviroids.

Objectives:

- Limited monitoring the overwintering and appearance of RB-TSWV in Central California the 2020.
- Develop, validate and transfer rapid diagnostic tests for RB-TSWV.
- Evaluate the response of a range of tomato germplasm to RB-TSWV with sap inoculation in the greenhouse and in a field trial to identify potential tomato entries/lines and cultivars with resistance.
- Outreach for extending information on RB-TSWV and the IPM strategy for management

Methodology and Results:

- ***Limited monitoring of the overwintering and appearance of RB-TSWV in Central California for the 2020 growing season.***

Winter weed survey: In 2020, we conducted surveys for weeds that could allow RB TSWV to overwinter in the absence of infected tomato plants. In surveys from areas of Fresno with known outbreaks of RB TSWV, e.g., Cantua Creek, we did not detect RB-TSWV or WT-TSWV) infections in any of 47 winter weed plants examined based on symptoms or detection with TSWV immunostrips. The winter weeds tested were mostly groundsel, prickly lettuce, goosefoot and sow thistle and some showed symptoms of necrosis. Thus, we found **no evidence of winter weeds infected with TSWV in 2020**. This is also in agreement with results of surveys conducted in 2017-2019 in which little or no TSWV infection was detected. Thus, although winter weeds can't be completely ruled out as early season inoculum sources for RB TSWV, they do not appear to be an important source in Central California.

Surveys and testing of virus samples: Processing tomatoes in California are affected by >10 viruses. Furthermore, in a given year the incidence and important of viruses varies and symptoms of virus infection are not always sufficient for diagnosis. Thus, even though this project was mainly on RB-TSWV, we received or collected on our 27 July survey nearly 300 processing tomato samples for virus testing in 2020. This included samples received for testing from Fresno, Kern, Kings, Merced, San Joaquin, Stanislaus, Yolo and Colusa Counties and there were multiple viruses detected in 2020. Table 1 shows show the number of samples received from each county and the tests that were performed, based on a preliminary assessment of the symptoms.

Table 1. Total tomato samples tested for the detection of different pathogens (based mostly on initial diagnoses based on symptoms

County	Total Samples	Number of samples tested for each pathogen:						
		TSWV	BCTV	ToNSV	Tobamovirus	CMV	Cmm	Other*
Fresno	139	83	48	0	2	3	9	0
Kern/Kings	13	7	13	0	0	0	0	0
Merced	27	27	0	0	0	0	0	0
Stanislaus	4	0	4	0	0	0	0	0
San Joaquin	49	16	19	0	16	5	0	11
Yolo/Colusa	64	54	0	6	0	0	0	13
Total	296	187	84	6	18	8	9	24

*This category includes other factors such as rusted mites and abiotic diseases.

Note: The sum of the number of samples in each category does not necessarily coincide with the total of samples per county, because a sample may have been tested for more than one pathogen.

In 2020, 5 different viruses were detected infecting processing tomatoes in California. **Tomato necrotic spot disease caused by ToNSV appeared in fields in Fresno, San Joaquin and Yolo early in the season (May-June)** and can be confused with TSWV. The presence of this virus was confirmed by sequencing in samples from San Joaquin and Yolo Counties. We have previously established that ToNSV does not cause economic losses to processing tomatoes in California, and this was the case in 2020. Interestingly, this virus has recently been identified associated with necrotic spot disease of tomatoes in Ohio.

In Fresno, **symptoms of curly top also appeared early** in the season in some fields, and reached incidences as high as 10-20%. These samples were confirmed to be infected with BCTV with both the PCR test and BCTV LAMP test, including in the small ripe fruit that are characteristic of the disease. Outbreaks of curly top occurred later in the season in Stanislaus and San Joaquin Counties and BCTV infection was confirmed with PCR and LAMP tests.

Spotted wilt appeared in late April-early May in multiple fields in Fresno County, and somewhat slowly increased in incidence, such that in our 27 July survey the incidence in survey fields were still relatively low (5-10%). **Much greatly spread occurred later in the season and impacted fruit quality in some fields. In 2020, RB-TSWV was the prevalent strain in spotted wilt samples from Fresno and Merced, and it was detected for the first time in Monterey and San Joaquin Counties.** In the **Northern Counties** (Yolo, Colusa, Sutter), spotted wilt appeared in mid-to late May in some fields, but did not reach high incidences or impact yield**. Importantly, RB-TSWV was not detected in samples from these counties** and, consistent with these results, the Sw-5 gene continues to provide resistance in these locations. What was unexpected was the failure of the WT-TSWV RT-PCR test to detect these strains in the Northern Counties, whereas the LAMP test for WT TSWV did detect these strains. Thus, the LAMP test may be a more robust test than RT-PCR.

Finally, during 2020, we made an effort to look for symptoms of tobamovirus infection due to concerns about ToBRFV. No tobamovirus symptoms were observed in processing tomato fields, though a sample of tobamovirus-like symptoms in leaves of tomato plants in an experimental trial at UC Davis. These symptoms were confirmed to be caused by a tobamovirus with AgDia immunostrips, but RT-PCR and sequencing showed that the virus was *Tomato mosaic virus* (ToMV). A processing tomato fruit sample also was received, which had tested positive for tobamovirus infection with the immunostrip test, and was shown to also be infected with ToMV. was received and confirmed to be infected with a tobamovirus.

Table 2. Results of tests conducted for the resistance-breaking (RB) and wild-type (WT) strains of *Tomato spotted wild virus* (TSWV) and the mild (in sugar beet) and severe strains of *Beet curly top virus* (BCTV)[a]

County	Total Samples[2]	RB-TSWV		WT-TSWV		BCTV (PCR)			
		RT-PCR (+)	RT-PCR (-)	RT-PCR (+)	RT-PCR (-)	Severe	Mild	Mix	Negative
Fresno	131	64/83[b]	18/83	0/83	83/83	13/48	19/48	9/48	7/48
Kern/Kings	20	0/7	7/7	0/7	7/7	11/13	0/13	0/13	2/13
Merced	27	17/27	10/27	0/27	27/27	0/0	0/0	0/0	0/0
Stanislaus	4	0/0	0/0	0/0	0/0	2/4	1/4	0/4	01/4
San Joaquin	35	13/16	3/16	0/16	16/16	2/19	14/19	0/19	3/19
Yolo/Colusa	78	0/30	30/30	0/30	30/30[a]	13/48	19/48	9/48	7/48
Total	295	94/163	68/163	0/163	163/163	41/132	53/132	18/132	20/132

[a]Samples tested for TSWV and BCTV by RT-PCR and PCR, respectively.

[b] Number of positive and/or negative samples/number of tested samples.

[c] Samples with spotted wilt symptoms from Yolo county that were positive with the TSWV immunostrip but negative for RB- and WT-TSWV by RT-PCR. These samples did test positive for WT-TSWV in the LAMP test (see Table 2)

Objective 2. Develop, validate and transfer rapid diagnostic tests for RB-TSWV:

Through the support of the CTRI, we have developed two rapid diagnostic tests for RB-TSWV: the RT-PCR and the LAMP tests (Figure 1). However, both of these tests were based on the use of purified RNA as a template and this is costly and requires a fully equipped laboratory. Therefore, we evaluated numerous buffers to look for an extraction method that would provide an RNA template for the RT-LAMP test that was rapid and simple and did not require a sophisticated laboratory. Therefore, in 2020, we evaluated 7 types of buffers, many of which had to reported to be able to provide a RNA template suitable for PCR or LAMP tests. Most of these did not provide a suitable template. However, the hard work of Dr. Tomas Melgarejo led to the identification of the glycine buffer method (Panno et al., 2019), which provided a template suitable for the RT-LAMP test for RB-TSWV, but also for detection of BCTV in the BCTV-LAMP test.

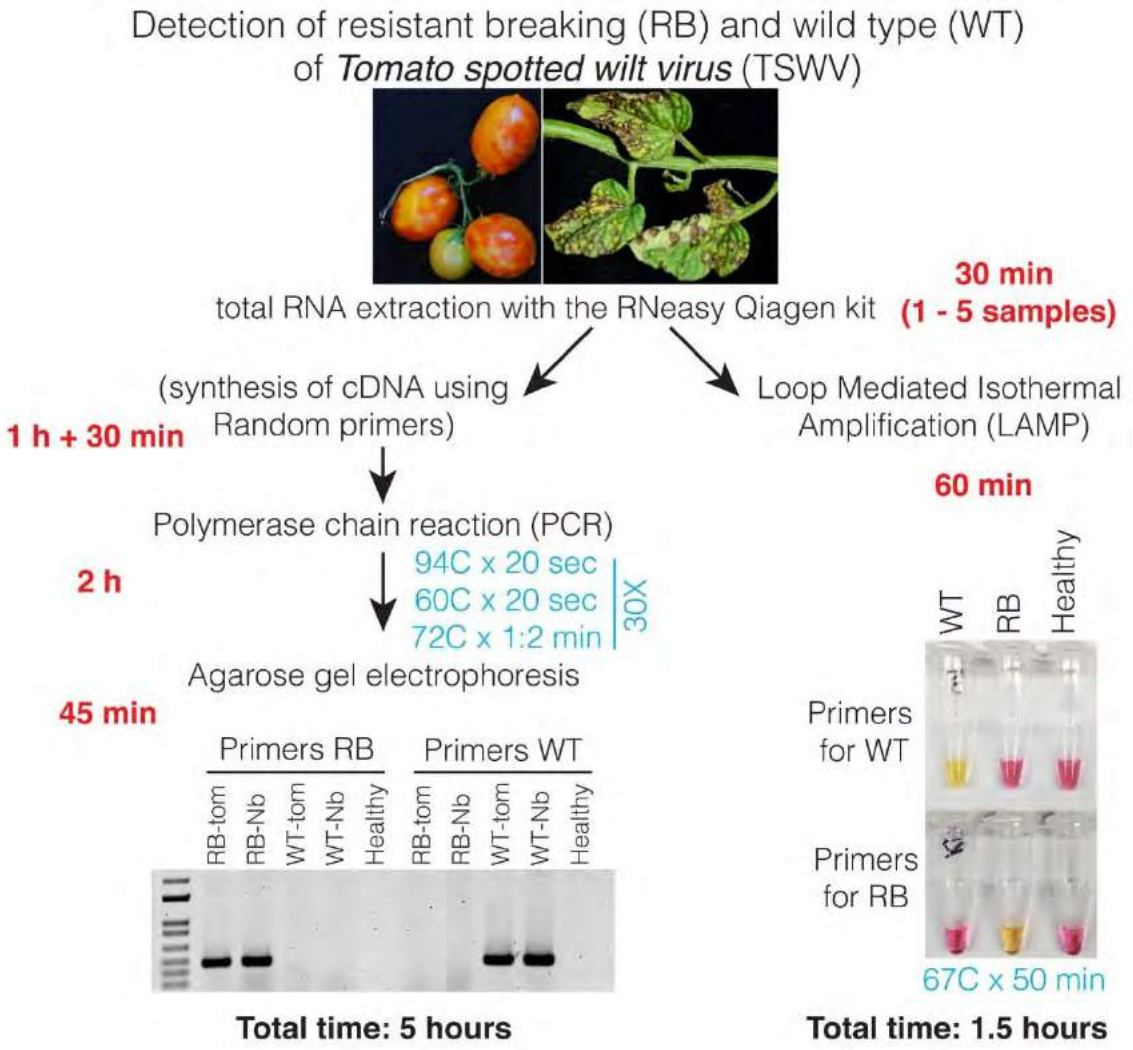

Figure 1. Side-by-side comparison of the RT-PCR (on the left) and RT-LAMP (on the right) tests for the rapid and specific detection of resistance-breaking (RB)- and wild-type (WT)-TSWV strains

We next performed validation tests. Results of the validation tests performed with the RT-TSWV and BCTV LAMP tests are shown in Table 3. For RB-TSWV 16 tomato samples from Fresno with TSWV-like symptoms were tested by RT-PCR and 11 were positive for RB-TSWV infection, where 15 were positive with the RT-LAMP. This suggests the RT-LAMP maybe a most sensitive method for RB-TSWV than is RT-PCR. For WT-TSWV, we noted that the RT-PCR test, which use primers developed from the sequences of isolates from Fresno, was not detecting non-RB-TSWV strains from Yolo County, whereas these strains were detected by the WT-TSWV RT-LAMP, which involved the generation of different primers. Thus, the WT-TSWV RT-LAMP is able to detect a wider diversity of WT-TSWV strains. Finally, the glycine buffer template preparation method was evaluated in the BCTV LAMP was slightly better than the PCR detection method. **Thus, our validation tests conducted thus for show that the rapid template method and the RT-LAMP and LAMP performed as well or better than the RT-PCR.**

Table 3. Validation of the LAMP assay (template extracted by glycine buffer) comparing to the RT-PCR (template extracted by Qiagen kit)

Buffer	Number of tomato samples tested for TSWV and BCTV											
	RT-PCR for RB-TSWV		LAMP for RB-TSWV		RT-PCR for WT-TSWV		LAMP for WT-TSWV*		PCR for BCTV		LAMP for BCTV	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Glycine buffer	11	5	15	1	0	12	11	1	18	5	19	4

*WT-TSWV was detected only by LAMP assay on tomato samples collected in Yolo county.

Note: The following buffers were assayed:

1. 0.5 N NaOH (sodium hydroxide) + 1 mM EDTA
2. GEB2 buffer from Agdia
3. PBST buffer (10 mM potassium phosphate; 150 mM sodium chloride, 20 g/L polyvinil-pirrolidone and 5 ml/L tween-20)
4. 0.1 M Tris-HCL buffer, pH 8.0
5. Glycine buffer (1 mM EDTA, 0.05 M Sodium chloride and 0.1 M glycine)
6. 1% SDS (sodium dodecyl sulfate)
7. Sucrose buffer (50 mM Tris-HCL pH 7.5, 300 mM NaCl and 300 mM Sucrose

Objective 3. Evaluate the response of a range of potentially resistant tomato germplasm to the RB-TSWV with mechanical inoculation and perform a field trial to test the potential of these varieties to prevent RB-TSWV infection: Because the RB-TSWV strain overcomes the *Sw-5* gene, we initiated efforts to search for sources of resistance. In 2018, we conducted two types of tests: 1) mechanical (sap) inoculation tests in the greenhouse and 2) a field plot at the Westside Field Station.

Mechanical transmission: We have a very robust inoculation protocol for screening tomato materials for resistance to RB TSWV that allows for effective screening of materials for resistance. Unfortunately, all tomato cultivars or lines tested to date have been susceptible, including materials reported to be resistant to RB TSWV strains in Europe.

Therefore, in 2020, we tested two materials from ISI (reportedly resistant to RB TSWV in Europe), and also performed an inoculation test to confirm known Sw-5 resistance in a commercial variety. Plants of the ISI 25033 and ISI 18205 varieties developed symptoms slower than the susceptible control, but eventually developed a necrotic tip dieback that turned into a systemic infection in 91% (22/24) and 89% (24/27) of inoculated plants, respectively, with a small proportion of plants showing milder or no symptoms. Furthermore, similar observations were made regarding these varieties in the field plot, especially for 25033, but both varieties had some plants with systemic infection. Thus, neither variety showed high levels of resistance to RB TSWV.

Although wild tomato species have been the source of resistance to TSWV, e.g., the Sw-5 gene from *S. peruvianum*, identifying and introgressing such resistance is a long, time-consuming and expensive process. However, we continue to actively work with seed companies to facilitate identification of resistance to RB TSWV (see leveraged funds). An encouraging note is that there has been some promising evidence of resistance to the California RB TSWV in tomato germplasm.

Field plot at the Westside Field Station to evaluate breeding line and variety response to *Tomato spotted wilt virus* in 2020. This plot was established to assess whether acyl sugar lines, the ISI varieties 25003 and 18205, and control lines with and without the Sw-5 gene. All entries were seeded and grown for 6 weeks at West Side Transplants Huron, California commercial transplant facility. Transplants were mechanically planted on 22 May onto 60-inch beds in a Panoche clay loam soil at the University of California West Side Research and Extension

Center. All irrigations were through trickle irrigation tape injected at a depth of 10 inches. Commercial practices in terms of nutrition, and pest management were followed except for exclusion of insecticides from the pest management program. Expression of virus-like symptoms were rated through the season and representative samples were tested for Tomato spotted wilt virus with AgDia immunostrips and by RT-PCR in the Gilbertson Laboratory. Samples that had BCTV-like symptoms were tested by LAMP by Tom Turini and by PCR in the Gilbertson Laboratory. Plants were evaluated for virus symptoms on 12 and 25 Jun, 17 and 24 Jul, and 18 Aug. Percentages of affected plants were calculated based on plant counts taken on 12 Jun. Analysis of variance was performed on square root transformed data and Least Significant Difference was calculated. Back transformed means are presented.

As noted in Objective 1, curly top was the first virus to appear in the field plot. Thus, we initially evaluated the plot for curly top (following the confirmation of BCTV infections in plants with these symptoms. As shown in Table 4, although disease incidence ranged from ~8-35%, there were no significant differences at the last reading due to high levels of variation. The moderately resistant line 20 performed among the better materials for BCTV, but did not stand out. It did not appear that the acyl sugar lines had any major impact on curly top disease, and these lines were among the most susceptible to BCTV.

Table 4. Response of breeding lines and varieties to infection by *Beet curly top virus*, in the field plot in Fresno County 2020

	12 Jun	25 Jun	17 Jul	24 Jul	18 Aug
N6366	1.03	3.95	8.62	9.22	8.14
ISI-25003	1.69	6.60	8.82	8.82	8.82
ISI -18205	5.49	7.81	8.40	8.90	8.90
191065-010	0.98	3.17	8.67	9.85	9.85
Line 20 (12)	1.06	2.74	10.09	10.51	10.51
191080-001	0.36	0.96	4.66	10.99	10.99
191172-001	0.00	2.45	12.05	12.63	12.63
AB0311	1.87	5.19	9.90	12.69	12.69
191069-003	0.00	2.64	13.77	13.77	13.77
141278-012	0.15	6.20	17.47	18.98	18.98
131192-039	0.00	4.36	19.40	20.83	20.83
181213-001	1.36	7.51	24.37	32.31	32.81
171002-2	1.50	6.60	32.01	35.57	35.57
LSD 0.05	NS	NS	3.69	13.37	NS
CV (%)	28.44	27.48	35.68	31.78	41.77

In terms of RB-TSWV (confirmed as the prevalent stain in the area), the virus did not appear until later in the season and then started increasing in mid-July. Thus, the disease incidences ranged from 4-15%, which represents

moderate disease pressure (Table 5). In this trial, none of the lines or varieties were free of infection or symptoms, indicating the absence of strong resistance such as that conferred by the Sw-5 gene to WT-TSWV strains. The acyl sugar lines showed a wide range of responses, having 4 of the 5 lines/varieties with lowest rates of disease and two with the highest rating. This points to a beneficial effort of acyl sugars, but that the effect does not always prevent infection and may be genetically complex. The ISI 25003 variety had the third lowest incidence of spotted wilt (~6%), which is consistent with this variety having delayed disease development, but ultimately getting infected and not showing strong levels of resistance. Interestingly, the commercial Sw-5 and non-sw-5 varieties had almost the same disease incidence of ~13.5%.

Table 5. Response of breeding lines and varieties to the resistance-breaking strain of *Tomato spotted wilt virus* in the field plot in Fresno County, 2020

	12 Jun	25 Jun	17 Jul	24 Jul
191069-003	0.00	0.15	2.66	4.13
181213-001	0.00	0.59	0.87	4.15
ISI-25003	0.00	1.29	3.94	6.02
141278-012	0.30	0.30	1.28	6.79
131192-039	0.00	0.15	2.88	8.06
ISI -18205	0.00	1.04	0.40	8.31
171002-2	0.00	0.48	2.60	9.04
191172-001	0.00	1.08	5.70	11.36
Line 20 (12)	0.52	1.59	4.59	11.57
AB0311	0.00	2.02	8.42	13.62
N6366	0.13	0.29	8.13	13.76
191065-010	0.00	3.84	7.74	14.96
191080-001	0.00	4.24	13.83	15.38
LSD 0.05	0.64	3.00	3.62	0.91
CV (%)	441.94	118.74	65.12	21.75

Objective 4. Outreach for extending information on RB-TSWV and the IPM strategy for management: A main effort continues to be running the thrips degree day (DD) model through the ANR website. Unfortunately, there does not appear to be any new effective insecticides for thrips control on the horizon and, given the establishment and spread of RB-TSWV, we believe that targeting the early (2^{nd}-3^{rd}) generations of thrips that it will slow appearance of viruliferous adult thrips, which can only develop on TSWV-infected plants. Growers and PCAs can access the website: http://ucanr.edu/sites/TSWVfieldriskindex/Thrips_Population_Projections/ and identify when the appearance of these generations is predicated and implement management accordingly. For example, for Fresno in 2020, the period for the 2^{nd}-3rd generation was between 1 April to 1 May, whereas in Yolo County

the period was between 1 May and mid to late June. Thus, the predicated time for spraying these generations varies depending on geography and climate (temperature).

In terms of other activities, COVID-19 negatively impacted some of our typical outreach activities, such as grower and professional meetings. However, we have been able to be active in getting information out on both RB-TSWV and ToBRFV. Here are some examples:

- Participated in virtual grower meeting held by Tom Turini
- Provided information to Farm Advisors network, including a virus update
- Published a scientific journal article summarizing 10 years of CTRI-funded research on IPM for thrips and TSWV
- Gave invited talk on 'Epidemiology of ToBRFV' to the USDA PROCINORTE virual meeting 9/20
- Gave invited virtual talk on 'Tomato tospoviruses in the United States'

Discussion

The California RB TSWV strain is now the predominant strain in Fresno and Merced Counties and is continuing to spread, with detections in Monterey and San Joaquin Counties. It appears that RB-TSWV has not reached the Northern Counties, where WT-TSWV strains are prevalent and the *Sw-5* gene is still effective. Thus, it is important to continue to map the spread of RB-TSWV, and the development of the rapid RT-LAMP test should allow for better and more precise mapping of the spread of RB-TSWV.

The development of the RT-LAMP for RB-TSWV provided the experience and knowledge to rapidly develop RT-PCR and RT-LAMP tests for ToBRFV that allow for rapid and specific detection of this invasive virus. In 2020, we received tobamovirus leaf and fruit samples that were positive with the TMV immunostrips (which only indicates a tobamovirus infection) and we were able to use these new tests for ToBRFV to quickly rule this virus out. In these cases, the viruses were subsequently identified as ToMV. We also continue to be active in working with the seed industry to harmonize the seed testing and management efforts to minimize the impact of the virus. I will note again that ToBRFV was not found in California processing tomatoes in 2020, and the evidence continues to suggest that ToBRFV is most a problem in protected culture where plants are touched on a frequent basis.

The identification of the glycine buffer method to prepare DNA and RNA templates for LAMP/RT-LAMP tests could make this method much more user friendly. This method involves grinding tissue in the buffer, heating at 90-95 C for 10 minutes and using this extract in the LAMP/RT-LAMP. For the 2020 growing season, the LAMP assay was successfully used by Tom Turini to detect BCTV, so we are optimistic that we can also transfer the RT-LAMP tests for RB-TSWV and ToBRFV for the 2021 growing season. We also will be working with TriCal diagnostics to see about transferring this testing capacity to a commercial testing company.

While we have been able to develop effective tests to detect RB-TSWV, the virus is proving difficult to manage with the Sw-5 no longer effective. Furthermore, many of the varieties are highly susceptible following the loss of Sw-5. A major challenge continues to be the identification of effective resistance. Although not substitute for the Sw-5 gene has yet to be identified, there are some reports of promising resistance in some tomato germplasm. Our mechanical inoculation screening method is very effective and we have been able to leverage some funding to assist in screening or provide expertise in the method. As mentioned, having to go back to wild relatives to search for new sources of resistance is time-consuming and expensive, so it is important to continue screening a wide range of materials to identify resistance.

We understand that growers are frustrated with the loss of Sw-5 resistance, which was extremely robust. However, there remains many components of the IPM program that can still be used. More emphasis on sanitation during the winter and effective management/removal of bridge crops. Early in the season, target the 2nd-3rd generations of thrips based on DD model predictions in order to slow the spread of disease. Though often written off as impractical, the removal of TSWV-infected plants early in the season has been shown to slow the spread of tospoviruses. Aggressive thrips management should be considered if virus incidences are high (e.g., 5-

10%). Following harvest, the santitation effort would resume. By combining these aspects of the IPM program it should be possible to obtain reasonable management of RB TSWV, while efforts to develop an effective resistant varieties continue.

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 spotted wilt-like symptoms for RB-testing. We thank Paul Scaroni (White Seed Co.) for providing seeds of ISI varieties.

Leveraged funds: We have been able to leverage some funding from seed companies to help screen tomato germplasm for resistance to RB TSWV. These were single year projects with potential for renewal. In 2020, we received $12,500 for these activities.

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Key Takeaway(s) of this project:

Efficacy of seven insecticides each in separate conventional and certified organic experiments were evaluated for efficacy against beet leafhopper (*Circulifer tenellus*) at the University of California West Side Research and Extension Center in Fresno County.

- In the conventional insecticide trial, insect counts were lowest in the Baythroid and Warrior II treatments, which were not significantly different than the Sevin and Malathion treatments.
- There were no significant differences among the treatments in the certified organic trial.
- Progress has been made and additional similar trials, along with these results, will provide a basis for decisions regarding beet leafhopper control in the future.

Introduction: The beet leafhopper (*Circulifer tenellus*) is the vector of *Beet curly top virus*, which has been responsible for tremendous crop losses in tomatoes. Symptom expression on tomatoes includes cupped dull green leaves with swollen purple veins and small distorted prematurely red fruit. Plants infected at early stages of development will not produce fruit. Low incidence of disease can be tolerated, but during seasons with high beet leafhopper population densities and high virus titers, nearly all plants in a field may be infected.

In winter and spring seasons, this leafhopper survives primarily on natural vegetation in the foothills: In spring as the vegetation dries, it migrates into crops on the valley floor. There are multiple generation in spring and summer that migrate within the production area. Tomato is not a suitable host for the beet leafhopper, and it does not colonize the plants as it does beets or many weed species. However, it will feed on tomatoes and transmit *Beet curly top virus*. The virus is semi-persistent in the insect, so it will transmit the virus after minutes of feeding and the insect retains the virus for all or most of its life. In California, the disease is most common in western Fresno, Kings and Kern counties but also occurs in other areas with less frequency and intensity. To reduce population densities of migrating beet leafhopper carrying the virus, areas that harbor the virus and its vector are treated with malathion, an organophosphate insecticide, prior to migration into the cropped areas in spring.

Currently, the insecticide used for beet leafhopper treatments of non-cropped areas or rangeland is limited to malathion, which has widely been used for mosquito abatement and exotic pest eradication. Availability of an additional chemistry would reduce the risk of compromised efficacy of malathion due to resistance development and would provide an alternative should regulatory restrictions be imposed on malathion. Furthermore, if efficacious materials with organic certification were identified, high risk organic rangeland that cannot currently be addressed could be treated reducing the threat to the tomato industry in those production areas. For additional materials to be available for this purpose, efficacy data is needed.

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

Provide information on performance of conventional and certified organic insecticides against beet leafhopper.

Methodology and Results:

At the University of California Research and Extension Center in Fresno County, seed of a *Beet curly top virus*-tolerant sugar beet variety was sown into a Panoche clay loam soil on January 23, 2020. The crop was sprinkler irrigated to emergence and all subsequent irrigations were furrow. Beets were selected as the test plant because seed is available, it can be cultivated easily, and it will support resident beet leafhopper populations. Specific treatments and rates of formulated product per acre for the conventional and for the certified organic insecticide comparisons were as follows:

Conventional	Organic
Baythroid 2.6 fl oz (cyfluthrin)	Pyganic 2 pints (pyrethrins)
Mustang 3.0 fl oz (zeta-cypermethrin)	Spray Oil 3.125 pints
Warrior II 1.6 fl oz (lambda-cyhalothrin)	Sil Matrix 2 pints (potassium silicate)
Admire Pro 1.6 fl oz (imidicloprid)	Trilogy 2 pints (clarified hydrophobic extract of neem oil)
Sevin SL 32 fl oz (carbaryl)	Entrust SC 8 fl oz (spinosad)
Success 4 fl oz (spinosad)	Venerate 1 gallon per acre
Malathion 5EC 32 fl oz	Grandevo 3 lbs/acre (48 oz/acre)
Untreated control	Untreated control

The experimental design was a four-replication randomized complete block. Each plot was 70 ft long and three 30-inch beds wide. All treatments were applied with a CO_2-pressurized sprayer at 30 psi in the equivalent volume of 40 gallons per acre. The sprayer boom was equipped with four Teejet 8003 EVS. The area in the center 50 ft of each plot of the center bed was sampled for leafhopper.

Although beet leafhopper was detected on cards early in the season, the population densities in the sugar beets did not reach levels sufficient for initiation of an efficacy study until 1 July. At that time, on average, 2.5 beet leafhopper counts per ten sweeps with a 15-inch diameter net were recorded. The conventional insecticide comparison treatments were applied on 13 July, and the trial for comparison of organic insecticides was treated on 14 July. On 17 July, both conventional and organic plots were swept, and Beet leafhopper counts were recorded. Analysis of Variance was performed and Least Significant Difference at a probability of 0.05 is presented.

There were significant differences in beet leafhopper counts among treatments in the conventional trial, but treatments were all similar in the comparison of certified organic insecticides (Tables 1 and 2). In the conventional insecticide comparison, no beet leafhoppers were captured in the Baythroid and Warrior II treated plots, and less than one per plot was captured in the Sevin and Malathion treatments while the average per plot count was 3.5 in the untreated control (Table 1). The organic trial was placed on the south side of the trial, which had lower counts in the untreated control (1.75 beet leafhoppers per plot). Pyganic, Spray oil, Trilogy Venerate and Grandevo averaged less than 1 leafhopper per plot, but all treatments were similar P=0.05 9 (Table 2).

Table 1. Comparison of conventional insecticide efficacy against beet leafhopper (*Circulifer tenellus*) densities in sugar beets in Fresno County, 2020.

Insecticide trade name and rate per acre (active ingredient)[z]	Beet leafhopper (10 sweeps)[y]
Baythroid 2.6 fl oz (cyfluthrin)	0.00
Warrior II 1.6 fl oz (lambda-cyhalothrin)	0.00
Sevin SL 32 fl oz (carbaryl)	0.25
Malathion 5EC 32 fl oz	0.50
Mustang 3.0 fl oz (zeta-cypermethrin)	2.00
Admire Pro 1.6 fl oz (imidicloprid)	2.25
Success 4 fl oz (spinosad)	2.75
Untreated control	3.50
$LSD_{0.05}$[x]	**1.89**
CV (%)	**91.35**

[z] All materials were applied in the equivalent volume of 40 gallons per acre with a CO_2-pressurized sprayer on 13 July.

[y] A 15 inch diameter sweep net was passed ten times per plot on 17 July and the number of beet leafhoppers were recorded.

[x] Means appearing above the Least Significant Difference are the statistically similar at P=0.05 if their difference is less than that number.

Table 2. Comparison of certified organic insecticide efficacy against beet leafhopper (*Circulifer tenellus*) densities in sugar beets in Fresno County, 2020.

Insecticide trade name and rate per acre (active ingredient)[z]	Beet leafhopper (10 sweeps)[y]
Trilogy 32 fl oz (neem oil)	0.25
Grandevo 3 lbs/acre (48 oz/acre)	0.50
Spray Oil 50 fl oz	0.50
Pyganic 34 fl oz (pyrethrins)	0.75
Venerate 128 fl oz per acre	0.75
Entrust SC 8 fl oz (Spinosad)	1.00
Untreated control	1.75
Sil Matrix 32 fl oz (potassium silicate)	2.00
$LSD_{0.05}$[x]	**NS**
CV (%)	**103.2**

[z] All materials were applied in the equivalent volume of 40 gallons per acre with a CO_2-pressurized sprayer on 14 July.

[y] A 15 inch diameter sweep net was passed ten times per plot on 17 July and the number of beet leafhoppers were recorded.

[x] Means appearing above the Least Significant Difference are the statistically similar at P=0.05 if their difference is less than that number.

Discussion:

Beet leafhopper transmits *Beet curly top virus,* which has capacity to cause devastating losses to processing tomatoes and other crops. Conventional and certified organic insecticides were compared for efficacy against this vector to provide options to the malathion treatment that is currently being used to treat non-cropped or rangelands that harbor the virus and vector.

Under the conditions of these studies, population densities were sufficient to see differences among treatments in the conventional insecticide comparison (Table 1). Insect counts were lowest in the Baythroid and Warrior II treatments, which were not significantly different than the Sevin and Malathion treatments. The other treatments

including Mustang, Admire Pro and Success were similar to the untreated control. However, the Mustang treatment was statistically similar to the Malathion treatment, which was the standard.

In the comparison of the certified organic materials, differences were not significant (Table 2). Numerically, Trilogy, Grandevo, Spray Oil, Pyganic and Venerate treatments had between 0.25 and 0.75 beet leafhoppers per ten sweeps, average densities in the Entrust treatment was `1.0, 1.75 in the untreated control and 2.0 in the Sil Matrix treatment.

One season of data is not sufficient to make conclusive statements. These field trials represent a substantial increase in the recent work on this topic in this region, but seasonal variability in densities and movement can influence results. The conditions of this study included a late season, relatively low-density population in which two pyrethriods, Baythroid and Warrior II, and a carbamate, Sevin, performed similar to the malathion standard. A minimum of three years of similar studies will better represent the range of conditions possible in this production area.

Acknowledgements: Raphael Solorio (Superintendent) University of California West Side Research and Extension Center for planting and maintenance of the sugar beets.

This project as leverage for other dollars: Generally, little interest among chemical companies was expressed although I received an unrestricted gift of $1000 each from Certis USA and from Bayer Agrochemical. Additional interest was expressed in discussion with chemical company representatives in terms of the 2021 studies and I expect at least $2000 from industry in contributions for next year's work on this topic.

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CONTROL STRATEGIES FOR F. FALCIFORME, A NEWLY RECOGNIZED AND WIDESPREAD CAUSE OF PREMATURE VINE DECLINE

CASSANDRA SWETT & BRENNA AEGERTER

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KEY TAKEAWAYS AT THIS DATE

- Across the three field trials, N 6428 and H 5608 were both consistently top performing cultivars. These cultivars are recommended for F. falciforme management. Strong performers from UCD trials to examine in commercial fields in 2021 include H 1776, UG 4014, SVTM 9025, and SVTM 9016.
- HM 58801 was less consistent, performing well in UCD trials but with only moderate yields in both commercial field trials. HM 58841 was the reverse, performing well in commercial trials but less strongly in UCD trials, although this cultivar performed better in 2019 UCD trials.
- Consistently poor performers included H 1310, which while decent in the San Joaquin trial (likely because, as an F3 cultivar it did well under Fusarium wilt pressure), was among the lowest yielder in Fresno, and was poor performer in 2019 UCD trials. HM 3887, the worst cultivar in UCD trials, was a middle ranking cultivar in commercial trials, as was HM 4909. AB 311 was consistently poor in commercial trials (not tested at UCD). It is recommended that these cultivars be avoided for F. falciforme management.
- Despite significant variation in both vine decline and yields, 85-100% of plants in all cultivars developed rot in the stem (above and/or below ground). This indicates that resistance development efforts should focus on traits such as vine decline, and that rot development, while temping to use as a phenotype due to rapid and easy production in greenhouse assays, will be misleading.
- We are using this work to inform synergistic efforts to develop resistance screening protocols for breeding companies, to help improve genetic resistance traits across a wider range of commercial cultivars.
- In one 2019 tomato field with F. falciforme, one year after rotation out of tomato we found stem rot and stunting in the sunflower, but F. falciforme was not recovered. We have established two additional F. falciforme survey fields in San Joaquin County—this year we collected data on vine decline incidence, and next year we will monitor for disease in the rotation crops planted.
- F. falciforme was recovered from stem rot of nightshade in on commercial field in San Joaquin County, as well as in the F. falciforme-infested field at UC Davis, indicating that nightshade is a host and may be important to control to prevent F. falciforme build up.
- Controlled host range trials indicate that F. falciforme pathogenic to tomato can also cause stem rot in corn, safflower and sunflower—all common rotations with tomato which may both suffer economic losses from this pathogen and also contribute to inoculum load build up in the soil.
- However, cotton, cucumber, melon, pumpkin, pepper, wheat and cowpea did not develop stem rot—these crops may be better rotations for F. falciforme infested-fields since they may not suffer losses and could also suppress soil inoculum load increases.
- Based on three separate field trials over two years we have established that K-Pam is effective in reducing losses from F. falciforme at the 30 gal/A rate and is an economical management tool based on the relative cost of application vs. yield gains. This is perhaps the first effective management tool established for F. falciforme in the state, which can be widely adoptable by the industry.

INTRODUCTION

In the last four years we have documented Fusarium falciforme as a growing threat to the processing tomato industry. This pathogen is present in all major tomato producing counties, causing premature vine decline in up to 75% of plants in commercial fields resulting in at least a fivefold increase in damaged fruit. At the start of this project there were no known management options for *F. falciforme*. Short term, we are finding that chemical control may provide a means of curbing losses which can fit into an IPM program; Velum and Kpam appears to be the most promising for management (Paugh, Aegerter and Swett 2020), and also appears to work against Fusarium wilt, providing a potential co-management tool when both pathogens are present in a field. However chemical treatments do not prevent disease and must be complimented with other management tools.

In 2018 and 2019 we identified several putatively tolerant, as well as several highly susceptible cultivars; we continued these efforts in 2020 to both assess field-stability of putatively tolerant cultivars across years and sites, and expand the number of cultivars. At this point, we have several strong candidate cultivars which can be used by growers for management, as well as several highly susceptible cultivars which can be avoided. We are expanding these efforts to include trials in Colusa and Stanislaus Counties in 2021, and to include a cultivar demonstration trial run by AgSeeds.

Appropriate selection of crops for rotation is likely important to manage *F. falciforme* soil inoculum loads, but which rotation crops should or should not be grown and the duration of rotation out of tomatoes is unknown at present. Certain crop rotations have been shown to increase soil inoculum loads more than others; for instance, rotation with corn, fescue and soybean increase inoculum loads of *F. virguliforme* and soybean losses, whereas sorghum and wheat reduce inoculum loads and soybean losses (Rupe et al., 1997; Kolander 2010, Navi and Yang 2016). Based on 2020 studies, F. falciforme isolates pathogenic on tomato also appear to cause crown rot in several other hosts, including corn, sunflower, safflower and nightshade. In addition to posing a management problem in alternate hosts, these crops/weeds likely build up inoculum, increasing losses in tomato.

THE MAIN *GOAL* AND *OBJECTIVES* OF THE FUNDED PROJECT:

Main goal. Following the 2019 field season, CTRI and others asked us to prioritize development of management methods for *F. falciforme*. In response, we developed this multi-PI project to rapidly develop an effective integrated management strategy for *F. falciforme* in processing tomato, combining use of currently available tolerant cultivars, cultural practices and chemical control.

Objective 1. Develop resistant (or tolerant) cultivar recommendations for growers

1.1 Evaluate a new suite of up to ten commercially available cultivars under controlled (inoculated) field conditions, with non-inoculated controls; emphasis on F3 cultivars (Swett).

1.2 Conduct multi-location trials of seven or eight of the top performers in 2019 at two F. falciforme-infested commercial fields, one in Fresno (Turini) and one in San Joaquin (Aegerter) counties.

Objective 2. Assess effective and ineffective crop rotations

2.1. Assess the ability of *F. falciforme* isolates from tomato to infect the common rotation crops cotton, corn, melon, safflower, sunflower, wheat and crops from which *F. falciforme* has been previously recovered (pepper, pumpkin, cowpea, hemp).

2.2. Follow *F. falciforme* across crop rotations in grower fields with known *F. falciforme* incidence and then assess disease incidence in F3 tomato.

Objective 3. Screen fungicides (Velum, K-Pam) for efficacy in controlling Fusarium falciforme when applied through the drip.

METHODOLOGY AND RESULTS

Objective 1. Develop resistant (or tolerant) cultivar recommendations for growers

1.1 Evaluate a new suite of up to ten commercially available cultivars under controlled (inoculated) field conditions, with non-inoculated controls; emphasis on F3 cultivars (Swett).

We evaluated 14 commercial cultivars, including 6 F3 lines. These were planted in May and disease began to develop in late June. We collected data at 10, 8, 6, 4 and 2 weeks pre-harvest as well as harvest data, September 23-25. By six weeks pre-harvest, disease has developed in all cultivars in between 3 and 30% of plants, indicating that we had good uniformity in exposure to the pathogen and good conditions for disease development. Supporting this inference, by harvest, 85-100% of plants in all cultivars developed stem rot; levels similar to 2019 trials, and which support the previous conclusion that there is no variation in resistance to the rot component of this disease, only vine decline and associated fruit damage. This is consistent with other closely related pathogens, such as F. virguliforme, the cause of soy bean sudden death, and provides important insight into the traits future resistance development efforts should focus on, such as vine decline, as well as non-focal traits, such as rot (Lenandro et al., 2018, Weems et al. 2015). We are using this work to inform synergistic efforts to develop resistance screening protocols for breeding companies, to help improve genetic resistance traits across a wider range of commercial cultivars.

In 2020, N 6428 was the top performing cultivar, based on vine decline incidence both pre-harvest and at near-harvest (7% of plants). This translated into the highest marketable yields (65 kg/plot, or 65 tons/A) and among the lowest amount of fruit damaged by sunburn and rot. Other strong performers for 2020 based on yield included HM 58801, H 5608, H 1776, UG 4014, SVTM 9025, SVTM 9016 and N 6434 which all yielded above 50kg/plot. Of these, all except H 5608 and UG 4014 had less than 33% vine decline at harvest; for H 5608 and UG 4014, vine decline incidence at harvest was not a good indicator of performance, with 40 and 48% vine decline respectively. However, these two cultivars both performed well based on biomass of fruit with sun damage (0.72-0.90kg) and rot (1.67-1.91kg). This suggests that while F. falciforme can have severe effects on certain cultivars, field holding traits which delay fruit decay allow more susceptible cultivars to perform well under pathogen pressure.

The worst performer for 2020 was the susceptible check, HM 3887 which had among the highest incidences of vine decline (up to 100% in some plots, average of 45% of plants / plot), the lowest fruit biomass, and the highest amount of sun damaged fruit. Other poor performers in 2020, with similar yields to HM 3887 (43-47 kg/plot) were HM 58841, HM 4909, H 8504 and HM 5235. Statistically, there were no significant differences between cultivars based on any of the measured variables.

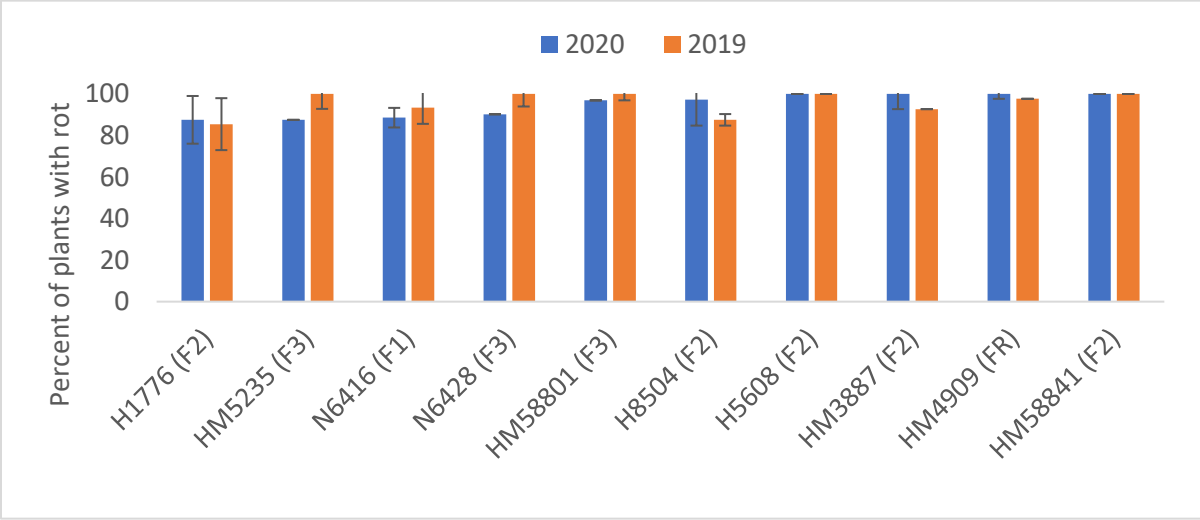

Figure 1. 85-100% of plants develop stem rot by harvest in all cultivars, in both 2019 and 2020 trials at UCD.

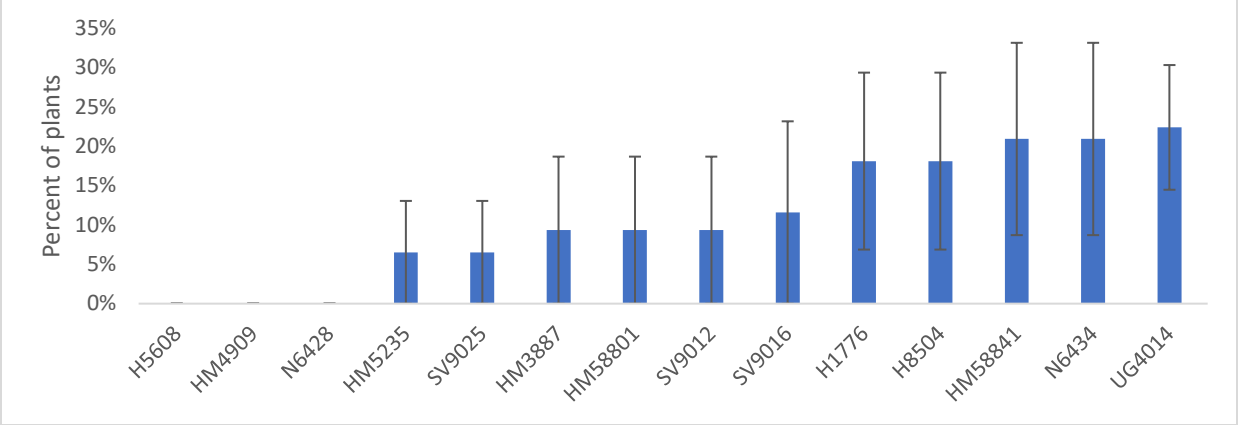

Figure 2. Incidence of premature vine decline six weeks pre harvest, UCD trial.

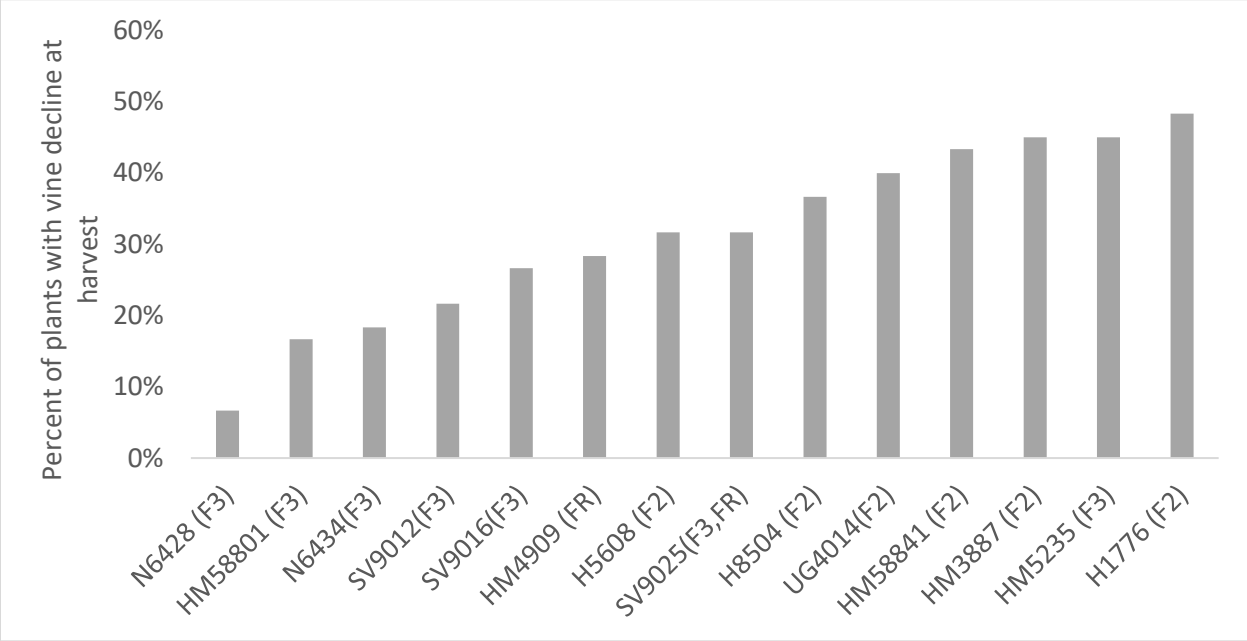

Figure 3. Percent of plants with vine decline at harvest, UCD trial.

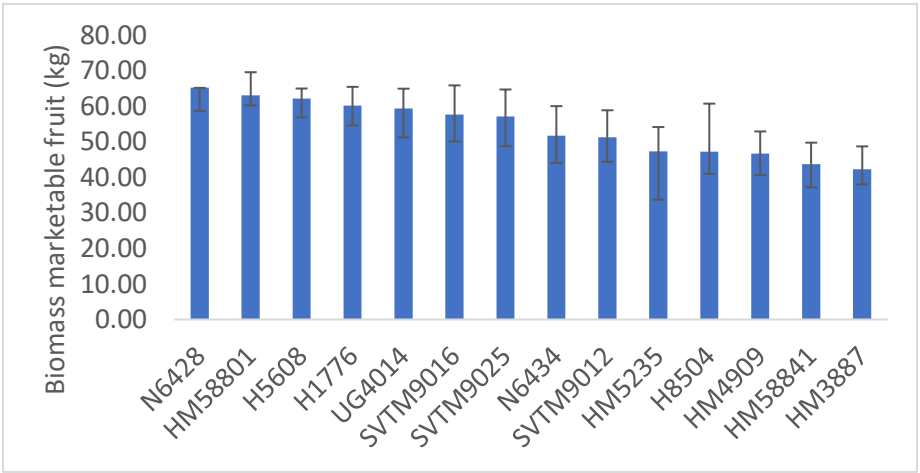

Figure 4. Biomass of marketable red fruit per 2m plot, UCD trial

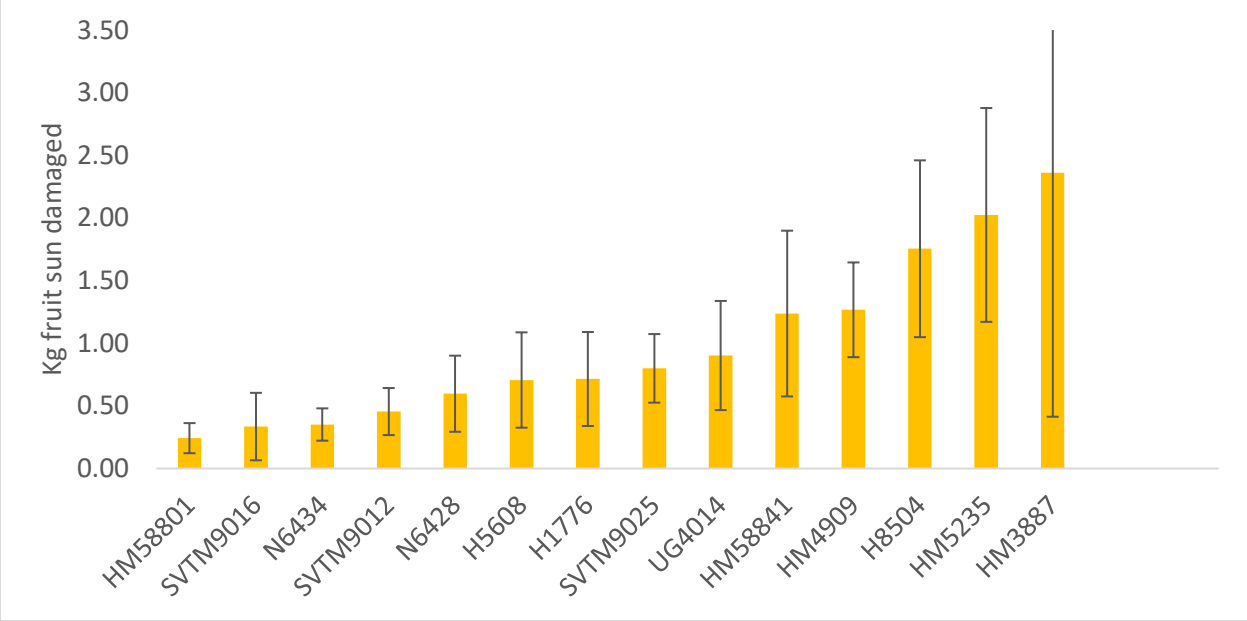

Figure 5. Biomass of sun damaged fruit (may also have rot), UCD trial

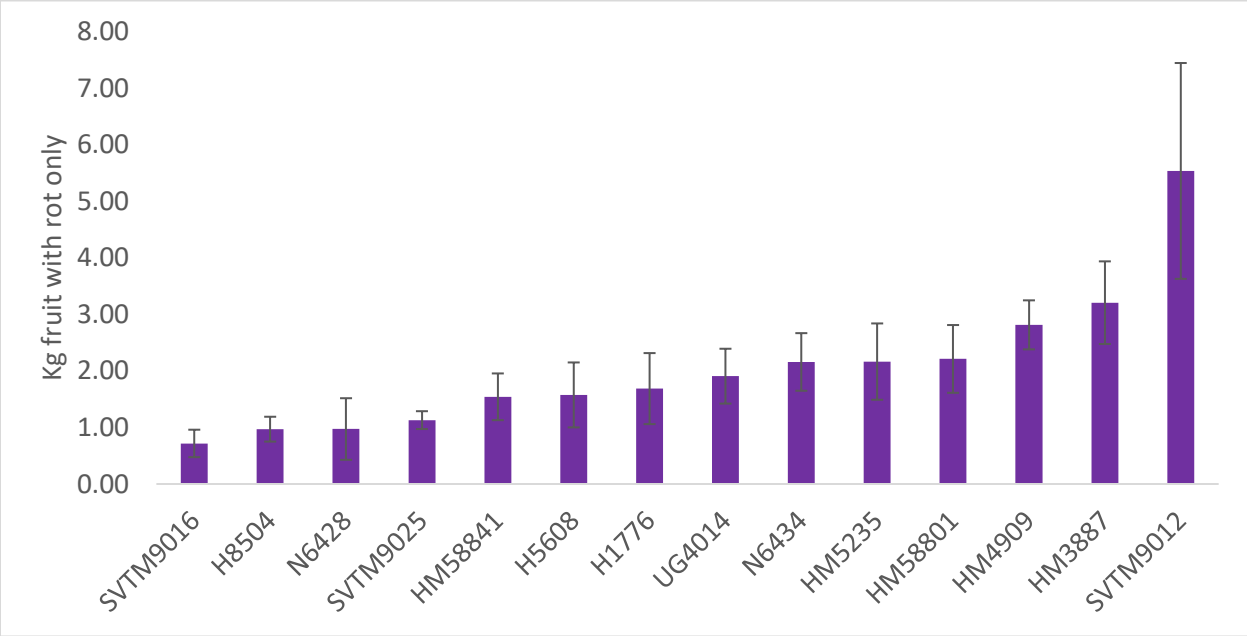

Figure 6. Biomass of fruit with only rot symptoms (no sunburn or end rot), UCD trial.

Table 1. Cultivar performance rankings based on two years of UC Davis field trials, with evaluation of consistency in tolerance and yield performance across years

	Performance category		FF-driven yield reduction		Total Markeable Yield (kg/plot)		Vine Decline Incidence at harvest		Tolerance	Yield
Cultivar	2019	2020	2019	2020	2019	2020	2019	2020	consistent?	consistent?
H1310 (F3)	Highly S, low yield	NT	61%	NT	6.77	NT	60%	NT	NA	NA
H1428 (F2)	Highly S, mod yield	NT	41%	NT	10.57	NT	56%	NT	NA	NA
H1776 (F2)	S-Tolerant, high yield	S-Tolerant, high yield	24%	0%	12.48	60.18	43%	48%	YES	YES
H5608 (F2)	S-Tolerant, mod yeild	Highly S, high yield	37%	6%	10.15	62.14	60%	32%	NO	YES
H8504 (F2)	S-Tolerant, high yeild	Highly S, high yield	14%	12%	16.73	47.19	28%	37%	NO	YES
H9663 (F2)	Highly S, low yield	NT	62%	NT	4.88	NT	82%	NT	NA	NA
HM3887 (F2)	Highly S, mod yield	Highly S, mod yield	46%	6%	9.05	42.24	39%	45%	YES	YES
HM4909 (FR)	S-Tolerant, mod yield	S-Tolerant, mod yield	23%	0%	10.85	46.72	49%	28%	YES	YES
HM5235 (F3)	S-Tolerant, mod yeild	S-Tolerant, mod yeild	27%	0%	8.74	47.28	47%	45%	YES	YES
HM58801 (F3)	S-Tolerant, mod yeild	S-Tolerant, high yeild	27%	0%	9.29	63.10	41%	17%	YES	YES
HM58841 (F2)	S-Tolerant, high yeild	Highly S, mod yield	0%	31%	13.95	43.67	60%	43%	NO	NO
N6416 (F2)	Highly S, low yield	NT	50%	NT	7.29	NT	70%	NT	NA	NA
N6428 (F3)	S-Tolerant, high yield	S-Tolerant, high yeild	28%	0%	15.30	65.19	55%	7%	YES	YES
N6434(F3)	NT	Highly S, high yield	NT	14%	NT	51.68	NT	18%	NA	NA
SV9012(F3)	NT	S-Tolerant, high yeild	NT	0%	NT	51.24	NT	22%	NA	NA
SV9016(F3)	NT	S-Tolerant, high yeild	NT	0%	NT	57.69	NT	27%	NA	NA
SV9025(F3,FR)	NT	S-Tolerant, high yeild	NT	0%	NT	57.12	NT	32%	NA	NA
UG4014(F2)	NT	S-Tolerant, high yeild	NT	0%	NT	59.40	NT	40%	NA	NA

Red:	Tolerant with high yields in both years
Orange:	Tolerant with high yields in at least one year
Yellow:	Tolerance and moderate yields in both years

Objective 1.2 Cultivar evaluation trials in commercial fields

Two trials were conducted in commercial fields. In San Joaquin County, a trial was established in a commercial field of processing tomatoes which was naturally infested with both Fusarium wilt race 3 and *Fusarium falciforme*. The planting configuration was a single plant row on 60-inch centered beds. Subsurface drip irrigation was used, with a single line of low-flow drip tape centered on the bed and buried approx. 10 inches. The trial area was managed by the grower similarly to the rest of the field. Plot size was a single bed (5 feet) by 100 feet and the experimental design was a randomized complete block with four replications. Transplants were grown at California Masterplant in Tracy and were mechanically transplanted on May 14th. The trial was machine harvested on October 2nd (141 days).

In Fresno County, a trial was established within a commercial cotton field which had been in tomatoes in 2019 and had *Fusarium falciforme* problems. Because it was part of a cotton field, the cultural and pest management practices were not standard for commercial processing tomatoes. Plot size was a single bed by 25 feet and the experimental design was a randomized complete block with four replications. Transplants produced by West Side Transplants were hand planted into double line 80-inch beds on May 21st. Beet curly top virus symptoms were present by July 6th and onset of Tomato spotted wilt virus was present at substantial levels so that rating above

ground symptoms was difficult. The trial was hand harvested on 15th and 16th September (118 days) because the cotton field that it was in was scheduled to be defoliated. Many of the entries had high percentages of green fruit.

Disease evaluations were conducted multiple times. At the San Joaquin site, both Fusarium falciforme and Fusarium wilt were apparent, thus complicating disease assessments as it is nearly impossible to undertake an accurate field diagnosis based on foliar symptoms without destructively sampling the vine. The two diseases were rated separately when possible, but often it wasn't possible to discriminate and thus the more general category of "uncertain Fusarium disease" was used. At the Fresno site, there was no Fusarium wilt, but the foliar evaluations were hampered by a significant amount of virus symptoms. At harvest, destructive sampling was used to determine which plants had crown rot symptoms.

Among the entries, there were five cultivars with resistance to Fusarium wilt race 3 ("F3 cultivars") and seven which were susceptible to race 3 ("F2 cultivars"). Although our primary goal was to evaluate cultivar performance when challenged by Fusarium falciforme, the presence of race 3 at the San Joaquin County trial necessitates that these two groups of cultivars be discussed separately.

Among the F3 cultivars, the three which ranked highest in terms of yield were HM 5235, N 6428 and N 6434. These tended to have lower disease as well, although disease incidence and yield are not always well correlated. Among the F2 cultivars, the top-ranked in terms of yield were HM 58841, H 5608, DRI 319 and HM 3887. HM 3887 had the highest levels of Fusarium falciforme incidence at the Fresno site, but is tolerant of Fusarium wilt race 3 which helped it at the San Joaquin site. HM 58841, which has done well in Fusarium falciforme trials on campus, had significant disease levels at both sites, but yielded well despite this fact. H 5608 had low Fusarium falciforme levels at both sites and yielded well. DRI 319 had low falciforme incidence at the Fresno site. Among the lowest yielding cultivars were AB 311, HM 58801, H 1310, HM 4909 and H 8504. These tended to have higher disease levels, but again, that relationship doesn't hold in all cases. In the San Joaquin trial, F3 cultivars ranged from 57 to 72 tons per acre, whereas F2 cultivars ranged from 50 to 62 tons per acre, indicating an advantage of growing F3 cultivars in fields co-infested with F. falciforme and Fusarium wilt.

Table 2. Yield and soluble solids performance in fields naturally infested with Fusarium falciforme (both sites) and Fusarium wilt race 3 (San Joaquin site only).

		San Joaquin trial			Fresno trial			Combined locations
Cultivar	Fusarium resistance	Yield (t/ac)		Rank	Yield (t/ac)		Rank	Soluble solids (° Brix)
HM 58841	F2	61.6	b	2	56.9	a	1	5.24
H 5608	F2	61.0	bc	3	46.8	ab	2	4.68
N 6434	F3	72.3	a	1	29.0	cd	5	5.05
N 6428	F3	59.8	bcd	5	42.8	abc	3	4.93
HM 3887	F2	60.8	bc	4	39.2	bcd	7	5.46
DRI 319	F2	56.1	def	7	44.7	abc	6	5.31
HM 5235	F3	52.6	efgh	9	48.9	ab	4	5.23
H 1310	F3	56.7	cde	6	28.8	cd	10	5.04
H 8504	F2	53.8	efg	8	42.1	abcd	8	5.14
HM 4909	F2, Forl	52.1	fgh	10	36.1	bcd	9	5.65
AB 311	F2	49.6	gh	11	25.4	d	12	5.54
HM 58801	F3	48.5	h	12	35.3	bcd	11	5.15
	Mean	57.1			39.7			5.20
	LSD value	4.48			17.29			0.2715
	P value	*<0.0001*			*0.0275*			*<0.0001*
	CV (%)	*5.46*			*30.30*			5.23

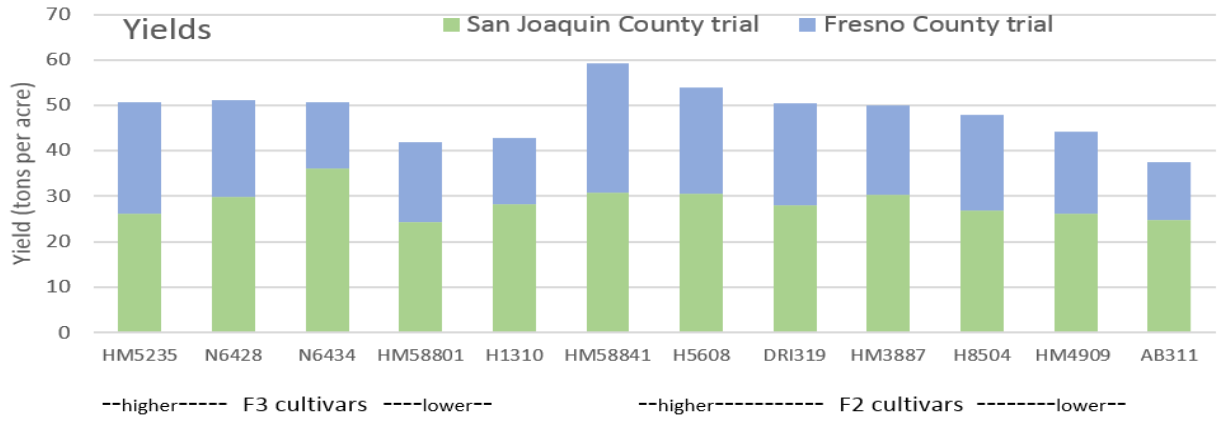

Figure 7. Yield performance of cultivars evaluated in both a San Joaquin and Fresno field infested with F. falciforme. Top performers across both trials include H5608, N6434 and HM58841. Yields are the average of the two trials, with the two colors indicating the proportion contributed by each trial (e.g., N 6434 did better at San Joaquin trial than at Fresno trial).

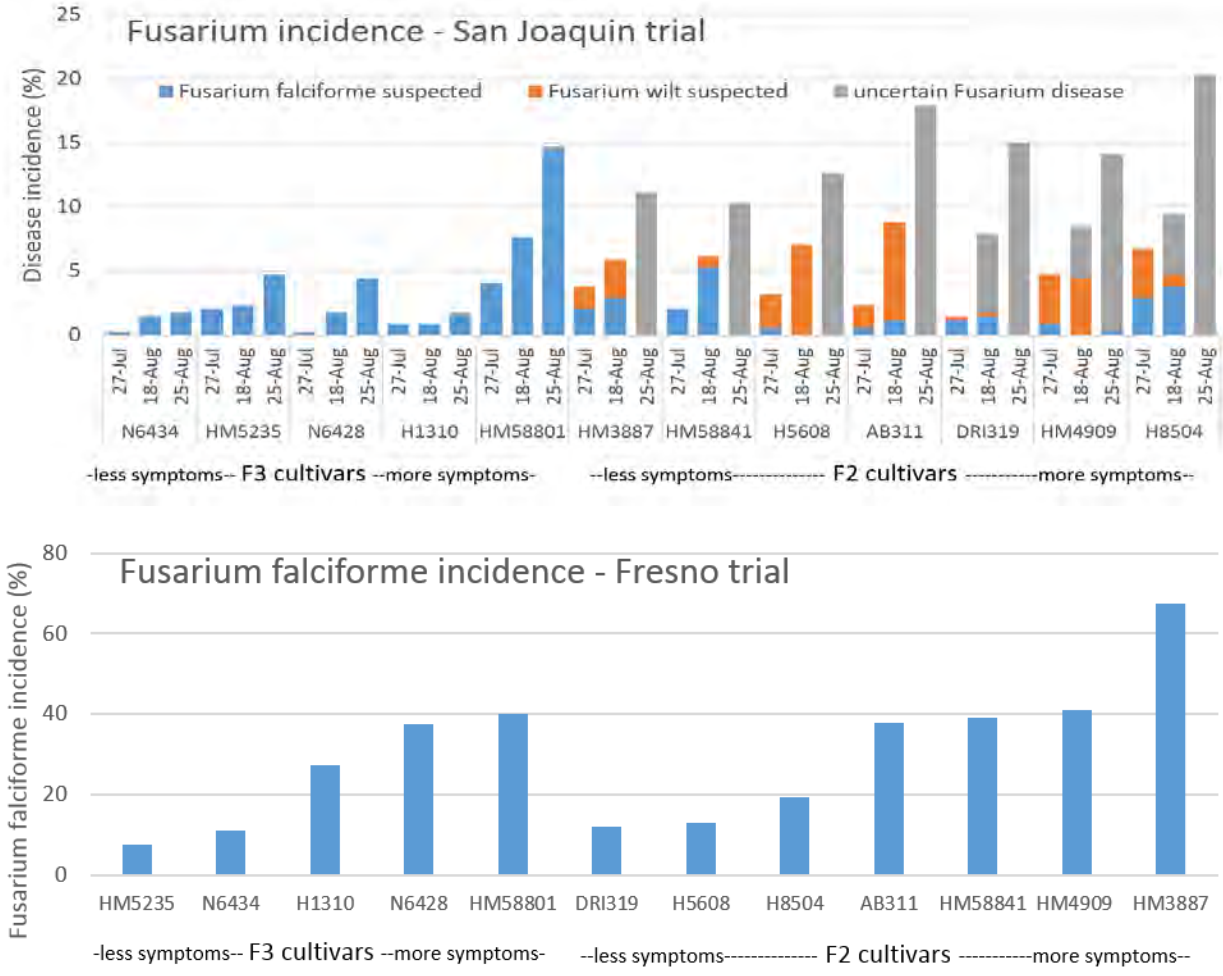

Figure 8. Fusarium falciforme estimated incidence (based on foliar and rot symptoms) in San Joaquin (top) and Fresno (bottom) trials. F. falciforme was confirmed in both fields via laboratory diagnosis. Fusarium wilt was also confirmed in the San Joaquin field.

Objective 2. Assess effective and ineffective crop rotations

2.1. Assess the ability of *F. falciforme* isolates from tomato to infect the common rotation crops including crops from which *F. falciforme* has been previously recovered

In the field, we inoculated pumpkin, muskmelon and cucumber with the same isolate by dip inoculating 6 week old transplants. In each of three rows, we planted ten plants of each crop. Four months after planting, we cut into the stems of five plants per crop per row. No crown rot was observed in any plants.
In a separate greenhouse trial, we inoculated the following crops in the greenhouse with a F. falciforme isolate from tomato: cotton, corn, cowpea, sunflower, wheat, safflower, pepper and hemp. Tomatoes were included as a positive control. These plants were inoculated by wounding the foot of ~3 week old seedlings (8 plants / crop) and dipping in a spore suspension of a single F. falciforme isolate which we had recovered from and documented pathogenicity on tomato (CS 109). We monitored disease development over nine weeks. Stem rot developed in corn, safflower and sunflower, while cotton, pepper, wheat and cowpea did not develop stem rot—these crops may be better rotations for F. falciforme infested-fields since they may not suffer losses and could also suppress soil inoculum load increases.

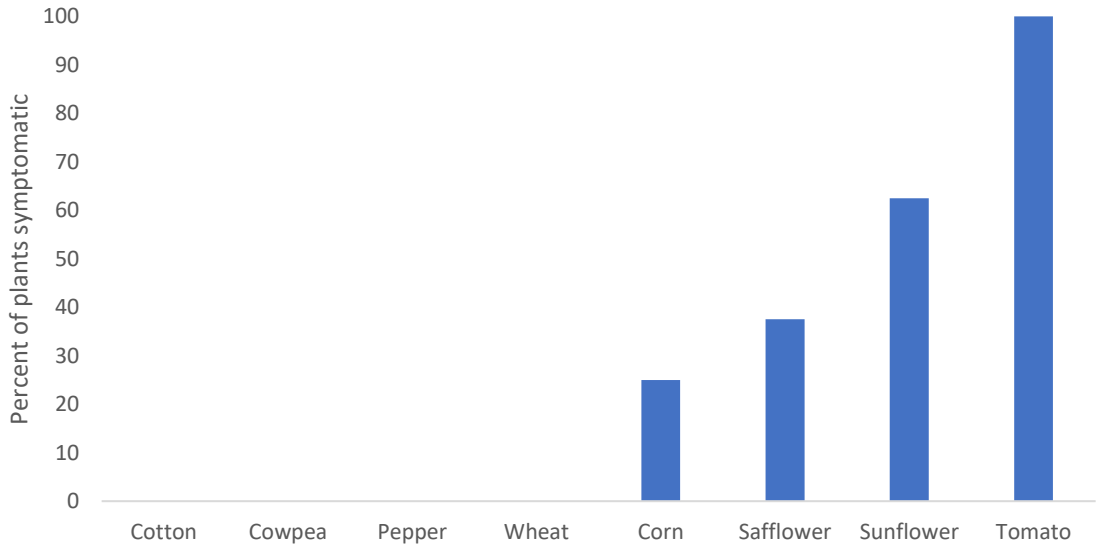

Figure 9. Seven common rotation crops were inoculated with a Fusarium falciforme isolate from tomato, and evaluated for disease development nine weeks later. This shows the percent of plants within each crop that developed any disease symptoms. Non inoculated controls remained healthy.

Table 3. Symptom notes for each crop

Crop	Principal symptoms observed
Corn	No external symptoms. Brown discoloration and sour smell of both internal and external tissue of stem node at soil line.
Cotton	None seen.
Cowpea	None seen.
Pepper	None seen.
Safflower	No external symptoms. Dark brown to reddish streaking present in cortex and sometimes vasculature near crown.
Sunflower	No external symptoms. Brown discoloration and/or necrosis of pith in crown and stem.
Wheat	None seen.
Tomato	External symptoms consisting of foliage dieback, wilt, and/or a necrotic lesion present at crown. Internal lesion was also present at crown, often extending over 6-inches up stem and accompanied by necrosis in pith.

Figure 9. Stem rot in safflower (left), sunflower (middle) and corn (right) caused by a F. falciforme strain pathogenic on tomato

2.2. Follow *F. falciforme* across crop rotations in grower fields with known *F. falciforme* incidence and then assess disease incidence in F3 tomato.

We have established four survey fields thus far, two in Yolo and two in San Joaquin County. In each field we documented F. falciforme vine decline incidence. We are focusing on F3 cultivar fields in most counties, to avoid confounding effects of Fusarium wilt. In 2020 we struggled to add fields due to COVID-associated logistical challenges. In 2021 we aim to add at least one Fresno field, and ideally three fields total to our survey.

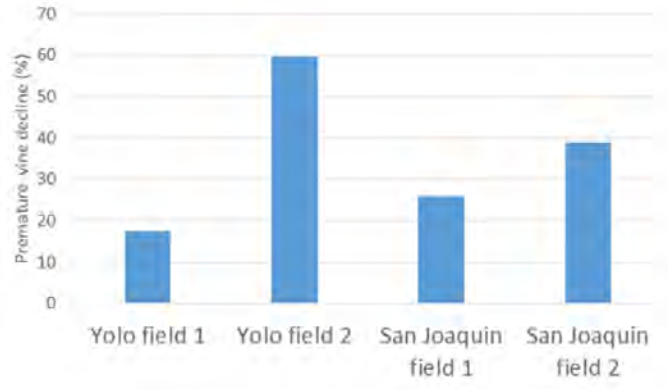

Figure 10. Premature vine decline incidence due to F. falciforme at four survey sites, with two new San Joaquin fields added in 2020.

We surveyed for F. falciforme in one sunflower field in Yolo County with pre-established F. falciforme incidence in tomato, evaluating three 100 ft transects randomly selected within the infested side of the field. Incidence of stem rot, stunting and decline symptoms were quantified and the fungi associated with these symptoms were evaluated to determine if F. falciforme is present. While stem rot and stunting were observed in sunflower (Figure 2), F. falciforme was never recovered. We were unable to survey the second Yolo site due to COVID-associated logistical challenges, but plan to get information on the rotation crop used and survey the 2021 rotation crop.

Figure 11. Sunflower field planted at Yolo survey site 1, which had F. falciforme vine decline in 18% of tomatoes in 2019. Stem rot and stunting were observed in sunflower, but F. falciforme was not found. We will revisit this site annually to evaluate all rotation crops and, ultimately, re-assess F. falciforme vine decline to determine effects of the rotation system on disease losses.

F. falciforme was recovered from stem rot of nightshade in on commercial field in San Joaquin County, as well as in the F. falciforme-infested field at UC Davis, indicating that nightshade is a host and may be important to control to prevent F. falciforme build up. Further trials are needed to establish this as a host.

Figure 12. Stem rot of nightshade in an F. falciforme infested commercial tomato field

Objective 3. Screen fungicides and metam for efficacy in controlling Fusarium falciforme when applied through the drip line.

Although two fields trial were originally proposed, only a single trial in San Joaquin County was conducted, due to the difficulty of finding a suitable site in Fresno County (all commercial fields with confirmed *Fusarium falciforme* problems in 2019 were rotated out of tomatoes for 2020). A trial was established in a commercial field of processing tomatoes (cv. 'SV8011TM') which was naturally infested with both Fusarium wilt race 3 and *Fusarium falciforme*. The site is located on Roberts Island in the Sacramento-San Joaquin River Delta (lat 37.884° long -121.381°) and the soil type is an Egbert silty clay loam. The planting configuration was a single plant row on 60-inch centered beds. Subsurface drip irrigation was used, with a single line of low-flow drip tape centered on the bed and buried approx. 10 inches. The trial area was managed by the grower similarly to the rest of the field. Plot size was a single bed (5 feet) by 100 feet and the experimental design was a randomized complete block with four replications. For the experimental fumigation application, K-Pam (metam potassium) was injected into the drip tape at the head of individual rows on April 16th, over a period of approx. four hours. The field was mechanically

transplanted on May 13th. The same day, the transplant plugs were treated with a fungicide drench at a volume of 150 ml per plug by pouring the fungicide solution at the base of the stem (equivalent to 290 gallons per acre). Although this volume of water is lower than would be applied by a mechanical transplanter, we were not drenching the soil in-between the plugs, thus we were able to apply less water and still saturate the soil surrounding the plug. For the later experimental applications made via the drip irrigation system at three and five weeks after transplanting, fungicides were injected into the tape at the head of each row to treat the full row length of 800 feet. Injections were done early in the irrigation set and took place over an approx. 60- to 70-minute period. See the treatment list below for fungicide rates and application dates (Table 1). The plots were evaluated at regular intervals beginning 8 weeks after transplanting when Fusarium symptoms were first observed. When possible, symptoms of Fusarium wilt and *Fusarium falciforme* were recorded separately, based on a visual assessment of the foliage, without cutting into the stems, crowns or roots. However, without destructive sampling of the crown tissue and laboratory confirmation of the pathogen, it is not possible to distinguish the two diseases with any confidence. Thus, analyses are presented for the total incidence of Fusarium diseases and no attempt is made to draw any conclusions about the efficacy of the treatments on one particular disease or the other. On September 21st, a small section of each plot was harvested by hand and fruit were graded and weighed. Analysis of variance was conducting using PROC GLM (SAS ver 9.4). Disease incidence did not differ between treatments at the 5% significance level, although they were nearly significant at 12 weeks after transplanting (Table 2, Figure 1). Yield was likewise not statistically different between treatments. Metam-potassium at the 30-gallon rate seems to have had a small impact, with lowest disease levels at 8 and 12 weeks after transplanting and yield which was more than 10 tons higher than the non-treated control. However, without statistical significance, we have no confidence in these conclusions. However, coupled with our observations from other trials evaluating metam-potassium for Fusarium diseases of tomato, we think believe that this product shows the greatest promise for providing partial control of Fusarium diseases and reducing yield losses.

Table 4. Chemical programs evaluated and treatment rates and timings

application timing(s) relative to transplant date	pre-plant	planting	3 wk	5 wk
	Apr 16	May 13	Jun 2	Jun 16
Product (active ingredient)	Product application rate (per acre basis)			
Velum One (fluopyram)		6.84 fl oz drench	6.84 fl oz drip	6.84 fl oz drip
Rhyme (flutrifol)		7 fl oz drench	7 fl oz drip	7 fl oz drip
Miravis (pydiflumetofen)		13.7 fl oz drench	13.7 fl oz drip	13.7 fl oz drip
K-Pam (metam potassium)	15 gallons			
K-Pam (metam potassium)	30 gallons			
Non-treated control				

Table 5. Impact of fungicide programs on tomato Fusarium disease incidence and severity and fruit yield in processing tomato.

	DISEASE INCIDENCE RATINGS (% OF PLANTS WITH FUSARIUM SYMPTOMS)									
Date of disease evaluation	*8-Jul-20*			*5-Aug-20*			*25-Aug-20*	*3-Sep-20*	Fruit	Market
weeks after transplanting	*8 weeks*			*12 weeks*			*15 weeks*	*16 weeks*	biomass	yield
Treatment	*F.o.l.*	*F.f.*	Both diseases	*F.o.l.*	*F.f.*	Both diseases	Both diseases	Disease severity rating	(ton/ac)	(ton/ac)
Non-treated control	4.41	0.88	5.29	17.65	1.47	19.12	30.59	5.8	50.84	49.09
Velum (fluopyram)	6.76	0.00	6.76	22.35	1.18	23.53	31.47	6.0	49.11	47.43
Rhyme (flutriafol)	4.71	0.00	4.71	14.12	0.29	14.41	25.00	4.3	55.84	54.65
Miravis (pydiflumetofen)	5.29	0.29	5.59	13.53	1.18	14.71	23.24	4.5	57.71	55.22
K-Pam 15 gal (potassium N-methyldithiocarbamate)	4.71	0.29	5.00	14.12	0.59	14.71	29.12	4.0	55.41	53.76
K-Pam 30 gal (potassium N-methyldithiocarbamate)	2.65	0.29	2.94	11.47	0.29	11.76	27.06	3.8	62.97	59.79
Mean	4.75	0.29	5.05	15.54	0.83	16.37	27.70		55.31	53.32
P value			*0.6401*			*0.0595*	*0.6204*		*0.1601*	*0.3058*

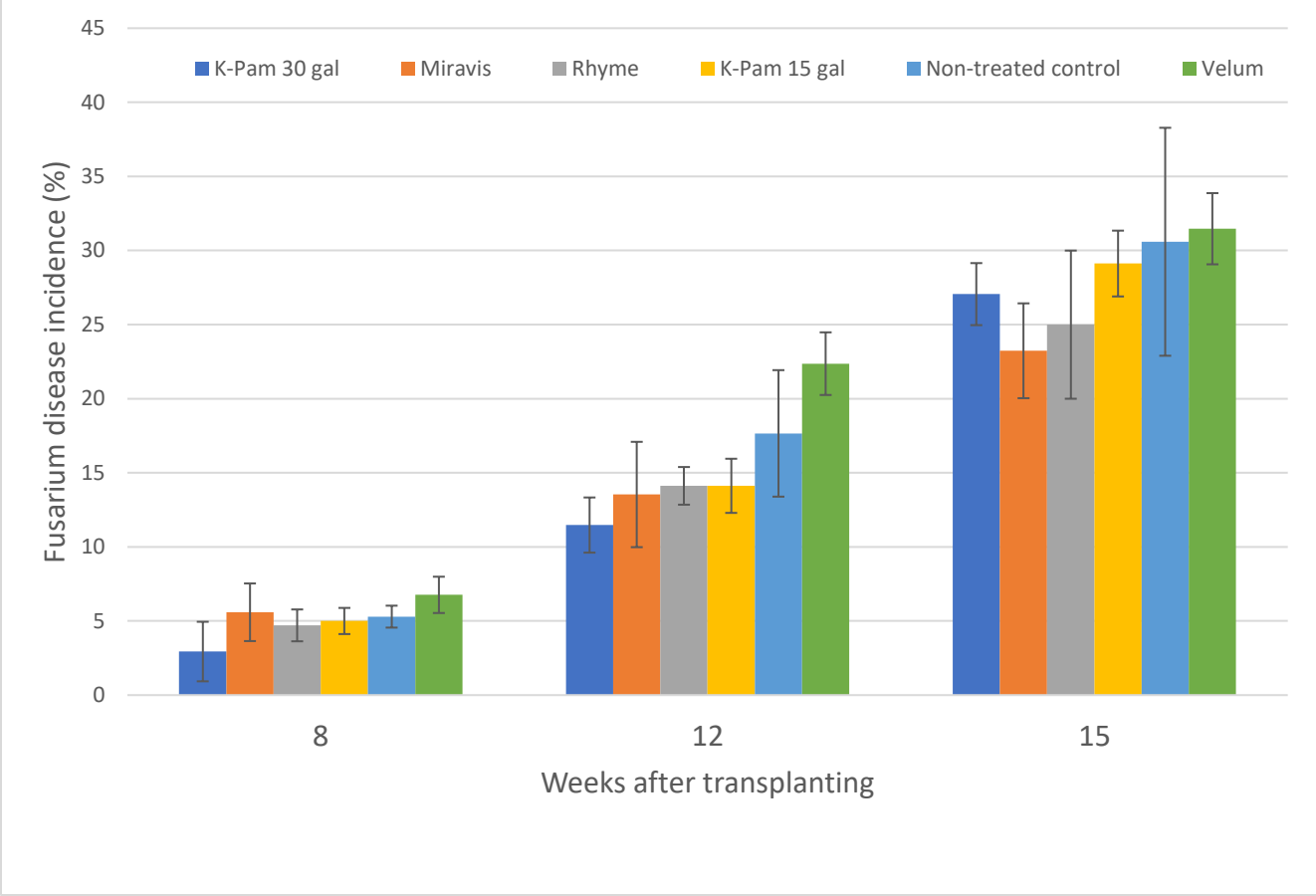

Figure 13. Impact of chemical programs on incidence of Fusarium diseases in processing tomato.

DISCUSSION

Over the last three years we have established that there is potential to use partially resistant cultivars to reduce losses and to improve tolerance traits in commercial materials. At this point, we have evaluated 20 different cultivars, establishing two as strong performers, ~5 to 8 as moderate performers and ~8 to 10 as highly susceptible (depending on the trial). We have found that cultivars do not perform consistently between years or sites--to get a clear picture of performance potential requires testing in replicated trials repeated across years in fields known to be infested with F. falciforme. This is a service which Cooperative Extension is ideally equipped to do since we have advisors in all major processing tomato counties and via Swett lab efforts have been able to identify F. falciforme infested fields in each of five counties, which will be included in 2021 cultivar trials. We are ideally set up to collaborate to develop consistent datasets and characterize pathogen profiles in fields with multiple pathogens. In 2020 we diagnosed F. falciforme in several TS&L and AgSeeds cultivar demonstration plots. We are planning to partner with AgSeeds in 2021 to use one of their F. falciforme-infested non-replicated demo plots in Colusa County to examine performance of the 100+ cultivars in their trial, to help inform cultivar selection in 2022 screening efforts. Ideally, these partnerships will be expanded in future years to include additional sites and collaborations. Brenna and I will continue to provide joint leadership to coordinate these statewide efforts.

The San Joaquin trials highlighted one of the major challenges with pathogen management using genetic resistance—the presence of multiple pathogen management targets. Overall, the F3 cultivars in this Fusarium wilt-F. falciforme co-infested field tended to do better, and the top performing cultivar was an F3 line. To provide meaningful management tools during this transition to use of F3 cultivars, it is going to be critical to include F3 lines in field trials so growers have options to manage both pathogens.

Appropriate selection of crops for rotation is likely important to manage *F. falciforme* soil inoculum loads, but which rotation crops should or should not be grown and the duration of rotation out of tomatoes is unknown at present. Many Fusarium crown rot pathogens have wide host ranges, including as F. *oxysporum* f. sp. *radicis lycopersici* (Fusarium crown and root rot of tomato), which causes crown and root rot in over a dozen other crops

(McGovern 2015) and the soybean sudden death pathogen *F. virguliforme*, which can cause crown rot in diverse legumes and asymptomatically colonizes corn and ryegrass (Kolander 2010). Based on greenhouse trials, F. falciforme strains that are pathogenic on tomato also appear to cause disease in several agronomic crops. We need to expand this work to examine a wider range.

Our studies with Fusarium wilt indicate that the ability for a pathogen to extensively colonize a given crop does not necessarily mean this crop will increase disease loss in tomato. For instance, cotton was extensively colonized by the Fusarium wilt pathogen but resulted in the lowest disease incidence in tomato after a one year rotation. Multi-year field trials to examine effects of these different crops will thus be critical to actually understanding their effect on F. falciforme development in tomato. Commercial field surveys can provide important insights into effects of multi-year rotations, but are not controlled or replicated, limiting their usefulness. Thus, we plan to compliment these efforts through controlled multi-year field trials at UC Davis starting in 2021.

We would like to conduct host range evaluations with cool season rotation crops in the winter (lettuce, wheat, garbanzo) as well as additional warm season crops (eg. alfalfa, potato, garlic, sweet potato). Our goal is to use these trials to develop controlled field trials to assess effects of different rotation crops on F. falciforme development when planted to tomato, similar to trials our lab has conducted for Fusarium wilt. However, with the uncertainty regarding the pandemic, we are hesitant to commit to conducting a trial of this scale in 2021.

It appears that weeds such as nightshade may also be hosts to F. falciforme, developing stem rot. As part of field trials at UC Davis, we are planning to establish non-weeded plots in which to examine a wider range of weeds as hosts in our F. falciforme-infested field. This can help determine whether management of certain weeds will be important to F. falciforme management in tomato.

Based on two years of study, in three separate field trials, we have established the potential to use K-Pam and possibly Miravis and Velum (applied through the drip) to suppress Fusarium falciforme. This is perhaps the first effective management method established for F. falciforme which will be widely adoptable by the industry. However, fungicides will not be the silver bullet, and additional management efforts using tolerant cultivars and potentially other methods will be needed to effectively control this disease. We are providing this information at regional meetings and via publications (Plant Health Management Reports), which we have already started providing to growers interested in using this strategy. We will continue to provide this information via various outreach methods in 2021; we do not foresee conducting further chemical evaluation trials unless additional promising materials emerge on the market.

Acknowledgements

For cultivar trials we would like to acknowledge Dino and Ronnie Del Carlo (Del Carlo Farms) and Kevin Ruble (Woolf Farming) for providing sites for cultivar screenings, as well as Gene Miyao for consultation support for San Joaquin and UC Davis trials. The Swett lab would like to thank TS&L for kindly donating transplants, to help us adapt to COVID-shutdowns, Zach Bagley, Gene Miyao and Kamyar Aram for helping with tomato harvest, farm managers Bryan Pellisier and Alexa Sommers for all their work in field prep, maintenance and harvest assistance and all Swett lab members who assisted with cultivar trials through the season and at harvest. For the UC Davis cultivar trial, matching was provided by HM Clause, Seminis and United Genetics. For crop rotation trials, we would like to thank Harlan Farms as well as Gene Miyao for assisting with coordination of this trial. For the fungicide trial, we would like to again thank Del Carlo Farms for providing a trial site; matching was provided by AMVAC, Syngenta, and FMC.

This project as leverage for other dollars:
This year we were awarded a NIFA-AFRI grant for $1 million over four years; this translates into ~$80,000 per year for four year to my program directly (since it is a multi-PI grant, where I am the lead PI). This award is primarily to develop diagnostic tools for Fusarium pathogens, but also include characterization of the F. falciforme host range. This grant started June 1, 2020.

Further, we have been awarded a CDFA-SCBG for $202,998 over the next 2.5 years to evaluate cultivar resistance in tomatoes and assess the host range of F. falciforme isolates from all affected crops. This is a single PI grant which translates into $75,000 year for my program starting November 1, 2020 through October 31, 2022, and $9,500 for the last six months to April 30, 2023.

We were able to secure $5,200 from industry collaborators to help support the UC Davis cultivar trials in 2020. We were also able to secure $4,000 in industry support for the chemical trials.

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Appendix 1

Additional ongoing research in *F. falciforme* IPM (no funding requested)

Is soil moisture / irrigation management important to reducing *F. falciforme* losses?

Two years of irrigation trials in a *F. falciforme*-infested field at Russell Ranch indicate that irrigation management can influence vine decline, foot rot and yields. Contrary to expectations based on similar pathosystems, disease appears to be promoted under low soil moisture and suppressed under high soil moisture (Figure 2). In 2020 we initiated controlled field studies to examine effects of different irrigation / soil moisture regimes in *F. falciforme* inoculated plants; the first year of this trial focused on optimizing methodologies. We will be adapting this study in 2021 with the objective of evaluating whether irrigation system management can reduce *F. falciforme* losses, and potential to combine irrigation-based management with use of tolerant cultivars to optimize management. No funding is requested for this project.

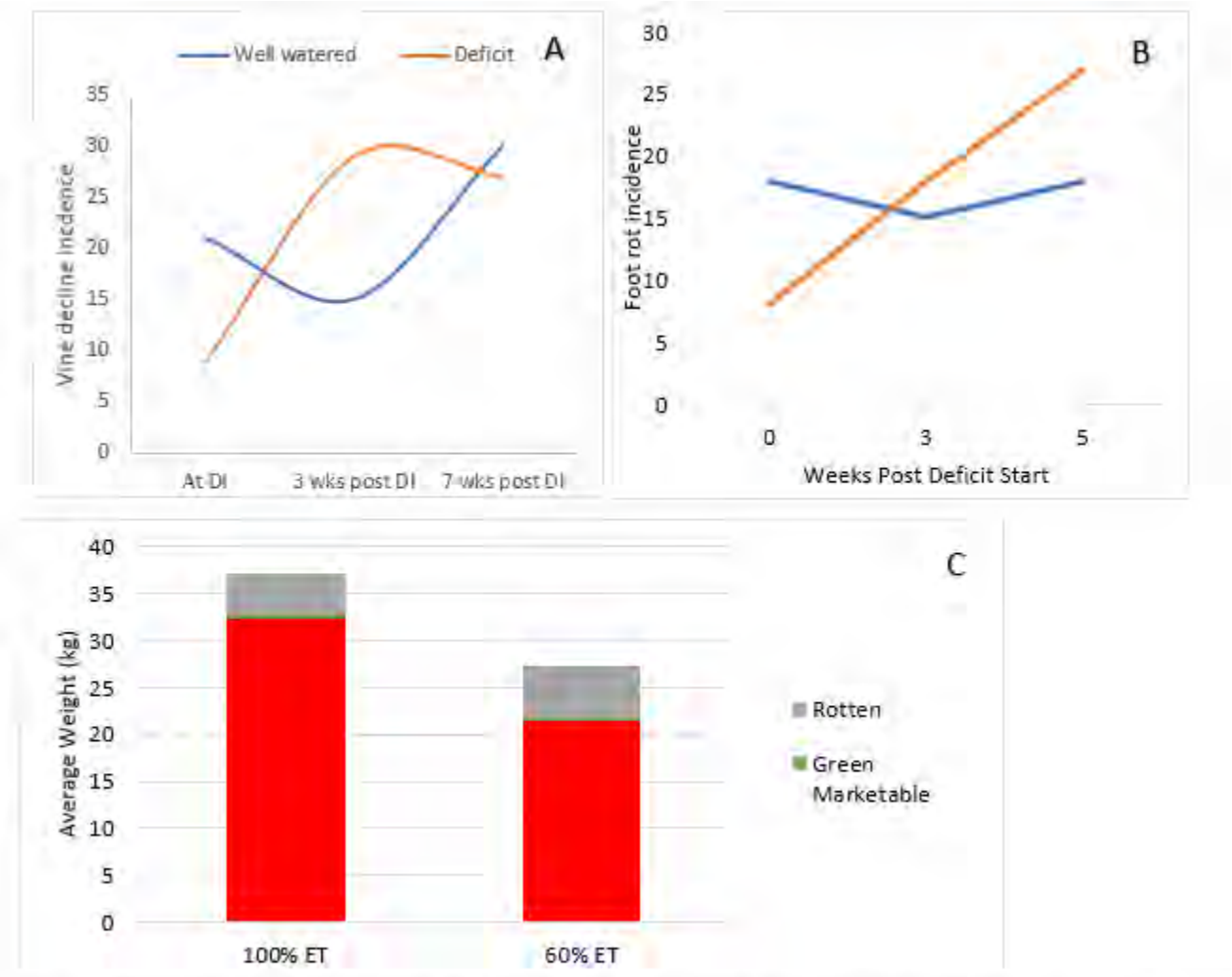

Figure 2. Effects of deficit irrigation at 60% ET vs. well-watered at 100% ET on (A) vine decline, (B) foot rot development and (C) yields in the second year of a two year consecutive trial in a *F. falciforme*-infested field.

DEVELOPING ACCURATE, RAPID AND COST EFFECTIVE TOOLS FOR DIAGNOSIS AND PREDICTIVE MONITORING OF FUSARIUM PATHOGENS OF TOMATO

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- Processing tomato growers, statewide

Key Takeaway(s) at this date:

- Bioinformatic-based identification of potential diagnostic regions for Fol R3 and subsequent set lab analysis:
    - 127 FOSC isolates phenotyped. Most are Fol R3 and several were ambiguous and are being retested.
    - 176 new FOSC isolates were saved in 2020.
    - We are currently phenotyping 57 isolates, with an emphasis on non Fol R3.
    - A subset of characterized isolates will be included in genome-based analysis. The remainder will be used in wet-lab testing following in-silica screening.
    - We hope to complete the genomic DNA library for bioinformatic analysis by 2021
    - We have assembled a reference genome collection of ~70 characterized F. oxysporum forme speciales.
    - Preliminary analysis of diagnostic regions for Fol has been initiated based on an analysis of genomes from 20 isolates and this reference collection; we are awaiting additional isolates to finalize this analysis
- Developing tools for diagnosing F. solani pathogens
    - We have curated a collection of 150 tomato-associated F. solani-type isolates from 2017-2020 as well as historical collections. This includes 115 isolates in 2020 from 93 farms.
    - 104 of these were tentatively identified as F. falciforme. We also identified 11 FSSC isolates from tomato which were not non-F. falciforme.
    - To aid us in efforts to capture the diversity of FSSC pathogens Gene Miyao collected tomatoes from ~70 tomato fields in 2020 which are “historical Fusarium foot rot” fields.
    - To characterize diversity, we worked to develop a protocol for biological analyses of tomato-associated FSSC isolates based on virulence profiles in differential cultivars (Eg. phenotyping). Through over five trials we have established a method.

    - Preliminary trials comparing F. falciforme and historical F. solani f. sp. eumartii indicate F. falciforme virulence based on mortality, but Fse was more virulent based on stem rot abilities. Further trials are needed.
    - These efforts will be furthered by host range analyses, which we are working on via characterizing the F. falciforme host range. Thus far, corn, safflower and sunflower appear to be hosts (see F. falciforme proposal).
    - Diversity will also be evaluated genetically in 2021.
- We have also identified at least four other putatively pathogenic Fusarium species, including several FSSC species. These are not documented as tomato pathogens but have been documented as crown rot pathogen of other crops. We are characterizing these as pathogens, to determine whether they need to be added to our diagnostics efforts.
- Developing predicative monitoring tools.
    - Tissue bating results indicate that we get better detection with a larger sieve, which includes larger tissue fragments. Overall, they support the hypothesis that tissue can be a reservoir for Fol.
    - Putative *Fusarium* colonies were present at 3/9 sampling points in a high disease incidence plot and at 2/9 sampling points in a low incidence plot, suggesting that we should be capable of detecting the pathogen even at lower levels using this sampling strategy.
    - Molecular testing is needed to confirm identity as Fol for both trials
    - These sampling strategies will be repeated for winter sampling in the absence of tomato plants.

Introduction:

Fusarium wilt, caused by *F. oxysporum* f. sp. lycopersici (Fol) race 3 has been a number one pathogen threat for many processing tomato growers in recent years. Resistant cultivars are the best tool for management but many fields planted to Fusarium wilt race 3 resistant (F3) cultivars are still suffering major yield losses. While this is due in part to issues with F3 resistance gene stability, most F3 diagnoses are are Fusarium crown/stem rots caused by *F. falciforme* and *F. oxysporum* f. sp. *radicis lycopersici* (Forl). Reflecting this, in 2020, 50% of all F3 samples were diagnosed with F. falciforme (40%) and Forl (10%). This reflects that, in addition to Fusarium wilt, these other Fusarium pathogens pose ongoing management challenges. All of these diseases relay primarily on pathogen-specific management methods, from cultivar selection to chemical control and crop rotation. At present, growers either submit plant samples for diagnosis to the Swett lab, to the CDFA lab, or to a private lab. The Swett lab is the only lab that uses Fol specific molecular methods to diagnose this disease. All other labs use species-based identification and symptoms. These diseases are very challenging to diagnose. They look like each other and like many other diseases, including Verticillium wilt and bacterial canker. Even more advanced molecular methods like PCR diagnostics are flawed, as documented by recent studies where we have demonstrated generation of both false positive and false negatives. These misdiagnoses are costly, since they lead the grower to waste money on costly management methods that do not work, resulting in economic losses from yield reductions. Although there are accurate diagnosis tools, these all rely on inoculating plants and waiting for symptom development, a process which takes ~4 months and is not timely enough for growers to make management decisions.

We need timely, accurate, management-informing diagnosis tools. Ideally, these tools would be easy to use and adaptable to the field. To this end, the first objective of this project is to use a whole genome approach to develop accurate, rapid, widely available, and cost-effective molecular-based diagnosis tools for all Fusarium pathogens of tomato. The end goal is a field-ready multi-pathogen detection tool that can be used by a range of practitioners (eg. diagnosticians, crop advisors) to simultaneously diagnosis all Fusarium pathogens of tomato. Previous years efforts have focused on Fusarium wilt race 3; in 2020 we expanded our efforts to *F. falciforme* as another high impact pathogen. Critical to the development of accurate diagnostics tools is a comprehensive, characterized isolate collection which can be used to validate prospective diagnostics regions; this needs to include both pathogens and non-pathogen look-alike isolates. These isoaltes need to come from a wide geographic range, in order to capture the genetic diversity of the pathogen/ non-pathogen groups. Thus, the major focus of the early stages of this project have been to build a comprehensive, characterized collection of pure-cultured isolates from across the state. We have partnered with Florida to get a representative collection from both states. From there, we are working to build our

library of tomato-associated Fusarium genomes. At present, we have successfully sequenced the genomes of over 15 isolates, and have identified several putative diagnostics regions for Fol race 3.

It would be a major asset for growers to be able to determine whether their management methods, such as rotation crops, fumigation, or other soil treatments successfully reduced pathogen loads prior to planting tomato. In cases where growers are going into new fields, it would be helpful for they to know if there are any Fusarium pathogens present which need to be managed. This could help in selecting cultivars, pre-plant chemical treatments, or simply deciding not to plant tomato. Although there are soil testing tools for Fusarium pathogens of other crops, there are no existing tools for quantifying any Fusarium tomato pathogens in soil. Molecular tools are a must since there are many look-alike non-pathogenic Fusariums in the soil. The second goal of this project is thus to develop predictive monitoring tools to enable practitioners to determine risk of planting tomatoes/certain cultivars. In 2020 we continued to emphasize soil monitoring tools for Fusarium wilt race 3. Downstream we aim to expand efforts to seed and water, and to other Fusarium pathogens. Goals for this year were to: (1) develop soil sampling strategies and (2) identify Fol race 3 diagnostic regions (synergistic with objective 1) to develop a quantitative PCR (molecular) method for detecting and quantifying Fol race 3 in soil. We have made significant progress in establishing that tissue baiting methods offer promise as a means to detect the pathogen at hot spots; further sampling studies are in process and we ask for support to complete these studies.

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

Main goal: Our main goal is to develop in-planta diagnostics tools and predictive soil monitoring tools to help growers select the right management strategies for high impact Fusarium diseases of tomato

Objective 1. Develop molecular-based multi-pathogen diagnostics tool for Fusarium pathogens of tomato
1.1. Build a comprehensive phenotyped collection of Fusarium oxysporum isolates associated with tomatoes for genetic analysis.
1.2. Build a genomic DNA library of *F. oxysporum* isolates from tomato and use to identify genetic regions unique to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) race 3 and Fol (all races).
1.3. Screening these unique gene regions for generation of false negatives and false positives (bioinformatic and wet lab validations) and start to develop quantitative PCR methods for Fol race 3.
1.4. Extend diagnosis efforts to include *F. falciforme* [Fusarium solani species complex (FSSC) pathogen]: pathogen curation and development of basic identification methods.

Objective 2. Develop predictive monitoring tools for Fusarium wilt race 3 in soil
2.1. Analysis of hierarchical sampling strategies
2.2. Optimizing Fol R3 detection using soil tissue extraction
2.3. Developing a molecular-based quantification tool for Fol race 3 in soil.

Methodology and Results:

Objective 1. Develop molecular-based multi-pathogen diagnostics tool for Fusarium pathogens of tomato

The major focus on our current efforts is to develop better diagnostic tools for Fol R3 and FSSC pathogens, including F. falciforme; as part of Fol R3 effort, we also hope to elucidate improved molecular diagnostic methods for Forl. As part of our statewide surveys, we are also monitoring for other Fusarium pathogens of tomato. In the

last two years we have found several additional Fusarium species associated with stem rot on processing tomato: Fusarium brachygibbosum, F. redolens, F. equiseti, F. keratoplasticum and F. accuminatum. Pathogenicity trials are underway to determine if these are pathogenic on tomatoes.

Fol race 3 diagnostic tools		
Task	**Goal**	**Status and notes**
1.1 Build phenotyped collection of *F. oxysporum* isolates (non-pathogen, Forl)	45 isolates phenotyped; phenotyping of 51 additional isolates underway	Complete phenotyping work for 51 isolates by early summer 2021; may need to repeat some trials
1.2 Building a genomic library of *F. oxysporum* from tomato in California and Florida	Genome sequenced and annotated and library created for 20 isolates; sequencing underway for 13 isolates; 14 additional isolates being retested and DNA extraction planned for early 2021; final set of isolates from 2020 diagnostics to be determined	Genomic DNA analysis for 14 isolates done by mid 2021 and for final two sets (currently being phenotyped) by the end of 2021
1.3.1 Identifying unique regions of the Fol and Fol race 3 genome	Building FOSC database—have ~70 formae specialis; goal for 80-90 of the 106 known f. sp. Bioinformatic validation conducted for initial set of 20 FOSC tomato genomes	Bioinformatic analyses will be completed once all genomes are completed. Covid delays continue.
1.3.2 PCR, RPA and TaqMan qPCR development	Planning to initiate once bioinformatic validation is done. Covid delays continue.	Planning to initiate once bioinformatic validation is done. Covid delays continue.
1.3.3 Wet lab validation of regions	Screening isolates in 1.3.1 using methods developed in 1.3.2.	Planning to initiate once bioinformatic validation is done. Covid delays continue.

Objective 1.1-1.3: Developing diagnostic tools for Fol race 3

In our efforts to develop a large isolate collection for bioinformatic and wet lab validation, in 2020, we saved pure cultures of 176 FOSC isolates from tomatoes. These isolates were all analyzed by SIX3 PCR and species identity analysis via elongation factor sequencing is underway (see below tables); for some we are expanding on SIX analysis to repeat SIX3 and also conduct SIX1 PCR (Tables __ and __) as well as conducting elongation factor gene sequencing to double check identity as Fusarium oxysporum.

We are currently phenotyping 51 isolates, including several re-test isolates from previous years which we deem important to our analyses. Collaborator Martin requires genomes of at least ten additional non-pathogens, five Forl, and three Fol R1 and R2 for bioinformatic validation. The remaining characterized isolates will be used in wet-lab validation of unique diagnostic regions identified in bioinformatic analysis.

Advancing our efforts towards genomic library curation and bioinformatic analyses of unique genomic regions, overall, we continue to build the reference database, and this year added ~30 new formae specialis, bringing the total to ~70 of the 106 known f. sp's.

We have curated 20 FOSC tomato genomes, including 17 Fol race 3 and 3 non-pathogens. We are working to add 14 new isolates which we have characterized phenotypically in early 2021 and additional isolates currently being phenotyped in later 2021. To develop an RPA tool, we have purchased an RPA machine and are currently being trained on it's use in anticipation of developing our own RPA protocols.

Table 1. 127 isolates have been phenotyped. Several reveal false negative (blue) and false positive (red) results from molecular-based diagnosis methods

SIX	No Isol Total	FolR1	FolR2	FolR3	Forl	Non pathogen	Ambiguous
(+)	64	4	2	45	2	5	6
(-)	31	2	0	2	6	9	12
No Six ID	21	2	0	6	0	5	8
Total	**127**	**8**	**2**	**53**	**8**	**19**	**26**

Table 2. Expanded molecular testing of phenotyped isolates; note that all non-pathogenic isolates were in this case six negative, although one Forl isolate remained six positive. All of these isolates are being repeat tested in phenotyping trials prior to genome sequencing.

SIX	No Isol Total	FolR3/4	Forl	Non pathogen	Ambiguous
FOSC conf	9	2	1	3	3
FOSC to conf	1				1
SIX 1(+)		2	1		4
SIX 1(-)				3	
SIX 3(+)		2	1		4
SIX 3(-)				3	
Total isolates	**10**	**2**	**1**	**3**	**4**
Total PCR tests		**4**	**2**	**3**	**8**

Table 3. Initial molecular analyses of 2020 diagnostic isolates to be included in phenotyping; Fusarium wilt isolates were tentatively called race 4 because they all came from F3 cultivars

SIX	FW race 4?	Forl	FolR4?/Forl
FOSC conf		1	1
FOSC to conf	4	9	1
SIX 1(+)			1
SIX 1(-)		1	1
SIX 3(+)	4		2
SIX 3(-)		10	1
Total isolates	**4**	**10**	**2**
Total PCR tests	**4**	**11**	**5**

Objective 1.4: Developing diagnostic tools for F. falciforme

***F. falciforme* diagnostic tools**		
Task	**Description and status**	**Competed by**
1.4.1 Building a library of *F. falciforme* isolates: isolate curation	Three years of isolates curated; continue to curate in 2020	Underway; no CV19 delay. Have curated 16 isolates thus far. Gene Miyao conducted a survey of ~10 Yolo farms with F. solani/foot rot.
1.4.2 Develop phenotyping method for *F. falciforme*	Develop a method to differentiate FF from other Fusarium's by fall 2020	Done. Have developed an inoculation method that results in symptoms; differential cultivars segregate from Fol and Forl.

1.4.1. Isolate curation. We have curated a collection of 150 FSSC pure cultured isolates from tomatoes in California, from dozens of farms. This included 115 isolates in 2020 from 93 farms. 104 of these were tentatively identified as F. falciforme based on homology with FSSC group 3+4. However, we also identified 11 non-F. falciforme isolates which were most homologous with FSSC 5 AKA true F. solani (2 isolates), FSSC 2 AKA F. keratoplasticum (4 isolates) (Coleman 2016), as well as two apparently unnamed phylogenetic species, FSSC 5-6 (1 isolate) and FSSC 20 (1 isolate).

To aid us in efforts to capture the diversity of FSSC pathogens in the region in general, and specifically to help us address the question about the relationship between what was called F. solani f. sp. eumartii and the current phylogenetic species referred to as F. falciforme. Gene Miyao collected tomatoes from ~70 tomato fields in 2020 which are "historical Fusarium foot rot" fields. This accounts for the large isolate numbers in 2020. We are planning to characterize all of these isolates phylogenetically to both determine the diversity of F. falciforme populations and also relationships with both these other FSSC isolates and historical F. solani f. sp. eumartii isolates.

Table 7. Expansive surveys' of historical foot rot fields in 2020 revealed up to four additional FSSC species associated form foot rot

County	No isolates	No FSSC 3+4	Other	No fields	Cultivars
Colusa	3	3		1	H1662(F3)
Fresno	12	11	1: FSSC 2-j	5	H1428, N6415
Merced	7	7		2	
San Joaquin	8	6	1: FSSC 5-6; 1: FSSC 5-g	5	
Yolo	82	76	3: FSSC 2-j; 1 FSSC 5-g; 1 FSSC 1-d	47	HM58801, HM3887, N6428, 9025, HM58841, 9011, HM4909
Sutter	3	1	2: FSSC 20-d	1	SV9025
Total	**115**	**104**	**11**	**93**	

Table 8. 150 FSSC tomato isolates curated from tomatoes across four years

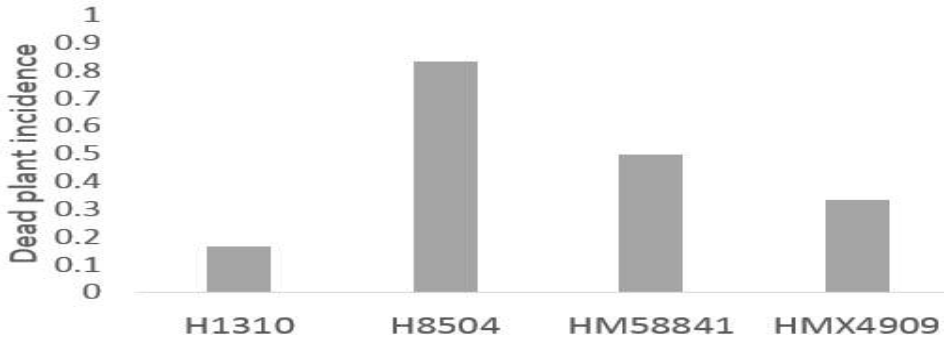
Year	County	No. Isolates saved
2017	Fresno	2
	Kern	2
	San Joaquin	6
	Yolo	10
2017 Totals	*4 counties*	*20*
2018	Fresno	3
	Merced	2
	San Joaquin	1
	Yolo	6
2018 Totals	*4 counties*	*12*
2019	San Joaquin	8
	Sutter	3
	Ventura	1
	Yolo	2
2019 Totals		*14*
2020	Colusa	3
	Fresno	11
	Merced	7
	San Joaquin	6
	Yolo	76
	Sutter	1
2020 Totals		*104*
Total		**150**

1.4.2. Assessing biological diversity of FSSC isolates from tomato

Although genetic differences provide valuable insight into relationships, they can misrepresent biological diversity. In some cases, like with F. oxysporum pathogens, organisms that are genetically similar are biologically different (cause different diseases in different hosts). In other cases, organisms might be phylogenetically distinct but cause the exact same symptoms in the same hosts, making them ecologically the same species or strain. We thus are complimenting our genetic analyses with biological analyses of F. falciforme and FSSC diversity in diseased tomatoes. This is being done by (a) evaluating differences in virulence across a range of cultivars—in other words, phenotyping, and (b) by evaluating difference in host ranges.

1.4.2.1. Developing an F. falciforme inoculation and phenotyping method. The first component of a good phenotyping method is to have meaningful phenotypes. We have found that vine decline is the most meaningful indicator of variations in virulence in the field, so we have been working to develop a greenhouse assay that leads to decline symptoms. We initially were struggling to develop an inoculation method in the greenhouse that would result in vine decline symptoms over a reasonable trial window (9 weeks or less). We evaluated several different methods that resulted in no decline symptoms: stem wound (no decline), plug dip with root wounding (no decline), plug dip with stem wound (no decline) (Figure 1). Finally, plug dip with foot wound made below the soil line resulted in vine decline by 9 weeks post inoculation (Figure 2).

Figure 1. Four separate inoculation trials were unable to results in canopy symptoms. These were all conducted without stem wounding.



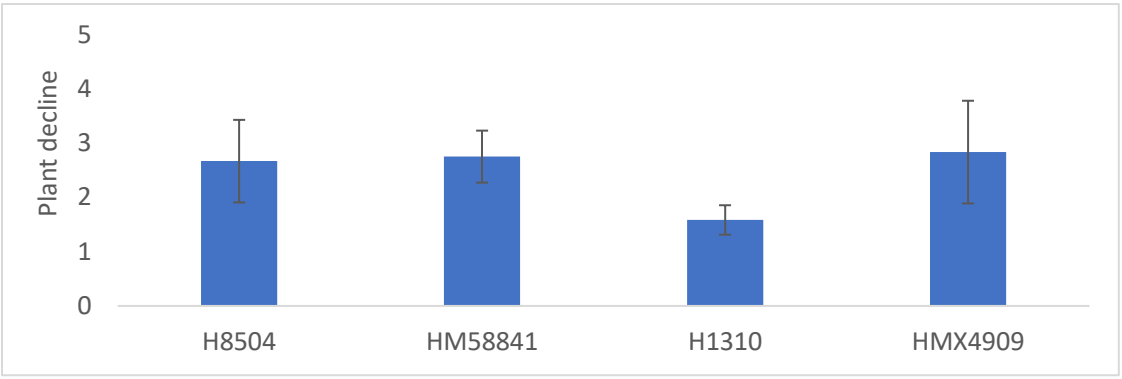


Figure 2. Results from the first inoculation method (dip inoculation of a seedling with a foot wound) which resulted in vine decline symptoms.

Figure 3. Incidence of canopy decline in two phenotype development trials, showing canopy and crown rot symptoms resulting from inoculation.

The next step has been to develop a method which gives us vine decline levels similar to what is seen in the field, to enable us to profile F. falciforme using highly susceptible and more resistant cultivars. Initial efforts were based on only one year of data and led to poor cultivar selection, which gave us ambiguous results wherein cultivars that were supposed to be more resistant (HM58841) had higher vine decline than those that were supposed to be more susceptible (H1310 (Figures __ and __). After the 2020 field season, we now have two years of replicated field data, based on which we have selected a different suite of cultivars which have performed consistently across years, which we are looking at for developing phenotyping methods.

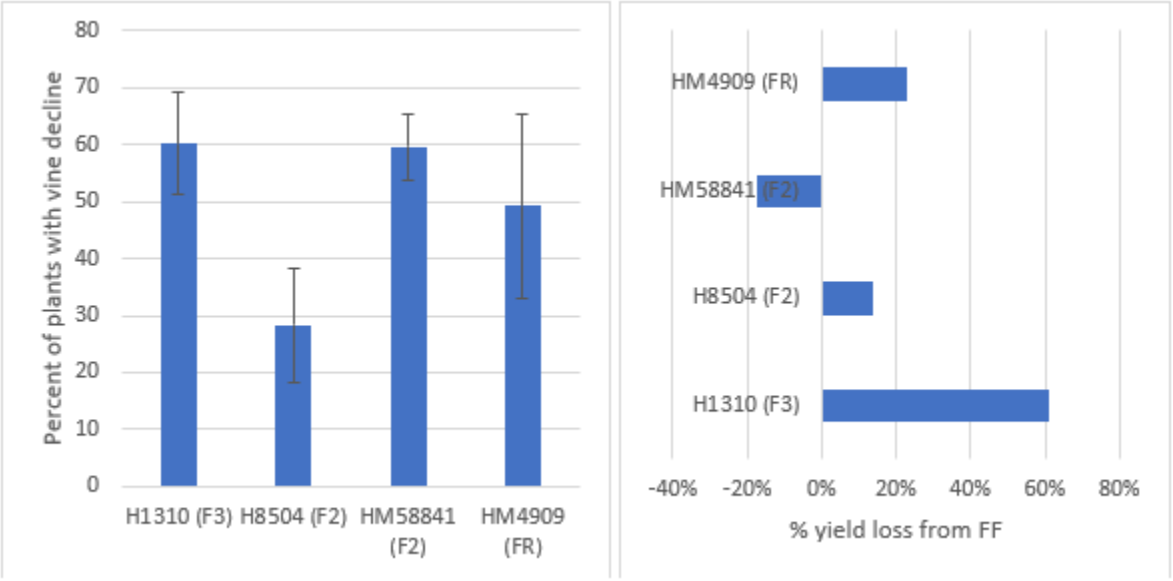


Figure 4. Preliminary phenotype development efforts resulted in vine decline levels that did not corroborate with field vine decline levels or corresponding yield loss.

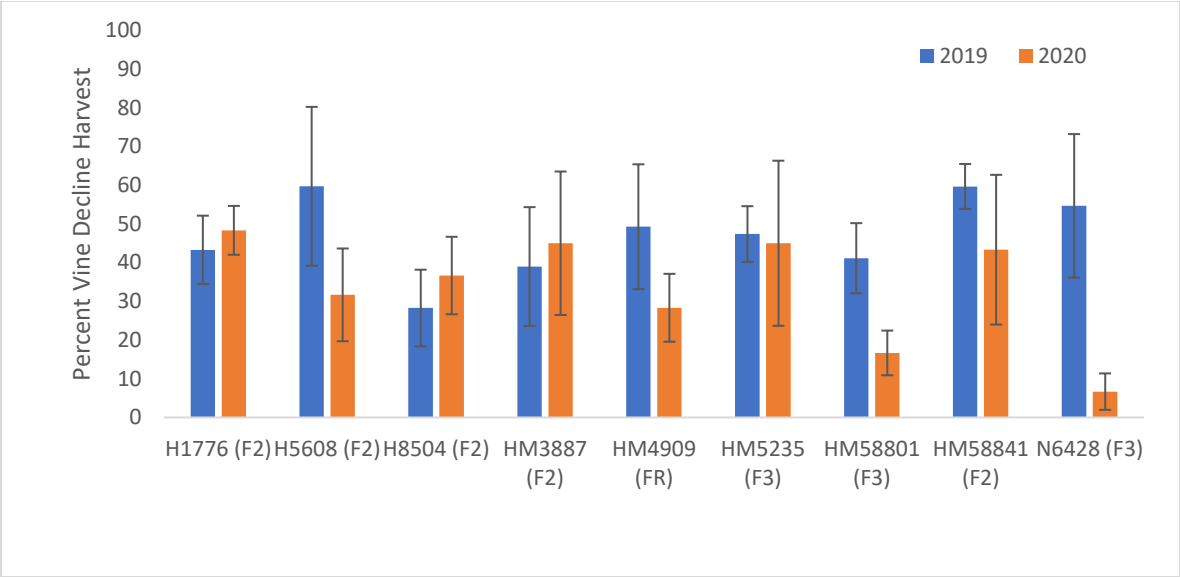


Figure 5. Vine decline data for two years of field trials; many varieties did not perform consistently. However, H1776, HM5235 and HM3887 appear to be consistently susceptible and both H8504 and HM58801 appear consistently more resistant. N6428 did not perform consistently, but had very low vine decline in 2020 (and also in 2018, data not shown).

We are also adapting the method, testing both a slurry inoculation method and a spore suspension method and are allowing the trial to run longer. In initial efforts we added N6428, which has been a fairly consistently resistant cultivar and H9036, which was highly susceptible. The spore suspension dip inoculation method resulted in ambiguous resolution of cultivars that were supposed to be more resistant (orange bars), intermediate (blue) and highly susceptible (red). However, the spore slurry soil inoculation method resulted in a clear segregation of difference in vine decline in the more resistant cultivar N6428 (none) and the more susceptible lines (10-25%), although there was not clear segregation between the intermediate (blue) and highly susceptible (red). There was also a detectable difference in the incidence of crown rot that followed these same patterns. Based on this, we are conducting a replicated trial using the spore slurry method, including additional cultivars, which we are hoping will represent the final stage of methods development. However, we believe we have developed baseline methods for F. falciforme phenotyping and have started to use this method to see if we can differentiate FSSC isolates.

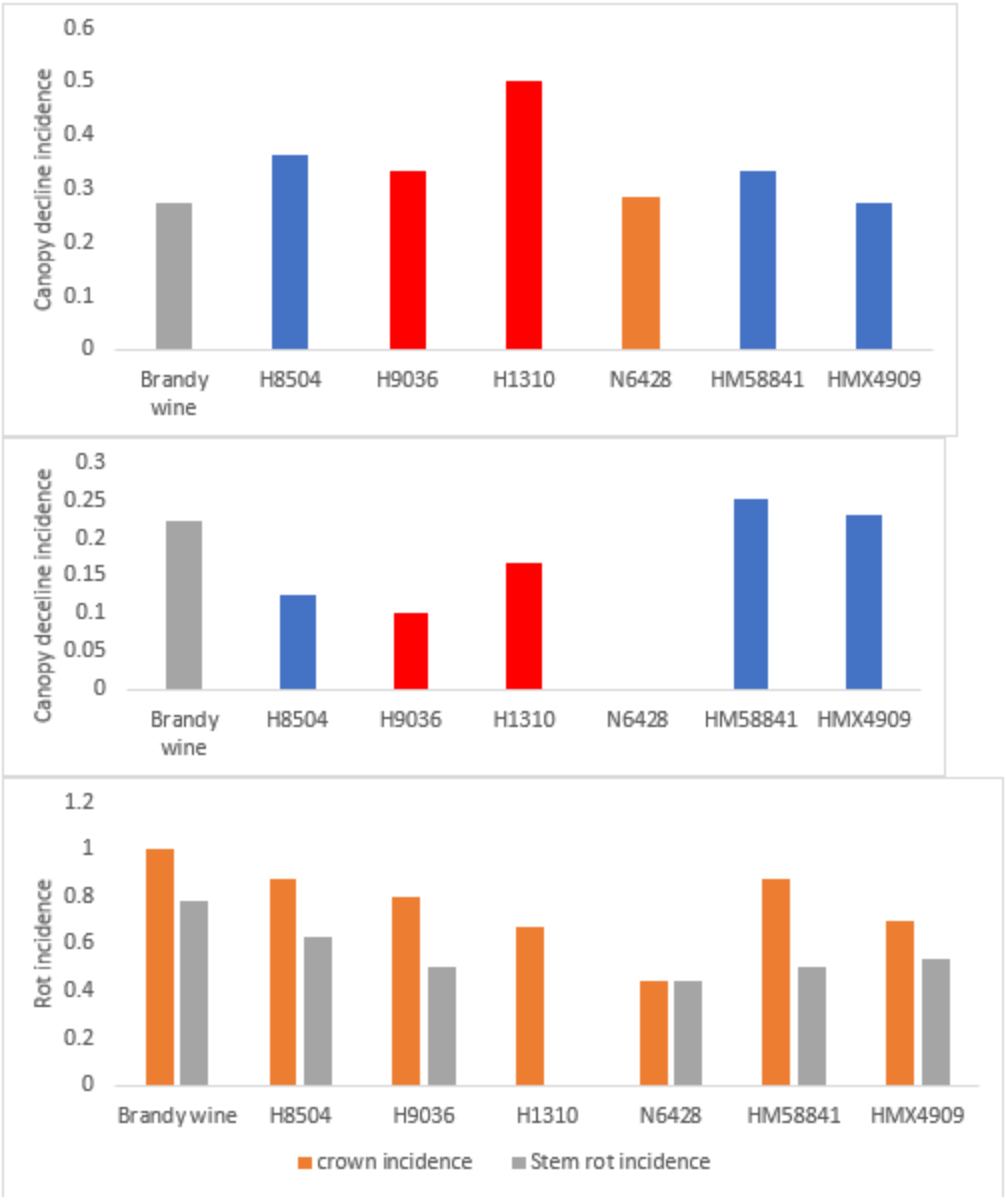


Figure 6. Percent of plants with canopy decline 9 weeks post inoculation using (A) a spore suspension dip or (B) a spore slurry soil inoculation method. We included seven cultivars, one considered more resistant (orange bar), three with intermediate resistance (blue), two highly susceptible (red), and one (grey) that we included to evaluate potential as a susceptible control. (C) Rot incidence using the spore slurry method.

Characterizing the diversity of FSSC tomato pathogens in California

To lend insight into the current identity of what we previously called F. solani f. sp. eumartii and its relationship with F. falciforme we initiated greenhouse trials using F. falciforme and historical Fse isolates, to determine if there are differences in virulence and cultivar susceptibly profiles. The spore dip inoculation method described above was used for this trial.

F. falciforme and historical Fse isolates were not different based on decline, but F. falciforme appears more virulent based on abilities to kill more H8504 plants and cause mortality in HM58841 where Fse could not. Interestingly, although Fse is reported NOT to cause stem rot, in our trials it caused extensive stem lesions, much longer than those caused by F. falciforme.

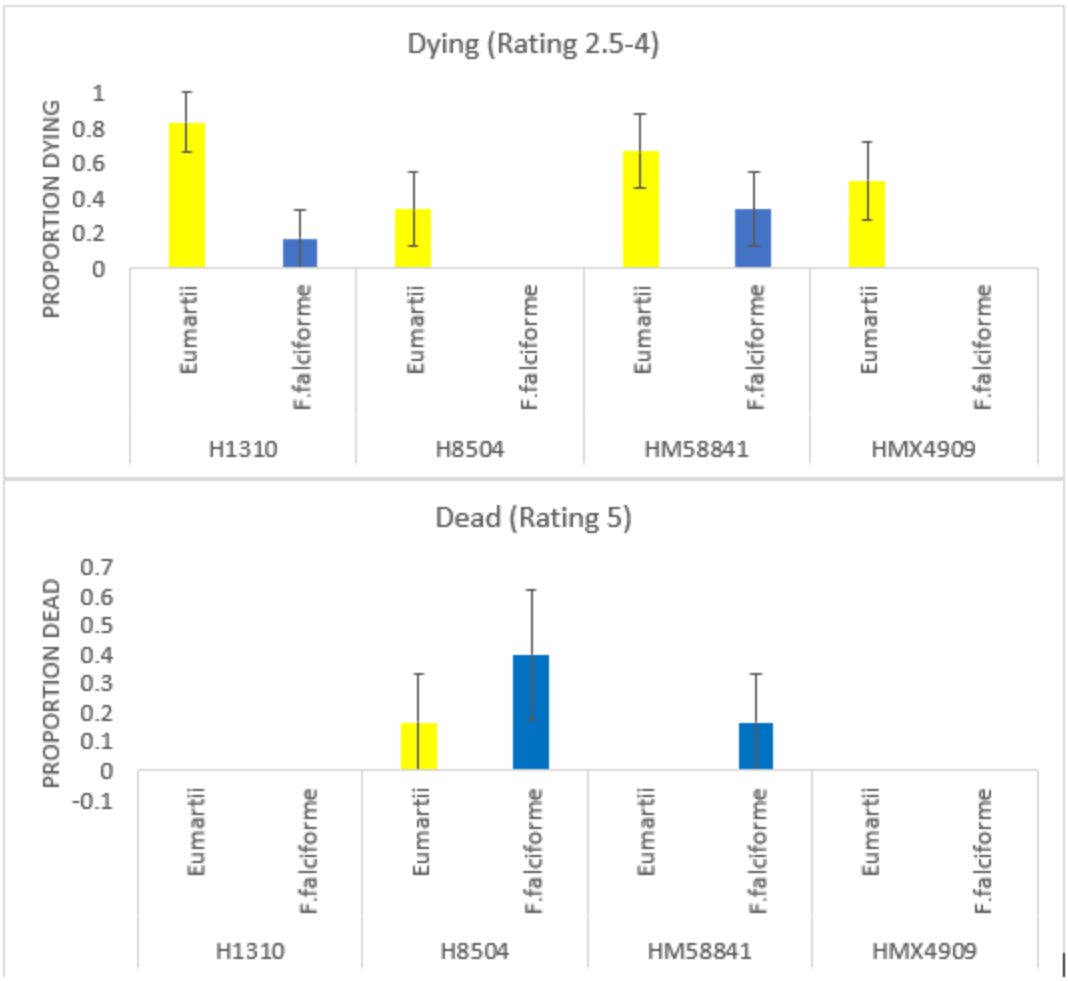


Figure 7. Comparing virulence and cultivar susceptibility between F. falciforme and historical Fse isolates based on percent of plants with early decline (top) and that died (bottom) during the trial.

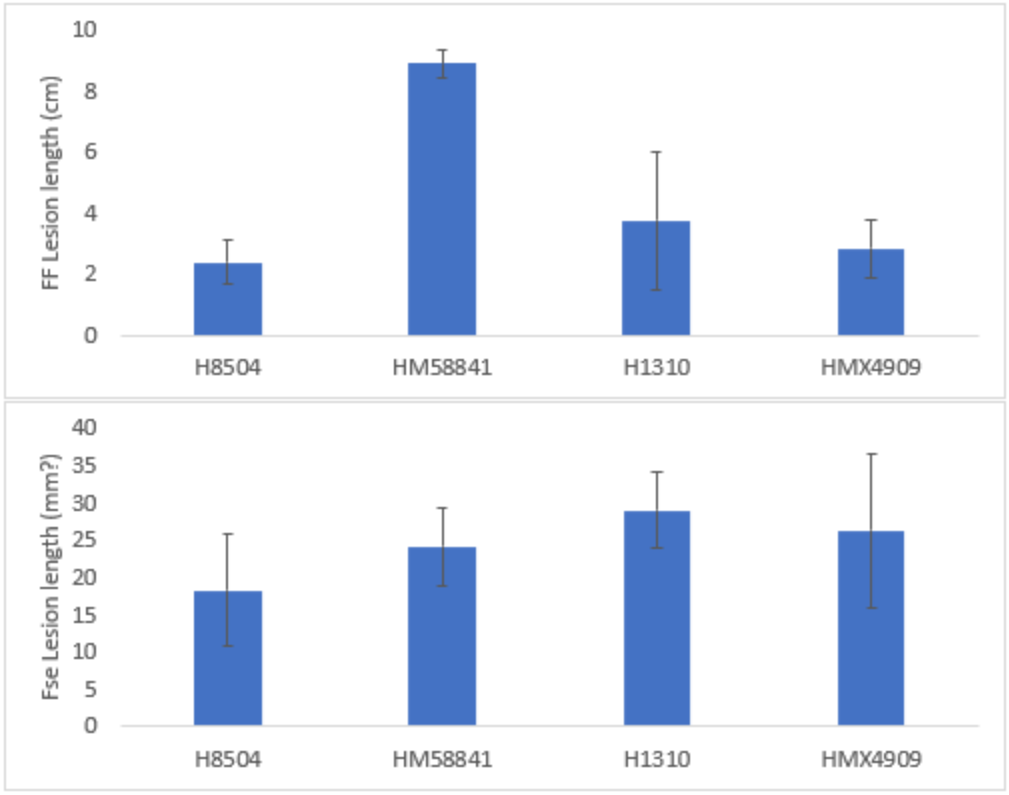


Figure 8. Lesion length produced following inoculation of F. falciforme and Fse.

We are also conducting a large-scale phylogenetic analyses under the guidance of Dave Geiser who, due to covid-19, has no staff to do this work himself. As part of both effects, we conducted an FSSC survey for Yolo county, wherein Gene Miyao collected 5-20 plant samples from each of ~45 fields (separating out different cultivars) where he and Mike Davis historically found what they called F. solani f. sp. eumartii. We are including these isolates in our phylogenetic analyses with the aim of resolving the identity of what we previously called Fse.

Objective 2. Develop predictive monitoring tools for Fusarium wilt race 3 in soil

Task	Goal	Status
Identifying and validating diagnostics regions for Fol race 3	See above 1.3.1 and 1.3.3. Aimed to complete by Dec 2020	Delayed due to CV19 as described above in 1.3.1 and 1.3.3
Developing a PCR/qPCR diagnostics assay for soil and seed detection	See above 1.3.2. Aimed to compete by June 2020	Delayed due to CV19 as described above in 1.3.2.
Developing soil sampling strategies	Evaluating tissue baiting methods and hierarchical sampling strategies. Complete by Dec 2020	Underway-delayed by CV19 but anticipate completing by the end of the project period
Developing qPCR for soil detection thresholds	Awaiting development of qPCR assay; initiating in 2020 and completing in 2021	Should still be able to complete in 2021
Provide methods to labs-ensure labs are well trained	Workshops; protocols developed	Held a Laboratory diagnosis workshop in Feb 2020

Developing soil sampling strategies: Evaluating tissue baiting methods and hierarchical sampling strategies

To evaluate the efficacy of tissue baiting and hierarchical sampling strategies for detecting Fol race 3, we collected soil in a tomato field infested with this pathogen. For tissue baiting, approximately 500 grams of soil was collected twice using trowels about 6 inches away from the crown of six diseased and six healthy tomato plants. A random selection of tomato tissue (primarily roots) that was unable to pass through 8-mm and 1.6-mm mesh size sieves was incubated on selective culture media. Fungal colonies emerging from tissue that morphologically resembled *F. oxysporum* were subcultured and will be confirmed as Fol race 3 through PCR in early 2021. For hierarchical sampling, the centerline of two perpendicular transects was randomly positioned in one location in a field with high disease incidence and in two locations in a field with a low disease incidence. At each hierarchical sampling site, soil samples were collected as two 1-cm diameter cores at 0 to 14 cm depth at the centerline and at 5 ft and 10 ft away from the centerline at each cardinal direction. Hierarchical sampling was conducted pre-incorporation of tomato plants in October and post-incorporation in December, 2020. Soil samples were sieved to <4-mm size and dilution plated onto selective media. Fungal colonies that resembled *F. oxysporum* were subcultured for morphological identification and will later be confirmed as Fol race 3 via PCR in early 2021.

For samples collected in early October, putative *F. oxysporum* was isolated from tomato tissue in 4 out of 5 baiting samples at both healthy and diseased plant sites. *F. oxysporum* was more frequently recovered from tissue samples that were >8mm-size than between 1.6- and 8-mm size.

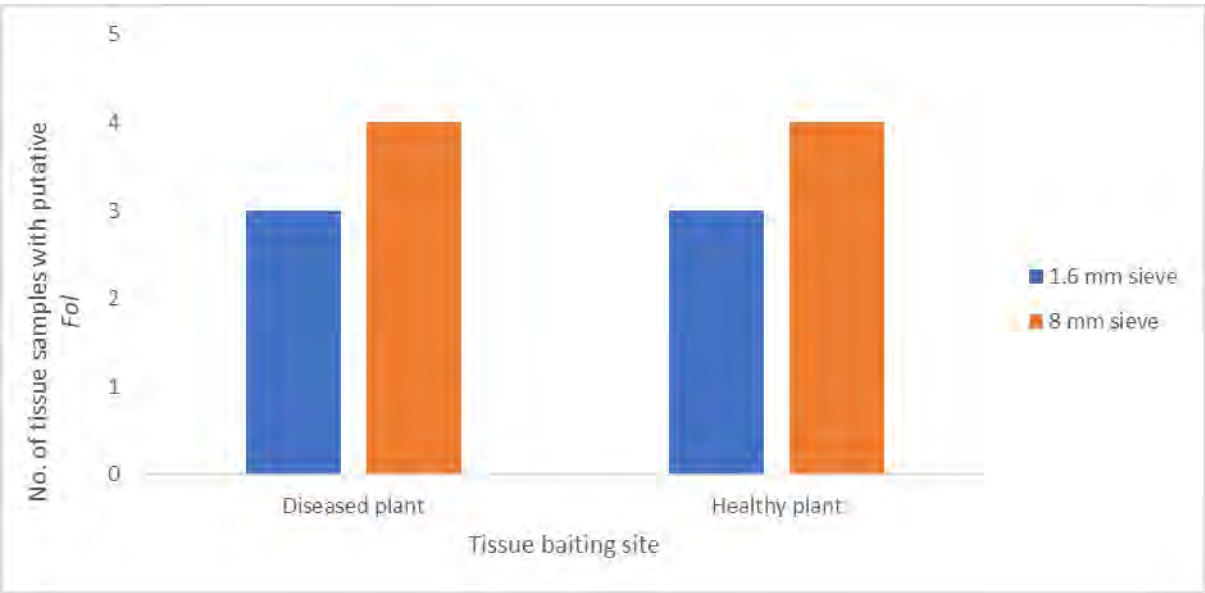


Figure 9. Number of tissue samples with putative Fol colonies out of five total samples.

Soil assays were completed for two out of three hierarchical sampling sites for samples collected in early October. Putative *Fusarium* colonies were present at three sampling points in a high disease incidence plot and at two sampling points in a low incidence plot, out of a total of 9 sampling points per hierarchical sampling site. This suggests that we should be capable of detecting the pathogen even at lower levels using this sampling strategy.

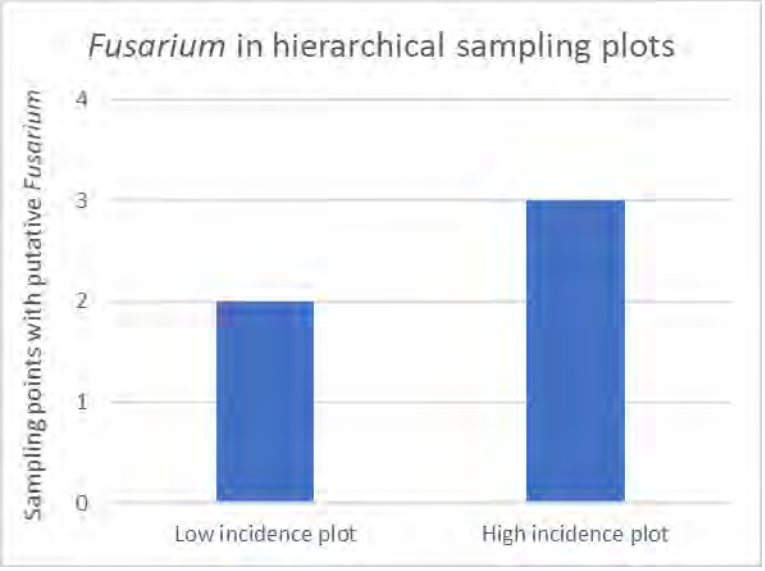


Figure 10. Putative Fol was detected in both low and high disease incidence plots in at least 2/9 samples, using our soil sampling method.

Discussion:

Isolate characterization efforts for genome library. 127 FOSC isolates phenotyped. Most are Fol R3 and several were ambiguous and are being retested. 176 new FOSC isolates were saved in 2020. We are currently phenotyping 57 isolates, with an emphasis on non Fol R3. Genomes have been sequenced and annotated for 15 Fol R3 isolates and preliminary analysis of unique genomic regions in comparison with the full FOSC genomic library (~70 f. sp.) indicates that there are several candidate diagnostic regions. However, we need the non Fol isolates above to determine whether they are truly unique. Including Forl isolates can also facilitate screening for unique diagnostic regions for this pathogen as well.

We have curated a collection of 150 FSSC pure cultured isolates from tomatoes in California, from dozens of farms. This included 115 isolates in 2020 from 93 farms. 104 of these were tentatively identified as F. falciforme. We also identified 11 FSSC isolates from tomato which were not non-F. falciforme. To aid us in efforts to capture the diversity of FSSC pathogens Gene Miyao collected tomatoes from ~70 tomato fields in 2020 which are “historical Fusarium foot rot” fields. We are planning to characterize all of these isolates phylogenetically to both determine the diversity of F. falciforme populations and also relationships with both these other FSSC isolates and historical F. solani f. sp. eumartii isolates.

Since genetic analyses are limited in detecting diversity, we are conducting biological analyses of tomato-associated FSSC isolates. We have spent over a year developing methods to evaluate virulence based on differences in disease development across a suite of cultivars (phenotyping trials). We have tested over five different methods, many of which did not work, but this year we have been successful in establishing the basic parameters for this protocol. Using our new method, we have started by comparing F. falciforme and historical F. solani f. sp. eumartii isolates. In preliminary trials we detected greater F. falciforme virulence based on mortality, but Fse was more virulent based on stem rot abilities. We have also initiated efforts to characterize FSSC isolates diversity based on host range. To this end, our objectives dovetail into those in our F. falciforme management project, by evaluating the host range of F. falciforme pathogenic on tomato. This synergy will continue with efforts in 2021.

We have also identified at least four other putatively pathogenic Fusarium species, including several FSSC species. These are not documented as tomato pathogens but have been documented as crown rot pathogen of other crops. We are characterizing these as pathogens, to determine whether they need to be added to our diagnostics efforts.

Tissue bating results indicate that we get better detection with a larger sieve, which includes larger tissue fragments. Overall, they support the hypothesis that tissue can be a reservoir for Fol, but molecular testing is needed to confirm identity as Fol. Putative *Fusarium* colonies were present at 3/9 sampling points in a high disease incidence plot and at 2/9 sampling points in a low incidence plot, suggesting that we should be capable of detecting the pathogen even at lower levels using this sampling strategy. These sampling strategies will be repeated for winter sampling in the absence of tomato plants.

We foresee this project continuing for several years. We now have a four year NIFA grant which can help support our major research activities and would likely just be looking to CTRI to assist with grower collaborations and to help cover costs of unforeseen projects that put us beyond our NIFA budget.

As a parallel project, we are currently working to evaluate phylogenetic relations and compare virulence traits of isolates we currently call F. falciforme and isolates curated by Mike Davis that he called F. solani f. sp. eumartii. This will help us understand whether there is just one or multiple F. solani pathogens affecting tomato, and is critical to diagnostic tool development.

This project as leverage for other dollars:

CDFA-SCBG. **$168,000**. PI-Swett. Developing innovative detection tools and cultural solutions to minimize economic damage of Fusarium wilt in tomato. Awarded 2019, 2.5 year grant. **$35,000** for 2020, which is being used to cover costs of a project scientist (not included in this budget) and supplies.

NEW NIFA-AFRI Tactical and Applied Biosciences grant. **$1.0 million over four years.** Lead PI-Swett. Pathogenomics-Based Development of Crop-Specific Diagnostics Tools for Emerging and Expanding Fungal Diseases in the U.S. Start date: June 2020. ~$80,000 per year for four year to my program directly. This award is aimed at developing tools for diagnosis and soil, water and seed detection for Fusarium pathogens of tomato.

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DISEASE DIAGNOSIS, PATHOGEN MOVEMENT / EMERGENCE MONITORING, NEW PATHOGEN IDENTIFICATION AND FUSARIUM WILT RACE 4 MONITORING IN SUPPORT OF THE PROCESSING TOMATO INDUSTRY

CASSANDRA SWETT

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- Processing tomato growers, statewide

Key Takeaway(s) at this date:

- We received a record 173 tomato samples in 2020—more than any year previously.
- Across the state, this was a year for high Fusarium wilt, F. falciforme, root knot nematode, Verticillium, southern blight, TSWV and curly top based on our monitoring efforts. We also saw Phytophthora root and crown rot in several fields as well as necrotic streak virus.
- We saw a lot of multiple pathogen infections. For instance, Fusarium wilt + F. falciforme, Fusarium wilt + nematode, southern blight + Fusarium wilt, Fusarium wilt + Verticillium, F. falciforme + Verticillium.
- We recovered at least four putatively new Fusarium pathogens from tomato stem rots in 2020; some of these have also been recovered in previous years.
- In cumulative race 4 monitoring efforts over the last three years we have tested a total of ~20 possible Fol race 4 isolates. So far, all isolates have been Fol race 3 or unusual Forl isolates. **We have not detected Fol race 4.** Three isolate have proven difficult to characterize (as they caused disease in F3 plants in our tests)—we are retesting these.
- 12 putative Fol isolates were recovered from F3 samples this year. These might be Fol race 4, but it is more likely that they are Fol R3 isolates and disease development in F3 cultivars is related to stress (a phenomenon we have documented), high inoculum loads, or infection of an off type individual.
- In total, 15 putative race 4 isolates are currently in testing.
- We were able to offer a tomato wilt and crown rot laboratory diagnostics workshop in February, before the shut-down (16 attendees); this was very well received.
- We are been presenting these results to CE advisors at several ANR meetings, to aid in diagnostics, and to growers via online meetings throughout the year. We published an article in several national and regional newsletters addressing Fusarium diagnosis (emphasizing Fusarium wilt). We also used this information to provide a short re-cap on the disease status for California tomatoes at the Tomato Disease Workshop online meeting.

Introduction:

Disease diagnosis support offered by our lab represents a critical service which enhances many research and extension activities in support of the processing tomato industry. Our lab routinely receives about 120-170 tomato disease samples per year. Tomatoes comprise about 50% of the total samples received by our lab; this year, it was closer to 75%. For diseases that are not readily diagnosable in the lab, we also visit field sites with the advisor and grower(s)/PCA for field-based observations and more detailed information and sampling. In addition to aiding growers in management decisions, these activities enable us to both better understand California tomato pathosystems and diagnostic challenges. In the last two years we have characterized and increased awareness about F. falciforme as a severe tomato disease, and detected several potential new pathogens, such as Curtobacterium flaccumfaciens, found for the first time causing tomato leaf spot in 2019. Our efforts have also enabled detection of pathogen expansions or increased activity including documentation of Fusarium wilt expansion to Kern county, documentation of the geographic range affected by F. falciforme, increase of Southern blight incidence in the northern central valley (see Light proposal for work based on this). Every year we provide this data; while overall outcomes are impossible to know, with consistently irregular spring, summer and fall weather conditions, every year we see new diseases and new pathogen expansions. We are currently the only lab to offer definitive testing for Fusarium wilt of tomato—this includes use of molecular diagnosis tools and routine race phenotyping using differential cultivars. We are one of the only trained labs which routinely conducts molecular-based identification of fungi, and this has enabled us to detect unrecognized pathogens such as F. falciforme; we are at present the only lab trained in diagnosing F. falciforme. We are the only lab in the western region that offers this level of precision in diagnosis. We keep detailed records of all our submissions (cultivar, field incidence, unusual conditions) and these are critical for developing and informing research projects conducted by ourselves and others and for publishing information on new and emerging diseases, to bring the California situation to the global community. We work actively to enrich CTRI with a diverse group of researchers by informing, encouraging and collaborating with other researchers at UCD and in ANR.

Through these efforts we have identified several diagnostic tool shortcomings, which we are working to improve. We are working to inform the diagnostics community of the various diseases which require testing, the tools available, and their limitations. In 2020 this involved preparation of a 100 page tomato disease laboratory diagnosis workbook and a one day training workshop held at UC Davis. We also provide diagnosis support tools to CE advisors. For instance every year we purchase and provided Agdia test strips to CE advisors for common virus and bacterial diseases as well as selective media to farm advisors conducting their own diagnoses. Our lab fills a critical function in supporting and training new and experienced farm advisors alike in diagnosis. Every year we do farm calls with most to all tomato farm advisors in the state, to visit fields that we are concerned about, and we typically hold an in-season farm advisor field day and roundtable discussion which serves as a way for the advisors to share their experiences and observations for that year with each other and for researchers at UC Davis to connect with the advisor community. I routinely invite CTRI to join all meetings to be kept abreast of the issues. In alternate years we have also started offering a continuing education tomato disease diagnosis and management field day open to the whole industry as well as UC researchers. Finally, diagnosis efforts are critical to outreach services, both for providing information on new diseases and photodocumenting symptoms of EVERY sample that comes in to the lab--these images have proven critical to UC IPM disease updates. We also use incidence data from diagnosis to provide indication of the relevance of diseases to the industry, informing the level of coverage needed in online resources.

The Main Goal and Objectives of the funded project:

Main goals: To enable growers to make informed management decisions and provide accurate information on pathogens present in the state.
Objective 1. Provide comprehensive laboratory-based diagnostics support to growers in managing diseases of tomato, monitoring the movement / emergence or re-emergence of pathogens across the state.

Objective 2. Monitor for Fusarium wilt race 4: Fol identification, race phenotyping, I3 resistance gene test.

Objective 3. Outreach and diagnostics service enhancement. Develop in field diagnostics guidelines for wilt and crown rot identification, provide in-service training for farm advisors, provide laboratory diagnosis training for farm advisors, and both private and public diagnosticians and provide diagnostics tools for farm advisors and growers

Methodology and Results

Objective 1. Provide comprehensive laboratory-based diagnostics support to growers in managing diseases of tomato, monitoring the movement / emergence or re-emergence of pathogens across the state.

1.1 Provide comprehensive laboratory-based diagnostics support to growers in managing diseases of tomato

Laboratory diagnosis efforts consists of a combination of tissue incubation, tissue isolation onto non selective and selective media, use of diagnostic kits (eg. Agdia immunostrips), use of PCR-based diagnosis (for Fusarium wilt), and use of molecular based sequence analysis to identify fungi to species and bacteria to genus level. With the increasing difficulty in identifying similar looking species, molecular-based sequencing is increasingly necessary and we often have to sequence 1-2 genes from 3 to 5 different fungal isolates / sample. Further, we offer race and forme speciales phenotyping for Fusarium wilt race 3 and Forl (Fusarium crown and root rot) as a routine service to growers that have not previously had Fusarium wilt.

This year we received 173 tomato submissions. As each submission consisted of about 3-6 plant samples, we processed about 700 plant samples. From these efforts, we made 216 diagnoses, reflecting the fact that many submissions had multiple diseases. We also curated over 200 fungal isolates for research projects (eg. Fusarium projects).

This was a year for high Fusarium wilt, F. falciforme, root knot nematode, TSWV and curly top; we also detected higher than usual levels of Verticillium wilt. Bacterial canker levels were normal. We also saw Phytophthora root and crown rot in several fields as well as necrotic streak virus, which is atypical. We received a number of samples with multiple disease diagnoses. We received tomato samples from all the major tomato-growing counties, with the greatest number of samples from Yolo county, indicating that Gene Miyao is only pretending to retire.

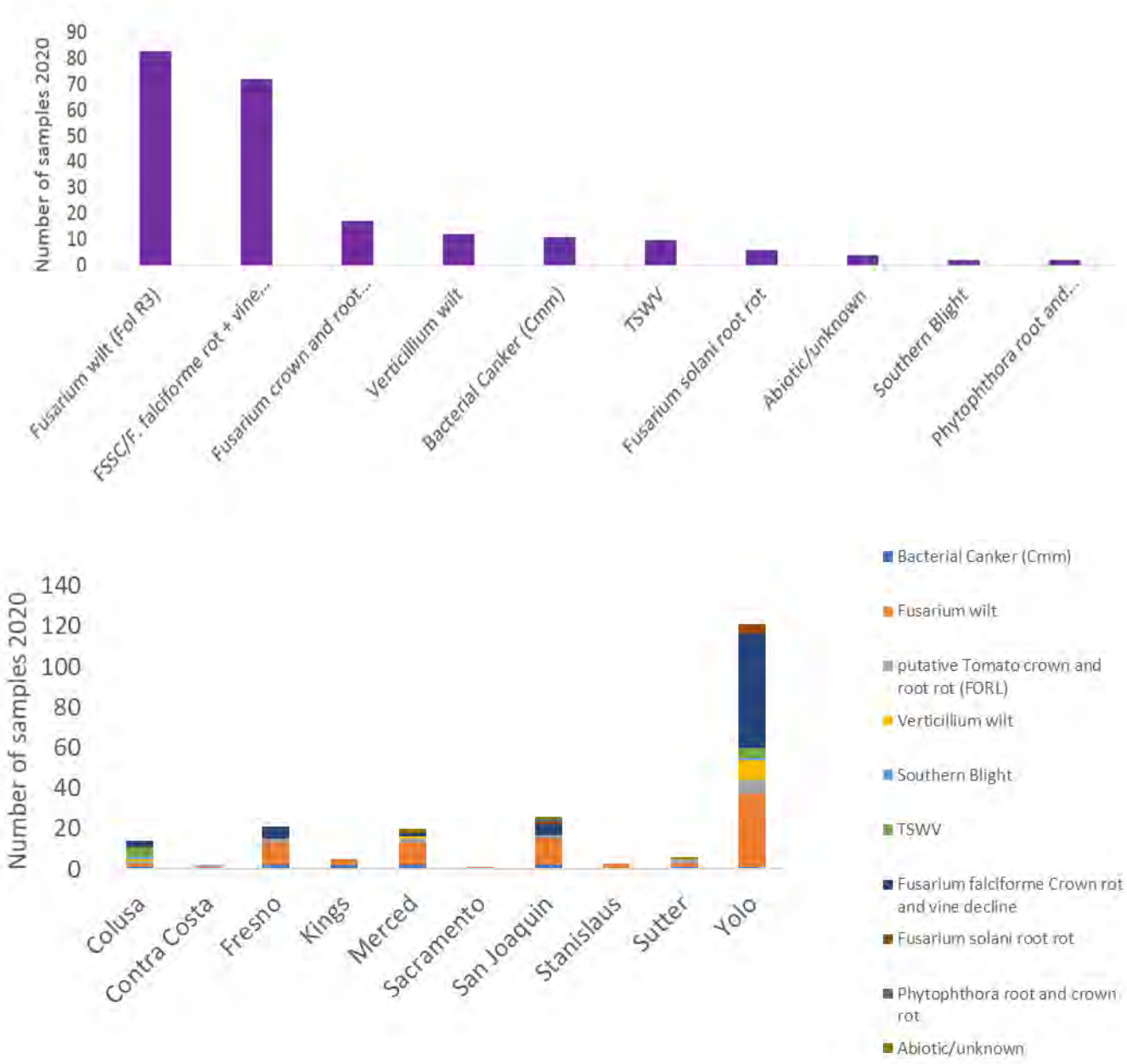

Figure 1. Top: number of samples diagnosed by disease and; bottom: by county and disease.

1.2. Monitoring the movement / emergence or re-emergence of pathogens across the state

We provide monitoring data to advisors and the industry at our field days; this information is also disseminated through annual newsletter articles. This involves maintaining a detailed diagnostics database and conducting regular analytics. This year we identified at least three potentially new tomato pathogens, associated with stem rot and vine decline symptoms—F. keratoplasticum, F. redolens, F. brachygibbosum and F. solani (FSSC 5). These have all been described as rot pathogens of other crops, although not of tomato (Rentería-Martínez et al. 2019; González 2020; . Beyond this, we have found several other Fusarium species associated with stem rot and vine decline, which are typically saprophytes (F. equisitii, F. accuminatum) and two undescribed species for which we have no information (FSSC 20, FSSC 1).

Table 1. Fusarium species associated with tomato stem rot and vine decline symptoms over the last two years, which are known to cause rot in other crops.

Species	Counties	No Fields	CS number (pure culture)
F. brachygibbosum	Yolo	2	CS 748, 749,
F. keratoplasticum	Yolo, Fresno	3	CS 862, CS 863, CS 864, CS 881
F. redolens	Yolo	1	in process
F. solani (FSSC 5)	San Joaquin, Yolo	2	CS 958, CS 989

1.3. Curating new pathogens for pathogen characterization. New pathogens (to tomatoes and/or to the state) are continuously coming through our lab. As one of our functions we curate these isolates to our culture collection and work with researchers or members of the Swett lab to characterize these new diseases. The above pathogens were saved to the culture collection by establishing a single hyphal tip for each of three isolates from each sample (submission). We saved a total of ~30 isolates that represent potentially new pathogens to the state—pathogenicity tests are needed to determine pathogenicity.

Objective 2. Monitor for Fusarium wilt race 4: Fol identification, race phenotyping, I3 resistance gene test.

We received 42 F3 tomato samples in 2020, representing ~24% of all samples and 40% of samples with known resistance status. From these F3 samples, 12 fields were diagnosed as tentative Fusarium wilt. The remainder of samples were diagnosed primarily as F. falciforme, likely reflecting the similar symptoms and also the economic impacts of this pathogen which are being revealed as F3 cultivars are more widely planted. Three isolates from each of these ten fields have been saved as pure cultures to the culture collection.

15 tentative Fol isolates are currently being screened in race 4 testing, including 3 from previous years with ambiguous results and 12 from 2020 (1 isolate from each field). Prior to testing we are both re-confirming SIX results (which were all positive for these isolates) by retesting the single hyphal time culture for both SIX 1 and SIX 3 gene regions. We are also conducting EF sequence analysis since most of our Fusarium wilt diagnosis are conducted based on morphology-based identification. In cases where there are multiple pathogens present, we have in the past found that from the initial diagnosis to the phenotyping trials, we find that the isolate identity is not what it should be. This will remove this error.

Since we have had issues in the past with F3 cultivars not holding up well against Fol race 3, we are including three different F3 cultivars to reduce the chances of getting confusing results. We are also including a Forl differential, since we have found that Forl can be SIX positive, and without this differential we have in the past thought we had race 4.

Table 2. F3 cultivars make up ~36% of known tomato cultivars and ~21% of total tomato samples, to date.

FW R status	**No samples**
F1	2
F2	62
F3	42
unknown	66
Total	**172**

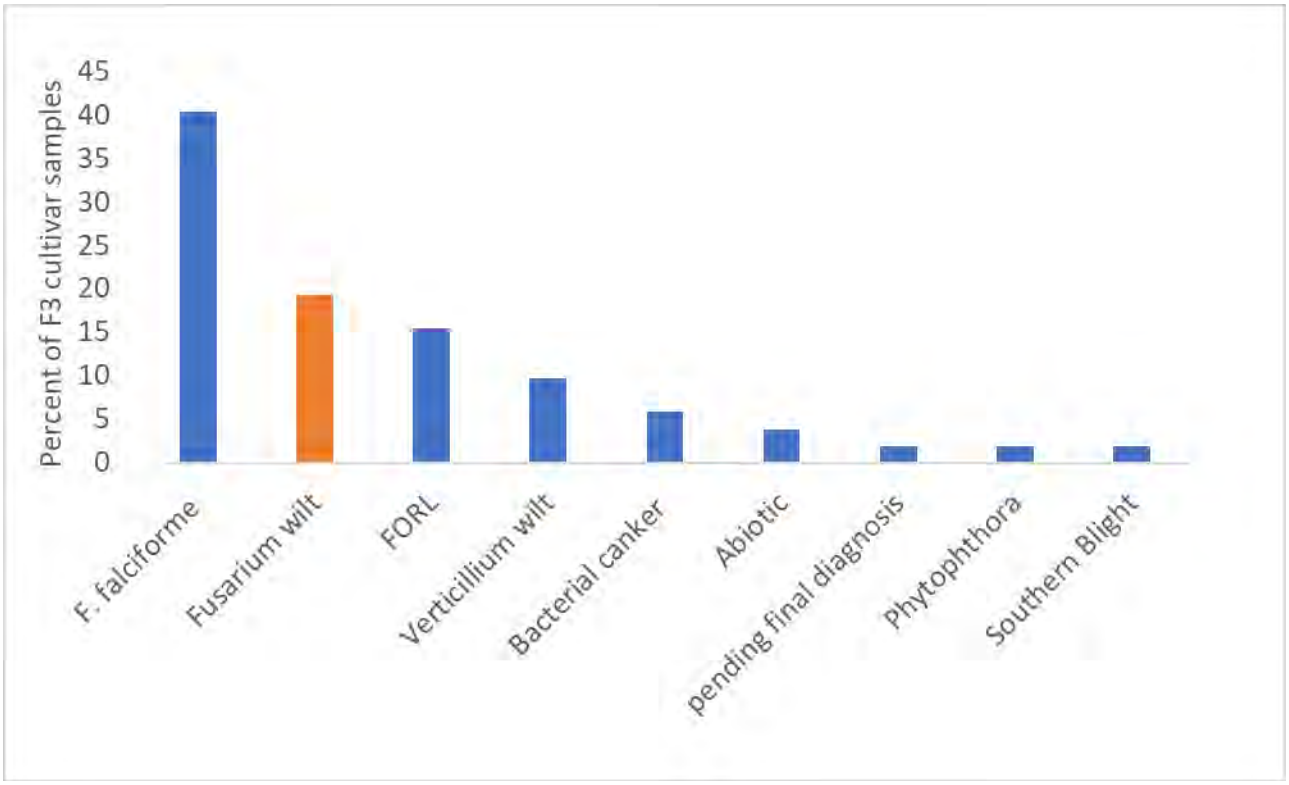

Figure 2. Diagnoses of F3 samples based on the percent of total samples (52); 20% were diagnosed as Fusarium wilt.

Table 3. 15 tentative Fol isolates currently being screened in race 4 testing, including 3 from previous years with ambiguous results and 12 from 2020

	Initial Diagnostics Information							Repeat of molecular anlaysis on pure cultures			
CS #	Diagnostics (DS) #	Diagnosis year	Location	Tomato type (proc, fresh, heirloom); (F status)	Disorder	Disease Diagnosis	SIX results	SIX 3 Retest Results	SIX 1 retest results	Confirmed seq ID (EF1&2)	BLAST Results – first match
CS 804,80	DS 1152020-1,2	2020		F3			(+)				
NOT SAVE	DS 1132020	2020		F3		?	?				
CS 806, 8	DS 1162020-1 or 3	2020		F3		Fosc?	(+)?				
CS 803	DS 1112020-7	2020		F3		Fosc?	(+)?				
CS 759, 7	DS 0942020-2	2020		F3		Forl?	?				
CS 756	DS 0642020-2	2020		F3		Fol?	(+)?				
CS 677	DS 0662020-2	2020		F3		Fol?	(+)				
CS 788 (a	DS 0762020-3,5	2020		F3		Fol?	(+)				
CS 818	DS1502020-1	2020		F3		Fol?	(+)				
NOT SAVE	DS 1912020-1 or 2	2020		F3		Fol?	(+)				
NOT SAVE	DS 2022020-1,2,5 or 6	2020		F3			awaiting ID				
CS784	0752020-1	2020	Sutter	Tomato (H1662); F3)		Fusarium wilt and Fusarium crown and root rot (Forl)	(+)				
38	**962017**	**2017**	**Yolo**	**tomato**	**(symptom description same as disease diagnosis)**	**Fusarium wilt**	**(+)**	(+) 11/30/20	(+) 11/30/20	YES 11/30/20	**Fusarium oxysporum f. sp. lycopersici isolate PLM905 translation elongation factor 1-alpha (tef1) gene, partial cds YES 12/7/20**
28	**1092017**	**2017**	**Merced**	**tomato**	**(symptom description same as disease diagnosis)**	**Fusarium wilt**	**(+)**	(+) 11/30/20	(+) 11/30/20	YES 11/30/20	**Fusarium oxysporum f. sp. lycopersici isolate PLM905 translation elongation factor 1-alpha (tef1) gene, partial cds YES 12/7/20**
315	1402018	2018	Sutter	H1662 (F3)	vd of crown and stem, cortical discoloration	Fusarium wilt on F3,	(+)	(+) 11/30/20	(+) 11/30/20	YES 11/30/20	**Fusarium oxysporum f. sp. lycopersici isolate FOL1 translation elongation factor 1-alpha gene, partial cds YES 12/7/20**

Objective 3. Outreach and diagnostics service enhancement. Develop in field diagnostics guidelines for wilt and crown rot identification, provide in-service training for farm advisors, provide laboratory diagnosis training for farm advisors, and both private and public diagnosticians and provide diagnostics tools for farm advisors and growers

For 2020, we were able to hold a disease diagnosis and control laboratory workshop. We prepared a 100 page workbook and gave 7 presentations which included two guest speakers, Shree Thapa on bacterial canker PCR-based diagnosis and Johanna Del Castillo from my lab (on phenotyping methods and molecular identification). There were 22 attendees--16 professional diagnosticians and 6 diagnosticians-in-training. Attendees included farm advisors Brenna Aegerter and Tom Turnini, diagnosticians from California Seed and Plant Lab and TriCal Diagnostics, as well as from HM Clause, Nunhems and Heinz, and diagnosticians-in-training in the Plant Pathology department. This event was very well received and we have been asked to offer it again.

We are been presenting these results to CE advisors at ANR meetings, to aid in diagnostics and monitoring efforts. I also presented this work at the seed central meeting in December and used this information to provide a short re-cap on the disease status for California tomatoes and the Tomato Disease Workshop online meeting. I have been talking with individual growers and am planning to present at grower meetings through the spring. Regarding Fusarium wilt diagnosis, we included diagnostic information in an Fusarium wilt article published in local and national newsletters. I am also using information collected on Fusarium falciforme via the diagnostics lab to prepare the first full length publication documenting this pathogen in California.

Discussion:
We received more tomato samples in 2020 than any year previously, despite covid-19. This annual effort has proven critical to document statewide pathogen movement, monitoring resistant cultivar efficacy, and to detect new and unusual diseases. We plan to provide this service to the industry in 2021. In adapting to covid-19 restrictions we are hiring three full time technicians to help us meet the challenges of working in a personal-restricted environment. This is increasing the costs of diagnostics; most of these costs are being offset by other grants, but this did result in a slight budget increase for CTRI in 2021. Over the last couple of years we have been recovering other Fusarium pathogens from stem rot symptoms, and these recoveries increased in 2020 due to more intensive sampling. Four of these species are described as crown rot pathogens in other systems. It would be informative to evaluate these for pathogenicity, to determine if there are other contributors to stem rot and vine decline. Let's hope not!

Thus far Fol race 4 has NOT been confirmed in the state. Although every year we recover Fol from F3 cultivars, thus far all cases have been Fol race 3. Likely these are cases of off types in the field or possibly stress-induced resistance breaking. From 2020 monitoring efforts we have recovered Fol from 12 F3 cultivars samples; we are testing these plus three previously tested, ambiguous isolates in 2020-2021. As more growers plant F3 lines across the state, we continue to increase our ability to detect Fol race 4 populations that are potentially present but otherwise undetectable.

Southern blight continues to be a persisting problems across the state. While a perennial issue in the south (Kern co), southern blight periodically has severe impacts in more northern regions (eg. Yolo, Colusa). Since I started, SB was bad in 2017 and again this year in the northern central valley, likely due to warmer summer temperatures. We are excited to work with CTRI in 2021 to develop better management methods for this disease in tomato. Verticillium also continues to be a problem. This year we identified both V. dahliae and V. longisporum from cultivars with Verticillium resistance. It could be informative to work with CTRI in the future to understand whether we have a resistance breaking strain or strains of these species, in order to facilitate development of improved management tools.There was also a widespread occurrence of root knot nematode in cultivars with RK resistance, suggesting that these are resistance breaking strains or new species of Meloidigyne. We have been working with Dr. Amanda Hodson to characterize these and are excited to partner with CTRI and statewide growers for our 2021 project to better understanding the distribution of root knot resistance breaking populations in the state, to facilitate improved management.

Although in management research we focus on single pathogens, in reality most fields host disease complexes. For instance, in diagnostics this year there were multiple submission with Fusarium wilt + F. falciforme, Fusarium wilt + nematode, F. falciforme + Forl, southern blight + Fusarium wilt and Verticillium + Fusarium wilt. Our F. falciforme cultivar trials in 2021 should help in developing co-management strategies with Fusarium wilt. However, more work in this area is needed.

This project as leverage for other dollars:

NEW NIFA-AFRI Tactical and Applied Biosciences grant. **$1.0 million over four years.** Lead PI-Swett. Pathogenomics-Based Development of Crop-Specific Diagnostics Tools for Emerging and Expanding Fungal Diseases in the U.S. Start date: June 2020. ~$80,000 per year for four years to my program directly. This award is aimed at developing tools for diagnosis and soil, water and seed detection for Fusarium pathogens of tomato. A portion of this is being used to cover diagnostics.
CDFA-SCBG. **$168,000**. PI-Swett. Developing innovative detection tools and cultural solutions to minimize economic damage of Fusarium wilt in tomato. Awarded 2019, 2.5 year grant. **$35,000** for 2020, part of which is being used to diagnose Fusarium wilt of tomato.
National Plant Diagnostic Network. In 2020 NPDN provided $5,000 in general funds and $14,000 to fund new molecular equipment purchases. Based on the approximation that tomato disease diagnostics represented 75% of total operations this year, this provided **~$14,250** in support of tomato diagnostics.

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González, V., García-Martínez, S., Flores-León, A., Ruiz, J. J., Picó, B., & Garcés-Claver, A. (2020). Neocosmospora keratoplastica, a relevant human fusarial pathogen is found to be associated with wilt and root rot of Muskmelon and Watermelon crops in Spain: epidemiological and molecular evidences. *European Journal of Plant Pathology*, 1-8

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Key Takeaway(s) at this date:

- We are 21 months into our study of Fol R3 survival in soil; it remains in soil at detectable levels, indicating that rotations out of tomato need to occur for at least 21 months.
- After two years of trials, we have strong data to support the recommendation to avoid peppers and melons as rotations with tomato. Rotations with cotton, onions, wheat and beans appear to be the lowest risk; sunflowers are intermediate.
- Warm season weeds appear to contribute to a small extent to Fusarium wilt, but contribute less than the high risk rotations.
- In the last few years, we have seen several fields new to tomato that still had high Fusarium wilt levels. If these growers had had the ability to test their soil for Fol R3 in advance of planting, they could have avoided major losses. While we work to develop molecular-based soil testing methods which will be available in the medium term, we have also made progress in developing a less sensitive soil testing method, which can function in the short term to inform growers whether they have high Fol R3 inoculum loads ("lethal fields"). Lethal field studies in 2020 tell us that we are able to detect Fol R3 in soil by planting infested soil to susceptible and resistant cultivars, and evaluating symptom development over 6 weeks. We hope to beta test this soil analytical method to CTRI members in 2021 free of charge and look to CTRI to less their members know this service is avilable.
- Based on physiochemical analyses of soils from Fusarium wilt outbreak fields, salinity appears to be a correlative with disease risk. Growers may be able to use salinity testing to tell them whether they have a high risk field, and should take steps to manage Fusarium wilt.
- Threshold trials are underway to determine the level above which we need to be able to detect the pathogen in molecular-based soil tests under development (see Fusarium diagnostics tool report). We will have results from field trials by early 2021. In greenhouse trials, we identified issues with contamination associated with long durations which we are addressing in follow up studies.
- This information is being disseminated through various scientific and outreach meetings, has been published as an article in both national and local newsletters (readership of ~8,000 in total), and is being prepared for publication in Plant Disease.

Management suggestions based on these studies:

- In order to reduce survival of Fol R3 in soil, rotate out of tomato for at least 21 months and select rotation crops that are associated with lower FW risk (i.e., cotton, beans, onion, wheat). Avoid rotating with melons, peppers and potentially sunflowers.
- Avoid planting F2 tomato in fields with both saline soil and a history of FW, even if incidence of FW remained low in the past.
- If unsure about your site history or have been out of tomatoes many years, submit soil samples to the Swett lab for testing. As these assays take ~2 months total, plan accordingly. This assay will tell you if you have high Fusarium wilt loads, but may not detect lower levels.
- If you know the site has a history of Fusarium wilt, have your soil tested for salts; if you have high salt levels you are likely at higher risk of Fusarium wilt.

- In cases of fields you have established to have high Fusarium wilt risk some options are to: grow an F3 cultivar (N6428 is one with strong yields), wait to plant tomato (rotating with a low risk crop, see above), apply K-Pam (spring application; at least 30 gal/A), or if you are already committed to an F2 cultivar, apply fungicides via the drip (Miravis is recommended) at planting and at 3 and 6 weeks post planting.

Introduction:

Fusarium wilt, caused by *Fusarium oxysporum* f. sp *lycopersici* (Fol) race 3 is an increasing threat to the industry following the recent and ongoing geographic expansion across California. This pathogen is highly unpredictable and, once in a field, can remain a persistent nuisance or "blow up" and affect up to 100% of the field. The unpredictability of epidemics is a major fear factor associated with this disease. Although cultivar resistance is available, there remain critical needs for soil management methods to reduce the risk of losses from Fusarium wilt. In addition, unmitigated, inoculum load increases pose significant risk for race 4 emergence; a risk with clear historical precedent, as race 3 evolved in California directly from race 2 populations--if race 2 populations had been actively managed, race 3 emergence may have been significantly prolonged or even prevented.

Although Fusarium wilt of tomato has been in California for the better part of the last century, no one has studied its ability to survive in the soil. Studies of Fusarium wilt in lettuce indicate that *F. oxysporum* f. sp. *lactucae* only survives at economically significant levels for about 9 months (Gordon and Koike 2015) while the Fusarium wilt pathogen of cotton (f. sp. *vasinfectum*) persists at high levels for over three years (M. Davis). Such variability strongly suggests that host influences longevity of the fungus in the soil. These studies also make it clear that we cannot guesstimate Fol survival patterns based on studies on other pathosystems—studies must be done specifically with Fol on tomato. Currently, crop rotations for Fusarium wilt management in tomato are conducted without any science-based information. Thus the time out of tomato (2-7+ years) and selection of appropriate crops are not science-based. Information on rotation crop timing and crop selection will help reduce Fusarium wilt enhancement and race 4 emergence. We also propose to characterize environmental conditions that lead to rapid Fusarium wilt epidemics and develop detection tools for lethal fields, to help demystify which fields will have high Fusarium wilt losses if planted to a susceptible cultivar.

In 2019 we initiated efforts (with CTRI support) to determine inoculum risk thresholds under controlled conditions; this work is ongoing and we request support to continue studies in the greenhouse and also conduct field studies to assess inoculum loads found at diseased and healthy plants in infested fields. We also initiated a long-term infested microplot study in the field where we are monitoring inoculum loads following incorporation of infested tomato tissue (January 2019). As of July 2019, inoculum loads remain high in these plots and we request support to continue monitoring. We hypothesized that long-term pathogen persistence may be enhanced by cryptic "Typhoid Mary" hosts, which are colonized by Fol R3 during the growing season and maintain or even increasing population loads over time. We have characterized host status of 16 different cash and cover rotation crops. We initiated rotation crop trials in 2018; CTRI provided support to continue these trials in 2019. In 2020 we request support to complete these trials, completing warm and cool season crop studies. We further hypothesize that rates of tissue decomposition and ability to sporulate on tissue will determine the degree to which these different crops perpetuate inoculum loads. To evaluate these more nuanced aspects of inoculum dynamics we initiated controlled trials where we incorporated infested crop residue into at defined levels, which we concluded this year. Based on this work, we have a suite of crop rotations which we can recommend for use or avoidance in managing Fol.

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

Main goal: develop information on how to manage the survival-stage of the Fusarium wilt disease cycle.

We aim to address the following questions:

1. What is the target inoculum load we are aiming for? Understanding Fol R3 threshold inoculum loads needed to result in economically significant losses.

1.1. Dose response under controlled conditions

1.2. Establishing range in soil inoculum associated with diseased and neighboring healthy plants

2. How long does it take to reach non-significant levels and what are factors that influence population decline? Understanding survival biology of Fol R3 in tomato tissue over time

3. What rotation crops should and should not be grown? Quantifying relative contribution of rotation crops to soil inoculum loads and Fusarium wilt development:

3.1. Evaluate soil population persistence in non-tomato warm and cool season crop rotations under field conditions over time

3.2. Evaluate Fusarium wilt development in warm season rotation trial (2019) and cool season trial (in 3.1) when planted to tomato

3.3. Factors influencing inoculum dynamics: Rates of tissue decomposition and ability to sporulate on tissue will determine the degree to which these different crops perpetuate inoculum loads

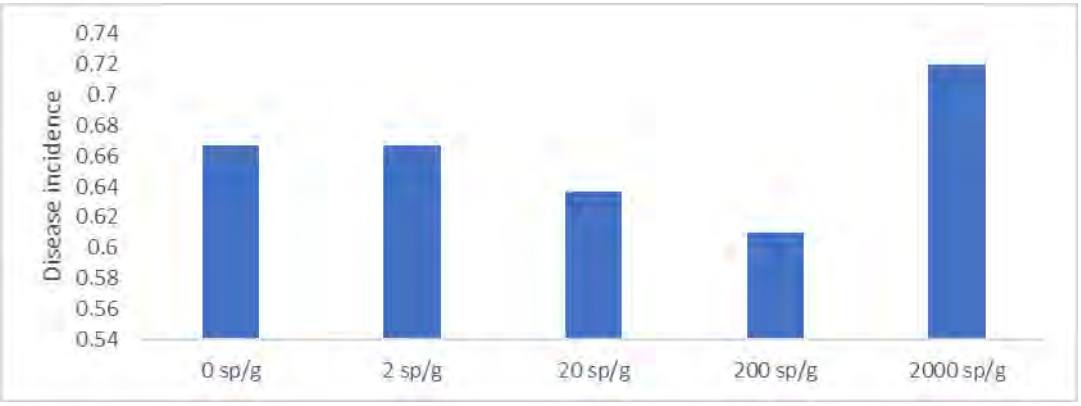
4. Characterizing traits and developing tools to detect "lethal" Fusarium wilt fields

Methodology and Results:

1. What is the target inoculum load we are aiming for? Understanding Fol R3 threshold inoculum loads needed to result in economically significant losses.

1.1. Dose response under controlled conditions

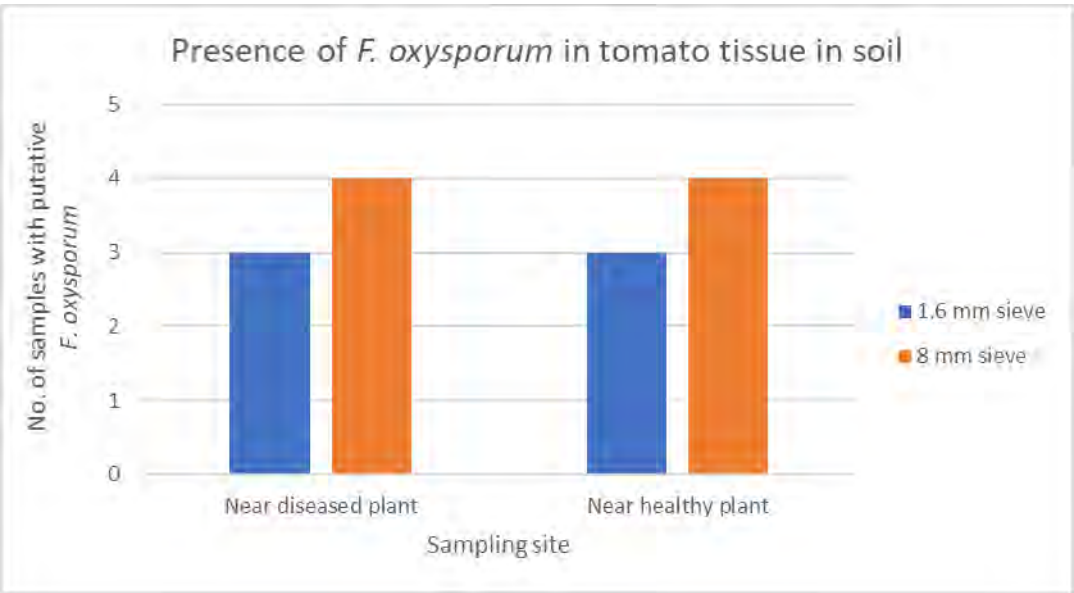
Based on this study, disease development was observed even at the 0 sp/g level, indicating contamination. We did see an increase in disease incidence at 2000 spore/g, indicating dose-dependent effects. This trial ran about six months, in order to detect disease at the lower inoculum levels. We believe that the long duration allowed for cross contamination to occur. We were planning to redo the trial with a shorter study window and more rigorous cross contamination prevention measures when COVID shutdowns were imposed. We hope to initiate this follow up trial in early 2021. That said, the ability for Fol R3 to cause disease at levels which occur solely from cross contamination indicate that disease development occurs at low inoculum loads.



1.2. Establishing range in soil inoculum associated with diseased and neighboring healthy plants

To establish the inoculum density range associated with disease in tomato plants, approximately 500 grams of soil was collected twice using trowels about 6 inches away from the crown of six diseased and six healthy tomato plants in a plot infested with Fol R3. To estimate pathogen inoculum density, each soil sample will be sieved to <4mm and assayed via dilution plating on selective media. Putative Fol race 3 colonies on soil dilution plates will be confirmed as Fol race 3 via PCR. We expect to complete soil assays for pathogen inoculum density in early 2021. To determine whether Fol R3 can be detected in tomato tissue located near diseased plants or healthy in soil, a

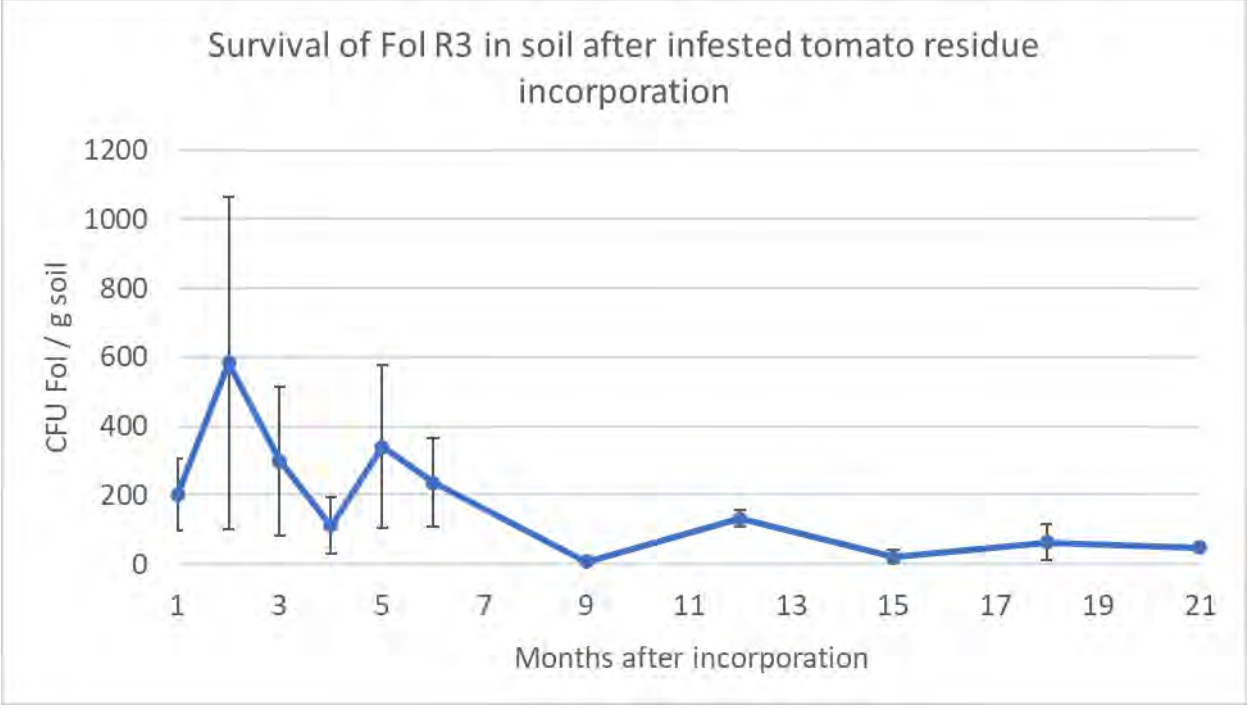
random sample of tomato tissue (primarily roots) that were unable to pass through 8- and 1.6-mm mesh sieves were incubated on selective media. Cultures of putative *F. oxysporum* from tomato tissue are awaiting confirmation as Fol R3 via PCR. *F. oxysporum* was recovered from tissue at the same frequency from sampling sites near both diseased and healthy plants.



2. How long does it take to reach non-significant levels and what are factors that influence population decline? Understanding survival biology of Fol R3 in tomato tissue over time

In January 2019, tomato residue infested with Fol R3 was incorporated into three microplots at a depth of 0 to 14 cm in a field plot with no previous history of FW. Microplots were 3-ft x 3-ft with a 2-ft length buffer between each microplot. An additional microplot with no infested residue was established as a negative control. Soil samples were collected every month until 6 months after incorporation and thereafter every three months until present time. For each sampling, five 1-inch diameter soil cores were sampled randomly and bulked together per microplot. We continue to perform soil dilution plating of samples to estimate pathogen inoculum density.

We are currently conducting assays every six months. We continue to detect Fol R3. However, inoculum loads are significantly lower than earlier time points.

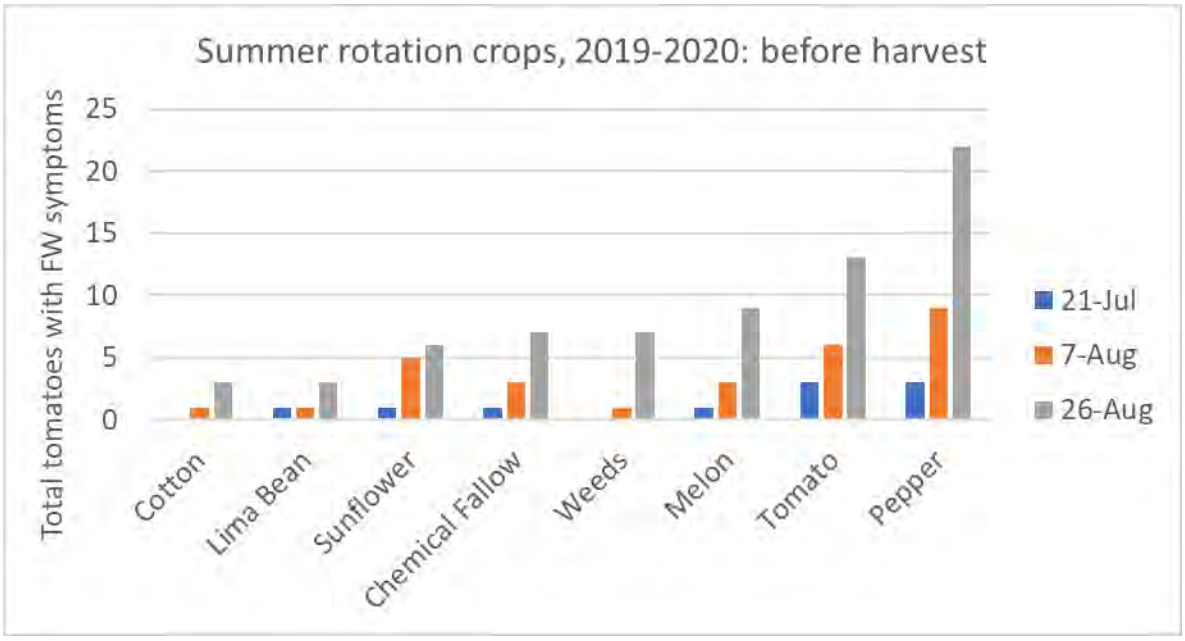


3. What rotation crops should and should not be grown? Quantifying relative contribution of rotation crops to soil inoculum loads and Fusarium wilt development:

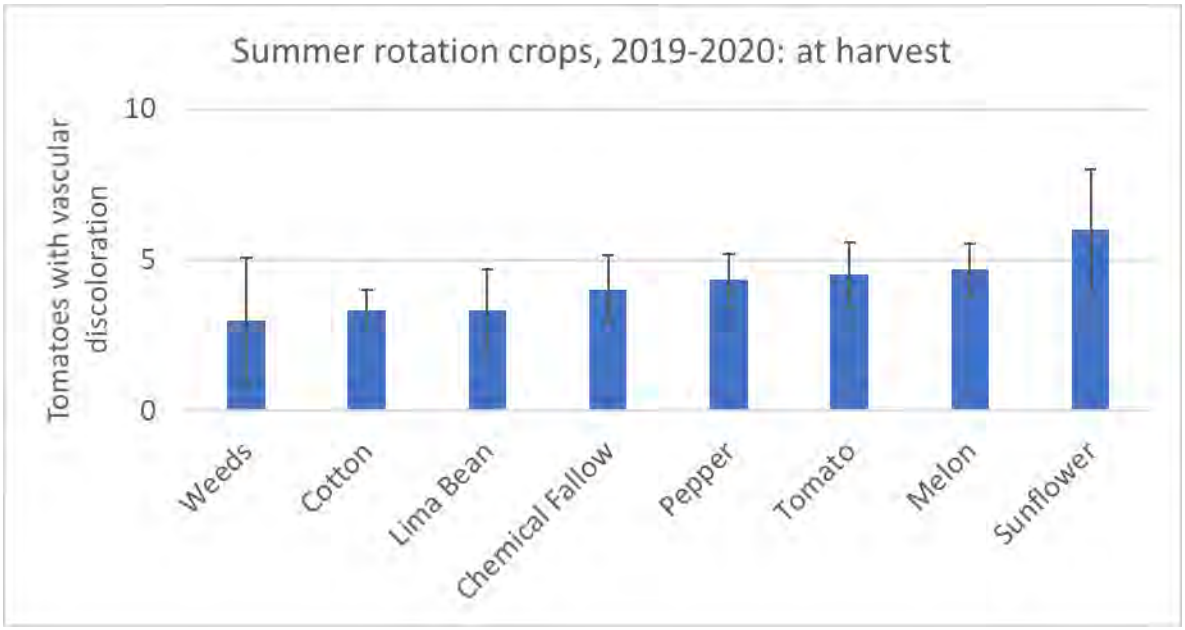
In May 2019, summer rotation crops and tomato, for comparison, were planted to a field plot that was artificially infested with Fol race 3 and then incorporated into soil in October 2019. In October 2019, winter rotation crops were planted to a different field plot that was artificially infested with Fol race 3 and then incorporated into soil in April 2020. Soil samples were collected at incorporation and at 3 and 6 months after incorporation for summer rotation crops and at incorporation for winter rotation crops. In May 2020, both summer and winter rotation crop plots were planted to tomato. FW symptoms in the canopy were evaluated at three time points before harvest time. For the final evaluation at harvest (October 2020), vascular discoloration was checked in plants to confirm disease symptoms as FW. To assess the contribution of inoculum to soil relative to tomato for a wider range of crops, we set up a greenhouse trial where 10 rotation crops and tomato infected by Fol race 3 were incorporated into soil in pots. Soil samples were collected at 2 and 4 months after incorporation of crops into soil, and assays of soil samples for pathogen inoculum density will be completed in early 2021.

We have conducted both field trials to evaluate disease impacts of rotation crops and greenhouse trials to evaluate effects of different rotation crops on inoculum loads in soil. Soil samples have been collected for the latter, but molecular confirmation of Fol R3 colonies in assays have been held up by COVID shutdowns; we hope to have these completed in early 2021. For the greenhouse trial, all tested rotation crops (corn, cotton, broccoli, fava bean, wheat, rice, melon, sunflower, pepper, hairy vetch) were found to contribute some amount of Fol R3 to soil thus far; as anticipated, Fol R3 has been most frequently detected in soil with tomato residue. Compared to control soil (no crop added), broccoli and hairy vetch decomposed significantly fastest, whereas pepper, tomato, and melon decomposed significantly slower.

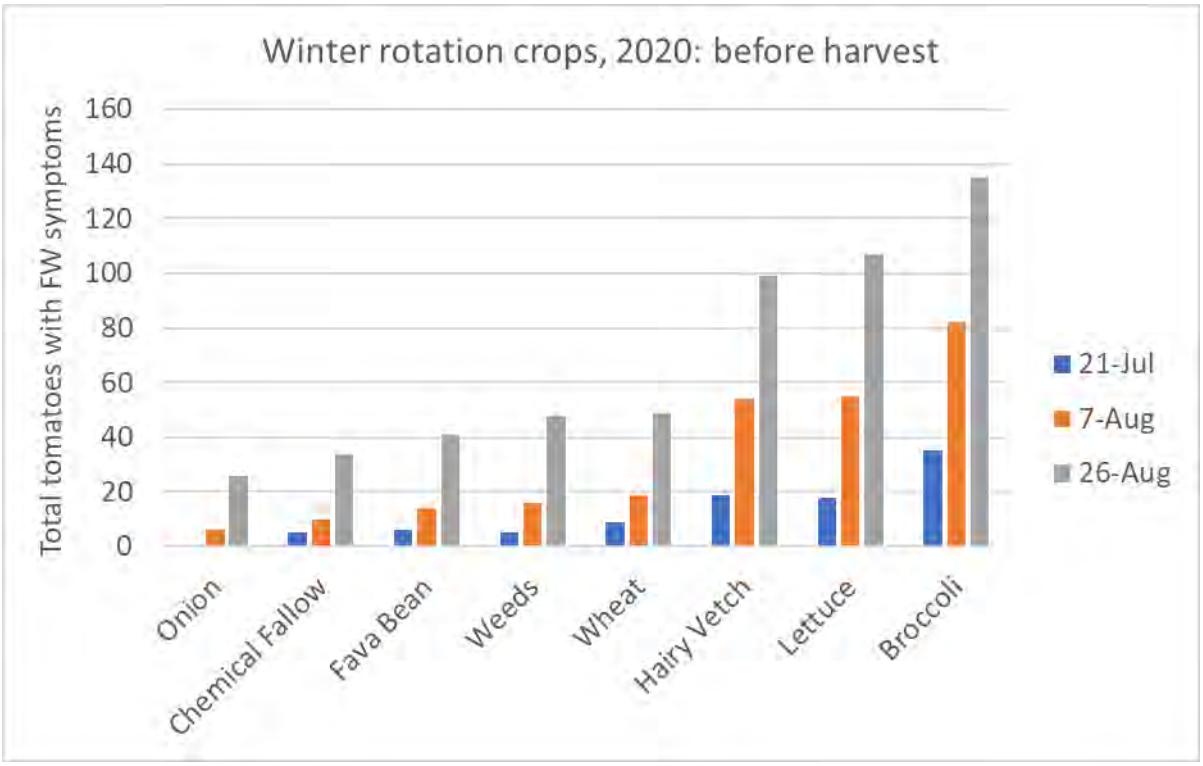
Warm season rotation crops. Soil assays are still underway and are expected to be completed in early 2021. Fusarium wilt evaluations were completed in October 2020. Based on both canopy disease and final evaluation data, disease incidence has been highest in fields previously grown to tomato, sunflower, melon, and pepper and lowest with lima bean and cotton. These results are consistent with 2019 trials and suggest that cotton and beans are lower risk crops, whereas pepper, melon and sunflower are intermediate to high risk rotation crops. In addition, plots with weeds were intermediate in Fusarium wilt levels, suggesting that weeds may contribute to inoculum loads. Interestingly, chemical fallow was actually higher than lima bean and cotton, which was not expected as fallow is typically suppressive.

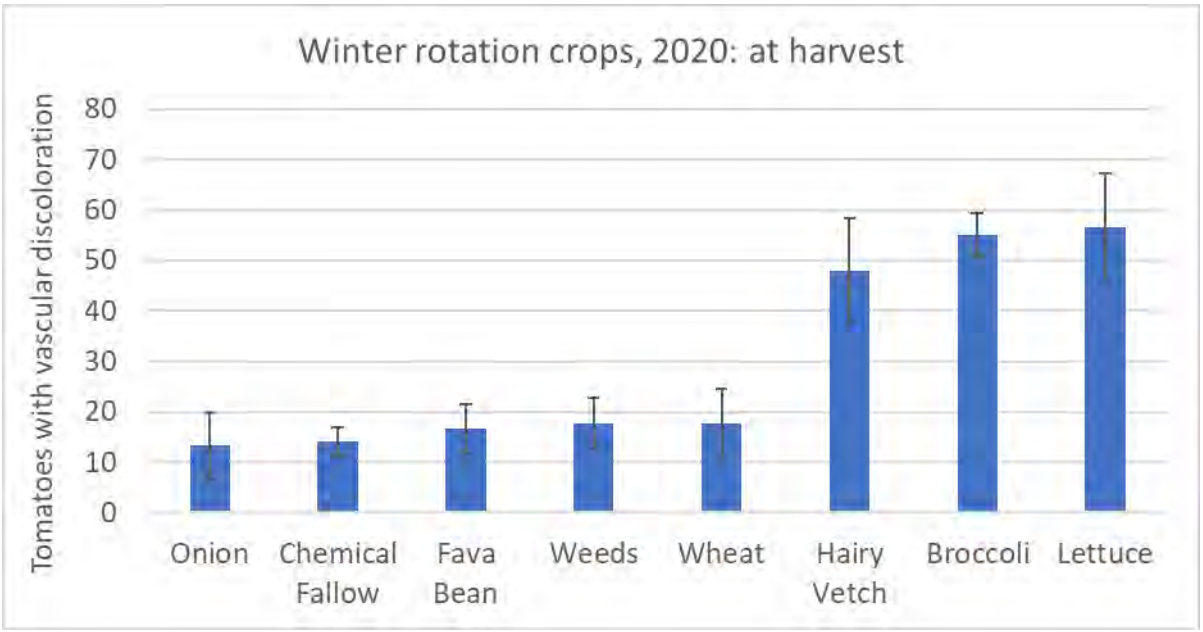


FOL DETECTED IN: 1 pepper (crown), 1 melon (crown), 2 cottons (crown), 2 tomatoes (crown and root)



Cool season rotation crops. Soil assays are still underway and are expected to be completed in early 2021. Based on canopy disease and final evaluation data, Fusarium wilt was lowest in chemical fallow, which was expected, as well as in onion, fava bean, weeds and wheat plots; these results are consistent with 2019 trials. Disease was higher in lettuce, as well as in hairy vetch and broccoli plots. These results are somewhat incongruous with 2019 data, where hairy vetch incidence was lowest.





4. Characterizing traits and developing tools to detect "lethal" Fusarium wilt fields

We have conducted physiochemical analysis for five tomato fields with severe Fusarium wilt; a severe field is one where at least 75% of plants were diseased (Table 3). We also evaluated four fields with low Fusarium wilt levels (30-50% of plants diseased). Based on this, we found that two of the fields with high Fusarium wilt levels also had significantly higher salinity (EC) levels and a significantly higher sodium adsorption ratio. Also, within each region, those fields with higher Fusarium wilt had significantly higher sodium levels and tended to have higher calcium levels. These results suggest that salinity is a potential predictor of Fusarium wilt risk.

For three lethal fields, we are testing the ability to detect Fol R3 following seeding of soil collected from the field, and placed in pots in the greenhouse. In tests of the first two fields (one in Colusa and one in Sutter), we were able to detect Fusarium wilt in plants grown in one of the two field soils. Of note, this was started in February,

right before the COVID shut down and these plants received very little care for about four months, which stunted their growth, making it difficult to detect Fusarium wilt. Trials from the third field soil are underway. Despite challenges, these preliminary results indicate that we can detect Fusarium wilt in field soils using this method.

Discussion:

Progress towards addressing issues

- By the end of 2020 we expected to be able to provide more definitive information on inoculum loads over which disease development occurs and below which disease is not observed to occur. We determined that a relatively low inoculum load (below 20 CFU/g detection threshold of soil assays) may cause economically significant losses. We also established that disease incidence was higher under higher inoculum loads, indicating a dose-dependent effect.
- By the end of 2020 we aimed to determine whether two years out of tomato is sufficient to significantly reduce inoculum loads. We found that rotating out of tomatoes for at least 21 months may be necessary to reach non-significant pathogen levels in soil.
- By the end of 2020 we anticipated having definitive rotation recommendations based on replicated field and greenhouse trials for eight cash crop rotations (cotton, melon, sunflower, pepper, wheat, onion, lettuce, and lima bean as well as three cover crops (broccoli, vetch, fava bean. This compliments greenhouse trials where we also have information on effects of corn and rice rotations. Rotation crops were shown to influence population decline, with onion, fava bean, lima bean, wheat, and cotton associated with lower disease incidence and broccoli, hairy vetch, lettuce, sunflower, pepper, and melon with higher incidence. Rotation crops were found to decompose at different rates in soil, which may also be a factor that influences pathogen levels.
- We developed a “grow out”bio assay for detecting Fol in infested commercial field soil, which worked to detected Fol in one out of two lethal field soils; this assay shows promise as a tool for detecting lethal FW fields. We also found that “Lethal” FW fields were found to have more saline soil (EC and SAR) than lower FW risk fields.
- We have also established that salinity may be a co-factor for Fusarium wilt risk—further analyses (through beta testing, below) can help us establish the consistency of this association. The main question we need to address is whether there are soils with high salinity and Fusarium wilt which are sustaining only low losses. If so, salinity may not be a strong indicator of risk.
- This information is being disseminated through various scientific and outreach meetings, has been published as an article in both national and local newsletters (readership of ~8,000 in total), and is being prepared for publication in Plant Disease. We will work on adding this content to the Vegetable resource information center and eventually UC IPM Fusarium wilt of tomato webpage.

What’s next for this project?:

- We would like to continue to evaluate survival of Fol R3 in our established plots over time. This is a legacy study and one that will likely never be conducted again, and provides critical insight into the duration that growers need to rotate out of tomato to reduce Fol to non-detectable levels.
- We would like to continue to study the threshold inoculum levels at which disease occurs, since we think we have overcome initial technical issues and this is a critical question.
- Crop rotation studies have lended important insight into the cryptic roles that certain rotation crops play in increasing Fusarium wilt risk. There are still several important crops including cotton, alfalfa, rice, safflower, garlic, potato and hemp which remain unexamined in the field since they require special agronomic practices.
- Lethal field studies tell us that we may be able to detect these fields both with greenhouse “grow outs”; we would be interested in offering this as a pilot testing service to a small number of growers to see if it helps predict risk.

Acknowledgements:

We would like to thank the 11 growers who provided soil for our lethal field testing trials, as well as the managers at Armstrong, Bryan Pellissier and Alexa Sommers. In addition, thanks are due to the many growers who guided us in crop rotation selections for this study.

This project as leverage for other dollars:

NIFA-Postdoctoral Fellowship Award. **$164,912.** PI-Paugh. Advancing systems-based approaches for co-management of soil health and plant disease. September 1 2020-August 31 2022. Kelley (postdoc) secured a two year post doc to work on survival of both Fusarium wilt and southern blight in tomato. This will cover her salary starting in September. Note that this is Kelley's award and she will take it with her when she gets a job.

CDFA-SCBG. **$168,000**. PI-Swett. Developing innovative detection tools and cultural solutions to minimize economic damage of Fusarium wilt in tomato. Awarded 2019, 2.5 year grant. **$35,000** for 2020, which is being used to cover costs of a post-doctoral researcher on this project and supplies.

CDFA-SCBG. **$128,000.** PI-Scow; Co-PI Swett. Integrating compost into conventional processing tomatoes to improve soil health and water management. Awarded in 2018. Runs through April 2021. Used to supposed post-doctoral researcher and supplies.

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Cooperating Personnel: David Bodine (Ag Seeds) and Johnathon Deniz (TS&L), Brenna Aegerter (San Joaquin County) and Zheng Wang (Stanislaus County)

Key Takeaway(s) of this project:
Within Fresno, Kings and Merced counties, twenty-one commercial variety trials were evaluated for *Tomato spotted wilt virus* over three years and forty-six entries were compared: Resistance-status of the virus was determined.

- Lowest TSWV levels were observed in HM5522, BQ 400, SVTM1082, SV8011TM, N2428, HM4521, HM58801, BQ403, HM58841, H1428, HM58871, N6416, H5608, UG16609, HM5235 and SVTM9000, were among those expressing the lowest incidence of symptoms.
- Highest levels of TSWV expression were in HM 7885, N6420, HM8163, HM5369, N6366, AB0311, H1293, BOS0811, HM5511, H5508, DRI319, H2401, BQ273 and HM58811.
- In all areas tested, the TSWV present could break the resistance gene in tomato.

Introduction: *Tomato spotted wilt virus (TSWV)* caused substantial damage through 2003-2009. Regional evaluations of epidemics and integrated approaches to management were established. A single gene resistance (Sw5) became heavily utilized since 2011 in high-risk areas and the gene has been widely applied in all California production areas since. Currently, there are no other approaches to TSWV resistance commercially available for tomatoes in California. In 2016, very high incidence of *TSWV* symptoms occurred in varieties with Sw5-resitance. In 2016; the presence of a SW5 resistance-breaking strain of TSWV was confirmed in symptomatic resistant varieties in three production areas within Fresno County (Batuman et al. 2017). The distribution of the resistant-breaking strain within Fresno County included most production areas by September 2017. In September and October, it was also reported in Merced and eastern Contra Costa County. In 2018 and 19, it reoccurred throughout Fresno and Merced Counties.

Antidotally, differences in symptom incidence and severity in fields where multiple varieties were grown were apparent. With knowledge of which of the Sw5 varieties express symptoms most severely under field conditions, varietal selections can be made in high-risk situations to mitigate chances of economic loss.

With cooperation with Ag Seeds and TS&L, twenty-one industry trials in commercial fields were evaluated for relative incidence and character of TSWV symptoms from 2018 through 2020. Based on that data, there were differences among entries in incidence. In three trials each season, representative symptomatic samples were tested for resistance-breaking status in Robert Gilbertson's laboratory. In all trials tested, the Sw5 resistance-breaking virus was detected. Although they have not had active involvement due to lack of detection of the virus in their production areas, both Brenna Aegerter and Zheng Wang, who are Advisors in Stanislaus and San Joaquin Counties, have been assessing their areas for indications of the resistance breaking strains with intentions of assisting with this study should it be needed.

The arrangement with seed companies is providing capacity to assess susceptibility of current varieties to resistance breaking *TSWV* over a large geographic area under Central Valley conditions with very little investment and I intend to continue this effort. The data gained with a few days of my time a year will allow me to update the variety risk assessment as varieties change.

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

- Quantification of relative susceptibility of processing tomato varieties to *Tomato spotted wilt virus*.
- Identify Sw5-resistance status of TSWV present in three of the trials evaluated to represent distinct production areas

Methodology and Results:

In collaboration with commercial companies that conduct variety trials, TS&L and Ag Seeds, TSWV symptom expression among varieties was compared. By mid-season, seed company representatives provided trial maps. All trials evaluated were grown within commercial fields on subsurface drip irrigation with the field variety being a Sw5 variety. Trial size varied from 80 to 120 entries; however, we were not provided with variety information for entries at earlier stages of development and some were not included in all trials. For purposes of this report, the focus is on 46 varieties.

Varieties analyzed include the following:

resistance	variety		resistance	variety		resistance	variety
Sw5	AB 0311		No Sw5	H2401		Sw5	HM5522
Sw5	BOS 0811		Sw5	H5508		Sw5	HM58811
Sw5	BP 13		Sw5	HM 3887			N 2428
Sw5	BP 16		Sw5	HM 4521		No Sw5	N 6366
Sw5	BQ 273		Sw5	HM 4885		Sw5	N 6415
Sw5	BQ 400		Sw5	HM 4909		Sw5	N 6416
Sw5	BQ 401		Sw5	HM 58801		Sw5	N 6420
Sw5	BQ 403		Sw5	HM 58841		Sw5	N 6426
Sw5	BQ 413			HM 58871		Sw5	N 6428
Sw5	DRI 319		Sw5	HM 5900		Sw5	N 6441
No Sw5	H 1015		No Sw5	HM 7885		Sw5	SV 2756TM
Sw5	H 1293		Sw5	HM 8163		Sw5	SV 8011TM
Sw5	H 1428		Sw5	HM5235		Sw5	SVTM 1082
Sw5	H 1662		Sw5	HM5369		Sw5	SVTM 9000
Sw5	H 5608		Sw5	HM5511		Sw5	UG 16609
Sw5	H1776						

Decision to evaluate trials was based on presence of levels of TSWV symptoms of 5% incidence in the most severely impacted varieties. Field trials included in the analysis are six fields from 2018 (two in the Five Points area, two in Huron area, one near Mendota and one near Dos Palos in Merced County), seven fields from 2019 (two in Huron, two in Five Points, one in Cantua Creek, one in Mendota, one near Lemoore and one near Dos Palos) and eight from 2020 (one in Corcoran, one in Huron, two in Five Points, two in Helm, one in Firebaugh and one in Los Banos).

Severity evaluations: The trials were evaluated one time per field within two weeks of projected harvest. Total plants per plot were recorded. Tomato spotted wilt symptoms were categorized based on symptom character/severity as follows: a) shoot dieback - expression on young shoots and only the youngest fruit, b) fruit symptoms - expression is limited to color irregularities on red fruit and mild distortions; c) systemic symptoms - chlorosis, necrosis of foliage and majority of fruit expressing symptoms; d) severe - severely stunted or dead plant

with TSWV- symptoms (Figure 1). Representative symptoms were tested with TSWV AgDia immunostrips in the field. Incidence is presented as a percentage of the total plants per plot. Analysis of variance was performed on data from 46 entries, and the Least Significant Difference P=0.05 ($LSD_{0.05}$) is presented.

1 shoot dieback

2 fruit symptoms with few foliar symptoms

3 systemic symptoms through leaves and fruit

4 collapse

Figure 1. Categorization of TSWV symptoms, 2018-2020.

Under the conditions of all trials evaluated over the three-year study, most symptoms were either mild or moderate and there were few observations of severe symptoms (Figure 2). Entries HM5522, BQ 400, SVTM1082, SV8011TM, N2428, HM4521, HM58801, BQ403, HM58841, H1428, HM58871, N6416, H5608, UG16609, HM5235 and SVTM9000, were among those expressing the lowest incidence of symptoms p=0.050 (Table 1). Entries that statistically had the highest levels of TSWV expression included HM 7885, N6420, HM8163, HM5369, N6366, AB0311, H1293, BOS0811, HM5511, H5508, DRI319, H2401, BQ273 and HM58811 (Table 1). All other varieties had intermediate levels of disease (Table 1).

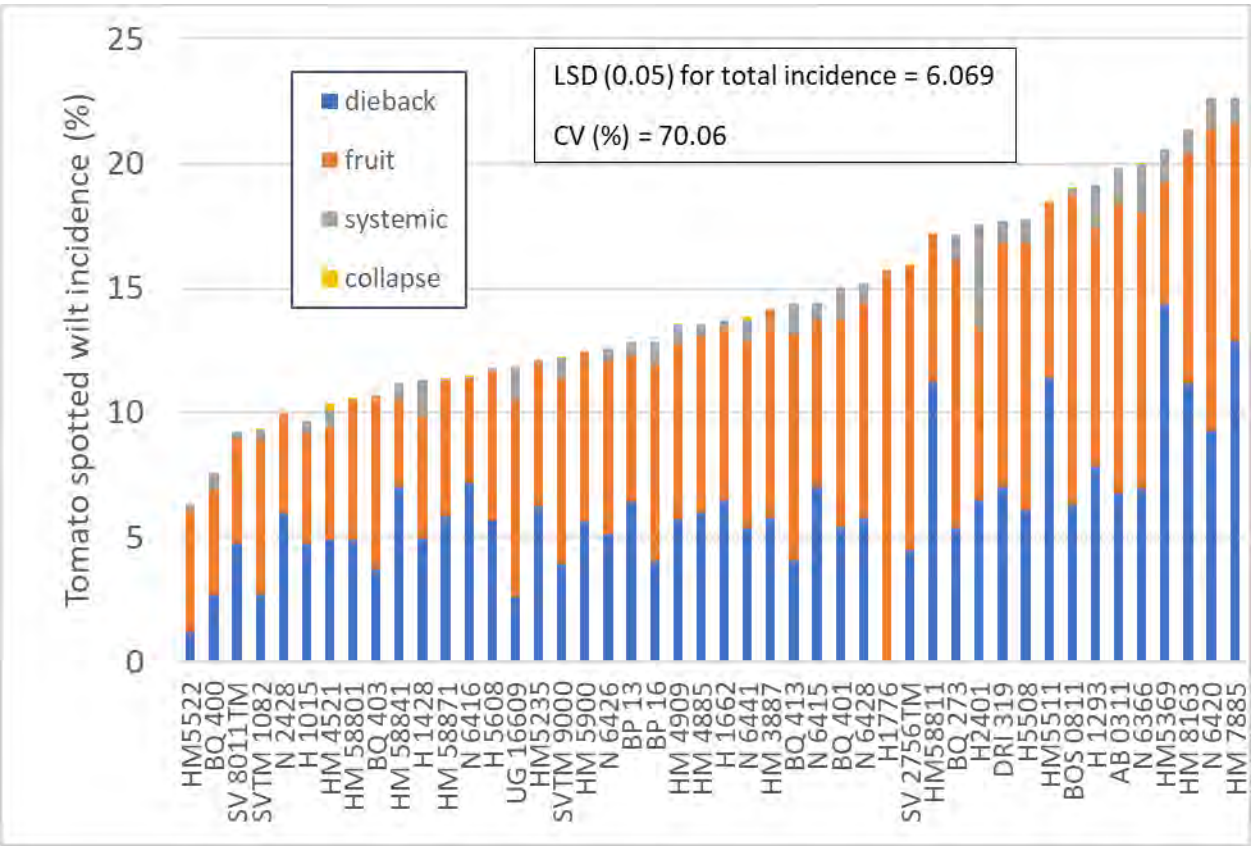

Figure 1. *Tomato spotted wilt virus* expression in forty-six entries over twenty-one sites from 2018 to 2020.

Table 1. Incidence of *Tomato spotted wilt virus* on forty six entries over twenty-one sites with varietal use information included.

Variety	Use	Total incidence	Variety	Use	Total incidence	Variety	Use	Total incidence
HM5522	inter	6.339	HM5900	inter	12.463	HM58811	thick	16.974
BQ400	early	7.579	N6426	thick	12.589	BQ273	inter	17.15
SV8011TM	inter	9.241	BP13	early	12.823	H2401	thick	17.572
SVTM1082	thin	9.317	BP16	inter	12.824	DRI319	thin	17.718
N2428		9.506	HM4909	inter	13.539	H5508	thick	17.743
H1015	early	9.693	HM4885	thick	13.567	HM5511		18.262
HM4521	inter	10.36	H1662	thick	13.704	BOS0811	thick	19.016
HM58801	inter	10.579	N6441	inter	13.876	H1293	pear	19.15
BQ403	early	10.675	HM3887	inter	14.13	AB0311	thin	19.808
HM58841	inter	11.171	BQ413	early	14.363	N6366	thin	19.956
H1428	thick	11.313	N6415	thick	14.428	HM5369	pear	20.618
HM58871	inter	11.336	BQ401	inter	15.047	HM8163	pear	21.362
N6416	early	11.415	N6428	inter	15.172	N6420	pear	22.641
H5608	thick	11.814	H1776	thick	15.278	HM7885	pear	22.679
UG16609	inter	11.834	SV2756TM	thick	15.783			
HM5235	inter	12.041						
SVTM9000	early	12.226				$LSD_{0.05}$		6.069

Testing for Sw5-resistant *Tomato spotted wilt virus*: In three trials annually, representative samples were collected and sent to Robert Gilbertson's laboratory for race determination for three shoots in each of four to nine entries. In 2018, samples were collected from Five Points, Huron and Merced trials. Samples were collected from trials in

Five Points, San Joaquin production area in Fresno County and Dos Palos in 2019. In 2020, Huron, Helm and Firebaugh-area fields were sampled. Entries submitted in 2018 and 2019 included N6366, UG19406, BQ413, UG16609, HM5900, H1293, N2420, BOS0811 and AB0311, although the first five entries listed were omitted from the 2019 Dos Palos collection. In 2020, Entries submitted included H1293, BQ413, UG16609, AB0311, H5608, N6472 and SVTM9016. In all samples, the Sw5 resistance breaking TSWV was detected.

Discussion:

In 2020, *Tomato spotted wilt virus* was at a higher incidence in many of the production areas in Fresno, Kings and Merced counties than in the recent past and most of the isolates tested, both in Sw5 tomatoes and in other hosts, tested positive for the resistance-breaking virus. It is likely that Sw5-resitance breaking TSWV is now established in these production areas. Management will be most effective with an integrated strategy that will include sanitation and avoidance of uncontrollable sources and well-timed thrips management. While an alternative resistance is not immediately available, information regarding relative susceptibility and expression among varieties represents a means of reducing risk in areas with a history of the issue, particularly if there are notable contributing factors that would indicate a likelihood of high virus levels.

Due to the addition of eight trials, there were a few entries that shifted dramatically as compared to the 2019 report, but most were very consistent with the findings from last year. As data is being added, confidence is increasing. A few patterns were apparent. All pear varieties included were among those with the highest disease levels. In addition, most entries lacking Sw5 (three of four varieties) were among those with highest levels of disease. However, because of the ubiquitous nature of Sw5-resistance breaking virus in the areas evaluated, it is likely that the higher TSWV levels in varieties without Sw5 is due to other genetic characteristics rather than the contribution of the resistance gene.

Acknowledgements: The involvement of AgSeeds and TS&L was crucial to the generation of useful information.

- David Bodine from AgSeeds provided maps and progress reports on specific trials of interest, but with certainty, there were other critical company personnel involved.
- Jonathon Deniz from TS&L provided maps and more specific trial information through the season, but there were certainly other company personnel that made this possible.

This project as leverage for other dollars: The generation of this information was possible with very little additional expense because the focus of the effort was to collaborate with industry and evaluate existing trials to produce supplemental information.

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BRANCHED BROOMRAPE IN CALIFORNIA PROCESSING TOMATO SYSTEMS RESEARCH UPDATE

MOHSEN MESGARAN & BRAD HANSON

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THE MAIN *GOAL* and CORRESPONDING *OBJECTIVES*: The overarching goal of this work, which was started in 2019, is to develop actionable, cost-effective solutions for rapid detection, containment (sanitation), management, and eradication of branched broomrape in the processing tomato production systems of California.

The specific objectives addressed in the first year (2019) of the project were:

- Evaluate the crop safety of *DSS-PICKIT* technique under California conditions;
- Determine the cardinal temperatures for seed germination of broomrape;
- Calibrate a thermal time model for prediction of the parasitism dynamics.

The specific objectives addressed in the second year (2020) of the project were:

- Rapid identification of broomrape infected tomato plants ;
- Continue field evaluation of DSS-PICKIT technique to support herbicide registration efforts;
- Field validation opportunity for remote sensing efforts.

In addition to the above PICKIT adaptation plan, studies have continued in exploring the potential effectiveness of biosolarization in seedbank depletion of small weed seeds. Another study in collaboration with a group of scientists in Germany is conducting a genetic analysis of California branched broomrape.

Introduction:

The parasitic broomrapes (*Orobanche* and *Phelipanche* spp.) are considered as one of the most disrupting weeds in many economically important crops (Jain & Foy, 1989). Lacking chlorophyll, broomrapes entirely survive on uptaking water and assimilates from the roots of their hosts and therefore can cause severe yield losses or, in case of heavy infestations, even total crop failure (Hershenhorn et al., 2009). Studies in Israel show that at high infestation levels (~100 shoots m^{-2}), Egyptian broomrape (*P. aegyptiaca*) can cause yield losses as high as 70 ton ha^{-1} in the processing tomato. In a semi-commercial field in Israel, effective management of Egyptian broomrape (~95% control) increased the tomato yield by 40 ton ha^{-1} and the net revenue by \$4,731 ha^{-1} (Eizenberg & Goldwasser, 2018). The annual losses in tomato due to broomrapes in Israel and Turkey are estimated at \$5 and \$200 million, respectively (Hershenhorn et al., 2009). Up to 80% crop loss due to branched broomrape (*P. ramosa*) has been reported in tomato in Chile. About 30% of tomato growing areas in Greece were once thought to be infested to branched broomrape, with an estimated yield loss of 25% (Parker, 2009).

The re-emergence of branched broomrape in California is particularly concerning to the tomato industry as: 1) the experience in other regions of the world has established extreme vulnerability of the tomato crop to branched broomrape parasitism (Hershenhorn et al., 2009), (2) broomrapes are highly likely to establish and spread in California because of the similarity of California's climate to the native range of species, (3) the availability of a wide range of hosts (e.g. carrot, sunflower, safflower) in California, (4) broomrapes produce copious number of minute seeds (0.2 to 0.4 mm) that can easily disperse via machinery and irrigation water in highly mechanized and irrigated cropping system of California, (5) high seed longevity (~40 years) allows the parasite to persist even in the absence of any hosts, and (6) the major part of the parasite's lifespan occur underground, making it

inaccessible to conventional means of weed control such as cultivation and contact herbicides (Hershenhorn et al., 2009). The spread of broomrape in California will further constrain the export of produce out of the state (and country), severely affecting the bottomline of growers.

In the wake of the potential threats posed by this parasitic weed to California tomato industry, there is an urgent need to develop short- and long-term strategies for effective management, containment and eradication of broomrape. Fortunately, we can leverage the decisions support system, known as *PICKIT*, developed by Israeli scientists over the past 25 years, for effective management of Egyptian broomrape in tomato (Eizenberg & Goldwasser, 2018). Implementation of *PICKIT* over 33 commercial tomato fields (400 ha) in Israel, gave 95% Egyptian broomrape control with tomato yields ranging from 115 to 145 tons ha^{-1}. The *PICKIT* system uses a GDD-based phenological model to precisely time the application of PRE and POST herbicides. However, the adaptation of *PICKIT* in California tomato cropping system requires some modifications and further evaluations because: (1) the current *PICKIT* model has been optimized based on the growth and development of Egyptian broomrape and therefore needs to be re-calibrated for branched broomrape, (2) the herbicides found to be most effective in *PICKIT* (sulfosulfuron and imazapic: Eizenberg et al. 2012) have not been registered for use in tomato in California, and (3) differences in soil and climate conditions, crop variety, and management practices can affect the phenology of parasite and herbicide efficacy necessitating the reassessment of *PICKIT* under California tomato growing conditions.

Methodology and Results:

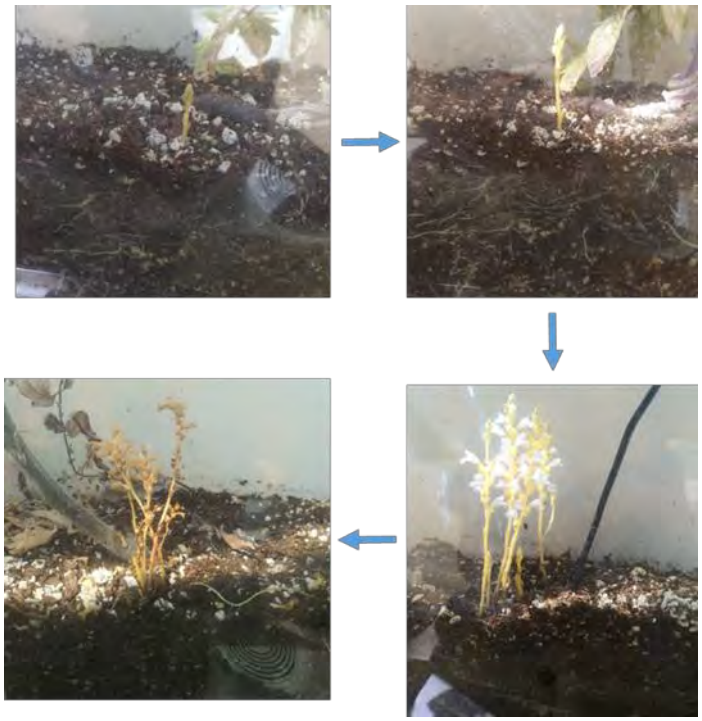
A. **Crop Safety Trials**: Tomato safety trials to evaluate the crop safety of the *DSS-PICKIT* system have been conducted with supplemental funding from the USDA-IR4 program in spring 2019. Two trials were initiated in May and June-planted tomatoes and a third rotational crop safety experiment was established in June 2019 and was planted to rotational crops in 2020. Regular crop injury evaluations and plant vigor ratings did not reveal any visible crop injury or developmental delays. Fruit yield data did not suggest negative impact of the DSS-PICKIT on tomato. The plantback experiment suggested some rotational crop issues with sulfosulfuron will need to be addressed if the herbicide programs are registered. In 2020, a third crop safety trial was conducted at the UCD campus and a broomrape control study conducted in a grower field. Thus far, the DSS-PICKIT techniques for broomrape control in tomato appear safe under California production conditions. The sulfosulfuron component of this program was prioritized for herbicide residue testing in the IR4 program with first field trials conducted during 2020 and continuing in 2021. Unfortunately, the grower field trial results indicated that broomrape control with the DSS-PICKIT programs was not as effective as anticipated, possibly due to differences between the phenology of branched broomrape in California and Egyptian broomrape in Israel where the program was developed. Additionally, potential barriers to registration of one of the herbicide components (imazapic) in California became apparent after multiple discussions with the registrant. For 2021, changes to the proposed treatment regime will be made in an effort to address broomrape growth and emergence patterns in California as well as a change in chemical focus from imazapic to imazamox which does not have the same regulatory barriers in the state.
B. **Seed Collection**: Broomrape seed collection from greenhouse propagated plants was scheduled for May to July, 2019. However, the planned soil collection from an infested field site was delayed by winter soil conditions and delays in the CDFA permitting process. Soil was collected in June 14, 2019 and branched broomrape plants were propagated at the Contained Research Facility (CRF) of UC Davis. Matured seeds were collected during the last week of October 2019 and are being stored in dark at room temperature for future uses.
C. **Seed Germination:** Seed germination represents the first step in the progression of broomrape parasitism and the success of soil applied herbicides in controlling germinant (i.e. germinated but unattached seeds), to large extent, depends on the precise prediction of germination timing. The timing of germination also dictates the progression of other phenological events including attachment, emergence, flowering and maturity. Once chemo-stimulants are released from the roots of the host to the rhizosphere, the

germination process is mainly governed by the soil temperature. One of our objectives in this project is therefore to model the germination responses of branched broomrape seeds to temperature as the first step in calibration of *PICKIT.* The seed germination studies were scheduled to commence by August but were delayed until first week of November as a result of delayed seed collection and permits explained above. Seed germination were tested under two temperature condition (10 and 20 C) unlike the initial plan of testing a wide range of temperatures (4, 8, 12, 16, 20, 24, 28, 32, and 36 C). This due to limited growth chamber in the CRF. About 100 seeds of branched broomrape were placed on moistened filter paper in a 5-cm diameter Petri dish and kept in dark at 20 C for a week as pre-conditioning, and then moistened with a solution (10 ppm) of GR_{24} (a synthetic germination stimulant). Using one growth chamber, the 10 and 20 C constant temperatures were tested in a sequential manner, with three replications of Petri dish containing the pre-conditioned seeds treated with germination stimulant. Observation of seed germination using a microscope lasted two weeks for each temperature. The results showed that about 55% of the seeds germinated under 20 C compared to about 10% germination at 10 C. Relatively higher germination under 20 C was not surprising as a previous study suggested 18 to 23 C as the optimum germination temperature in a similar parasitic species, Egyptian broomrape (Kebreab and Murdoch 1999). It is important to evaluate temperature higher than 20 C in order to conclude on the optimal temperature for branched broomrape.

Due to limited space in the CRF, the study was conducted only with 25/18 C (day/night) temperature unlike the initial plan of four different temperature regimes of 20/12 C, 23/15 C, 26/18 C, and 29/21 C (day/night). However, a data logger (HOBO, Bourne, MA) was buried at the depth of 10 cm within four of the six rhizotrons to record hourly and daily temperature aimed to calculate thermal time in growing degree days (GDD). The rhizotrons were inspected visually and by taking photos of tomato roots, through the transparent glass, for the presence of broomrape attachments and development of tubercle.

Results from First Study: Emergence of shoots over time, flowering and maturity were also observed. The observation lasted for about 14 weeks (96 days) after tomato emergence. Observation indicated that branched broomrape emergence coincided with early blossom stage of tomato which was about 10 weeks (70 days) after tomato emergence, and broomrape flowering commenced about 4 to 7 days after emergence. Seeds were formed about 4 weeks after emergence (2 to 3 weeks after flowering). Very few branched broomrape tubercle were observed and these were observed at the first flush of broomrape emergence.

A 1-cm long tubercle on tomato root observed at the first flush of branched broomrape emergence



***Results from Second Study*:** A second set of rhizotron study commenced in late May 2020, when ~ 10 cm tall tomato seedlings were transplanted into the rhizotrons. The aim of the second trial is for repeatability of first trial and to detect the attachment and development of the tubercle on tomato root. Early tubercle were first found at 504 GDD (equivalent to 37 days after transplanting tomato [DAT]). The tubercles were still being formed until 826 GDD (61 DAT). Shoot started emerging from the tubercle at 746 GDD (equivalent to 55 DAT). The emergence of multiple shoots from a single tubercle commenced at 773 GDD (equivalent to 57 DAT). The first above ground emergence of branched broomrape was about 840 GDD (62 DAT) and coincided with the early blossom stage of the tomato plants. Above ground emergence of broomrape lasted up to 1416 GDD (105 DAT). Broomrape flowering commenced about 3 days after above ground emergence and mature (black) seeds were formed about 4 weeks after emergence (~ 3 weeks after flowering).

***Results from Third Study*:** The rhizotron study was further conducted for the third time. This study commenced in late August 2020, when ~ 10 cm tall tomato seedlings were transplanted into the rhizotrons. This is to confirm results from previous studies. The tubercle were detected earlier at 430

GDD (equivalent to 32 days after transplanting tomato [DAT]) than in the previous study. Tubercles were detected on tomato roots until 693 GDD (60 DAT). Shoot started emerging from the tubercle at 690 GDD (equivalent to 52 DAT). The emergence of multiple shoots from a single tubercle commenced at 754 GDD (equivalent to 52 DAT). The branched broomrapes started emerging above ground at about 933 GDD (71 DAT). The relatively delayed emergence above the ground was due to depth of tubercle below the ground, which was up 10 cm depth, compared to a maximum of 5 cm depth in the previous study. Broomrape flowering commenced about 5 days after above ground emergence and the first set of mature (black) seeds were being formed at 5 weeks after emergence (~ 4 weeks after flowering). The growing degree days (GDD) of the observed phenological dates in these studies were calculated based on air temperature in the greenhouse, and the GDD based on soil temperature may be slightly different from above.

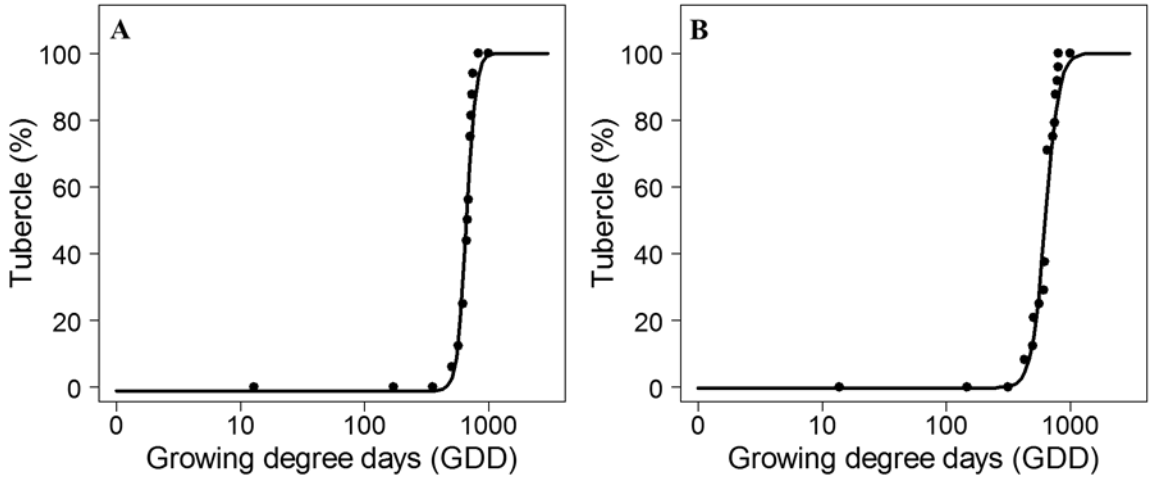
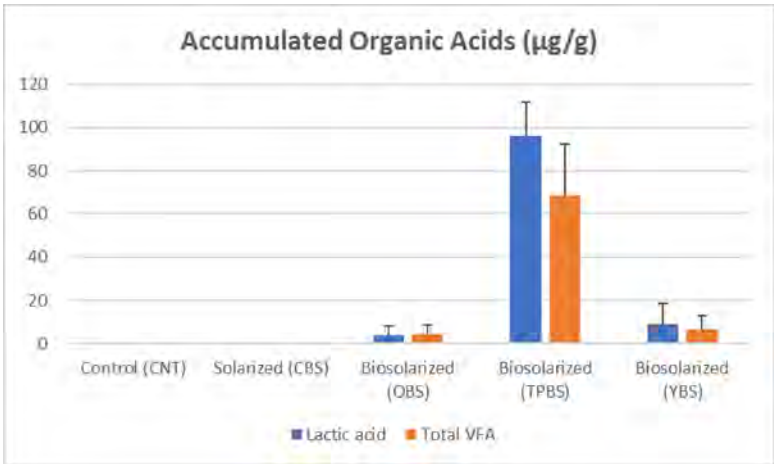
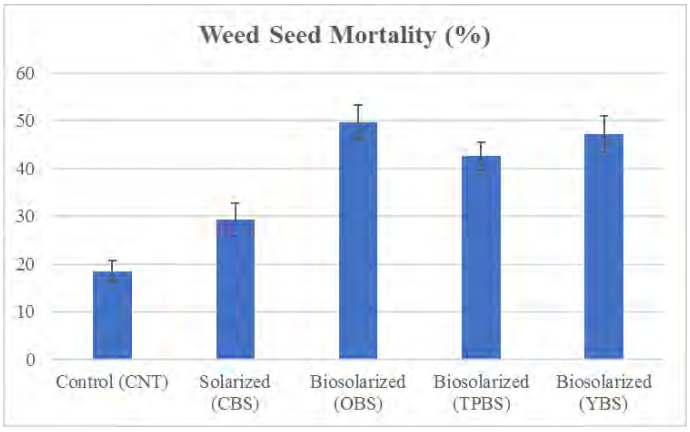


Figure: Branched broomrape tubercle attachment over thermal time, during two different studies (A and B)

E. Biosolarization Study: The biosolarization study was conducted as planned in summer 2019. Treatments were incorporated to the soil prior to the solarization: early development tomato plants (YBS), late development tomato plants (OBS) and tomato pomace (TPBS). Non-amended (CBS) and control (CNT) plots were included. The tomato pomace was spread on the soil surface and the tomato plants were first flail mowed before all plots were mechanically incorporated to a depth of 5 cm with a rototiller. Individual plots were 1.5 m wide and 3.7 m long and each treatment was replicated six times. The evaluated weed seeds were redroot pigweed (*Amaranthus retroflexus)*, common lambsquarters (*Chenopodium album*), and field bindweed (*Convolvulus arvensis*). Weed seeds were buried following incorporation of plant debris, prior to plastic tarp installation. For each weed species, there were 2 cloth bags (~ 0.3 litre in size) containing 100 seeds each, buried in a depth of about 5 cm in each plot. The buried seeds were collected after two weeks for viability test. All the tested treatments with organic amendment showed accumulation of organic acids (e.g lactic acid and total volatile fatty acid [total VFA]) that are the target bio-pesticides that are expected to have an important role in the inactivation of broomrape seeds in the soil. Results showed that the incorporation of tomato pomace generated greater amounts of lactic acid and total VFA compared to those generated by tomato plants.



Biosolarization partly killed the seeds of the evaluated weeds; the level of mortality varied with the type of organic amendment. Treatments with organic amendments provided greater weed seed mortality (42 to 50%) compared to those without organic amendments (18 to 29%), averaged across weed species. The next step would be to confirm if the detected levels do inactivate broomrape seeds.



F. **Genetic analysis of California branch broomrape:** We joined an international effort aimed at exploring the genetic diversity of broomrape species across the globe. We sent plant materials of branched broomrape obtained from our CRF experiment to Dr. Susann Wicke (Institute for Evolution and Biodiversity, University of Muenster, Germany). The genetic analysis will allow us to gain insight into the origin of California broomrape population i.e. where does California branched broomrape come from? This study was not part of our initial proposed plan.

Discussion: Parasitic broomrapes are notorious for their devastating impacts on various high value crops in many regions of the world and the re-emergence of branched broomrape (*Phelipanche ramosa*) in California is deemed as a big threat to the sustainability and profitability of tomato industry. California is lacking effective management solutions to cope with this difficult-to-control weed. The DSS-*PICKIT*, has been developed over two decades of research in Israel, which has been proven to provide successful management of "Egyptian" broomrape (*P. aegyptiaca*) in tomato. Our crop safety study suggested that the *DSS-PICKIT* system which includes a PPI application of sulfosulfuron followed by several chemigation treatments of low rates of imazapic at prescribed intervals caused no tomato injury or yield loss in California conditions. A field-level evaluation of this system is ongoing in a grower's field known to be infested with branched broomrape, results from this study will provide information on whether this *PICKIT* system is effective for branched control in California. The *PICKIT* system is largely based on a thermal time model that forecast the belowground development of parasite to precisely time

the application of PRE and POST herbicides. Efforts to develop this thermal time model for California condition is ongoing, however, preliminary results suggest that branched broomrape can have increased germination under 20 C temperature and its emergence aboveground is almost at the same time with early flowering stage of tomato. Our preliminary study suggests biosolarization as a potential tool for weed seedbank depletion. Efforts are being made to know the level of mortality this method can have on branched broomrape seeds; this information will help to know the contribution of biosolarization as a component of an integrated approach to managing broomrape in tomato.

What's next for this project: Efforts to develop a hyperspectral technology for rapid detection of broomrape parasitized tomato plants is still ongoing. The 2020 field location, with more than a thousand marked individual broomrape clumps, was used as a field validation site for a drone-based imaging systems; these data are being analyzed and will be used to supplement other rapid-detection aspects of the research. The field efficacy research will continue in 2021 with some changes to the chemigation protocol to adapt the DSS-PICKIT treatments in response to observations in 2020 and to refocus on herbicide components with greater likelihood of registration in California. The 2020 location will also be used for a field evaluation of soil fumigation research trial supported by separate research funding. Initial research on the seed mortality efficacy of ammonium compounds as equipment disinfestation techniques suggests that ammonium compounds such as alkyl dimethyl benzyl ammonium chloride (ADAC), didecyl dimethyl ammonium bromide (DDAB) and didecyl dimethyl ammonium chloride (DDAC) effectively killed branched broomrape seeds with 1% solution concentration when let sit for 20 minutes on seeds. A further study suggested the required concentration of the compounds for effective broomrape seed mortality depends on the duration of exposure of the seeds. With one hour exposure, 0.5% solution concentration of the compounds was sufficient to cause 100% seed mortality. In addition, we would like to conduct a study that evaluates a wide range of tomato varieties, to determine if there are differences among varieties with regard to hosting broomrape. Furthermore, we only evaluated the safety of the DSS-PICKIT techniques on a single tomato variety; it may be important to determine if there is differential response of California commercial tomato varieties to these techniques. We have also prepared a blog post (https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=43342), several cooperating personnel have given extension presentations on the topic during 2020, and we have submitted an extension-type article about the biology and management of branch broomrape the journal California Agriculture (Osipitan et al.).

This project as leverage for other dollars:

CTRI funds for this project are being successfully leveraged with other sources to address this important risk to the California tomato industry. Other funding sources include CDFA-PHPPS (CTRI-Bagley lead), USDA-IR4 program (Hanson lead), and most recently a CDFA-Specialty Crop Block Grant (Mesgaran lead-PI). Those additional sources of funding will likely total nearly $500k over the next three years.

Acknowledgements:

We are grateful to our grower collaborators, UC Davis CRF staff, and Gene Miyao for valuable inputs on various aspects of the project.

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WEED CONTROL AND COST-BENEFIT ANALYSIS OF AUTOMATED CULTIVATORS TO CONTROL WITHIN-ROW WEEDS IN PROCESSING TOMATOES

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Salinas, CA

Colusa County grower cooperator: Joe Wallace, Jim Wallace and Jennifer Sanders, Wallace Bros.
Merced County grower cooperator: Todd Diedrich, TD Farms, Dos Palos

Key Takeaways:

- High interest for within-row mechanical cultivators
- Larger plots in Colusa field would have been helpful to gain a better understanding of weed control
- Robovator provided very good weed control in Colusa field, but caused significant crop injury in Merced field
- Finger weeder provided excellent weed control in both fields, except for one plot in Colusa field with heavy bindweed
- Matrix and finger weeder treatments reduced costs and time for hand weeding in Merced, and Matrix and both cultivators reduced costs in Colusa

Introduction:

Conventional processing tomato weed management in California often includes pre-plant herbicides (trifluralin [Treflan] and/or metolachlor [Dual Magnum]), followed by cultivation, and hand hoeing. Rimsulfuron (Matrix) herbicide can also be used in conventional systems and can be applied either pre or post transplanting. Post-plant applications of Matrix can selectively remove nightshades if applied when the weeds are very young, no more than 2 true leaves, however, long plant-back restrictions may limit its use. Therefore, the use of hand crews is often needed to remove weeds that emerge in the plant row, where standard cultivation equipment is ineffective. Automated weeders, or robotic weeders, use cameras and computers to distinguish crops from weeds. They are equipped with either spray nozzles or cultivators to remove weeds within the crop row. Commercially available for about 10 years, these complex machines are very expensive but have shown promising results in transplanted crops in Salinas, CA and Yuma, AZ. Gaining popularity in the Central Valley is the finger weeder, a relatively simple and low-priced mechanical cultivator designed to remove weeds within the crop row. The system uses interlocking rubber fingers to remove small weeds in the plant row once transplants are established. Finger weeders can also be adapted to and added to existing cultivators and modified for individual grower needs.

While both robotic cultivators and finger weeders have been used and evaluated in many vegetable crops, there has been little research evaluating these tools in processing tomatoes and how well they may complement or replace a traditional herbicide program.

Main Goal and Objectives:
The main objective was to evaluate crop safety, weed control, time, and costs associated with using mechanical cultivators as part of a conventional weed management program in processing tomatoes.

Colusa Methodology and Results:
Project cooperators were the Wallace Bros and the field was located just north of Colusa, CA. The field was transplanted March 21 with variety BP13, double row, 60" beds. Plot size was 5 beds by 250 ft length, except for Control (Treatment 4) which was 5 beds by 100 ft length. Each treatment was replicated 3 times. The following treatments were evaluated:

1). Matrix at 2oz/A (grower standard) on April 14th
2). Robovator robotic cultivator (1 bed/pass), run April 16th
3). Finger weeder mechanical cultivator (5 beds/pass), run April 24th
4). No Matrix and no in-row cultivation in April.

The entire field, including trial plots, received a pre-plant application of Medal/Triflurex at 32 Floz/A and Diazinon at 64 Floz/A (March 18), standard cultivation to remove weeds outside of plant row (April 20), Medal/Triflurex lay-by at 21.3 Floz/A (April 26), and a finger weeder pass on May 13. Plant stands were assessed before and after cultivator passes. Weeds were counted before treatment, 24 hours, 2 weeks and 4 weeks after treatment in the center bed of each plot. Crews hand-weeded on June 26 and weeds were counted before and after. Cultivators and hand-weeding crews were timed as they moved through the field. Hand weeding times were determined by measuring the time for one person to hand weed the entire length of each plot on June 26. Plots were hand-harvested July 21 and mechanical harvest completed on July 24. Ten feet from the center bed of each plot was harvested and sorted for red, green and culled fruit. Steve Fennimore, UCCE Weed Specialist, provided the Robovator and the finger weeder was a 2020 purchase by the Wallace Bros. Weeds present in the field included nightshades, bindweed, lambsquarters, pigweed, thistle, and thorn apple. The field did not have particularly high weed pressure compared to the Merced field site.

Left: Steve Fennimore pulling Robovator through trial in Colusa. Right: 5-bed finger weeder.

Weed control results are shown in Figure 1. The Robovator and finger weeder did an excellent job of weed control on all plots. Because of the uniform spacing of plants, the Robovator worked very well and we saw very little crop injury (~4% in one plot). On average, the Robovator provided up to 85% control two weeks and 4 weeks after it was run in mid-April. The finger weeder provided 71% control on average 4 weeks post-treatment. It is worth noting that by plot, the finger weeder provided over 90% control at 2 and 4 weeks post-treatment in two of the plots. The third plot showed poor control due to heavy bindweed pressure, therefore bringing the average down. There was no significant difference between the cultivator treatments and the grower standard (Matrix) for weed control.

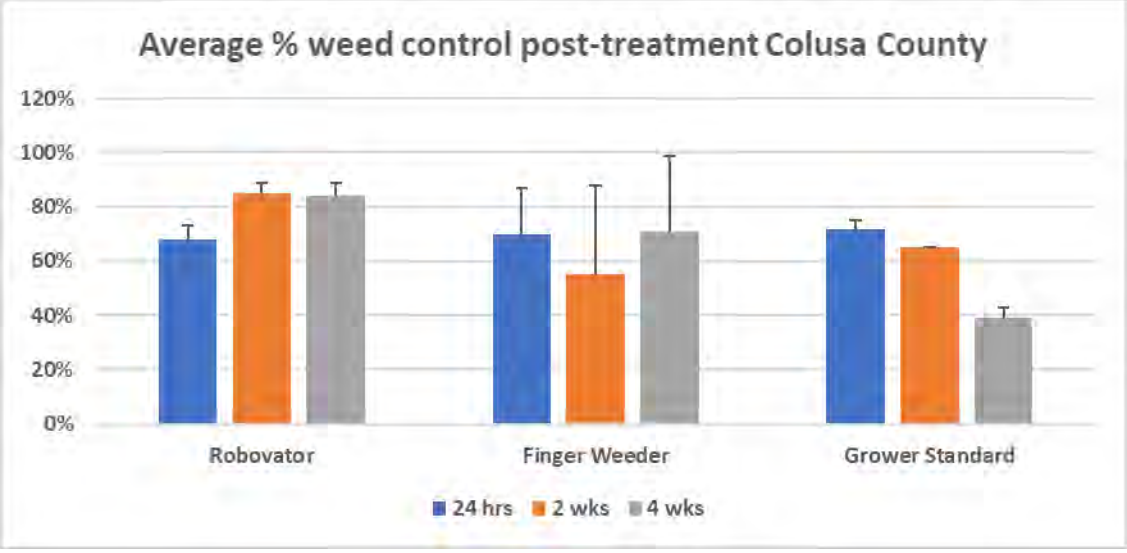


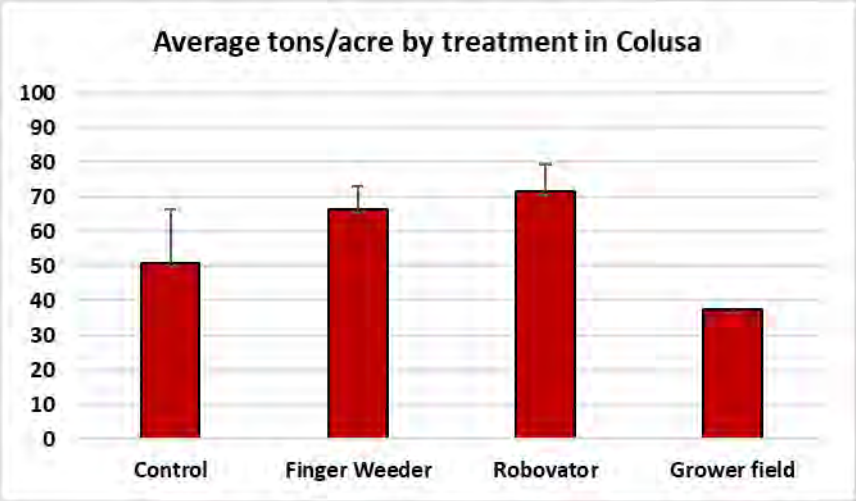
Figure 1. Average percent control for each treatment at 24 hours, 2 weeks and 4 weeks post-treatment at the Colusa field site.

Hand weeding times and costs were not significantly different between the Matrix (grower standard), finger weeder or Robovator treatments. However, all treatments decreased time and costs compared to the control plots (Table 1).

Table 1. Estimated time for 4 people to hoe 1 acre in Colusa field. Costs calculated based on $13.50/hour. Significance noted by different letters in last column.

Treatment		Hand hoe hours/A	cost $/A	
1	Matrix 2oz/A (Grower standard)	0:27	$24.30	b
2	Robovator	0:36	$32.40	b
3	Finger weeder	0:41	$36.90	b
4	No Matrix or cultivation	1:51	$99.90	a

Yield results are shown in Figure 2. There was no significant difference in yields between the control, Robovator and finger weeder plots, however there were numeric differences, with Robovator plots resulting in the highest yield. The field average was significantly lower than the treatment area, at only 37.5 tons/acre. The reason for this is unknown, though likely the area where our trial was located was at an advantage environmentally. Some of our control plots had areas of unhealthy plants due to waterlogging and blossom end rot. The lack of yield differences between treatments was likely due to hand weeding on June 26th.



Treatment	Tons/Acre ± StdErr
Control	50.96 ± 15.60
Finger Weeder	66.36 ± 6.58
Robovator	71.49 ± 7.80
Grower field (Matrix)	37.5

Figure 2. Yield ± standard error for treatments.

Merced Methodology and Results:

Project cooperator was Todd Diedrich with TD Farms, located near Dos Palos, CA. Soil type was Bolfar clay loam and El Nido sandy loam, deep and poorly drained. Soil sampling results are shown in Figure 4. The field was transplanted April 26, 2020, using SV1082, 2 lines per 72" bed. This was the second year for this drip installation. Treatments began May 8, and due to technical problems with the Robovator, were continued on May 12. Treatments targeted weeds at the cotyledon or the "white thread" stage of less than 2 true leaves. The following treatments were evaluated:

1). Matrix herbicide 2 oz/A on 30" band over-the-top on May 8 and May 22 (total 4 oz/A)
2). Robovator robotic cultivator on May 8 and 12
3). Stekatee finger weeder on May 8
4). No Matrix and no in-row cultivation.

All treatments were done on top of the grower's normal pre-plant herbicide program of Dual + Treflan (metolachlor + trifluralin) at label rates. Additionally, the grower followed his normal post-plant cultivation program, using standard shanks and cultivators to remove weeds on the outside of the beds on May 12 and layby. Plot size was 1 bed x 905 ft with 4 replications, using a randomized complete block design. Weed evaluations were made at three locations per treatment by counting emerged weeds along 15 feet within the 30" treatment band on May 8, 18, and 26, and Sept 2. Hand weeding times were determined by measuring the time for one person to hand weed the entire length of each bed on June 15. Harvest occurred on September 3. Plots were machine harvested and fruit transferred to a GT Cart for each plot. Harvest lengths ranged from 600 to 900 ft. Fruit samples were taken 1 day prior to harvest and submitted to the Ingomar PTAB grading station in Los Banos for fruit pH, SS, and color.

1. Matrix 2 oz/A fb 2 oz/A

2. Robovator (with Steve Fennimore, UCCE Salinas)

3. Stekatee finger weeder

4. No Matrix and no in-row cultivation (standard cultivation)

This was an excellent field site for this test, as the field had a history of heavy nightshade pressure (mainly hairy nightshade *Solanum physalifolium* , black nightshade, *Solanum nigrum*, and groundcherry, *Physalis* spp). Other weeds included pigweed, lambsquarters, barnyardgrass, and Johnsongrass. Dr. Steve Fennimore from UCCE in Salinas provided the Robovator; the Stekatee finger weeder was built by Sutton Agriculture in Salinas specifically for this test.

Weed control results are shown in Table 2. The Stekatee finger weeder did an excellent job of weed control on all plots with no crop injury. The number of weeds in the evaluation zones was significantly less than the Matrix and standard cultivation treatments on all evaluation dates. Weed control as compared to the standard cultivation was 89% 10 days after treating to 78% at harvest (Figure 3). We had difficulty, however, with the Robovator, as the system was not working correctly and crop injury was very high, exceeding 30% in some locations. Overall, the Robovator caused a significant reduction in the plant stand, about 20%. Where it worked properly, weed control was significantly better than standard cultivation, around 67%. However, there were large areas in this treatment that were skipped over to limit the amount of crop damage that was occurring, and therefore there were many areas with poor weed control and hand hoeing times not much different than treatment 4 (no Matrix herbicide and no cultivation).

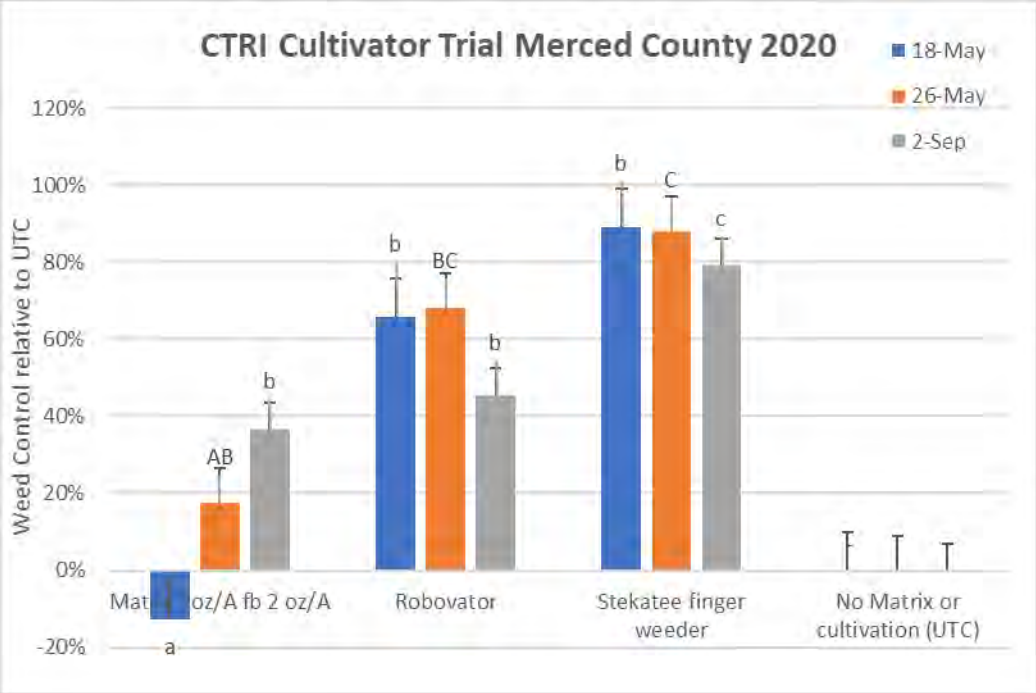


Figure 3. Weed control for Matrix herbicide and robotic/finger weeder cultivator treatments as compared to the standard cultivation (Treatment 4). Columns of the same color and letter are not significantly different using Fishers LSD comparisons at 95% confidence level.

Table 2. Yield results by weed control treatment. Merced County 2020

			PTAB		
	treatment	tons/A	pH	Solids	Color
1.	Matrix 2oz/A fb 2 oz/A	71.032	4.33	5.25	20.5
2.	Robovator	66.810	4.32	5.05	20.9
3.	Stekatee finger weeder	67.049	4.34	5.35	20.8
4.	No Matrix (UTC)	67.735	4.35	5.15	20.9
	Average	68.156	4.34	5.20	20.8
	LSD 0.05	3.01	ns	ns	ns
	CV%	2.8	0.94	4.66	1.97

Yields estimated from machine harvest 600 to 900 feet of each plot.

LSD 0.05. Least significant difference at the 95% probability level. NS = not significant.

CV = coefficient of variation

The problems with the Robovator taking out tomato plants were more likely operator error and not setting up the machine systems properly for this crop. Most plants at this location were leaning over from prevailing winds, and we couldn't get the knife actuator buffer zone properly adjusted. Subsequent testing in peppers in another location gave excellent results with almost no crop damage.

Matrix application initially showed little weed suppression at the first evaluation date on May 18, however, weed control improved after the second application and by the end of the season was significantly better than treatment 4, at 36% (Figure 3). Hand hoeing time was statistically the same as the finger weeder, and this treatment had highest yields at 71 tons/A.

Hand weeding times and cost was least for the finger weeder treatment at 49 minutes for a 4 person crew to weed an acre, followed by the Matrix treatment at 1:46 (Table 3). These times were not statistically different from each other, but they were significantly less than treatment 4, which required nearly 7.5 hours. Thus, these

two treatments reduced hand hoeing time and cost by 83% at this field location. The Robovator treatment had far longer hoeing times, for reasons explained above, and hand weeding time was reduced 37%. Hand weeding costs ranged from $44 (finger weeder) to $402 (no Matrix, no cultivation) per acre. Note these cost estimates are based on hand weeding times only, and do not include equipment or herbicide costs.

Yield and fruit quality results are shown in Table 3. Treatment #4 yields were similar to the finger weeder and Robovator, around 67 tons/A. The lack of yield differences between treatments were probably a result of all plots being hand weeded on June 15. There was no effect on fruit quality (pH, color, SS) from any treatment.

Discussion:

In Colusa, field variation and weed species influenced weed control and pressure, and impacted plot yields. There was poor bindweed control from cultivators and hand-weeding crews. Both in-row cultivators provided long-term control, especially 4 weeks post cultivator pass. The finger weeder was able to cover 5 beds and moved quickly through the field compared to the Robovator. All treatments reduced hand weeding costs and time compared to the control.

In Merced, the Stekatee finger weeder did an excellent job of weed control on all plots with no crop injury. We had difficulty, however, with the Robovator, as the vision system was not working correctly and crop injury was very high, exceeding 30% in some locations. Subsequent testing in peppers in another location gave excellent results with almost no crop damage. Matrix herbicide performed as expected, with good nightshade control and minimal crop injury. Treatment 4, no Matrix and no cultivation, had significantly more weeds than the other treatments. Matrix herbicide or the finger weeder reduced hand weeding time and cost by 83%.

We look forward to repeating this trial in 2021 to gain a better understanding of the Robovator and finger weeder and how they perform in different field conditions compared to post-emergent herbicides.

Acknowledgements:

Many thanks to Joe Wallace, Jim Wallace and Jennifer Sanders--Wallace Brothers in Colusa, CA; Todd Diedrich, TD Farms in Dos Palos; Steve Fennimore, Weed Management Specialist with UC ANR in Salinas; and CTRI for their help and support.

Table 3. Plant stand and weed control at 10 and 20 days after treatment and end of season, and estimated time and cost to hand weed treatments. Merced County, 2020.

	plants/A	# weeds per 15 ft (2)			Weed Control vs Treatment #4			hand weed (3)	
Treatment (1)	18-May	18-May	26-May	2-Sep	18-May	26-May	2-Sep	hours/A	cost $/A
1. Matrix 2oz/A fb 2 oz/A	8,591	57.3 a	42.1 ab	4.1 b	-13%	17%	36%	1:46	$ 95.40 c
2. Robovator	6,695	17.4 b	16.2 bc	3.5 b	66%	68%	45%	4:42	$ 253.80 b
3. Stekatee finger weeder	8,349	5.5 b	6.1 c	1.3 c	89%	88%	79%	0:49	$ 44.10 c
4. No Matrix or cultivation (UTC)	8,430	50.8 a	50.9 a	6.4 a	---	---	---	7:27	$ 402.30 a
Average	8,016	32.7	28.8	3.8	47%	58%	54%	3:41	$ 198.90
LSD 0.05	1,016	***	***	**	---	---	---	2:11	---
CV, %	15.3	50.1	54.2	35.4	---	---	---	37.1	---

1) Treatments applied May 8. Matrix applied again on May 22. All plots hand hoed on 15-June.

2) Least significant difference calculated on square root transformed data. Means in the same column with the same letter are not significantly different.

3) Estimated time for 4 people to weed 1 acre. Costs calculated based on $13.50 per hour.

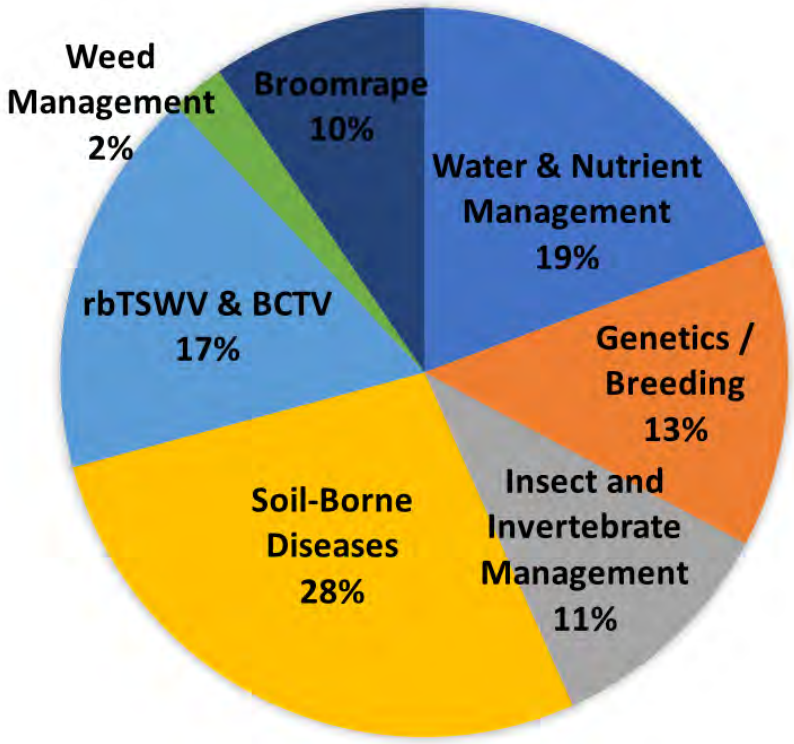
, * significant at 0.01 and 0.001 respectively

CV = coefficient of variation

2021 BOARD APPROVED RESEARCH - PROJECT LIST

2021 ANNUAL RESEARCH FUNDING: FINAL BOARD DECISIONS (DECEMBER 3, 2020)					
2021 TOTAL OF ALL PROPOSALS RECEIVED: $488,338					
2021 TOTAL AFTER FINAL BOARD DECISIONS: $371,006					
Agronomic/Water/Nutrient Management				$	71,663
2020 Start	How do different soil types impact physiology, yield, and quality under late-season deficit irrigation?	Mallika Nocco	UC Davis	$	13,698
2021 New	Satellite-based Precision Irrigation and Nitrogen Management for Processing Tomatoes	Isaya Kisekka	UC Davis	$	21,682
2021 New	Processing tomato quality assessment under three different compost application regimes	Radomir Schmidt	UC Davis	$	10,000
2021 New	Expanding the use of a tomato phyllosphere probiotic from the greenhouse to the field	Britt Koskella	UC Berkeley	$	26,283
Germplasm and Variety Development				$	50,000
1991 Start	C. M. Rick Tomato Genetics Resource Center	Roger Chetelat	UC Davis	$	15,000
2020 Start	Completion of Insect Resistance Source Line for Transfer Resistance to Insects and Insect Transmitted Virus Processing Tomato	Martha Mutschler	Cornell	$	15,000
2021 New	New transgenic resources for managing tomato spotted wilt virus resistance- breaking (RB) strains	Anna Whitfield	NCSU	$	20,000
Insect and Invertebrate Management				$	39,025
2019 Start	Consperse stink bug IPM Update	Tom Turini	UC Extension	$	1,727
2011 Start	Evaluation of Alternative Nematicides for the Control of Root-Knot Nematodes of Processing Tomatoes	Jaspreet Sidhu	UC Extension	$	2,000
2021 New	Diagnosing and co-managing the resistance breaking root knot nematode-Fusarium disease complex	Amanda Hodson	UC Davis	$	35,298
Pathogen Management				$	166,017
2017 Start	Disease diagnosis, pathogen movement / emergence monitoring, new pathogen ID and F4 monitoring for the CA processing tomato industry	Cassandra Swett	UC Davis	$	34,134
2021 New	Developing adaptive integrated MGMT strategies for southern blight in tomato: transferring tools from other CA annual cropping systems	Megan McCaghey	UC Davis	$	11,588
2018 Start	Control strategies for F. falciforme, a newly recognized and widespread cause of premature vine decline	Cassandra Swett	UC Davis	$	31,900
2020 Start	Control strategies for F. falciforme, a newly recognized and widespread cause of premature vine decline	Brenna Aegerter	UC Extension	$	11,913
2021 New	Developing best management sanitation practices for harvesters, to mitigate soil-borne pathogen spread	Cassandra Swett	UC Davis	$	12,500
2021 New	Monitoring beet leafhopper population in different sub-regions of processing tomato production in Stanislaus County	Zheng Wang	UC Extension	$	7,921
2020 Start	Beet leafhopper efficacy comparison	Tom Turini	UC Extension	$	5,300
2021 New	TSWV Varietal Response and Insecticide Efficacy	Tom Turini	UC Extension	$	9,495
2017 Start	Surveillance, rapid detection and MGMT of RB-TSWV and other viruses affecting processing tomatoes in CA	Robert Gilbertson	UC Davis	$	41,266
Weed Control and Management				$	44,301
2019 Start	Cost-benefit analysis of automated planters and cultivators in processing tomatoes	Amber Vinchesi-Vahl	UC Extension	$	8,887
2019 Start	Branched broomrape: doubling down on research for a critical CA pest	Brad Hanson	UC Davis	$	35,414
FINAL 2021 FUNDING DECISION				**$**	**371,006**

2021 Approved Project Funding			
Category	**Funding**		**%**
Pest Management	**$**	**205,042**	**55%**
Fusarium et al.	$	56,313	
TSWV	$	30,128	
BCTV	$	33,854	
Root-knot nematode	$	37,298	
Consperse Stink Bug	$	1,727	
Southern Blight	$	11,588	
Diagnostics Support	$	34,134	
Genetics	**$**	**50,000**	**13%**
University Breeding Projects	$	35,000	
TGRC-Rick Center	$	15,000	
Agronomic	**$**	**71,663**	**19%**
Product & Process	**$**	**-**	**0%**
Automation	**$**	**8,887**	**2%**
Weeding Technology	$	8,887	
Broomrape	**$**	**35,414**	**10%**
TOTALS	**$371,006**		**100%**



California Tomato Research Institute

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