FINAL PERFORMANCE REPORT

PROJECT INFORMATION- **SC-1920-13**

Project Title	Developing strategies for mitigating the impacts of freezing and cotton-root-rot on olive production in Texas			
Recipient Organization Name:	Texas Association of Olive Oil			
Period of Performance:	**Start Date:**	4/1/2020	**End Date:**	12/31/2021
Recipient's Project Contact				
Name:	Vijay J. Joshi			
Phone:	'830-988-9137			
Email:	'Vijay.Joshi@tamu.edu			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

Provide enough information for the reader to understand the importance or context of the project. This section may draw from the background and justification contained in the approved project proposal.

The Texas Association of Olive Oil (TXAOO) will work collaboratively with the Texas A&M AgriLife Research and Extension Center at Uvalde to develop strategies to minimize losses due to freezing and cotton root rot disease in olive orchards. With established regional adaptability, olive orchards in Texas now represent the fifth-highest acreage among fruit crops. However, with occasional freezing/frost, the trees are forced through cycles of the acclimation-de-acclimation process, often damaging orchards, especially so in young orchards. This damage often results in severe losses in yield and oil quality and even tree death in some cases. As a first step towards developing freeze-tolerant olive varieties for the state, we will use Texas-adapted varieties and non-regionally adapted germplasm to identify potential genetic and biochemical markers for evaluating the freeze tolerance using established techniques. Cotton Root Rot (aka Texas Root Rot), a widespread soil-borne pathogen in the southwest United States caused by *Phymatotrichopsis omnivora*, poses a significant challenge to producing Texas-adapted olive varieties as all are susceptible. A variety of trials is needed to determine the response of non-regional germplasm to CRR. If any tolerance is noted, it could provide valuable insight into overcoming challenges presented by this pathogen. The proposed project would enable olive growers in Texas to be better environmental and resource stewards for managing cold damage and disease in their orchards and provide risk information when making decisions regarding costly orchard installation at new sites. This project's outcome is expected to benchmark efforts to develop freeze and cotton root rot tolerant varieties for the Texas region.

ACTIVITIES PERFORMED

Address the below sections as they relate to the entire project's period of performance.

OBJECTIVES

Provide the approved project's objectives.

#	Objective	Completed?	
		Yes	No*
1	Objective 1: Devising strategies to minimize the freezing damage to the young and mature olive plants: (A) By optimal nutrient management and water balance and	X	

	(2) By evaluating the bioremediation potential of selected bio-stimulants (regional olive orchards and Texas A&M AgriLife Research and Extension Center; Uvalde)		
2	Maintain and correct any bugs with the online risk assessment tool for producers and screen a sub-set of olive accessions and varieties for genetic mapping	X	
3	Identifying molecular and biochemical markers for freezing and CRR tolerance using the US regionally-adapted olive varieties and globally sourced accessions obtained from the USDA Germplasm Resources Information Network (USDA-GRIN)	X	

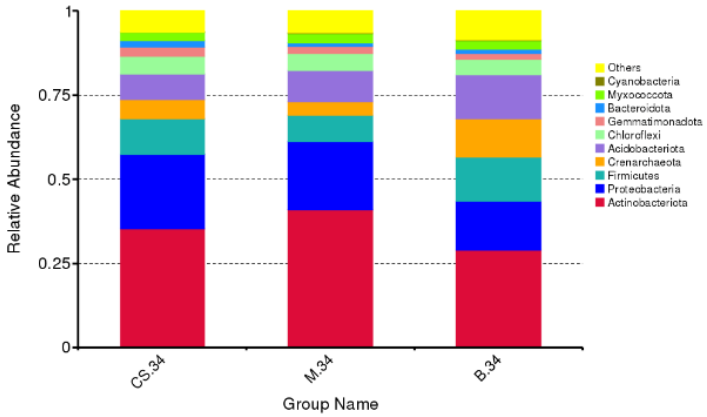
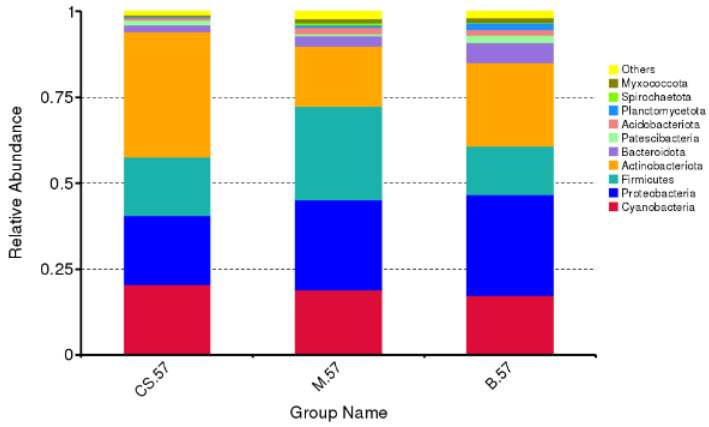
**If no is selected for any of the listed objectives, you must expand upon this in the challenges and lessons learned sections.*

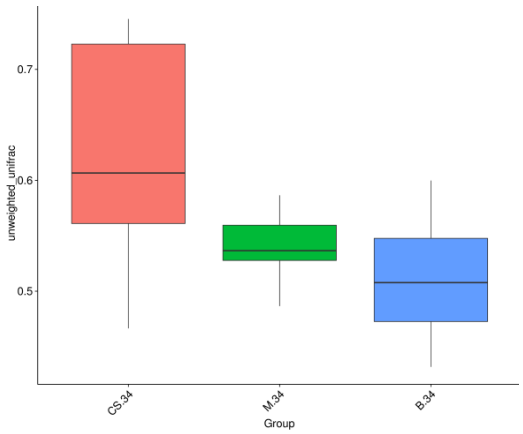
ACCOMPLISHMENTS

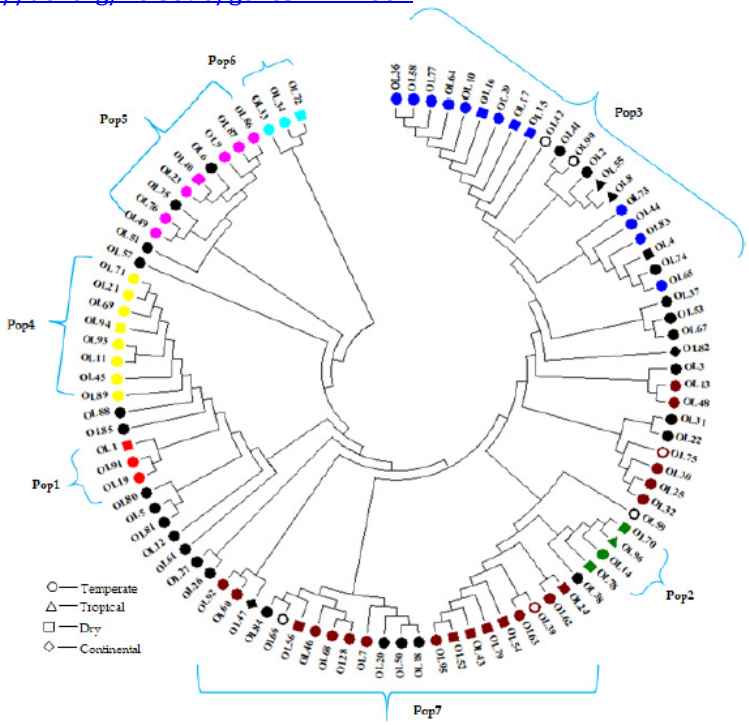
List your accomplishments for the project's period of performance, including the impact they had on the project's beneficiaries, and indicate how these accomplishments assist in the fulfillment of your project's objective(s), outcome(s), and/or indicator(s).

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	Soil and plant tissue sampling at different participating orchards, Carrizo Springs, Drippings Springs, Georgetown, Moulton, and Berclair, was conducted throughout the year from control and treatment (applied with a biostimulant, Lalitha-21) plant to analyze the nutrients. **Total amino acids:** The leaf tissue analysis of the varieties grown at different orchards showed variations in the accumulation of free amino acids and were genotype-specific. The highest accumulation of total amino acids was noticed in the variety Arbosana at Georgetown due to Lalitha-21 (bio-stimulant) application at the T1 (Mar-Apr) sampling stage. Variety Mission showed significant reductions in the accumulation of total amino acids at T1, T2 (May-Jun), and T3 (Sep) sampling stages, respectively, compared to their respective controls at Carrizo Springs. Likewise, variety Koroneiki showed significant reductions in the accumulation of total amino acids at the T1 sampling stage at Georgetown due to bio-stimulant application. Variety Arbequina and Koroneiki showed significant reductions in the accumulation of total amino acids at Carrizo Springs and Georgetown sampling sites, respectively, at the T5 stage **Foliar nutrients:** Nutrient analysis in the foliar samples were performed to assess the genotypic differences in the nutrient uptake. At Carrizo springs, variety Significantly higher NO3-N was observed in the varieties Arbequina and Mission at the T2 stage due to treatment than in the controls. Based on biostimulant treatment, the sodium content was significantly higher in the Arbequina at T0 and T3 stages on sampling at Carrizo Springs. Total phosphorus and calcium were significantly higher in the varieties Mission and Pendolino at the T1 stage at Drippings Springs, which was consistent in variety Pendolino at the T4 stage of sampling under treatment. Due to biostimulant treatment, there was significantly higher magnesium in the varieties Mission at T4 and Pendolino at T0 and T4 (Jan) stages. At Georgetown, variety Koroneiki showed significant reductions in total nitrogen (Total N) at T1, T3, and T4 and total phosphorous (Total P) at T1 and T4, respectively, due to treatment. Calcium content was significantly higher in the Arbequina variety at T0 and T3; however, it showed reductions in the variety Koroneiki at T0 and T1 sampling stages due to the application of biostimulants. Under treatment, total N was significantly higher in the Arbequina and Hojiblanca at T3, and variety Tosca at	Objective 1: Devising strategies to minimize the freezing damage to the young and mature olive plants <u>Outcome 4,</u> Indicator 1. A number of plant/see releases (i.e., cold-tolerant plants.) Outcome 4, Indicator 2.a. A number of growers/producers indicating the adoption of practices and technologies results in increased yields, reduced inputs, efficiency, economic return, and conservation of resources. adoption of recommended practices

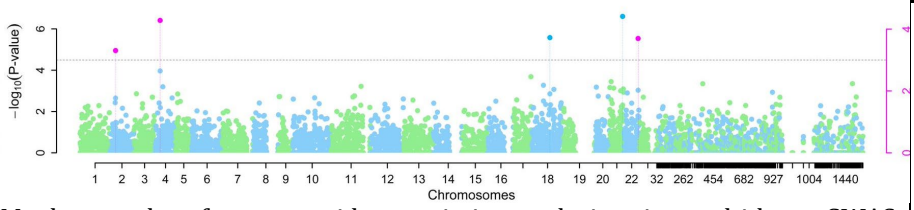
#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
	T0, T1, T2, and T4 stages at Moulton Variety Koroneiki showed mixed responses in the contents of NO3-N at Berclair. NO3-N was significantly higher at T1 and lowered at T3 due to the treatment. **Photochemical efficiency and electrolyte leakage:** The differences in chlorophyll fluorescence (quantum yield, QY), electrolyte leakage in the foliar samples, and nutrient analysis in the soil samples were performed at different stages of sampling. QY was measured in control and treated samples at different orchard sites to assess photosystem-II's photochemical efficiency (Fv/Fm). Electrolyte leakage (EL) was also evaluated to understand the differences between control and treated samples collected from orchard sites under freezing stress. Variety Pendolino and Mission showed significant lower QY at T4 and T5, respectively, at Drippings Springs and Carrizo Springs; however, significantly higher QY was noticed in the variety Arbosana at T3 and Tosca at T4 sampling stages, respectively, due to biostimulant, Lalitha-21 at Georgetown and Moulton orchard site as compared to their respective controls. No significant variations were observed in the electrolyte leakage between the varieties in control and treatment at the orchard sites. **Together, varieties Arbequina and Mission showed the best physiological traits to support enhanced freezing tolerance than other varieties**. **The biostimulants Lalitha did not significantly impact nutritional or physiological traits to support its role in enhancing cold tolerance. However, the long-term impact of the enrichment of selective microbial colonization on enhancing cold tolerance or improvement in yield or oil quality would require comprehensive study for the next five years. It is also imperative to underline the freezing damage caused by the winter storm Uri which may have induced substantial changes in the plant's physiological and soil microflora. The nutrient composition varied across locations and was influenced by soil types and seasonal variations.** **Soil and root-associated microbiome metagenomics analysis** Seasonal and environmental dynamics of rhizosphere and endosphere-bound bacterial and fungal communities associated with olive trees were performed using metagenomics. For this analysis, soil (rhizosphere) and root (endosphere) associated microbial analysis was performed by collecting samples during Spring, Summer, and Fall from three orchards; Carrizo Springs, Moulton, and Berclair, located in Texas. **Composition of microbial community analysis** Relative abundance: According to the taxonomic annotation results, top 10 taxa of each 3 olive orchard locations at each taxonomic rank (Phylum, Class, Order, Family, Genus) were used to form the distribution histogram to show relative abundance and their proportion in different classification levels. The relative abundance of taxa in the phylum for prokaryotic microflora (bacterial) and eukaryotic (fungal) was recorded for all 3 locations and 3 seasons. **The metagenomic analysis identified the differences in the microbiome across locations and seasons**. These differences suggest that the different managemental and environmental factors are responsible for differences across locations. For example, the illustrations below show the relative abundance of the prokaryotic microbiome in the soil and roots of the Arbequina.	

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
	 Prokaryotic taxa relative abundance in phylum in the soil samples across 3 locations (CS- Carrizo Springs, M- Moulton, B- Berclair)  Prokaryotic taxa relative abundance in phylum in the root samples of variety Arbequina across 3 locations (CS- Carrizo Springs, M- Moulton, B- Berclair) Beta diversity represents the explicit comparison of microbial communities based on their composition. Beta-diversity metrics thus assess the differences between microbial communities.	

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
	 Boxplot shows the difference in Beta Diversity indices across locations. The Carizzo Spring soil is significantly different in microbial community diversification than the other two locations based on Wilcox and Tukey tests. **The diversity in the microbial populations across locations suggests the role of management and soil types. Although the productivity of the orchards was significantly impacted due to freezing events, richness in the soil microflora at Carizzo Spring may provide clues for their role in defining yield or cold tolerance. Our research has identified microbial communities that are common across locations and unique species that are location-specific. The decision to use any new biostimulants in the future should be made based on the taxonomic distribution of particular microbiota in that location and stability across seasons. The data of metagenomic analysis of bacterial and fungal communities across locations and seasons in the olive orchards are in the process of publication in the journal Scientific Reports. The sequencing data will be available in the National Center for Biotechnology Information (NCBI) public repository.**	
2	Online Risk Assessment Tool Monitoring & Maintenance: The tool is functional on the Qualtrics platform. With the recent restructuring of AgriLife communications to AgriLife Marketing & Communications, Ideally, the new emphasis on marketing AgriLife Extension resources will provide new opportunities for distributing this tool. **CrrRAST is currently provided through the Texas A&M AgriLife Qualtrics platform here.** https://agrilife.az1.qualtrics.com/jfe/form/SV_5hXnE4nryKn3lFr	Objective 2: Maintain and correct any bugs with the online risk assessment tool for producers and screen a sub-set of olive accessions and varieties for genetic mapping (Texas A&M AgriLife Research and Extension Center, Uvalde).
3	Genomic DNA was isolated from over 96 USDA- GRIN olive accessions from leaves and sent to the Bioinformatics Resource Center, Biotechnology Center, the University of Wisconsin for sequencing using genotyping-by-sequencing (GBS)-generated SNP markers. **After quality filtering, 54,075 SNP markers**	Objective 3: Identifying molecular and biochemical markers for freezing

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
	were identified for the genetic diversity analysis. The average gene diversity (GD) and polymorphic information content (PIC) values of the SNPs were 0.244 and 0.206, respectively, indicating a moderate genetic diversity for the US olive germplasm evaluated in this study. The structure analysis showed that the USDA collection was distributed across seven subpopulations; 63% of the accessions were grouped into an identifiable subpopulation. The phylogenetic and principal coordinate analysis (PCoA) showed that the subpopulations did not align with the geographical origins or climatic zones. An analysis of the molecular variance revealed that the major genetic variation sources were within populations. These findings provide critical information for future olive breeding programs to select genetically distant parents and facilitate future gene identification using genome-wide association studies (GWAS) gene identification using marker-assisted selection (MAS) to develop varieties suited to production in the US. These results are published in the journal "Genes" https://doi.org/10.3390/genes12122007  Figure: Phylogenetic analyses of the 96 olive cultivars using the neighbor-joining method. Different colors depict the structure analysis generated populations. Legends indicate the climatic zones from where the accessions originated. Colors represent different subpopulations of the germplasm. **Genome-wide association analysis for cold tolerance:** Olive accessions were evaluated for their membrane stability using electrolyte leakage (EL) assays to find the molecular markers associated with freezing tolerance. The EL data was used for Genome-wide Association Analysis (GWAS).	and CRR tolerance using the US regionally adapted olive varieties and globally sourced accessions obtained from the USDA Germplasm Resources Information Network (USDA-GRIN)

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator



Manhattan plot of genome-wide association analysis using multi-locus GWAS (mrMLM; Mixed linear model) for electrolyte leakage in olive accessions. In the Manhattan plots, the x-axis indicates the physical positions of SNPs along each chromosome; the y-axis is the − log10 P for the association; the horizontal dashed line indicates the significant threshold (− $\log_{10}$ P =4.5).

The table below shows marker trait associations (MTAs) of electrolyte leakage subjected to identify favorable SNP alleles using different models.

Method	RS#	Chromosome	Marker position (bp)	SNP effect	LOD score	'-log10(P)'	r2 (%)	MAF
FASTmrMLM	S2_8043490	2	8043490	3.1617	3.231	3.9406	11.1601	0.1625
FASTmrEMMA	S2_8043490	2	8043490	3.54E-05	3.3815	4.1	2.11E-10	0.1625
FASTmrMLM	S4_3946210	4	3946210	-5.086	3.9986	4.7501	21.1154	0.1625
FASTmrEMMA	S4_3946210	4	3946210	-11.3776	4.6593	5.4412	26.9758	0.1625
ISIS EM-BLASSO	S4_3946210	4	3946210	-5.6353	4.2811	5.0462	25.8968	0.1625
FASTmrEMMA	S18_19069428	18	19069428	3.76E-05	3.724	4.4615	2.52E-10	0.1625
mrMLM	S21_16949494	21	16949494	-9.2377	4.4099	5.1809	35.4125	0.0449
mrMLM	S22_203576	22	203576	5.1766	4.1564	4.9156	15.7539	0.1282
FASTmrMLM	S22_203576	22	203576	3.447	3.235	3.9449	9.6994	0.125

The findings of this analysis **identified favorable SNPs associated with cold tolerance in olive accessions. These markers would benefit screening and developing elite olive varieties with preferred cold tolerance**. The outcomes of this study will be published in a peer-reviewed scientific journal.

Screening olive accessions for CRR tolerance (Cotton root rot resistance)
Olive cuttings were processed & established in rooting containers in preparation for inoculation with *Phymatotrichopsis omnivora*. Rooted cuttings were potted and inoculated in the greenhouse with a previously collected *Phymatotrichopsis omnivora* isolate. Woody plants in the same family as olive, Oleaceae, (Forestiera spp., including F. neomexicana; Fraxinus sp.) were grown from seed or cutting, maintained, and hardened off in the net house. At least 8 plants from each assession/variety were grown. Once seedlings were ~5" tall, they were transplanted from seedling trays to pots with field soil and maintained until adequately large for inoculation with *Phymatotrichopsis omnivora*. Plants were inoculated and monitored for disease symptoms.

Olive relatives screened for Po resistance listed by species. Highlighted accession had the highest survival rate (100%) and the least symptoms at 5 wk after inoculation.

Plant	Accessions			
Fraxinus pennsylvanica	Ames 29651			
Forestiera pubescens	W6 39606	W6 40460	W6 42803	W6 52126

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
	Forestiera neomexicana PI 596380 PI 495889 **Ames 27629**	

CHALLENGES AND DEVELOPMENTS

Provide any challenges to the completion of your project or any positive developments outside of the project's original intent that you experienced during this project. Also, provide the corrective actions you took to address these issues. If you did not attain an approved objectives, outcome(s), and/or indicator(s), provide an explanation in the Corrective Actions column.

#	Challenge or Development	Corrective Action or Project Change
1	Cochran Lab: Winter storm Uri set back the project timeline due to plant loss due to power outages (for heaters and heat mats) paired with extreme sub-freezing temperatures for an extended period. Seedlings required restarting and additional growth time from the original timeline. Dr. Cochran had hoped establishment during the warmer months would allow for earlier inoculation (concerning plant developmental stage), but the plants still required additional time to be ready for inoculation	A no-cost extension was requested and approved to allow for restarting plants & giving plants more time to develop prior to inoculation.
2	Cochran Lab: Some of the additional olive cuttings did not become symptomatic after inoculation. This was only a small portion, all from the same rep of inoculations, I believe it was an issue with the inoculum rather than true resistance since cuttings from the same variety (same parent plant) were symptomatic	I re-inoculated the non-symptomatic olives.
3	Joshi Lab: AgriLife center and growers experienced significant damage from the recent winter storm across the state. Many of the samples needed for metabolic and genotypic analysis and chemical stocks, including DNA/RNA in lab freezers, were exposed to ambient temperatures due to power outages for several days. The USDA accessions maintained in the net house for the past one and ½ years were also. We rescued some plants, but a power outage made it difficult to maintain the desired temperatures in the greenhouse. The project involved five olive orchard growers located across Texas. Many counties experienced significant damage from the last winter storm, with temperatures below 25F for more than 140 hours. Most growers experienced heavy damage to their trees; the sampled plants that received biostimulant treatments were damaged. We lost one location at Georgetown, Texas, as the orchard owner decided to sell off his property due to	The plant samples in the freezer that were exposed to room temperature were evaluated case-by-case based on the internal standards. The data were normalized based on the averages across usable samples. The compromised growth of plants has impacted the outcomes but based on the secondary parameters, we were able to extrapolate the best possible outcomes of the study. Despite the hurdles and backpressure caused by Covid-related issues, we accomplished the desired goals by working with available resources.

#	Challenge or Development	Corrective Action or Project Change
	COVId-19 and weather events. The project suffered in its flow due to occasionally losing employees during the Covid times.	

LESSONS LEARNED

Provide recommendations or advice that others may use to improve their performance in implementing similar projects.

Cotton Root Rot: Use field soil when working with Po, preferably from a site with known Po, then pasteurize or sterilize the soil. This way, researchers know with certainty that the soil has the required nutrients/conditions for the fungus. Using the plant material (sterilized cotton stems and colonized millet) worked very well as an inoculum substrate. When growing inoculum, incubate at 30-31C; this will accelerate fungal growth significantly.
Given the unexpected weather events and power outages, arrangements for a backup energy source to support plant growth in the greenhouse or protect important samples in the laboratory are strongly recommended!

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

Describe your plans for continuing the project (sustainability; capacity building) and/or disseminating the project results.

Co-PI Dr. Cochran plans to maintain the Po fungus in the laboratory for future work and make it available to other researchers. Additionally, she is experimenting with storage methods to maintain the fungus in long-term storage on dried plant material substrates and with ways to stimulate sclerotia production to increase the long-term survival of stored isolates. Dr. Cochran and Joshi will present a summary of results at the olive field day in Uvalde in the fall. Cochran lab will continue maintaining and monitoring the CrrRAST (Cotton Root Rot Risk Assessment Tool) survey system for submissions and consultations. New findings from the ongoing experiments will be shared at a conference in fall 2022/spring 2023.
Joshi lab will use the sequencing information to continue identifying molecular markers associated with different agronomic traits in olives. The new accessions showing increased tolerance to CRR will be used for further molecular characterization. Joshi lab will attempt to obtain additional funding from SCRI to undertake RNA-Seq analysis to understand cold tolerance and CRR tolerance.

BENEFICIARIES

Number of project beneficiaries:..... Enter Number of Project Beneficiaries

OUTCOME(S) AND INDICTATOR(S)/SUB-INDICATOR(S)

Provide the results of the project outcome(s) and indicator(s) as approved in your application and project proposal. The results of the outcome(s) and indicator(s) will be used to evaluate the performance of the Program on a national level.

OUTCOME MEASURE(S)

Select the Outcome Measure(s) that were approved for your project.

- ☐ **Outcome 1**: Enhance the competitiveness of specialty crops through increased sales
- ☐ **Outcome 2**: Enhance the competitiveness of specialty crops through increased consumption
- ☐ **Outcome 3**: Enhance the competitiveness of specialty crops through increased access
- ☑ **Outcome 4**: Enhance the competitiveness of specialty crops though greater capacity of sustainable practices of specialty crop production resulting in increased yield, reduced inputs, increased efficiency, increased economic return, and/or conservation of resources

- ☑ **Outcome 5**: Enhance the competitiveness of specialty crops through more sustainable, diverse, and resilient specialty crop systems
- ☐ **Outcome 6**: Enhance the competitiveness of specialty crops through increasing the number of viable technologies to improve food safety
- ☐ **Outcome 7**: Enhance the competitiveness of specialty crops through increased understanding of the ecology of threats to food safety from microbial and chemical sources
- ☐ **Outcome 8**: Enhance the competitiveness of specialty crops through enhancing or improving the economy as a result of specialty crop development

OUTCOME INDICATOR(S)

Provide the indicator approved for your project and the related quantifiable result. If you have multiple outcomes and/or indicators, repeat this for each outcome/indicator (add more rows as needed).

#	Outcome and Indicator	Quantifiable Results
1	**Outcome 4,** Enhance the competitiveness of specialty crops though greater capacity of sustainable practices of specialty crop production resulting in increased yield, reduced inputs, increased efficiency, increased economic return, and/or conservation of resources **Indicator 1.** A number of plant/seed releases (i.e., cultivars, drought-tolerant plants, organic, enhanced nutritional composition, etc.) 2 **Indicator 2.a.** Adoption of best practices and technologies resulting in increased yields, reduced inputs, increased efficiency, increased economic return, and conservation of resources. A number of growers/producers indicating adoption of recommended practices: 35	The genetic diversity and association analysis identified several accessions that can be incorporated into the genetic improvement program for the UC Olive variety improvement program. Based on the biochemical and nutrient analysis, varieties Arbequina and Mission showed a higher potential to tolerate freezing damages. • A number of cultivars with enhanced freezing tolerance based on biochemical assays: **2** Facebook Live presentation, Oct 1, 2021; 88 cumulative views on Plant Doctor Kim's Facebook Page. "Cotton Root Rot in Texas Olives & Woody Plants" A live presentation detailing what CRR is, risk factors, and mitigation efforts. The 2021 Field day at the Uvalde Center was not held due to Covid. The 2022 TxOAA meeting/field day was canceled and rescheduled for the fall out of sensitivity to Uvalde residents (school shooting incidence). We plan to present findings at that fall meeting, date TBD.
2	Outcome 5: Enhance the competitiveness of specialty crops through more sustainable, diverse, and resilient specialty crop systems. **Indicator 1:** Number of new or improved innovation models (biological, economic, business, management,	

	etc.), **technologies**, networks, products, processes, etc. developed for specialty crop producers, processors, distributors, etc.: **__1** **Indicator 5:** A number of new diagnostic technologies available for detecting plant pests and diseases: **1** __(Inoculation technology for disease screening) **Indicator 8:** A number of growers/producers that gained knowledge about science-based tools through outreach and education programs: **__35**	1 Survey tool for risk assessment and producer risk education. This interface also leads to increased producer support by putting users directly in contact with a plant pathology specialist if they choose. Successful inoculation was confirmed in woody crops in a greenhouse setting using potted plants in field soil. As an initial verification, 100% of pilot test plants of olive (Arbequina), a highly susceptible host (5 plants, replicated three times, n=15) died after inoculation with colonized cotton stems as of 5 wk after inoculation. 14 of 15 (93%) pilot test plants (5 plants, 3 rep, n=15) died after inoculation with colonized millet. Devising new inoculation methods that are functional and effective for large numbers of experimental units allows for research to be conducted in a much more efficient manner. Post inoculation: -All olive varieties declined after successful inoculation in pots -Most Oleaceae (olive family) relatives except *Forestiera neomexicana* declined and died. *F. neomexicana* did have chlorosis but did not die, even 3 months after inoculation. Twelve plants of *F. neomexicana* survived inoculation. Additionally, 3 plants of *Fraxinus pennsylvanica* survived inoculation as of 5 wk post inoculation. Facebook Live presentation, Oct 1, 2021; 88 cumulative views on Plant Doctor Kim's Facebook Page. "Cotton Root Rot in Texas Olives & Woody Plants" A live presentation detailing what CRR is, risk factors, and mitigation efforts. The 2021 Field day at the Uvalde Center was not held due to Covid. The 2022 TxOAA meeting/field day was canceled and rescheduled for the fall out of sensitivity to Uvalde residents (school shooting incidence). We plan to present findings at that fall meeting, date TBD.

DATA COLLECTION

Explain what data was collected, how it was collected, the evaluation methods used, and how the data was analyzed to derive the quantifiable indicator.

Olive Genotyping and Sequencing: We used 90 olive accessions obtained from the National Clonal Germplasm Repository (NCGR) at the University of California-Davis (UC-Davis) and samples of 6 regionally

popular olive varieties. For efficient rooting, the basal end of the cuttings was dipped in 1000 ppm indole butyric acid [30] for 10 s. After the IBA treatment, the cuttings were inserted in Deepot tree pots containing perlite and kept under mist (80 to 90% relative humidity) at the Texas A&M AgriLife Research and Extension Center, Uvalde, TX, USA. The intermittent mist system was operated as needed to maintain uniform moisture around the cuttings. The olive accessions originated from 18 countries. The cuttings with newly sprouted leaves were transferred to new pots for the subsequent management. The 18 countries were categorized into 5 major climatic zones based on the Köppen Climate Classification. Leaf samples were collected from the different accessions and were used for total DNA extraction. An ApeKI restriction enzyme was used to construct the DNA libraries for the GBS. The library construction and sequencing by NovaSeq 6000 (Illumina, San Diego, CA, USA) were performed in the Bioinformatics Resource Center, University of Wisconsin–Madison. The details of the bioinformatic analysis of the entire process are available online https://doi.org/10.3390/genes12122007

Soil and root metagenomics: Soil and root samples were collected throughout the year by visiting three orchards and processed using standard laboratory protocols before sequencing. The table below shows the sampling details.

Level 1	Organism	Bacterial or Fungal Microbiome																			
Level 2	Sample Type	Soil (Rhizosphere) - V3-V4									Root (Endosphere) - Arbequina										
Level 3	Season	Spring			Summer			Fall			Spring			Summer			Fall				
Level 4	Location	Carrizo Springs	Moulton	Berclair	Carrizo Springs	Moulton	Berclair	Carrizo Springs	Moulton	Berclair	Carrizo Springs	Moulton	Berclair	Carrizo Springs	Moulton	Berclair	Carrizo Springs	Moulton	Berclair		

Cotton root rot (CRR) screening: Pilot plants (alfalfa) were inoculated with either colonized cotton stems or colonized millet (20 plants each, 3 reps each). Plants were scored dead (with Po symptoms) or not dead at 5 wk after inoculation. After inoculation with the fungus, survival data were collected as disease symptoms (mild to moderate wilting of <50% tissue, or advanced wilting of >50% of leaf tissue and leaf death), and plant death over time post inoculation (3 weeks, 5 weeks). Visual assessments were performed to classify symptoms as (none; mild/moderate; advanced; or dead). Data were examined across reps for each accession/variety.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel		
Fringe Benefits		
Travel		
Equipment		
Supplies		
Contractual		
Other		
Direct Costs Sub-Total		
Indirect Costs		
Total Federal Costs		

PROGRAM INCOME (IF APPLICABLE)

Source/Nature (i.e., registration fees)	Amount Approved in Budget	Actual Amount Earned
1.		
2.		
3.		
Total Program Income Earned		

Use of Program Income *Describe how the earned program income was used to further the objectives of this project.*

ADDITIONAL INFORMATION

Provide additional information available (i.e., publications, websites, photographs) that is not applicable to any of the prior sections.

Publications(* corresponding author)

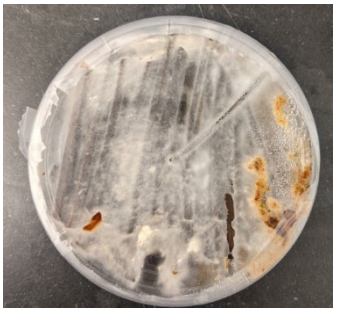
- Islam ASMF, Sanders D, Mishra AK, **Joshi V* (2021)** Genetic diversity and population structure analysis of the USDA olive germplasm using Genotyping-By-Sequencing (GBS). **Genes** 2021, 12, 2007.

- Additional publications on metagenomic analysis and GWAS of cold tolerance using electrolyte leakage will be available in the public domain when published.

Cotton root rot screening and culture deposition

Inoculum growing at 31C in the incubator prior to use. Colonized cotton stems (top shelf), and colonized millet in a mushroom spawn bag (bottom shelf).

500 ml bottle (left) and petri dish (Right) with sterilized cotton stems colonized with Po fungus. Petri plate is 10cm wide for scale.



Colonized millet chunk. The clumps of inoculum were broken up into small pieces (<5mm) prior to incorporation. Millet grains are approx.. 2-3mm. The white to beige material is the fungal hyphae.