FINAL PERFORMANCE REPORT

GRANT INFORMATION

AGREEMENT

AMS Agreement Number:	AM170100XXXXG002			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	9/29/2020
Award Amount:	$529,295.46			

RECIPIENT

Recipient Organization Name:	Oklahoma Department of Agriculture, Food & Forestry
Recipient's Point of Contact	
Name:	Jason Harvey
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REPORT

Report Type:	Final Report
Date Report is Submitted:	12/29/2020

GRANT ADMINISTRATION

Amount Requested	Direct and/or Indirect Expended to Date
$42,304.00	$32,301.18 Direct cost

Project Title	Integrated Cucurbit crop scheduling and pest and pollinator management			
Recipient Organization Name:	Oklahoma State University			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	9/29/2020

Recipient's Project Contact	
Name:	Eric J. Rebek
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PERFORMANCE NARRATIVE

PROJECT BACKGROUND

Summer squash is a popular vegetable crop both for commercial fresh market farms and home gardens. The primary insect pest of summer squash is squash bug, *Anasa tristis*, which overwinters in crop debris and protected areas in landscapes surrounding production areas. One method to manage this devastating insect pest is installing row covers. Row covers are used to exclude squash bugs and other insect pests from gaining access to squash. These exclusion devices are constructed from hardy insect netting materials, and they have been used with limited success on cucurbit crops. One obstacle to wider adoption of row covers is the exclusion of not only pests but also insect pollinators, which need access to flowers for successful pollination and fruit set. Thus, row covers must be opened for some time each day during bloom to ensure successful pollination by insects. However, this practice increases the potential for squash bugs to colonize the plants. In previous studies, we realized the potential for removing row covers two weeks after production of female flowers without inhibiting pollination while reducing abundance of squash bugs.

This project was conducted for two years at three replicated small garden sites in Oklahoma – Bixby, Langston, and Stillwater (2019) and Lane, Langston, and Stillwater (2020). We investigated the effect of timing of row cover removal at several time intervals (i.e., 1, 2, 3, and 4 weeks following 50% bloom of female flowers) on squash bug abundance and squash yield compared to a control (no row cover). Each plot measured 15 ft (4.6 m) and consisted of 6 squash plants ('Lioness' summer squash) grown from seed and spaced on 2 ft (0.6 m) centers. Row cover treatments and the control were replicated three times and assigned to plots as a completely randomized design. Row cover material consisted of DeWitt row cover 0.5 oz material (DeWitt Co., Sikeston, MO USA) draped over plots by attaching the fabric to metal hoops using large binder clips. Metal hoops were slid over rebar that was driven into the ground and excess fabric was buried under soil on all edges to prevent insects from crawling under the row cover material.

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*
1	Determine the effect of covering squash with insect-excluding row covers for different lengths of time on squash productivity following cover removal.	X	
2	Compare two squash types (summer and winter) for the effectiveness of insect exclusion for protecting squash from insect pests.		X
3	Determine effect of delayed row cover removal on the incidence of insect pest and pollinator species in squash following cover removal	X	
4	Sharing project results with Oklahoma farmers, market gardeners and the general public.	X	
5	Train Extension Educators and Master Gardeners in the use of row covers for insect pest management in squash crops		X

ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	We established row cover trials at three locations in Oklahoma – Bixby, Langston, and Stillwater (2019) and Lane, Langston, and Stillwater (2020). Squash plots were covered with row cover material, which was removed at 1, 2, 3, and 4 weeks after 50% bloom of female flowers to allow pollinator access.	These trial sites and the number of squash species vary from what was proposed in the original research proposal because of space availability. However, the trial sites still represent different growing environments and geographic areas of squash production within Oklahoma, and yellow summer squash is a popular species planted by Oklahoma gardeners and fresh market producers.
2	**Objective 1.** We recorded number of marketable and culled fruits, and the weight of marketable fruits (i.e., yield) at all three trial sites for each harvest date, approximately twice per week following fruit set.	Yield data relate directly to fruit productivity per plot and indicate relative success of pollination by insects since squash and other cucurbit crops are entirely dependent on insect pollinators for reproduction.
3	**Objective 3.** We documented insect pest and pollinator abundance and activity at all three trial sites approximately twice per week following plot establishment post-germination.	Abundance data for all squash bug life stages relate directly to the effectiveness of row covers in preventing squash bug access to squash plants.
4	**Objective 4.** Preliminary results from work at Bixby in 2019 were shared at the American Society for Horticultural Science annual meeting. This objective is partially complete and in progress.	We have more work to do with disseminating our research results with vegetable growers. Now that data collection and analyses are complete, we are prepared to share project results and

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
		recommendations with producers in Oklahoma and the Southwestern U.S. in 2021 and beyond.

CHALLENGES AND DEVELOPMENTS

#	Challenge or Development	Corrective Action or Project Change
1	**Objective 2.** The aim of comparing differences in squash bug abundance between two types of squash (summer and winter) was omitted in both years of the project due to lack of plot space.	Only enough research space was available to conduct the trial on 'Lioness' summer squash, a hardy, disease-resistant cultivar that is highly attractive to squash bugs.
2	**Objectives 4 and 5.** Aside from sharing some preliminary results with stakeholders at ASHS in 2020, the COVID-19 pandemic has impacted our ability to disseminate final results of the project due to cancellation of commodity meetings (e.g., 2021 OK/AR Horticulture Industries Show) and training sessions.	Final results of the project will be disseminated to stakeholders at grower meetings and in-service training sessions rescheduled or planned for in 2021, including the 2021 Oklahoma State Master Gardener Conference (to be conducted virtually) and the Hort Update monthly in-service training webinar. The final project report will be published in the 2021 OSU Vegetable Trials extension circular and a peer-reviewed publication will be prepared and submitted to a horticultural science journal.
3	Storms and high winds damaged row cover fabric in some plots at multiple sites, requiring repairs and/or replacement periodically. Openings in the row covers may partially explain why squash bugs were able to colonize certain plots protected by row covers.	In 2020, we amended the method of securing row cover fabric to frames to prevent storm and wind damage experienced the previous years. Specifically, row cover fabric was allowed more slack so binder clips were less likely to rip through the fabric in extreme weather. Yet, high winds commonly experienced in Oklahoma were still damaging to several row covers.

LESSONS LEARNED

We used a light row cover fabric, DeWitt row cover 0.5 oz material (DeWitt Co., Sikeston, MO), which presented a trade-off between durability and fruit quality. From a companion study, we learned that heavier fabric (1.0 oz row cover material) was better able to withstand high winds and storm activity, but fruits were more prone to mold and rot due to high humidity under heavy fabric. Thus, the choice of row cover material will depend on the prevailing climate and weather patterns where a grower is located.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

As mentioned above, project results will be disseminated to stakeholders at rescheduled or planned meetings and training sessions in 2021 and beyond. Additionally, full trial results for both years will be published in vegetable trial reports (2021) and a peer-reviewed manuscript

BENEFICIARIES

Number of project beneficiaries: 800

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

OUTCOME MEASURE(S)

- ☐ **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)

In order for vegetable producers to grow cucurbit crops in a sustainable, cost effective, and profitable manner, it is necessary to adopt effective and judicious insect pest management procedures. Oklahoma Cooperative Extension Horticulturists and Entomologists have worked with multiple approaches to mitigate the losses caused by cucurbit insect pests for over 25 years. One general approach we have taken is the use of exclusion tactics, a concept which requires consideration of not only exclusion of the pest species but also the provision of access to crops by pollinator insect species. The use of exclusion techniques is of interest to growers because they not only address the prevention of crop damage by insect pests, but they also offer potential to reduce the use of chemical insecticides in cucurbit crops. Several potential benefits here are the reduced need to apply insecticides (a production cost), potential reduction in consumer exposure to insecticide residues, and a reduced hazard to pollinator species of exposure to insecticides (i.e., once squash is producing fruit there is no good insecticide application window as flowering and fruiting occur simultaneously). Along with the research that has been conducted to develop the procedures and recommendations for using insect pest exclusion and pollinator

management, there has been a parallel outreach effort to disseminate this information to growers. This has included Extension Agent training sessions, presentations at the annual Oklahoma/Arkansas Horticulture Industries Show, presentations made at Master Gardener training events, personal visits with producers, and presentations made at sessions of the Oklahoma Market Garden School. Specialists who have worked with the development of insect pest exclusion have also visited with individual growers on the use of this insect pest management approach. Numbers provided in the "Outcome Indicators and Sub-indicators" are based on interactions with growers through these various communication venues. This said, the purpose of doing the field research funded by this Specialty Crops Research Initiative Block Grant is to develop the information needed to enable more concise recommendations for making efficient use of this concept of insect pest and pollinator management for cucurbit crops. Results of these trials from 2019-2020 will enable us to provide more concise recommendations on the use of this management approach, as well as any limitations that growers should be aware of. We anticipate that this information will contribute to an increase in adoption of this insect pest management approach, which in turn should reduce reliance on insecticides and production costs associated with their use.

#	Outcome and Indicator	Quantifiable Results
1	Outcome 4, Indicator 2.a,	Approximately 80 growers have indicated their intent to adopt recommended practices to exclude insect pests while allowing pollinator access to cucurbit crops. Following presentations of our trial results at the 2021 Oklahoma Master Gardener State Conference (to be held virtually on June 25) and the OK/AR Horticulture Industries Show in early 2022, we will provide a survey of attendees on their willingness to adopt this pest management approach based on our refined recommendations..
2	Outcome 4, Indicator 2b.	Approximately 40 growers have indicated a reduction in insecticide use based on our previous recommendations. As above, our survey of attendees at upcoming conferences will provide us an indication of the number of growers who are willing to reduce insecticide use upon adopting our refined recommendations.
3	Outcome 4, Indicator 2c.	Approximately 30 growers have indicated reduced costs per acre based on our previous recommendations. As above, our survey of attendees at upcoming conferences will provide us an indication of the number of growers who are likely to experience

		reduced production costs upon adopting our refined recommendations.
4	Outcome 5, Indicator 8.	Approximately 60 growers have gained knowledge about science-based tools through our previous outreach and education programs. As above, our survey of attendees at upcoming conferences will provide us an indication of the number of growers who will gain knowledge about this pest management approach based on our complete data set from two years of field trials..

DATA COLLECTION

In both years at all sites, squash bug life stages (eggs, nymphs, adults) were counted separately twice weekly from each plot once squash plants bolted. Squash yield, measured as total weight of marketable fruit per plot, was evaluated as fruit became available, typically once or twice per week. All data were pooled for each treatment and control by year and site, square root transformed to meet assumptions of normality, and analyzed using one-way analysis of variance (ANOVA) and Tukey's honestly significant difference tests at $\alpha = 0.05$ (PROC ANOVA, SAS 9.4).

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$20,000.00	$19,135.93
Fringe Benefits	$2,010.00	$1,217.03
Travel	$6,500.00	$191.98
Equipment	$0.00	$0.00
Supplies	$8,198.00	$3,029.17
Contractual	$0.00	$0.00
Other	$0.00	$0.00
Direct Costs Sub-Total	$36,708.00	$23,574.11
Indirect Costs		
Total Federal Costs	$36,708.00	$23,574.11

PROGRAM INCOME (IF APPLICABLE)

N/A

ADDITIONAL INFORMATION

N/A

Project Title	Evaluation of Pecan Root Systems to Increase Performance and Profitability of Pecans			
Recipient Organization Name:	Noble Research Institute			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	9/29/2020
Recipient's Project Contact				
Name:	Charles Rohla			
Phone:	580-224-6451			
Email:	ctrohla@noble.org			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

Pecans are the largest specialty crop in Oklahoma. Over the past 10 years there has been a lot of interest in planting pecans, in fact it is estimated that over 250,000 trees have been planted in Oklahoma over this time. With this increased interest in pecans and the large amount of investment it takes to bring an orchard into production there is a desire to increase the efficiency of newly planted trees to encourage the trees to start production earlier therefore, recouping investment costs quicker. The pecan industry is lacking relevant research concerning the root systems of pecan trees. The root system is the foundation of the orchard and a better understanding of the root system has the potential to increase tree performance, production and increase grower's profits. The development of a strong root system can increase the survivability, growth of trees and enhance production as the tree ages. This grant proposal addresses three different research studies to start developing a better understanding of the pecan root system. 1). Understanding how pecan trees grow in different soil types and the limitations of different soils on root development will assist growers planting new trees in these different soil types. Large horhizotrons (four wedge- shaped glass quadrants that extend away from the plant's root ball allowing measurements of roots) were built to be able to test the impact of different soil types on pecan root growth. Trees were harvested, and roots collected from each wedge to determine total root growth in the different soil types. 2). Understanding how the pecan root system grows and the size of the root system at different ages of trees will help growers know where water and nutrients should be placed to help with tree upkeep and growth. Large custom build containers were used to plant pecan trees in.

Trees were harvested after the first growing season to determine total root growth. 3). Root pruning of pecan trees to determine the impact on tree growth, production and quality was planned, however because of extreme rainfalls in the spring during the study period this study was not able to be conducted. This study will be continued within the Noble Research funding to complete this research.

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*
1	Evaluate the efforts different soil types have on pecan tree root development	X	
2	Correlate tree growth to root growth of newly planted trees	X	
3	Evaluate the efforts of root pruning on production and performance of pecan trees		X

ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	Horhizotron structures worked to evaluate pecan tree root systems. Additional research will be developed using these large horhizotron to understand root growth and modification of root systems that will benefit growers.	Object 1
2	There was an impact on root development due to the soil type that the roots were growing in. With this information additional research can be developed to have a better understanding of fertilizer and water placement for better efficiency and potentially lower input cost for growers.	Object 1
3	The large custom-made pots worked to grow pecan trees in. However, the pecan trees root systems reach the edge of the pots within 1 year, which was unexpected and could alter the root structure because of air-pruning the tips of the roots that reached the edge would encourage additional root development along that root.	Object 2

CHALLENGES AND DEVELOPMENTS

#	Challenge or Development	Corrective Action or Project Change
1	Weather problems	The excessive rains in the spring causes the root pruning to not be completed for objective 3. Therefore objective 3 project has not been completed at this time; however, the project will continue to determine the effect of root pruning on production and tree growth. We had planned to conduct the pruning prior to budbreak, but with the soil conditions at that time of year has shown us this time is not practical. We will be pruning roots in the winter after trees have gone dormant to allow adequate opportunity to complete the pruning prior to budbreak and spring rains.
2	Root growth more than expected	For objective 2, the root system grew more than what was expected. When this study is redone in the future, trees will be removed throughout the first year and not over years to determine root growth compared to top growth. Trees root systems had reached sides of the bags and therefore the root structure may have changed due to the tips of the roots being air-pruned at the edge of the bag. Air-pruning would encourage more lateral branching of the root and more fibrous root formation. The large containers were used to try to capture the entire root system to be able to collect total root growth from the trees. Doing this in the ground would be more difficult, however with new affordable technology available (ground penetrating radar) this type of study may be able to be conducted in the field and over a long period of time.

LESSONS LEARNED

Horhizotrons that were built for this project worked very well for tree root research. Additional studies are being developed to utilize these structures to continue research on pecan root systems. For the horhizotron study if different soil is used in the different compartments, you have to water each compartment differently. We used small micro-sprinkles that would water each compartment and had soil moisture sensors in each compartment to help us determine when to apply irrigation. Each soil type required different lengths of irrigation to maintain comparable soil moisture.

The large containers where custom built by High Caliper Growing Systems in Oklahoma City, Oklahoma. We learned that using a cattle panel to support the sides of the containers helped us fill the bags with soil. Without the panels the side walls were weighted down, and we were not able to pull them up to maintain the desired height. The high caliper containers are breathable therefore the sides dried out faster than the center of the container. Therefore, it takes a while to figure out how to water them to maintain consistent moisture throughout the container. We used two micro-sprinklers with a 50% wetting pattern on each side of the tree that would water just passed the edge of the container. This helped maintain moisture throughout the container.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

Information gained from these studies will be shared with pecan growers at the Oklahoma Pecan Growers Conference in 2021 (2020 conference cancelled due to Covid-19) and in the Oklahoma Pecan Growers newsletter and in Pecan South magazine. Results will also be shared at the Oklahoma State University Pecan Class and Noble Research Pecan Workshops.

At this time the results have not been published in a journal or newsletter, due to the fact that Noble Research Institute is transitioning to regenerative agriculture and all previous projects and external writing have been put on hold until the transition is completed. During Covid and the transition to regenerative agriculture, Noble Research Institute suspended all educational workshops (in person, webinars, etc.,) to facilitate all staff to be involved with the transition. Plans are to have articles ready to submit by fall of 2021. The results of this project is also included in the Noble Research Institutes 'Pecan Production 101: Establishing and Managing an improved variety pecan enterprise in the Southern Great Plains' (NF-HO-12-01 previous edition) updated publication that will be published by the end of 2021. This publication is used as an educational guide by Oklahoma State University Pecan Class, Noble pecan workshops, and by several other states for beginning pecan production courses (Mississippi, Arkansas, Tennessee, and Kentucky).

This work will continue as part of the research program at Noble Research Institute. We are currently developing additional research projects that will build on this research and information gained from this research to help pecan growers and researchers to gain a better understanding of pecan root systems and the development of the root system.

BENEFICIARIES

Number of project beneficiaries: 1,500

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

OUTCOME MEASURE(S)

- ☐ **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)

#	Outcome and Indicator	Quantifiable Results
1	Outcome 5. Indicator 7.	2
2	Outcome 5. Indicator 8.	None at this time do to Covid cancellation of conferences

DATA COLLECTION

Objective 1). Total tree and root growth was collected from the horhizotrons. Trees tops were separated at the ground level; therefore, top growth and root growth was harvested separately. Roots growing in each wedge of the horhizotron was collected separately. Root system was washed to remove soil without damaging the root system to keep as much of the root system intact as possible. All samples were dried to calculate dry weight. Roots were measured to determine number of roots, root length and diameter of roots.

Objective 2). Total tree and root growth was collected from the large containers. Trees were removed from the containers using water to wash the soil away from the root system to ensure that the root system remained intact. After entire tree were removed from container, tops were separated at the ground level, therefore, top growth and root growth was harvested separately. All samples were dried to calculate dry weight. Roots were measured to determine number of roots, number of tap roots that emerge from the planting cut, root length and diameter of roots. Tree height, trunk caliper and width of canopy was collected from the tree.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$45,611.00	$45,611.00
Fringe Benefits	$19,389.00	$19,389.00

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Travel	$0.00	$0.00
Equipment	$0.00	$0.00
Supplies	$0.00	$0.00
Contractual	$0.00	$0.00
Other	$0.00	$0.00
Direct Costs Sub-Total	$65,000	$65,000
Indirect Costs	$0.00	$0.00
Total Federal Costs	$65,000.00	$65,000

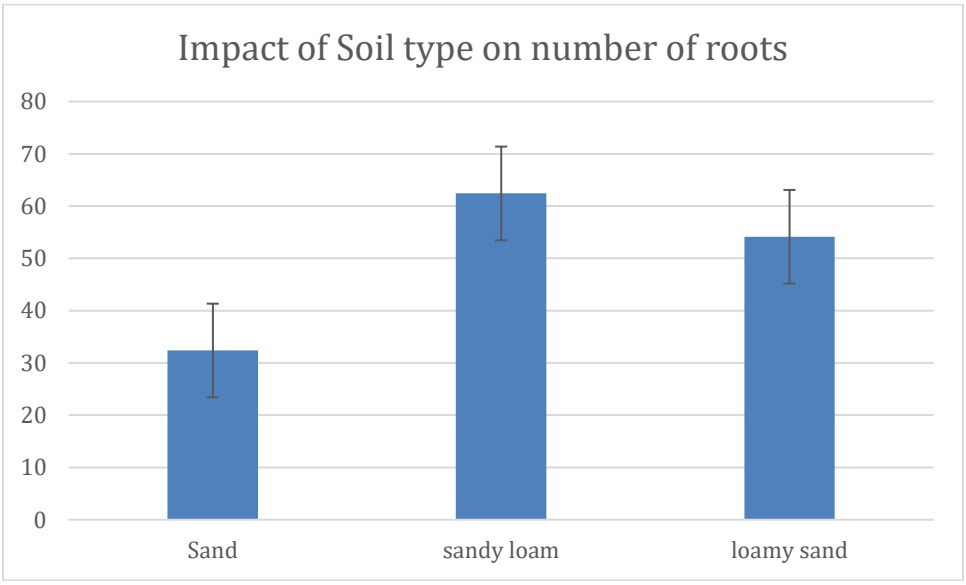
PROGRAM INCOME (IF APPLICABLE)

N/A

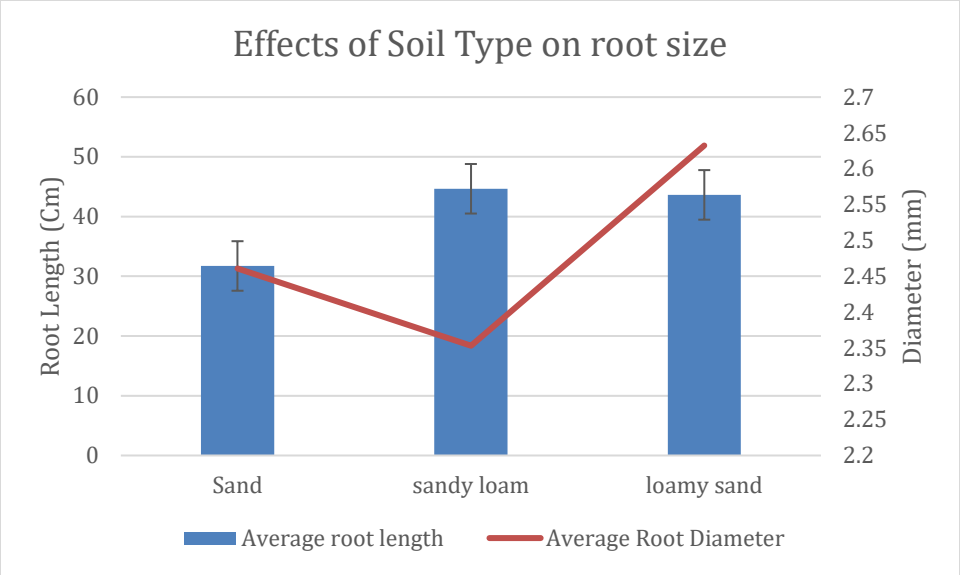
ADDITIONAL INFORMATION

Wedge of horhizotron showing root growth.

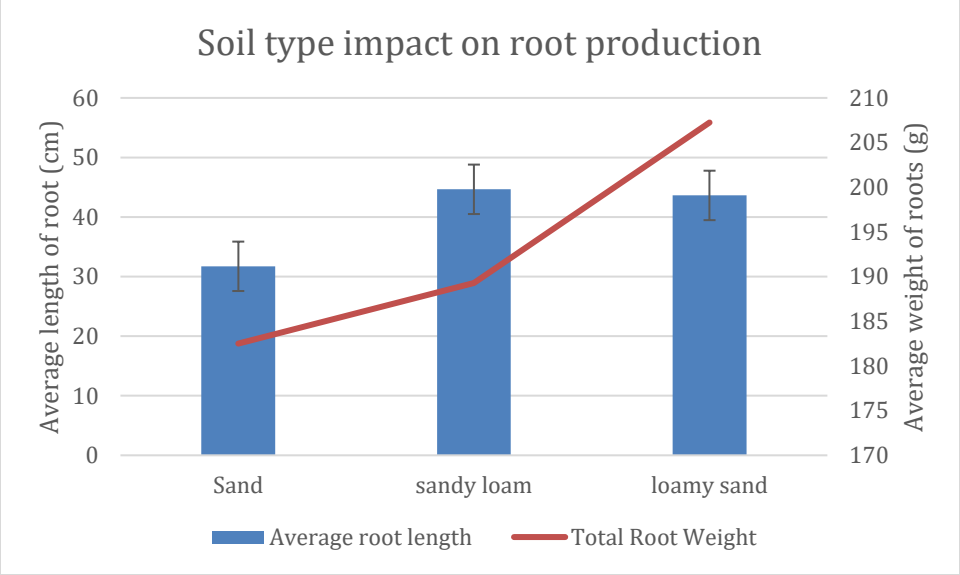
Roots collected from one wedge of the horhizotron. Each root was measured to determine length and diameter. Roots were dried to determine dry weight.



Sandy soils restricted number of roots. Sandy loam and loamy sand soils had significantly more roots developed.



Loamy sand soils form large roots while sandy loam soils had the smallest diameter roots. Sandy loam soil may encourage more fibrous root development which could improve water and nutrient uptake.

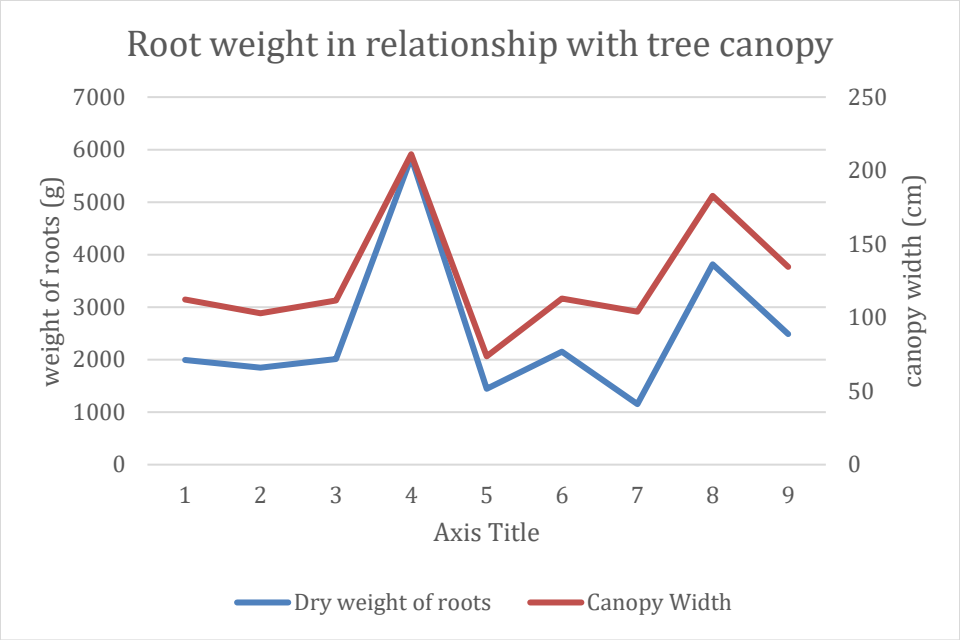


Loamy sand soils produced the largest root system, significantly larger than sandy loam or sand soils.

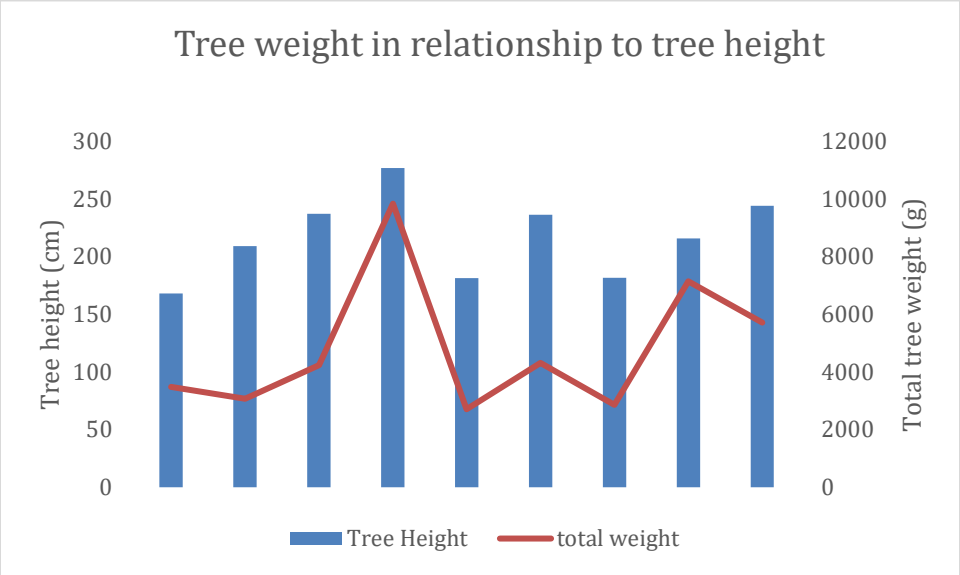
Pecan tree growing in the large container.

Root system of pecan tree that was harvested from the large container. Number of roots, number of tap roots, length of roots and root diameters were collected from all roots.

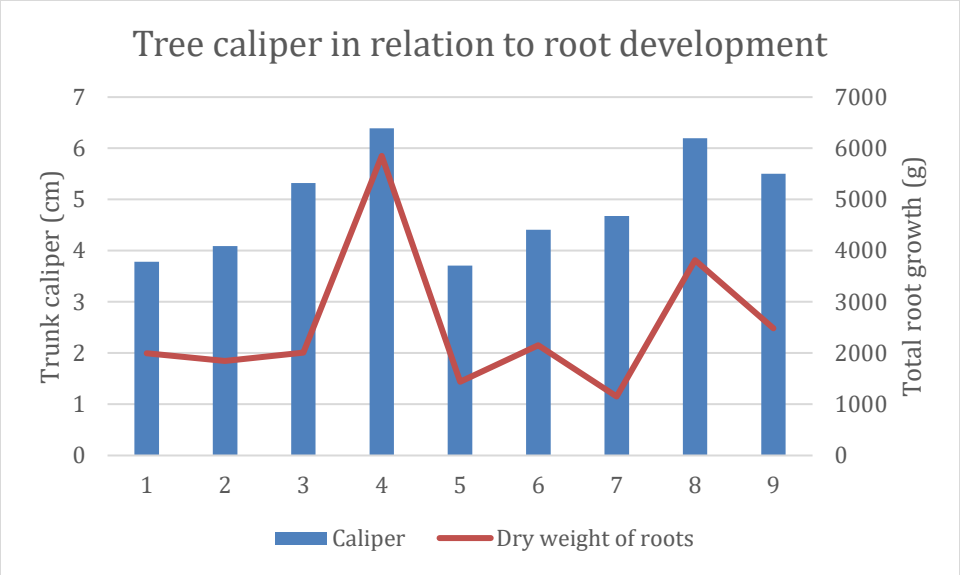
Top of the tree harvested from the large container. Tree height, trunk diameter and canopy width was collected.



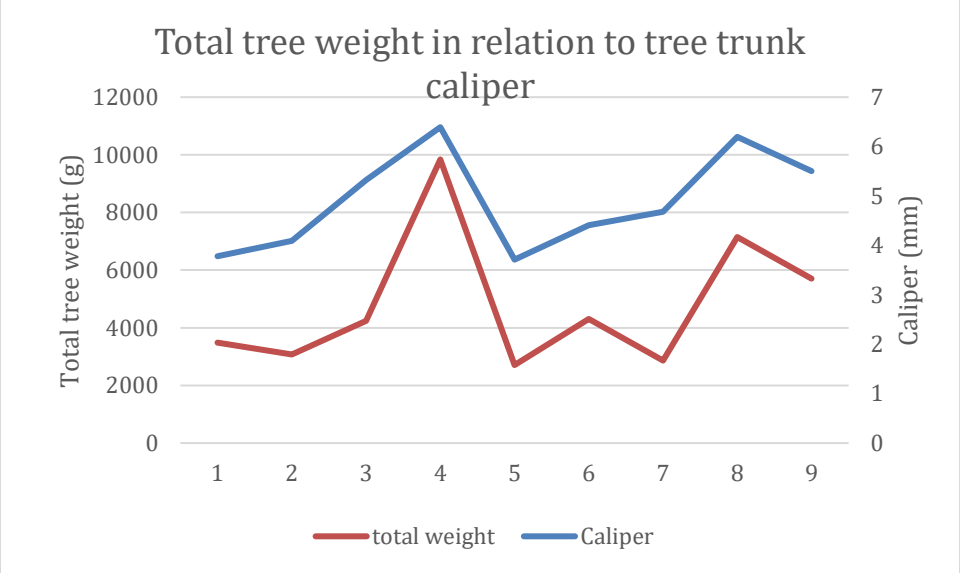
Total root weight was closely correlated to canopy width with the exception of one tree.



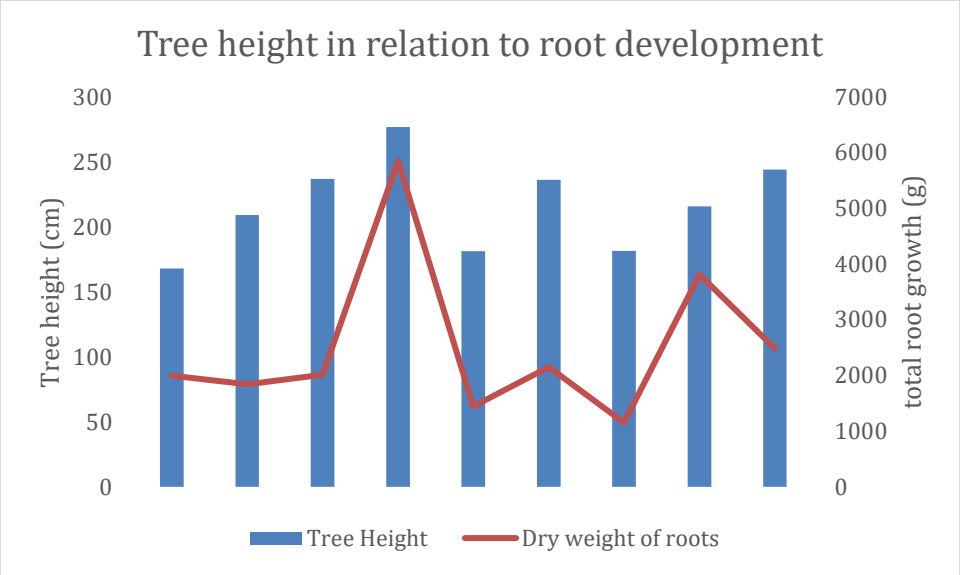
Tree height was not directly correlated to total tree weight.



Tree caliper in relationship to total root weight.



Total tree weight in relationship with trunk caliper.



Tree height in correlation with total root weight.

Project Title	Improving Pepper Establishment and Production Systems			
Recipient Organization Name:	Oklahoma State University			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	9/29/2020
Recipient's Project Contact				
Name:	Lynn Brandenberger			
Phone:	405-744-5408			
Email:	Lynn.brandenberger@okstate.edu			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

Pepper crops grown within the state of Oklahoma include peppers for fresh market including farmer's markets, restaurants, etc. and pungent peppers grown for capsaicin for flavoring salsas and sauces and other uses. The pepper industry within the state of Oklahoma annually accounts for approximately 500 to 600 acres for the combined fresh and pungent pepper acreage with an overall value ~2 million U.S. dollars. Pepper farmers face several challenges during the production cycle including high cost and time involved in transplanting, potential disease from greenhouse grown transplants, and competition from weedy species. Production soils within the state often have levels of organic matter less than 1% which has a negative effect upon seedling emergence and crop growth. During the past decade disease pressure from bacterial spot (*Xanthomonas vesicatoria*) has become an annual issue. Spread of this

disease may be from infected seed but may also be spread by infected transplants. Transplants are expensive ($300 per acre) and require significant amounts of hand labor to plant. Pepper is a warm season long-term crop requiring up to 80 days from transplanting to harvest for fresh types and longer for pungent types. During this extensive growth period the crop can suffer reduced growth and yield from weed competition from several species of warm season weeds. Fast growing weed species such as Palmer amaranth (*Amaranthus palmeri*), carpetweed (*Mollugo verticillata*), and buffalo burr (*Solanum rostratum*) are some of many aggressive weed species that Oklahoma pepper farmers are having problems with. Transplanting and hand-hoeing are major labor expenses for pepper farmers with costs ranging from 200 to nearly 1,000 dollars per acre.

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*
1	Determine the best management practices for adding organic matter to production soils through the use of cover crops.	X	
2	Determine the feasibility of establishing pepper crops through direct seeding rather than transplanting for both pungent and non-pungent pepper crops.	X	
3	Screening of potential weed control methods utilizing cover crops and herbicide technologies for weed control in commercial pepper production.	X	
4	Share research results with Oklahoma farmers, representatives of the IR-4 USDA project, and the general public.	X	
5	Training of a graduate student in applied research techniques while working on pepper establishment and production improvements.	X	

ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	Demonstrated that levels of organic matter can be increased in production soils through the use of cover crops.	During the past 3 years organic matter levels in our cover crop study plots have continued to rise and those levels are now higher than those in the fallow plots. This will help farmers safely increase the level of organic matter in their production fields.
2	Determined that the emergence of direct seeded pepper can be increased through the use of basic seed priming techniques that can be used by farmers when combined with certain cover crop treatments.	During year one and two of the study, greenhouse work was completed that increased the rate and emergence of pepper seedlings for both pungent and non-pungent pepper. This work was followed by field studies using the two different seed primers

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
		with three different cover crop treatments and the fallow treatment. The highest emergence was obtained in the wheat/crimson clover and the cereal rye, Austrian winter pea, tillage radish winter cover crop treatments.
3	Screening of different preemergence demonstrated the efficacy of several new herbicides for use in pepper production.	Two years of herbicide screening was accomplished and determined that some new materials have potential for controlling the emergence of weedy species both as pre-plant and post-plant applied materials. Results were shared with the IR-4 USDA project for future consideration.
4	Results were shared with farmers and industry representatives through various venues.	Presentations were given at the Oklahoma Horticulture Industry Show, Southern Region ASHS, and two separate field days to share information about cover crops and direct seeding pepper crops.
5	Graduate student completed studies on direct seeding establishment of pepper crops.	The student who worked on this project was able to share information at field days and professional meetings. She did complete her M.S. in Horticulture and defended her thesis in November of 2019. She is now employed and enjoying her position with the Bureau of Indian Affairs.

CHALLENGES AND DEVELOPMENTS

#	Challenge or Development	Corrective Action or Project Change
1	Pest problems during field research phase of the project.	We were able to determine that blister beetles were consuming the field study in 2018 and applied a pesticide to correct the problem.

LESSONS LEARNED

Recommendations include maintaining a regular meeting time with graduate students and setting deadlines for meeting project goals.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

Possibly disseminating the results of the cover cropping portion of the project through an extension bulletin or fact sheet

BENEFICIARIES

Number of project beneficiaries: 204

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

OUTCOME MEASURE(S)

- ☐ **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)

#	Outcome and Indicator	Quantifiable Results
1	**Outcome 4, Indicator 2.a.**	Due to the pandemic, we have had few opportunities to meet with growers/producers to survey producers to determine the number that have adopted practices from this research. Information was shared through the on-line publication of study results in the annual "Vegetable Trial Report" which is available at: https://extension.okstate.edu/fact-sheets/vegetable-trial-reports-2010-2019.html . There were no state trade meetings with producers this past year in-person or virtual and therefore we did not share through either venue. Future plans may include sharing information through newsletter articles and when we have field days.

2	**Outcome 4, Indicator 2. c.**	Due to the pandemic, we have had few opportunities to meet with growers/producers to survey producers to determine the number that have increased dollar returns per acre. Presentations were given at the Oklahoma Horticulture Industry Show (Grower conference) during 2018 and 2019, Southern Region ASHS in 2019, and two separate field days to share information about cover crops and direct seeding pepper crops.
3	**Outcome 5, Indicator 8.**	Knowledge was shared through field days at the Cimarron Valley Research Station and through presentations at professional meetings including the Oklahoma/Arkansas Horticulture Industry Show and the Southern Region meeting for the American Society of Horticulture. We were able to make contact with more than 200 clientele through these meetings. In addition, the results of the research were shared through the 2018 and 2019 Vegetable Trial Reports MP-164 which has been available through our department's website.

DATA COLLECTION

Greenhouse emergence trials were carried out using a randomized block design and results were analyzed using SAS v9.4 (SAS Institute Inc., Cary, NC). Recorded data included seedling emergence with data being subjected to an analysis of variance. Significant effects were further analyzed using the Probit procedure to determine differences in treatment means. Significance levels were at 0.05. The field studies were organized as a randomized complete block design (RCB) with four replications as a 2 x 2 x 4 factorial. Factors consisted of two planting dates, two seed priming treatments, and four cover crop treatments. Field data included plant emergence and whole-plant fresh and dry weights at the end of the season. These data were subjected to an analysis of variance. Plant count and yield significant effects were further analyzed using the GLIMMIX procedure, and soil organic matter data were also analyzed using GLIMMIX to determine differences in treatment means. All statistical tests were conducted at the 0.05 significance level.

Screening of herbicides for weed management in pepper included live crop-plant counts, crop injury ratings, crop height measurements, and efficacy ratings for weed control. All experiments were set up with three replications in a randomized block design and data were analyzed using Duncan's Multiple Range Test where significance levels were set at 0.05.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$41,588.00	$39,545.04
Fringe Benefits	$5,216.00	$7,187.41
Travel	$2,308	$1,086.83
Equipment	$0.00	$0.00
Supplies	$5,888.00	$4,780.59
Contractual	$0.00	$0.00
Other	$0.00	$0.00
Direct Costs Sub-Total	$55,000.00	$52,599.87
Indirect Costs	$0.00	$0.00
Total Federal Costs	$55,000	$52,599.87

PROGRAM INCOME (IF APPLICABLE)

N/A

ADDITIONAL INFORMATION

N/A

Project Title	Evaluation and Production Potential for Elderberry as a Potential Native Economic Crop in Eastern Oklahoma			
Recipient Organization Name:	Kerr Center for Sustainable Agriculture			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	9/29/2020
Recipient's Project Contact				
Name:	David Redhage			
Phone:	918-647-9123			
Email:	dredhage@kerrcenter.com			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

The project addressed two issues or needs: The need for perennial crops for permanent production systems that are more sustainable and better-adapted to the soil and climate conditions of the Mid-South and a need for greater diversity on area small farms particularly in the area of small fruits.

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*
1	Evaluate both new and current commercial varieties of Elderberries for production potential in Oklahoma	Yes	
2	Identify constraints to growing elderberries in Oklahoma	Yes	
3	Educate potential growers on Elderberry production and Marketing opportunities	Yes	

ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	Determine Bud break and cane emergence (2018, January 5th, 2019 February 13th, 2020 January 7th	Objective 1 Objective 2
2	Plant disease resistance and vigor can impact potential on yields. 2018-Little evidence of disease and plants were vigorous. 2019-Little evidence of disease, plants appeared to suffer from nitrogen deficiency, plant vigor was lower. 2020-Some Bacterial Spot, vigor heavily impacted by insects	Objective 1 Objective 2
3	Percentage Blossom Bloom- Attempting to estimate percentage in bloom per variety. Ranked varieties based on amount of flower development. Insect damage did not allow for good data collection in 2020.	Objective 1
4	Diameter and number of blooms-counting number of blooms proved to be unfeasible due to differences in bloom maturity and variable density.	Objective 1 Objective 2

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
5	1st harvest date-Growers may wish to plant different varieties to spread out harvest over time. The earliest harvest date 2018 was Bob Gordon on 7/23/18, in 2019 Ranch harvested 1st on 7/22/19. The last harvest date 2018 was 8/27/18 for 360 Farms' varieties. 2019 last harvest date was 8/19/19 for 360 Farms' varieties.	Objective 1 Objective2
6	Fruit Quality (Brix)-Sugar level may be relevant for winemaking. One of the new varieties varied in Brix level from 8 through 13 over the harvest period in 2018. High rainfall levels may reduce brix prior to harvest.	Objective 1 Objective 2
7	Fruit Yields-Important for potential end product development and budgeting. We have compiled the data for 2018 and 2019. The data for 2020 is separate since yields were so low.	Objective 1 Objective 2
8	Workshops-Conducted a workshop on July 13, 2019 at the Kerr Center. We were developing onsite workshops for 2020 but the COVID pandemic forced us to stop all onsite workshop planning in 2020.	Objective 3

CHALLENGES AND DEVELOPMENTS

#	Challenge or Development	Corrective Action or Project Change
1	Early Bud Break-Jan. 5, 2018, Feb. 13, 2019, January 7, 2020	Conducted hard prunes immediately after determining bud break even though plants in 2018 did not appear to be in hard dormancy.
2	2019 heavy rainfall levels leached nutrients. Plants exhibited nitrogen deficiencies up to and during harvest.	Conducted soil tests to determine nutrient levels. We increased fertilizer rates in 2020 to 120 lbs. actual N per acre using feather meal to compensate for nutrient losses.
3	Scale problems in 2018	New scale purchased at the end of 2018 worked for us in 2019 so we now have 100 berry weights. Weights were also recorded in 2020.

LESSONS LEARNED

Carefully evaluate what data you will be collecting. Measuring blossom size proved unreliable since we failed to take into account blossom density. The only way to properly measure blossom production for teas would be to harvest the blossoms alone but doing so would have eliminated the berry harvest. Attempt to anticipate problems such as insects and diseases and build into the project either some controls to implement or leave it alone

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

The planting remains in place. We will be working to identify controls for the eriophyid mite and work on better fertility management. One future issue may be woody plant encroachment into the planting rows. We will monitor and remove the invasive woody plants as needed. Onsite workshops will be conducted

BENEFICIARIES

Number of project beneficiaries: 29 workshop attendees, 2205 e-newsletter recipients

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

OUTCOME MEASURE(S)

- ☐ **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
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- ☐ **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)

#	Outcome and Indicator	Quantifiable Results
1	Outcome 4, Indicator 1-The project will be evaluating five new varieties selected by a private grower against one commercial variety to identify potentially new improved	Yields and brix readings were taken on the new varieties Dog Branch, Smokey, George Fork, South Fork and West Road along with two commercial varieties Bob Gordon

	varieties. Out of the five newly named varieties, we anticipate two may have commercial potential.	and Ranch. At this point we have three years' worth of raw data (provided in tables in the Discussion of Activities Performed Section below). Evaluation will need to be made using results from the first two years due to a yield loss in year three. Based on our trial results we were not able to recommend any of the five new varieties for commercial production. The most promising variety (Dog Branch) needs additional study. We can however recommend the two commercial varieties Bob Gordon and Ranch based on our location and data results.
2	Outcome 4, Indicator 3- Elderberries are native flowering plants. Additional planted acreage will increase habitat for native pollinators. Of the 100 potential growers who participate in the workshops, we expect 20 will establish quarter acre plantings of elderberries, resulting in five acres of pollinator habitat	A workshop was held on July 13, 2019 at the Kerr Center Horticulture Farm. COVID 19 prevented us from hosting two workshops in 2020.
3	Outcome 8 Indicator 6- Of the anticipated 100 attendees to workshops, we expect 80 to be potential beginning farmers and of those 15 will plant elderberries as a specialty crop.	The Kerr Center conducted a workshop on July 23, 2019. Twenty-nine individuals attended and twenty-six answered the survey. Seventy-three percent already grew elderberries and of the remaining individuals twenty-three percent indicated they were planning on planting elderberries as part of their farming operation. Workshop planning for 2020 was cancelled due to the COVID pandemic. While we considered virtual trainings, as a small non-profit we had no one on staff with the skill set to manage an online training. Based on federal guidance we hoped face to face trainings would open up in late summer/early fall of 2020, which did not happen. Additionally, the project lends itself to an in-person training technique bringing individuals to the test site to see each variety and evaluate them in the field. While we were unable to conduct trainings during the grant time-frame we are planning on maintaining the planting and

		hosting trainings in 2021if COVID guidelines are relaxed.

DATA COLLECTION

The study site consisted of two rows with ten plants of each variety. Each variety was replicated once per row for a total of twenty plants per variety. Since there was no gap between each variety, we only harvested from the center eight plants in each replication to eliminate the edge effect. The study evaluated plant health, disease resistance/tolerance and vigor though observation. Bud break was recorded. Diameter of the blooms was measured but we soon realized the diameter did not correlate with bloom density, so it was not a good measuring tool to determine feasibility for teas. Beginning and ending harvest dates were recorded. At harvest total berry weight, 100 berry count weights and total weight were taken.

Beginning and Ending Harvest Dates 2018

Variety	Trial 1 Beginning Harvest	Trial 1 Ending Harvest	Trial 2 Beginning Harvest	Trial 2 Ending Harvest
Ranch	7/23/18	7/25/18	7/23/18	7/25/18
Bob Gordon	7/23/18	8/6/18	7/23/18	8/6/18
Dog Branch	7/31/18	8/20/18	8/13/18	8/20/18
Smokey	8/13/18	8/20/18	7/31/18	8/9/18
George Fork	7/25/18	8/6/18	7/31/18	8/9/18
South Fork	7/25/18	8/20/18	7/25/18	8/20/18
West Road	7/25/18	8/27/18	8/6/18	8/17/18

Beginning and Ending Harvest Dates 2019

Variety	Trial 1 Beginning Harvest	Trial 1 Ending Harvest	Trial 2 Beginning Harvest	Trial 2 Ending Harvest
Ranch	7/22/19	7/30/19	7/22/19	7/26/19
Bob Gordon	7/26/19	8/5/19	7/26/19	8/5/19
Dog Branch	8/2/19	8/19/19	8/2/19	8/19/19
Smokey	8/12/19	8/19/19	8/12/19	8/19/19
George Fork	8/2/19	8/5/19	7/26/19	8/5/19
South Fork	7/26/19	8/19/19	7/26/19	8/19/19
West Road	7/26/19	8/19/19	8/2/19	8/19/19

Beginning and Ending Harvest Dates 2020

Variety	Trial 1 Beginning Harvest	Trial 1 Ending Harvest	Trial 2 Beginning Harvest	Trial 2 Ending Harvest

Ranch	7/28/20	7/28/20	7/28/20	7/28/20
Bob Gordon	7/28/20	8/3/20	7/28/20	8/3/20
Dog Branch	8/17/20	8/17/20	8/14/20	8/14/20
Smokey	8/10/20	8/17/20	8/14/20	8/10/20
George Fork	8/3/20	8/7/20	8/3/20	8/10/20
South Fork	8/3/20	8/10/20	8/3/20	8/10/20
West Road	8/7/20	8/7/20	No Yield	No Yield

Average sq. inches flower size by variety-2018

Variety	Trial 1 Average sq. inch/flower	Trial 2 Average sq. inch/flower	Average Both Trials
Ranch	74	98	86
Bob Gordon	93	89	91
Dog Branch	72	64	68
Smokey	60	60	60
George Fork	58	73	66
South Fork	95	101	98
West Road	119	101	110

Average sq. inches flower size by variety-2019

Variety	Trial 1 Average sq. inch/flower	Trial 2 Average sq. inch/flower	Average Both Trials
Ranch	72	68	70
Bob Gordon	82	88	85
Dog Branch	41	50	46
Smokey	47	47	47
George Fork	79	49	64
South Fork	57	73	65
West Road	73	72	73

Yields and Brix 2018

Variety	Total Yield	Yield/Plant-8 plants/rep	Average Brix	100 berry count weight/grams
Trial 1 Ranch	27 lbs. 4 oz.	3 lbs. 5.5 oz.	12.5	n/a
Trial 2	30 lbs.	3 lbs. 12 oz.	12.5	n/a
Trial 1 Bob Gordon	23 lbs. 8 oz.	2 lbs. 15 oz.	12.5	n/a
Trail 2	31 lbs.	3 lbs. 14 oz.	12.5	n/a
Trial 1 Dog Branch	19 lbs. 6 oz.	2 lbs. 6 oz.	10	n/a

Trial 2	20 lbs. 6 oz.	2 lbs. 8 oz.	11	n/a
Trial 1 Smokey	15 lbs.9 oz.	1 lbs. 15 oz.	9	n/a
Trial 2	19 lbs. 11 oz.	2 lbs. 7 oz.	12	n/a
Trial 1 George Fork	20 lbs. 15 oz.	2 lbs. 9 oz.	12	n/a
Trial 2	22 lbs. 11 oz.	2 lbs. 13 oz.	12	n/a
Trial 1 South Fork	34 lbs. 5 oz.	4 lbs. 4 oz.	11	n/a
Trial 2	22 lbs. 2 oz.	2 lbs. 12 oz.	11	n/a
Trial 1 West Road	18 lbs. 2 oz.	2 lbs. 4 oz.	11	n/a
Trial 2	15 lbs. 10 oz.	1 lb. 15 oz.	12	n/a

Yields, Brix and 100 berry count weight 2019

Variety	Total Yield	Yield/Plant-8 plants/rep	Average Brix	100 berry count weight/grams
Trial 1 Ranch	13 lbs. 9 oz.	1 lb. 11 oz.	13	8.04
Trial 2	33 lbs. 8 oz.	4 lb. 3 oz.	12	9.37
Trial 1 Bob Gordon	29 lbs. 5 oz.	3 lb. 11 oz.	11	7.74
Trail 2	38 lbs. 2 oz.	4 lb. 12 oz.	11	8.40
Trial 1 Dog Branch	22 lbs. 2 oz.	2 lb. 12 oz.	9.5	7.31
Trial 2	29 lbs.	3 lb. 10 oz.	11	8.16
Trial 1 Smokey	12 lbs. 6 oz.	1 lb. 9 oz.	10	5.85
Trial 2	19 lbs. 12 oz	2 lb. 6 oz.	10	6.40
Trial 1 George Fork	14 lbs. 12 oz.	1 lb. 13 oz.	10	7.04
Trial 2	12 lbs. 10 oz.	1 lb. 9 oz.	10	7.01
Trial 1 South Fork	28 lbs. 15 oz.	3 lb. 10 oz.	10	6.40
Trial 2	23 lbs. 6 oz.	2 lb. 15 oz.	9	7.40
Trial 1 West Road	21 lbs. 4 oz.	2 lb. 11 oz.	10.5	8.02
Trial 2	26 lbs. 10 oz.	3 lb. 5 oz.	11	9.44

Yields, Brix and 100 berry count weight 2020

Variety	Total Yield	Yield/Plant-8 plants/rep	Average Brix	100 berry count weight/grams
Trial 1 Ranch	6 lbs. 10 oz	13 oz	13	4.60
Trial 2	7 lbs. 0 oz.	14 oz.	13	7.60
Trial 1 Bob Gordon	3 lbs. 6 oz	3 oz.	11	5.83
Trail 2	11 lbs. 2 oz.	1 lb. 6 oz.	11	8.70
Trial 1 Dog Branch	1 lb. 4 oz.	3 oz.	10	6.40

Trial 2	2 lbs. 1 oz.	4 oz.	10	5.60
Trial 1 Smokey	2 lbs. 8 oz.	5 oz.	9	5.25
Trial 2	2 lbs. 3 oz.	4 oz.	9	6.00
Trial 1 George Fork	2 lbs. 4 oz.	5 oz.	11	7.85
Trial 2	2 lbs. 5 oz.	5 oz.	11	7.40
Trial 1 South Fork	1 lb. 12 oz.	4 oz.	11.5	6.65
Trial 2	1 lb. 0 oz.	2 oz.	11.5	6.30
Trial 1 West Road	3 lbs. 4 oz.	7 oz.	7	7.50
Trial 2	No yield	O oz.	7	0.00

Report on Workshop:

The Kerr Center conducted a workshop on July 23, 2019. Twenty-nine individuals attended and twenty-six answered the survey. Seventy-three percent already grew elderberries and of the remaining individual's twenty-three percent indicated they were planning on planting elderberries as part of their farming operation. Workshop planning for 2020 was cancelled due to the COVID pandemic.

Data Interpretation:

Yields were consistently higher for both Ranch and Bob Gordon. Observations indicated lower levels of mites on both Ranch and Bob Gordon. When looking at the other trialed varieties Dog Branch (sold as Oklahoma John) was the most consistent in yield. Shattering and uneven ripening were issues with Smokey, George Fork, South Fork and West Road. Ranch and Bob Gordon had the most consistent Brix levels between 11-13 percent brix. While we feel comfortable recommending Ranch and Bob Gordon to potential growers, the only other variety with potential is Dog Branch but more trial work is needed. The main issues are fertility management, insect management and more specifically, the eriophyid mite and conducting variety trials on different locations to determine how well adapted they are. Elderberries are proving to be very site specific in how well they do on a given location.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$25,099.16	$25,099.16
Fringe Benefits	$8,658.27	$8,658.27
Travel	$1,415.68	$1,415.68
Equipment	$0.00	$0.00
Supplies	$1,537.00	$1,534.08
Contractual	$2,079.89	$1,770.32
Other	$1,210.00	$290.00
Direct Costs Sub-Total	$40,000.00	$38,767.51
Indirect Costs	$0.00	$0.00
Total Federal Costs	$40,000.00	$38,767.51

PROGRAM INCOME (IF APPLICABLE)

N/A

ADDITIONAL INFORMATION

The link below opens to the location on our website where the pdf of the printed report is provided for those interested in the elderberry project:

https://kerrcenter.com/publication/elderberries-in-oklahoma

Project Title	Evaluation of fruits, vegetables, Herbs, and Ornamentals in Hydroponic Systems for Local Fresh Market Production			
Recipient Organization Name:	Oklahoma State University			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	9/29/2020
Recipient's Project Contact				
Name:	Dr. Bruce Dunn			
Phone:	405-744-6462			
Email:	bruce.dunn@okstate.edu			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

The human population is expected to reach nearly 9 billion people by 2050. The main challenge will be to supply local, safe products that are needed for a quality of life while maintaining a healthy planet. The major problems with growing crops in the field include soil-borne diseases, temperature fluctuations, water availability, and disease and pest infestations. Thus, in the last 10 years, there has been increasing interest in hydroponic or soilless techniques for producing greenhouse horticultural crops. Hydroponics is a technique of growing plants without soil using water and nutrient solutions. Crops grown using soilless methods include vegetables, herbs, fruit, and ornamentals, and is the fastest growing area of specialty crops. Today the hydroponics industry is a niche food production market that is dominated by only a few crops. Thus, this project evaluated the feasibility of other crops in hydroponic systems, establish methods to determine quality of lettuce which is one of the most common grown hydroponic crops, and there was a need to educate growers about soilless production.

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*
1	Evaluate alternative crops (fruits, vegetables, herbs, and cut flowers) for year-round production using hydroponics in a greenhouse to support and expand local fresh markets.	X	
2	Organize a conference to educate growers in the industry as well as new potential people that are seeking more information about hydroponic production.	X	
3	Evaluate modifications in postharvest practices necessary to maintain quality of crops grown with hydroponics and disseminate guidelines to complement production guidelines.	X	
4	Develop objective tests of quality for the most commonly grown crop (lettuce) to evaluate and fine tune improvements in hydroponic culture and postharvest handling.	X	
5	Disseminate research findings and learn from other growers or researchers to help solve common problems to advance the industry.	X	

ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	Experiments on blackberries, blueberries, raspberries, and ornamental grass have been completed for the Dutch bucket systems. Lettuce, collards, cilantro, and celery have been completed for the NFT systems.	Objective 1- Evaluated alternative crops (fruits, vegetables, herbs, and cut flowers) for hydroponic production

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
2	A one day soilless production conference occurred on July 10th, 2019. Speakers topics included soilless production systems, plant nutrition, food safety, post-harvest, leafy and fruiting crops, and pest and disease management in a controlled environment. Speakers included Dr. Bruce Dunn, Dr. John Damicone, and Dr. Eric Rebek from OSU/Stillwater, Richard and Jackie Tyler from Noah Farms in Oklahoma, Dr. Kimberly Williams from Kansas State University, and Dr. Stacy Tollefson from the University of Arizona. There were 80 people in attendance.	Objective 2- Organized a hydroponic conference
3	Information on post-harvest handling was discussed at the conference.	Objective 3- Discussed postharvest practices
4	Tests were developed and used for hydroponic lettuce grown in-house and for hydroponic lettuce obtained from hydroponic growers in the state.	Objective 4- Evaluated lettuce quality from growers in the state
5	The hydroponic systems have been shown off to potential and current growers. Those attending the conference heard eight different people talking about hydroponic production, some of which was results from the research. A presentation was given at the Tribal Alliance for Pollinator in November 2019 as part of a breakout day on hydroponics. Results from the lettuce quality studies were also presented at Southern Region ASHS in 2019. Lastly, recording from the conference are used in the Soilless Production class to help train students interested in hydroponic production and may later become growers.	Objective 5- Disseminated research findings

CHALLENGES AND DEVELOPMENTS

#	Challenge or Development	Corrective Action or Project Change
1	We lost several plantings of strawberries.	Initially, we planted in vertical towers then switched to NFT tables. Plants still died out after about 2 months. Once we planted the

#	Challenge or Development	Corrective Action or Project Change
		strawberries in a substrate, rockwool or hydroton, plants did not die out.

LESSONS LEARNED

- Lettuce, cilantro, and collards are all quick and easy crops to grow in NFT systems.
- If growing spinach, start the seeds in a cooler and after seeds have germinated move them under a mist bench.
- Celery took 2-5 months to finish out and would not be an economical crop to grow in NFT systems.
- Strawberries need a substrate, like hydroton or rockwool, when grown hydroponically or they will rot.
- Growers must plan to control spider mites with chemical or biological control in order for strawberry plants to thrive.
- Blackberry plants should be divided each year, as the roots will plug up the Dutch buckets otherwise and reduce yields in the second year as we observed.
- Blackberry canes should be pruned to five canes to reduce excessive vegetative growth.
- Blackberries show potential, but more research might show that plants would benefit from multiple vernalization cycles to induce more fruit production.
- 'PrimeArk' Freedom' would be recommended for greenhouse berry production.
- Under typical Oklahoma greenhouse conditions, raspberries do not produce enough for sustainable production and five plants per bucket would be recommended.
- Blueberries for the most part survived, but they did not thrive in the hydroponic system producing only a few berries. Plants showed iron deficiency and required foliar iron supplemental treatments.
- Purple fountain grass grew well in hydroponics and would need to be divided after 6 months of production as plants were severely root bound.
- To increase vase life of grasses floral preservative should be used.
- 'Rex', which is a butterhead type of lettuce, had the lowest sesquiteroene lactone levels in relation to bitterness and highest total sugar levels of all lettuce sampled.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

We are currently preparing two different manuscripts with one on production and the other on lettuce quality that will benefit current and future growers in the state and industry in general. We plan to apply for a grant through SARE to do a hands-on hydroponic workshop, as 100% of people indicated in the conference survey that they would be interested in attending such a workshop. Lastly, information from the research will continue to be disseminated through site visits, tours, classes, and future guest speaker presentations.

BENEFICIARIES

Number of project beneficiaries: 130

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

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; Indicator 2. a	Fourteen growers or potential growers have visited our production. We had around 80 attend our hydroponic conference and 87.2% of survey respondents indicated that they planned for future adoption of practices. Thus, we only report 75 of the 195 projected growers had adopted recommended practices. We had expected more people to attend the conference. The conference was recorded and some of the presentations are being used in the Soilless Production class that has 10 students each spring, so the outcome may be achieved but not within the grant period.
2	Outcome 5; Indicator 2	Although we only reached half of the projected 200 people, we had around 80 attend our hydroponic conference and 87.2% of survey respondents indicated that they planned for future adoption of

		practices, which is more than the 40 projected.
3	Outcome 5; Indicator 6	As part of the conference, Dr. Rebek and Dr. Damicone addressed how to combat insects and disease in greenhouse production. There was only 80 attending the conference, so we did not reach or train 200 as planned
4	Objective 7; Indicator 4	Postharvest handling and food safety were mentioned at the conference. There was only 80 attending the conference, so we did not reach or train 200 as planned.
5	Objective 7; Indicator 5	At least three classes a year are offered through Oklahoma State University for Good Agricultural practices certification. Only 23.9% of survey respondants indicated that they had already obtained a food safety certification. The program certifies around 100 growers a year but less than half of the 30 growers have the certification which is less than the 80% projected would become certified.
6	Objective 8; Indicator 6	As far as I know, there is only three new farms related to hydroponics food production; however, the same technology is being used throughout the state to grow other crops.
7	Objective 8; Indicator 7	There were only three and not 10 projected new socially disadvantaged farmers that went into hydroponic production. One new farm was started by Ekpe Udoh an African American farmer. The second farm is with the Chickasaw Nation. The third is Restore Farms, which is owned by a woman.

DATA COLLECTION

Seeds of lettuce (‘Tropicana’, ‘Green Forest’ and ‘Butterhead’), cilantro (‘Cruiser’ and ‘Calypso’), collards (‘Champion’, ‘Flash’, and ‘Tiger’), and celery (‘Tango’ and ‘Conquistador’) were ordered from Johnny’s Selected Seeds (Winslow, Maine). Seeds were sown in rockwool starter cubes size 1.5 cm × 1.5 cm × 1.5 cm (Gordan, Milton, Ontario) and transplanted into Hydrocycle 4” Pro NFT series (Growers supply, Dyersville, Iowa) at the Department of Horticulture and Landscape Architecture Research Greenhouses in Stillwater, OK. There were 10 plants per cultivar with three replications. Fertilizer used was Jack’s 5-12-26 (J.R. Peters, Allentown, Pennsylvania). Cultivars, planting dates, and harvest dates are given in Table 1. The EC of all the nutrient solutions was maintained at 1.5-2.5 mm/cm and

the pH was maintained at 5.5-6.5. The pH and EC of each solution was checked every third day. At the end of the study on lettuce, cilantro, and celery, data was collected on height, width, fresh weight, and root and shoot dry weight (plants cut at base and dried for 2 days at 134°F). There were 10 replicates per cultivar and the experiment was replicated 3 times.

Blackberries ('Apache', 'Triple Crown', 'Chester', and 'Prime-Ark Freedom') from W. Atlee Burpee & Co. (Warminster, PA), blueberries ('Bluecrop', 'Patriot', and 'Jersey', and 'New Hanover') from Indiana Berry & Plant Co. (Plymouth, IN), and raspberries (Heritage', 'Anne', 'Jewel', and 'Tulamagic') from W. Atlee Burpee & Co. (Warminster, PA) arrived as bareroots and all were 2 year old plants. Cultivars and plant material sources are given in Table 2. Plants were held for about 2 weeks in a cooler at 4.4°C then moved to the greenhouse on 27 March 2018. Plants were transplanted into a PolyMax Dutch Bucket System (Growers Supply, Dyersville, Iowa) at the Department of Horticulture and Landscape Architecture Research Greenhouses in Stillwater, OK. A single plant was transplanted in each bucket. There were 10 plants per cultivar and the experiment was replicated in a second greenhouse. The Dutch Buckets were placed 56 cm apart and the rows were 144 cm apart and arranged on opposite sides of the irrigation and drainage pipes. Water was provided to plants by one drip emitter, which supplied 1 gallon per hour of water. Buckets were filled with expanded clay pebbles (Mother Earth Hydroton, National Garden Wholesale Sunlight Supply, Vancouver, Washington). The water that drained away was recirculated from a 45-gallon capacity storage tank. On 14 June 2017 a 40% shade cloth was applied. Blackberry and raspberry canes were pruned to 30" and laterals of blackberries were pruned to 14". Both blackberries and raspberries were trellised. Crops were fertigated by Jack's 5-12-26 N-P-K at a rate of (0.504 lbs./50gal) Tap water was used to prepare the nutrient solution. The EC of all the nutrient solutions was maintained at 1.5-2.5 mm/cm and the pH was maintained at 5.5-6.5, except the blueberries which were kept at a pH of 4.5-5.5. The pH and EC of each solution was checked every third day. Blackberries were moved to a cooler set at 4.4°C for 3 weeks to induce vernalization mid-November, so berries will initiate flowering in the second year.

Several different bareroot strawberry varieties ('Eve', Sweet Summer', 'Seascape', 'Albion', 'TriStar', and 'Ozark Beauty') were purchased from Hirt's Garden (Medina, OH). Initially, plants were grown in a vertical hydroponic tower. Then in August plants were grown in a Hydrocycle 6" Pro NFT series (Growers supply, Dyersville, Iowa) at the Department of Horticulture and Landscape Architecture Research Greenhouses in Stillwater, OK. Plants were placed with the crown above the water and no substrate. For the 'Ozark Beauty' planted in 2019, plants were planted in a substrate of expanded clay pebbles or rockwool as a substrate. The water that drained away was recirculated from a 45-gallon capacity storage tank. Plants were fertigated by Jack's 5-12-26 N-P-K at a rate of (0.504 lbs./50gal) and monitored as described above. Predatory mites (Phytoseiulus persimilis) were applied on 10 January 2020. A single purple fountain grass plug was transplanted into a PolyMax Dutch Bucket System (Growers Supply, Dyersville, Iowa) at the Department of Horticulture and Landscape Architecture Research Greenhouses in Stillwater, OK. The Dutch Buckets were placed 56 cm apart and the rows were 144 cm apart and arranged on opposite sides of the irrigation and drainage pipes. Plants were fertigated by Jack's 5-12-26 N-P-K at a rate of (0.504 lbs./50g) and monitored as described above. Vase life treatments included bleach (20uL/L), floral preservative, sucrose (10g), bleach plus sucrose, floral preservative plus sucrose, and tap

water. Stems were cut to 2 feet and there were 10 stems per treatment that was replicated twice.

For quality analysis, lettuce samples were washed, frozen, and freeze dried. They were then ground with a UDY mill to pass a 1 mm screen and stored frozen in a brown bottle prior to extraction and processing for SL and for sugar determination. SL's (lactucin, 8-deoylactucin and lactucopicrin) were extracted in pairs with acidified methanol and then dried. Santonin was added just prior to extraction as internal standard. One pair was purified with an amino solid phase extraction column and used for HPLC analysis of free SL's. The other pair was treated with glucosidase enzyme to cleave bound sugars from the SL's, then dried and further purified prior to HPLC analysis as descried for free SL's for determination of total SL's. Bound SLs were calculated by subtracting the total SL runs from the free SL runs. SLs were separated using HPLC and quantitated relative to santonin as internal standard. Soluble sugars (glucose, fructose and sucrose) were extracted using 95 % boiling ethanol. Aliquots of the extract were then dried, reconstituted in water and analyzed by HPLC. Quantitation was by external standard against known quantities of glucose, fructose, and sucrose from standard runs.

The least significance difference method was used for comparing differences between treatment means. Tests of significance were performed at the 0.05 level. Data analysis was generated using SAS/STAT software (version 9.4; SAS Institute, Cary, NC).

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$34,828.00	$36,554.63
Fringe Benefits	$15,042.00	$15,915.13
Travel	$600.00	$473.00
Equipment	$0.00	$0.00
Supplies	$16,050.00	$14,322.24
Contractual	$2,480.00	$1,735.00
Other	$4,000.00	$4,000.00
Direct Costs Sub-Total	$73,000.00	$73,000.00
Indirect Costs	$0.00	$0.00
Total Federal Costs	$73,000.00	$73,000.00

PROGRAM INCOME (IF APPLICABLE)

N/A

ADDITIONAL INFORMATION

Overall Results:

Shoot dry weight was greater for 'Tropicana and 'Green Forest' lettuce, however fresh weight and root dry weight were not different than 'Butterhead' lettuce. For cilantro, 'Calypso' had greater plant width, fresh weight, and root and shoot dry weight than 'Cruiser'. 'Tiger' had greater shoot fresh weight and root and shoot dry weight than 'Champion' and 'Flash' collard. 'Flash' had greater plant width, shoot fresh weight, and root and shoot dry weight than 'Champion' collard. For spinach, greatest leaf number was seen with 'Harmony', however it was not different than 'Riverside' or 'Space'. 'Tango' celery had greater shoot and root dry weight over 'Conquistador'.

'Triple Crown' blackberry had the greatest number of weeks of production and the greatest average fruit weight per fruit at 5-8g. 'Chester' had the greatest number of fruits per picking and average fruit weight per picking. 'PrimeArk Freedom' had the greatest number of fruits and total fruit weight. For both the raspberries and blueberries, no cultivar produced more than 10 fruits. Strawberries did better in the nutrient film technique system compared to the vertical tower system, but spider mites have to be controlled and a substrate should be provided so the crowns do not rot out.

Floral preservative and floral preservative plus sucrose produced stems that remained green the longest. Floral preservative alone kept the stems alive the longest at 19 days. Adding only sucrose to the vase water resulted in the shortest vase life and would not be recommended.

Lettuce yield increased dramatically from the first to second harvests and increased more steadily at the final harvest. Lettuce sweetness remained constant or slightly decreased with advancing plant age at harvest. Total sugar content varied among lettuce varieties, but was highest in romaine, butterhead, and red oakleaf types. Bitterness, measured as concentration of SL's, increased slightly with increasing maturity. Red lettuce varieties were notably higher in SL's than green types. Assessment of quality for commercially grown hydroponic lettuces in OK revealed a difference in SL's and sugars between growers, especially between red colored lettuces. Even when harvests were conducted at younger plant ages, one grower produced product which was notably higher in SL's and lower in sugars than another grower.

The Soilless Protected Food Production Systems Conference was held on July 10, 2019 in Stillwater, OK. Speakers included faculty from University of Arizona, Kansas State University, Oklahoma State University, and industry people within Oklahoma. Topics included hydroponics, plant nutrition, marketing, organic production, crops, and disease and insect management. Of the 36 evaluations received from the conference, 97% reported the overall quality/usefulness of the conference as either good or excellent. When asked if they plan to adopt the recommended practices, 87.2% said yes. When asked if knowledge in detecting and responding to pests and diseases increased, 92.3% said yes. A total of 43.65 of respondents reported that they planned to get into specialty crop production. Only 23.1%

responded that they had obtained on-farm safety certification. All responded that they would attend a hands-on workshop related to soilless crop production.

Project Title	Development of Cold Hardy Bermudagrasses for Specialty Sod Production in Oklahoma			
Recipient Organization Name:	Oklahoma State University			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	9/29/2020
Recipient's Project Contact				
Name:	Justin Quetone Moss			
Phone:	405-744-5729			
Email:	Justin.moss@okstate.edu			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

Oklahoma State University evaluated and selected improved experimental bermudagrass (*Cynodon* spp.) genotypes for adaptability for golf course putting greens and specialty sod production in Oklahoma. Several Oklahoma golf courses have moved from creeping bentgrass (*Agrostis stolonifera*) to bermudagrass for putting surfaces over the past 15 years. However, bermudagrass putting greens are susceptible to winter kill from freezing winter temperatures in Oklahoma. Our goal was to develop and select bermudagrasses that could better withstand freezing temperatures in Oklahoma, but still produce an acceptable or high-quality putting surface. Bermudagrass genotypes were bred and developed at Oklahoma State University and were field tested for potential and performance as a putting surface in Oklahoma. This project allowed us to evaluate Oklahoma State University's top-performing bermudagrasses for possible use as putting greens in Oklahoma. We also developed a demonstration area at a regional turf sod producer's field site to assess the production capability and for possible specialization and use within the industry. Results of this work was disseminated to Oklahoma growers and industry professionals via various extension and outreach efforts.

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*

1	Develop bermudagrass genotypes with improved cold tolerance and high aesthetic quality under golf course putting green maintenance practices for use in Oklahoma golf courses.	X	
2	Evaluate sod/sprig production characteristics of experimental genotypes when being utilized for golf course putting green installations/renovations for local sod producers.	X	
3	Disseminate the findings through a peer-reviewed journal, Oklahoma Turf Research Foundation Annual Conference, and extension outreach events	X	

ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	Material transfer agreements were completed with off-site, non-OSU cooperators and on-farm testing nurseries were established and bermudagrass performance was evaluated.	**Objectives 1 and 2:** This was critical to the success of the project as we have proposed to test our experimental bermudagrass entries at off-site, non-OSU, on-farm locations. The testing nurseries were established from sprigs and establishment and growth data was collected. This on-site testing and demonstration is an important accomplishment for our project as it serves to meet project objectives and thus will work to enhance the competitiveness of specialty crops (sod production) through greater capacity of sustainable practices of sod production resulting in increased yield, reduced inputs, increased efficiency, increased economic return, and conservation of resources. This is also important for sod producer buy-in as these entries were evaluated on-farm in real-world production settings.
2	Turfgrass quality and performance data was collected throughout four growing seasons from the 2017 set of experimental bermudagrass testing nurseries in Stillwater, OK	**Objectives 1 and 2:** This data allowed us to test, obtain data, and select experimental bermudagrass entries with improved quality and traits that may enhance its competitiveness and potential use in the sod production and sprig production markets.
3	Turfgrass quality and performance data was collected throughout three growing seasons from the 2018 set of experimental	**Objectives 1 and 2:** This data allowed us to test, obtain data, and select experimental bermudagrass entries with improved quality and traits that may enhance its

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
	bermudagrass testing nurseries in Stillwater, OK	competitiveness and potential use in the sod production and sprig production markets.
4	Turfgrass quality and performance data was collected throughout two growing seasons from the 2019 set of experimental bermudagrass testing nurseries in Stillwater, OK	**Objectives 1 and 2:** This nursery allowed us to test, obtain data, and select experimental bermudagrass entries with improved quality and traits that may enhance its competitiveness and potential use in the sod production and sprig production markets.
5	Three bermudagrass genotypes were selected and entered into the National Turfgrass Evaluation Program National Warm-Season Putting Green Test which was established at 10 U.S. locations in 2019-2020.	**Objectives 1 and 2:** Data collected in this project allowed us to test, obtain data, and select experimental bermudagrass entries with improved quality and traits that may enhance its competitiveness and potential use in the sod production and sprig production markets. To this end, three selections (OKC 0805, OKC, 3920, and OKC 0920) were entered into a National germplasm evaluation program.
6	Project results were disseminated to key stakeholders and audiences.	**Objective 3:** This accomplishment to disseminate results to turf sod producers and turf industry stakeholders helped us to achieve our goal of reaching 350 turf industry professionals including Oklahoma sod producers and Oklahoma golf course managers.

CHALLENGES AND DEVELOPMENTS

#	Challenge or Development	Corrective Action or Project Change
1	The biggest potential challenge to our project was the weather or environmental conditions. This was not under our control, but we did our best to be prepared during inclement weather and extreme freezing temperatures during the project.	We monitored the weather and climate using local, on-site weather stations and the Oklahoma Mesonet system. At times, due to extreme freezing temperatures, we had to protect our test nurseries with specialized tarps known as “turf blankets” that are sold by golf course and sports turf product distributors. We also covered the nurseries during the first winter after establishment as plants were not fully established. We

#	Challenge or Development	Corrective Action or Project Change
		covered when the temperature fell below 28F. However, in order to test the freezing tolerance of the nurseries, we did allow nurseries to remain uncovered in the third winter (after two full growing seasons of data collection).
2	An additional challenge was the specialized management of putting greens in Oklahoma. This requires specialized equipment and mowers to maintain the cutting height in a range from 0.100 inch to 0.150-inch height of cut. (or roughly 1/10 of an inch to 1/8 of an inch height of cut).	We utilized a trained turfgrass manager and equipment operator for the specialized care of these nurseries at Oklahoma State University in Stillwater. For the off-site, on-farm nurseries, the sod producer cooperator had to buy a specialized reel mower to keep the height of cut below 0.250 inch for evaluation in a production setting.

LESSONS LEARNED

It is critical to have a good location for this type of research. The site must have the proper soil conditions, preferably a USGA specification putting greens mix for field research nurseries. This is why on-farm or on-golf course cooperators can be very important as they often already have these conditions. The disadvantage of the on-farm or on-golf course nurseries is that these field tests are not in production or not in play, and thus may not get the full attention of the on-farm crews. Also, it is important to have the needed equipment, such as specialized reel mowers, and preferably, a good and experienced mechanic to keep equipment properly maintained and running in good condition. This is always a challenge for this specialized turf and sod equipment.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

Data from the 2017, 2018, and 2019 field nurseries as well as the on-farm, off-site cooperator nurseries will continue to be collected through the 2022 growing season. This will allow us to collect additional data and make additional selections over time.

BENEFICIARIES

Number of project beneficiaries: 400

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

OUTCOME MEASURE(S)

- ☐ **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)

#	Outcome and Indicator	Quantifiable Results
1	**Outcome 4, Indicator 1**	We tested more than 100 OSU experimental bermudagrass entries against 4 industry standard bermudagrasses for performance, quality, and cold hardiness. It is expected that at least one of these OSU experimental bermudagrass entries will be developed showing improved traits compared to other experimental bermudagrasses and compared to the industry standard bermudagrasses.
2	**Outcome 4, Indicator 2. c.**	We completed and executed material transfer agreements and established field test nurseries to obtain data on OSU experimental bermudagrasses for potential for Oklahoma specialty crop sod production. While this testing is still ongoing, if it is determined that one of these experimental bermudagrasses is viable for specialty crop sod production, we expect at least a $0.05 per square foot of production return which would be an estimated increase of $2,178 per acre of production compared to common bermudagrass sod production.
3	**Outcome 5, Indicator 1**	We have tested more than 100 OSU experimental bermudagrass entries against

		4 industry standard bermudagrasses. Based on data received from the project thus far, we entered 4 OSU experimental bermudagrasses into the 2019-2024 National Turfgrass Evaluation Program warm-season putting green grass trial. This will enable us not only to get critical Oklahoma performance data, but also data from at other States. Assuming our selections perform well in the Oklahoma trials as well as in our region, we plan to release one new bermudagrass cultivar to sod producers via the Oklahoma Agricultural Experiment Station in the next 3-5 years.
4	**Outcome 5, Indicator 3**	It is assumed that at least one of our OSU experimental bermudagrass selections perform well for Oklahoma producers. While additional data is being collected, we plan to release one new bermudagrass cultivar to sod producers via the Oklahoma Agricultural Experiment Station. This will enable all sod producers in Oklahoma an opportunity to license or sub-license this cultivar for sod production in Oklahoma. We expect that up to five Oklahoma sod producers/farms will utilize the new cultivar in their business in the first three years following completion of this work and release of the cultivar.
5	**Outcome 5, Indicator 8**	We have presented results and outcomes of this research to sod producers and golf course industry professionals and managers at the recent Oklahoma Turfgrass Conference and Trade Show, the last three years held at Owasso, OK. We presented results and showed our field test nurseries to participants at our Oklahoma State University Turfgrass Research Field Day in September 2019. We have also presented preliminary results of this work to golf course industry professionals via the Oklahoma Chapter of the Golf Course Superintendents Association. The

		preliminary data from this project was synthesized and presented at the Annual Agronomy Society of America and Crop Science Society of America Annual Meetings in 2019 in San Antonio, TX and in 2020 via a virtual conference at the C-5 Turfgrass Science poster sessions to an audience of industry representatives, researchers, academics, and students. This has led us to reach over 400 event attendees (primarily from Oklahoma, but also includes professionals from other States) at these meetings, working towards our goal of ultimately reaching at least 350 Oklahoma sod producers and golf course managers and industry professionals.

DATA COLLECTION

Turf quality data was collected by one subjective mean (National Turfgrass Evaluation Program visual quality parameters) and one objective mean (Normalized Difference Vegetation Index) once monthly. Turf color data was collected once monthly as a visual rating as outlined by the National Turfgrass Evaluation Program (NTEP) data collection parameters. Spring green-up and fall color retention ratings were collected monthly during periods of transition. Ball roll data was collected once monthly for each nursery by use of a stimpmeter as recommended by the United States Golf Association. Disease susceptibility will also be rated according to the NTEP guidelines when symptomology was present or reported. Sod production characteristics were evaluated by a cooperating sod producer for production viability determined by the amount of material harvested per growing season reported as bushels per acre for sprig production and as square feet of sod for sod production. Additionally, establishment rate was evaluated according to NTEP guidelines by the researcher until the demonstration plots have reached 99% establishment. Basic data analysis was performed according to the date of data collection. Collected data was analyzed using statistical software (SAS 9.4). Analysis of variance was performed using the GLM procedure at the 0.05 significance level. When appropriate, mean separation tests were performed using the least significant difference (LSD) test at the 0.05 significance level. Although the time frame for this project is completed with this final project report, field data collection will continue through additional growing seasons for the nurseries established during this project. At the end of the data collection, analysis will take place using statistical software (SAS 9.4) and the generalized linear mixed model (GLIMMIX) procedure for repeated measures at the 0.05 significance level, along with appropriate mean separation tests.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$26,048.00	$35,670.01
Fringe Benefits	$11,250.00	$6,952.62
Travel	$5,200.00	$4,998.39
Equipment	$0.00	$0.00
Supplies	$8,500.00	$7,270.06
Contractual	$2,480.00	$0.00
Other	$4,002.00	$0.00
Direct Costs Sub-Total	$55,000.00	$54,891.08
Indirect Costs	$0.00	$0.00
Total Federal Costs	$55,000.00	$54,891.08

PROGRAM INCOME (IF APPLICABLE)

N/A

ADDITIONAL INFORMATION

Project Title	Development of shade tolerant turf-type Bermudagrasses			
Recipient Organization Name:	Oklahoma State University			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	9/29/2020
Recipient's Project Contact				
Name:	Charles Fontanier			
Phone:	405-744-6424			
Email:	Charles.fontanier@okstate.edu			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

Bermudagrasses possess very poor shade tolerance, thus being limited to full sun locations. The discovery or development of shade-tolerant bermudagrasses would make an important contribution to residential lawns, golf courses and other turfgrass industries, while creating a novel and highly marketable product for sod producers. At present, bermudagrass shade tolerance traits have been difficult to decipher and limited progress has been achieved on a national level. Much of the previous research at OSU and nationally has been conducted on common bermudagrasses (*Cynodon dactylon*) or interspecific hybrids of common bermudagrass and African bermudagrass (*C. transvaalensis*). Much less research has been conducted on the potential shade tolerance of African bermudagrasses. African bermudagrass is important because it has been widely used as one parent hybridizing with common bermudagrass for creating modern interspecific hybrid turf bermudagrass cultivars. This project was unique in that shade tolerance of African bermudagrasses were explicitly evaluated for use as a shade tolerant parent in future interspecific hybrid crosses or possibly as a new shade tolerant cultivar itself. To this end, ancillary measurements were made to identify plant morphological features that contributed to perceived shade tolerance in the selected populations.

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*
1	Screen African bermudagrasses from the OSU germplasm collection for their shade tolerance in comparison to current industry standards.	X	
2	Identify morphological and physiological features that potentially convey shade tolerance to bermudagrasses.	X	

ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	Pots of selected bermudagrasses were subjected to varying levels of shade for 8-12 weeks and various growth parameters measured.	Objective 1: Screen African bermudagrasses from the OSU germplasm collection for their shade tolerance in comparison to current industry standards.
2	Leaf and canopy architecture of selected plants were characterized and compared to observations on relative persistence under shade.	Objective 2: Identify morphological and physiological features that potentially convey shade tolerance to bermudagrasses.

CHALLENGES AND DEVELOPMENTS

#	Challenge or Development	Corrective Action or Project Change
1	The graduate student hired to complete the project endured personal trauma which required her withdrawal in May 2019.	Project activities were delayed slightly and then shifted to undergraduate student workers for basic upkeep until a new

#	Challenge or Development	Corrective Action or Project Change
		graduate student was hired. All objectives were met following the no cost extension.

LESSONS LEARNED

At the current funding level, the project was limited to greenhouse activities. Although our data provide useful information, a larger project with funding for field and greenhouse activities would provide more reliable information about the genotypes being tested. Morphological data alone was not always consistently related to shade tolerance. We also believe light use efficiency curves and pigment analyses may be required to gain a more complete picture of mechanisms driving apparent shade tolerance in these grasses.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

N/A

BENEFICIARIES

Number of project beneficiaries: 52 seed/sod producers in the state

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

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)

#	Outcome and Indicator	Quantifiable Results
1	Outcome 4, Indicator 1. Numbers of plant/seed releases: we anticipate selection of 4 plants from this immediate project with expectations that at least 1 new cultivar can be released through the life of the program.	Plants varied in relative shade response suggesting potential for selection of improved traits among this population. The outcome was achieved as indicated by selection of four plants (# 75, 82, 83, and 88) for future field evaluation and breeding efforts..

DATA COLLECTION

The study was conducted in the greenhouse under shade that reduced light to approximately 75% of full sun. 30 African bermudagrass plants having good turf quality were established in deep pots and subjected to the shade treatment for two 8-week cycles. Each entry was evaluated using visual ratings and digital image analysis to estimate green coverage. Image analysis of individual shoots was also used for quantifying leaf color, angle, shape, and size. Canopy vertical elongation rate was measured to determine if dwarfism traits were related to sustained green cover. Data were analyzed using a general linear model and means compared using Duncan's multiple range test. Results showed entry 75 having significantly greater dry mass under shaded conditions as compared to all but one other entry. Entries 82, 83, and 88 were also among the highest in percent green coverage and showed no reduction compared to their full sun controls. Surprisingly, canopy extension rate was not related to either green coverage or dry mass, while a larger leaf area showed promise as a predictor of higher shoot dry weight. Leaf angle was highly variable among entries but not consistently related to shade tolerance. OSU cultivars and Celebration tended to have lower leaf angle than top performing African bermudagrasses and TifGrand.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$19,500.00	$26,442.40
Fringe Benefits	$8,422.00	$2,232.91
Travel	$0.00	$0.00
Equipment	$0.00	$0.00
Supplies	$2,078.00	$1,324.69
Contractual	$0.00	$0.00
Other	$0.00	$0.00
Direct Costs Sub-Total	$30,000.00	$30,000.00

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Indirect Costs	$0.00	$0.00
Total Federal Costs	$30,000.00	$30,000.00

PROGRAM INCOME (IF APPLICABLE)

N/A

ADDITIONAL INFORMATION

N/A

Project Title	Electronic Diagnostic Nucleic Acid Analysis (EDNA) for accurate detection and discrimination of rose viruses			
Recipient Organization Name:	Oklahoma State University			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	9/29/2020
Recipient's Project Contact				
Name:	Francisco, Ochoa-Corona			
Phone:	405-744-9946			
Email:	ochoaco@okstate.edu			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

During the development of assays for detection of rose rosette virus (RRV) it has been evident that RRV is perhaps the most visible viral problem, however, RRV is not the only virus infecting roses in Oklahoma. In fact, according to the scientific literature reviewed during this project, the rose virome is composed by twenty-six viruses. A number of these viruses infect roses in the U.S. and worldwide. The appearance of these infectious virus-complexes add a holistic negative economic impact on roses and ornamental crops in general because symptoms do not develop during production stages and may not be identified until the plants reach the retailer. This cause restrictions on the commercialization and exportation of roses,

and frequently nursery growers are put on hold or rejected by retailers or by regulatory agencies. Rose viruses (rose rosette virus and other co-infecting viruses) has led to a significant decline of garden roses that had affected the rose industry and the landscape of cities, particularly Tulsa and Oklahoma City. Current diagnostics of rose samples is laborious and slow pace because is done one virus detection method at the time, either serologically or molecularly. As a consequence, timely return of diagnostics results to nurseries is challenging causing delays on proper disease management decisions at nurseries. The alternative approach proposed to solve this complex problem was to develop a method to screen all viruses at once in a single sample. The aim is to facilitate and improve the ability to produce virus-free rose nursery stocks. Also, monitoring foundation and propagative stocks in Oklahoma, which will boost the production of virus free plants and will make rose growers more competitive.
RRV is a virus species with RNA genome in the family *Emaravirus,* which is transmitted by the eriophid mite *Phyllocoptes fructiphilus* (Laney 2011). Mix infections of RRV with other rose viruses has led to a significant decline of garden roses that had affected the rose and the landscape industries as described above. While monitoring varietal resistance of RRV, diverse complex viral syndromes, different to rose rosette disease, were frequently found similarly to previous described (APS compendium). For example, RRV is frequently found infecting with blackberry chlorotic ringspot virus (BCRV) (Tzanetakis et al, 2007, Poudel et al, 2013,); prunus necrotic ringspot virus is reported to co-infect with arabis mosaic virus (ArMV) and apple mosaic virus (ApMV) (Milleza et al, 2007). RRV also mix infects with other viruses such as impatiens necrotic spot virus (INSV), tomato spotted wilt virus (TSWV), tomato ring spot virus (ToRSV), and other *Ilarvirus* such as tobacco streak virus (TSV), and BCRV mentioned above (Horst, K R. 1983). The symptoms of viral disease syndromes varies depending on the composition of the infecting virus mixture, infected rose variety, weather, plant age and nutrition. Moreover, the diversity of symptoms had caused confusion among growers, consultants and extension agents. In general, the presence of virus complexes cause a negative effect on roses besides rose rosette disease only. Most of these rose infecting virus mixtures spread through infected tissue during propagation, pruning and handling. Therefore, the development of the proposed assay will benefit in a first stage the propagation of rose stocks and will boost the production of virus free plants. The method will be subsequently used by rose breeders for tracking resistant progenies they may be breeding, and nursery monitoring the health status of their propagation stocks.

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*
1	To validate *in vitro* a broad detection protocol of EDNA-Rose for detection and discrimination of all reported viruses applicable to a single infected rose specimen.	Yes	
2	To standardize virus detection methods based on RT-PCR coupled to HRM for the most frequently found viruses infecting rose INSV,	Yes	

	TSWV, ToRSV, and viruses in the genus *Ilarvirus* such as PNRSV, ApMV, TSV, and BCRV. These methods will be used for confirmation purposes during and after the validation of EDNA-Rose.		
3	To survey rose propagation blocks in Oklahoma seeking virus free rose-individuals and/or high value varieties of interest for the rose industry to be selected to build a new clean foundation block and to contribute accessions to the National Clean Plant Network Roses (NCPN Roses).	Yes	
4	To transfer EDNA-Rose technology to plant diagnostics laboratories and diagnostic networks and rose stakeholders. For example, the Plant Disease and Insect Diagnostic Laboratory (PDIDL), the National Plant Disease Diagnostic Network (NPDN), the National Clean Plant Network Roses (NCPN Roses) and other states laboratories. Technology transfer to these audiences will include hands on training to developed labs procedures and software.	Yes	

ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	The accurate broad detection of EDNA-Rose MiFi®, was demonstrated with nine viruses that commonly infect roses in Oklahoma. EDNA-Rose MiFi® is a method that combines High Throughput Sequencing (HTS) with a bioinformatics pipeline (EDNA-Rose MiFi®). The method detected nine viruses that infect roses in Oklahoma. This method also has additional detection capacity for other 13 viruses because EDNA-Rose MiFi® was developed to detect 24 viruses. The number of rose viruses reported worldwide consists of 26 viruses. Two viruses were pulled out of the study because: a) there is no genome available at NCBI for the Rose color break virus, and b) the sequence of Rose necrotic mosaic virus is too short (probably incomplete) and does not generate e-probes.	**Objective 1**
2	Three Multiplex Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) coupled to High resolution Melting (HRM) were developed, and validated for the detection of eight viruses	Virus species detected by mRTqPCR-HRM are: Test A. *Impatiens necrotic spot virus* *Rosa multiflora criptic virus*

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
	that infect roses in Oklahoma. One additional virus was detected by RT-qPCR Virus species detected by mRTqPCR-HRM are: **Test A**. • *Impatiens necrotic spot virus* • *Rosa multiflora criptic virus* • *Tomato ringspot virus* **Test B.** • *Rose yellow vein virus* • *Blackberry chlorotic ringspot virus* • *Rose yellow mosaic virus* **Test C.** • *Prunus necrotic spot virus* • *Apple mosaic virus* **Test D** • *RRV* Test A, B, and C are additional contributions that can be used for either routine single or small group predetermined assays or confirmation of EDNA-Rose MiFi® results. A protocol for library preparation and processing of rose samples by two HTS, either Illumina or MinION were developed.	Objective 2
3	Surveys were conducted on two sites (OSU varietal block and Tulsa Rose garden) targeting selected rose material, Fifthteen rose varieties tested showed to be infected with multiple virus infections, which are common in rose germplasm in Oklahoma (Table 2). This finding was discussed with plant pathologist at and the multiple infections finding made in OK is apparently common in rose varieties in the U.S. The selected rose varieties were found infected with multiple virus combinations from two to five viruses. Ten out of 15 rose varieties were infected with three to five viruses and seven out of 15 with four to five viruses.	Objective 3

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
	This result offers a preview of the epidemiological complexity of the virus mix-infections in roses and the relevance of having developed a detection diagnostic method like EDNA Rose which will play a role in the virus-cleaning of rose varieties with economic value	
4	Hands on technology transfer was made to the Plant Disease and Insect Diagnostic Laboratory (PDIDL), which is affiliated to the National Plant Disease Diagnostic Network (NPDN), also to the the National Clean Plant Network Roses (NCPN Roses)Tier II.	Objective 4

CHALLENGES AND DEVELOPMENTS

#	Challenge or Development	Corrective Action or Project Change
1	Finding all 24 required reference positive control viruses for validation of the complete rose virome.	Validation *in-vitro* is not possible without reference positive controls (actual virus). To sort this situation, we validated the method with software assistance. The application Metasim is allowed to validate EDNA-Rose. Metasim simulates HTS runs while using the actual e-probes and virus databases created for this purpose. We also validated EDNA Rose with actual metagenomics outputs provided by Foundation Plant Services (FPS), University of California at Davis which were obtained from roses tested by HTS. We also created artificial positive controls.

LESSONS LEARNED

- This was definitively an ambitious project. Working 24 four viruses was not complicated *in-silico*, however, got complicated when trying to work with all of them *in-vitro*. Some of them were not reported in Oklahoma and required working out import permits from other countries or States. Few are reported in the literature but no vouchers are available in collections and scientists do not keep them for a while because high costs. We limited our demonstrations *in-vitro* to viruses found in Oklahoma.

- Synchronizing demonstrations and plant labs-time availability for technology transfer of EDNA-Rose technology to plant diagnostics laboratories turned to be a difficult task winter season seems to be amenable because the low accession of specimens to labs.
- The project got caught by the pandemic, which caused delays on personnel attendance, also on purchasing reagents and availability of numerous lab disposables like pipettes tips, which were suddenly all given priority to hospitals and suppliers put research orders on waiting lists.
- We learned how to navigate and manage the project under the pandemic created circumstances.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

We are planning to perform a virtual meeting during the pandemic to communicate the rose stakeholders located in Oklahoma the relevance and accomplishment of this project and how they can become users of this technology. We will make a second meeting in person with the same purpose after the pandemic.

BENEFICIARIES

Number of project beneficiaries: 400

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

OUTCOME MEASURE(S)

- ☐ **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)

#	Outcome and Indicator	Quantifiable Results
1	Outcome 5, Indicator 5	The Outcome for this project was met. There will be 2 diagnostic technologies available for detecting plant pests and diseases. However, this does not mean this research line is terminated. We will continue to work on the optimization and perfection of EDNA-rose focusing on increasing sensitivity and updating of e-probes. Also testing *in-vitro* viruses that were not tested or testing metagenomics files provided by scientists in other countries. The two new diagnostic technologies available for detecting plant pests and diseases are: 1. EDNA-Rose (Electronic Diagnostic Reverse Nucleic acid Analysis-Rose). Accessible at http://bioinfo.okstate.edu. Sign up and create an account. The new user can contact either Prof. Andres Espindola (andres.espindola@okstate.edu) or Prof. Francisco Ochoa Corona (ochoaco@okstate.edu) 2. Four Reverse Transcription Polymerase Chain Reactions (RT-PCR) coupled to High-Resolution Melting (HRM) for the most common viruses infecting roses in Oklahoma. Descriptive protocols will be provided to the scientific community through scientific articles which are under preparation.

DATA COLLECTION

METHODS

Part 1. EDNA-Rose MiFi®

EDNA-Rose MiFi® research was possible with the support of ODAFF-SCBG. EDNA-Rose MiFi® was developed to detect 24 plant viruses reported to infect roses in the U.S. and

worldwide (Dobhal et al, 2015, Goodwin et al, 2016). The list of viruses and additional data is available in Table 1.

Table 1. Virus species, genera, acronym, number of curated e-probes, and type of validation used for the 24 viruses that infect Rosa spp selected in this study. The virus species validated *in-vitro* are highlighted. The rest of the virus species were tested *in-silico* with software assistance Metasim.

List number	Virus species	Genus	Acronym	Number of e-probes	Type of validation
1	***Apple chlorotic leafspot virus***	*Trichovirus*	**ACLSV**	21	*Tested in-silico*
2	***Apple mosaic virus***	*Irlavirus*	**ApMV**	29	*Validated in-vitro*
3	***Apple stem grooving virus***	*Capillovirus*	**ASGV**	11	*Tested in-silico*
4	***Arabis mosaic virus***	*Nepovirus*	**ArMV**	30	*Validated in-vitro*
5	***Blackberry chlorotic ringspot virus***	*Irlavirus*	**BCRV**	12	*Validated in-vitro*
6	***Impatiens necrotic spot virus***	*Orthotospovirus*	**INSV**	20	*Validated in-vitro*
7	***Iris yellow spot virus***	*Orthotospovirus*	**IYSV**	21	*Tested in-silico*
8	***Prune dwarf virus***	*Irlavirus*	**PDV**	15	*Tested in-silico*
9	***Prunus necrotic ringspot virus***	*Irlavirus*	**PNRSV**	23	*Validated in-vitro*
10	***Raspberry ringspot virus***	*Nepovirus*	**RpRSV**	29	*Tested in-silico*
11	***Rosa cryptic virus* 1**	*Partitiviridae*	**RCV-1**	5	*Tested in-silico*
12	***Rose leaf curl virus***	*Begomovirus*	**RoLCuV**	7	*Tested in-silico*
13	***Rose leaf rosette-associated virus***	*Closterovirus*	**RLRaV**	59	*Tested in-silico*
14	***Rose rosette virus***	*Emaravirus*	**RRV**	22	*Validated in-vitro*
15	***Rose spring dwarf-associated virus***	*Luteovirus*	**RSDaV**	19	*Validated in-vitro*
16	***Rose yellow leaf virus***	*Tombusvirus*	**RoYLV**	10	*Tested in-silico*
17	***Rose yellow mosaic virus***	*Roymovirus*	**RoYMV**	24	*Tested in-silico*
18	***Rose yellow vein virus***	*Rosadnavirus*	**RoYVV**	17	*Tested in-silico*
19	***Strawberry latent ringspot virus***	*Secoviridae*	**SLRSV**	34	*Tested in-silico*
20	***Tobacco ringspot virus***	*Nepovirus*	**TRSV**	22	*Tested in-silico*

21	***Tobacco streak virus***	*Irlavirus*	**TSV**	19	*Validated in-vitro*
22	***Tomato ringspot virus***	*Nepovirus*	**ToRSV**	48	*Validated in-vitro*
23	***Tomato spotted wilt virus***	*Orthotospovirus*	**TSWV**	22	*Validated in-vitro*
24	***Tomato yellow ring virus***	*Tombusvirus*	**TYRV**	4	*Tested in-silico*

Field sample collection

Samples of rose tissue with characteristic symptoms of viral infection were collected during summer 2018. The samples were collected from varietal field trials. The field survey included plants from Payne County at Perkins and Tulsa County, Oklahoma. These two locations are hot spots where the rose rosette virus and its mite vector (*Phyllocoptes fructiphilus* Keifer) spreads endemically. Plant tissue from leaves and young stems were collected from 15 planted cultivars at open field. Cultivar selection was based on symptomatology in the field. Viral symptoms were confirmed in leaves and by excessive thorns in stems collected. A total of 50 mg of leaf tissue was stored in 1.5 mL nuclease-free tubes (3 tubes per cultivar) and saved at −80 °C until processing.

Oligonucleotide primer design

Specific primers were designed to target a region of the viral genome. Viral genomes from genes that encode the RNA-dependent RNA polymerase (RdRp) and the capsid protein genes of the virus were retrieved from the National Center for Biotechnology Information (NCBI). The sequences were aligned using MEGA6 (Tamura et al., 2013) and BioEdit (Hall et al., 2011) using the MUSCLE algorithm (Edgar, 2014). To group the virus sequences, a Maximum Likelihood phylogenetic tree was constructed with 1000 bootstrap values (Murshudov et al., 1997). Consensus sequences were used to design primers with a specific range of detection, as follow:

Primer3 software was used for primer design, this application takes in consideration the thermodynamic features of the sequences (Untergasser et al., 2012). To check the primer specificity, their sequences were uploaded to PrimerBLAST (Ye et al., 2012) software. Thermodynamic features were analyzed in OligoAnalyzer (Kuulasmaa, 2002) and Mfold software (Zuker, 2003) to ensure primers low energy free to form secondary and self-complementarity structures. Primer pairs were selected based on different amplicon sizes that allow product differentiation by High-Resolution Melting (HRM). uMeltSM was the software (Dwight et al., 2011) used to analyze and to predict the HRM.

Multiplex qRT-PCR HRM analysis

Multiplex RT-qPCR HRM assays were performed in 10 µl reaction volumes consisting of 5 µl HotStart. Master Mix (New England Biolabs, Ipswich, MA), 1 µl of LCgreen (Biofire, Salt Lake City, UT), 0.5 µl of each forward and reverse primer (7.5 µM), 2 µl of cDNA template, and 1 µl nuclease-free water (Ambion, Austin, TX, USA). Multiplex RT-PCR coupled with HRM was performed in a Rotor-Gene thermal cycler (QIAGEN, Hilden, Germany). The selected cycling parameters were: initial denaturation of 94ºC for 4 min followed by 35 cycles of denaturation at 94ºC for 20 s, annealing at 54ºC for 60 s, extension at 72ºC for 40 s, and a

final extension at 72ºC for 3 min. Finally, a 10 µl of the amplified PCR product was denatured for HRM from 65 to 99 ºC, temperature range. Positive, negative (non-template control; water) controls and healthy tissue (25ng) were included. A sensitivity assay from 100ng to 1 fg was performed.

Library preparation and sequencing

Twelve samples were selected based on the multiple viral infections found by RT-qPCR HRM. DNA libraries were prepared for Oxford Nanopore sequencing. RNA was extracted using the RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). dscDNA was amplified using NEBNext® Single Cell/Low Input cDNA Synthesis & Amplification Kit (New England Biolabs, Ipswich, MA). dsDNA was quantified by Quant-iT™ PicoGreen™ (ThermoFisher Scientific, Waltham, MA).

The metagenome library was synthesized according to the Oxford Nanopore protocol. Briefly, the library was prepared as the template strand was amplified by a transposase enzyme which synthesizes long fragments with adapters ready to be sequenced by MinIONTM (Oxford Nanopore Technologies, Oxford, UK). The library was kept on ice (-20 oC) until ready to load on the MinION deviceTM. Barcoding and ligation of the library were performed according to Oxford Nanopore (protocol SQK-LSK108). The barcoded libraries were equimolar-pooled before adding the adapters. The final library was stored at –20°C until MinIONTM sequencing Oxford Nanopore Technologies, Oxford, UK.

Sequencing was performed with the MinIONTM using the flow cell (FLO-Min106 R9.4; Oxford Nanopore Technologies, Oxford, UK). Platform Quality Check (QC) was performed to determine the number of active pores available in the flow cell. There were 1250 active pores after QC. After priming the flow cell following the per manufacturer's protocol, the pooled libraries were loaded onto the SpotON port of the flow cell using the Library Loading Beads (LLB; Oxford Nanopore Technologies).

Rapid virus detection using EDNA-Rose (MiFi®**) database**

The parameters selected while using EDNA-Rose Mi/Fi™ for assessment of the twelve metagenomics databases generated by MinION™ were: a) 90% percent identity and query coverage for sequencing. b) Hit frequencies between raw reads with E-probes were recorded for each of the sequenced varieties. c) Data of hit frequencies were analyzed with Tukey's HSD test and pairwise T-test at P value= 0.05.

EDNA-Rose aligned raw metagenomic reads and parsed those matching completely with the targeted infecting rose viruses (E-probe sets in the database). Also, EDNA-Rose generated a statistical correlation between the number of matches in each sample as a semi-quantification tool of the virus presence in the host.

Assembling the whole EDNA-Rose (MiFi®**) and associated methods**

As mentioned, this method (EDNA) combines High Throughput Sequencing (HTS) and bioinformatics as described (Stobbe et al 2013) and is intended for screening 24 viruses infecting roses in a single sample as shown in Fig. 1. The reason rose rosette virus (RRV) was selected as the relevant virus for validation of EDNA-Rose (MiFi®) is RRV and other co-infecting viruses had led to a significant decline of garden roses and caused losses in landscape

of cities affecting economically the rose industry, particularly in Tulsa and Oklahoma City. Moreover, RRV mix infects with other viruses randomly creating new mix infections in the field, which threatens to decimate the U.S. rose industry.

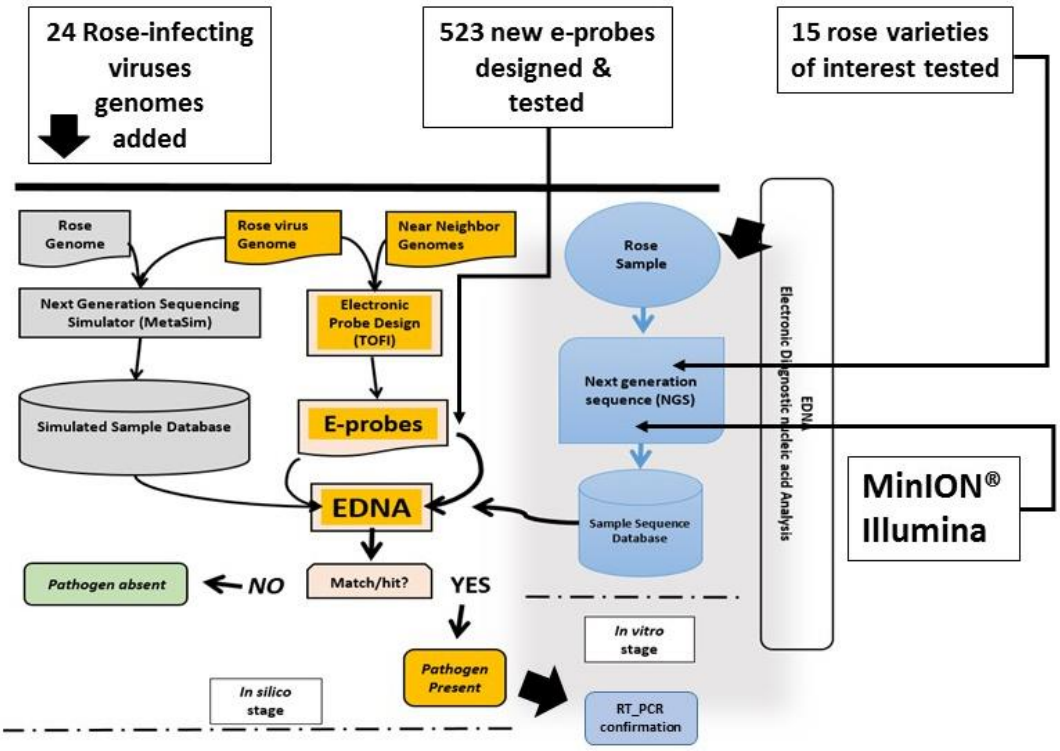

Fig. 1. Flow chart showing methods and steps followed during the development of databases, optimization, and validation of EDNA-Rose MiFi® as per objectives 1 to 3.

On the top of Fig. 1 is visible the production and curation of e-probe databases, including one containing all genomes to target 24 viruses, a database to host the rose genome, and a third database to keep the new-neighbor virus genomes (taxonomically related viruses). On left is the *in-silico* stage which comprises functionalization and testing of all databases and e-probes during optimization using the sequencer simulation software Metasim. On right is the *in-vitro* stage, to test this RNA was extracted from15 rose varieties, libraries prepared, and high throughput sequenced. The Oxford nanopore MinION® was used. The obtained metagenomes were checked for quality control and uploaded in the EDNA-Rose MiFi® pipeline. Bottom right shows the final reference check made with a comparative reference method, the reverse transcription polymerase chain reaction (RT-PCR). Three multiplex quantitative RT-PCR (mRTqPCR) methods able to detect 13 of the 24 rose infecting viruses were developed for validation purpose, results are shown in Fig. 2, next.

Part 2. Limit of detection of EDNA-Rose MiFi®

This study hypothesizes that the limit of detection of EDNA-MiFi® can be calculated for sensitive virus detection and quantification. A synthetic construct carrying the *arabis mosaic virus* (ArMV) RNA2 was synthesized *in-vitro* for this purpose. Also, electronic probes (E-probes) for the ArMV-RNA2 were designed *de novo* and curated for improved target specificity of these unique E-probe sequences. The new and highly specific E-probes were 20

and 60 nt long and were queried against 28 HTS metagenomes obtained by spiking purified genomic DNA from *Vitis vinifera* L. with a serially diluted gradient of the synthetic construct carrying the ArMV RNA2. The experiment simulated a range of virus abundance levels similarly observed during varying stages of virus infection. The limit of detection *in-vitro* of EDNA-MiFi® using 20nt long E-probes is 14.1 copies of the virus. EDNA-MiFi® rapidly queries the metagenome with sets of pre-determined plant viruses e-probes, allowing rapid and sensitive detection with no need for metagenome sequence assembly.

E-probe design for limit of detection experiments

ArMV is a virus in the genera Nepovirus and is infectious to rose, grapevine and other plant species. The ArMV genome is fragmented in two RNAs. The ArMV-RNA2 was selected as a model virus segment for this study and was sourced from the NCBI Genbank (NC_006056.1). E-probes of 20 and 60 nt length were designed using the EDNA- MiFi® MiProbe pipeline. The obtained target-specific E-probe datasets were uploaded to MiFi® to query the unassembled sequencing reads. The E-probe libraries of EDNA- MiFi® eliminate the E-probes prone to match to nonspecific targets creating false-positive results increasing specificity. Preliminary E-probes libraries were queried against the NCBI nucleotide database at E-value of 1 x 10-9. Meaning, the ArMV-RNA2 E-probe database went through further curation steps that made it highly specific before being upload once for all to MiFi® (Figure 2). Reverse sequences of the final selection of the curated E-probes were used as E-probe decoy set and were used as the internal control for statistically random matching. The use of decoy E-probes avoid false positives within the metagenome datasets (Espindola et al., 2015).

Statistical Analysis for limit of detection experiments

The pipeline EDNA- MiFi® allows counting high-quality hit alignments for each E-probe based on a set of metrics selected by the user ('hit counts') (Espindola et al., 2018). These 'hit counts' are transformed into a score to allow the use of parametric statistics. This transformation assigns to each E-probe a unique score that is compared with the negative control E-probes (Decoy E-probes). A pairwise T-test was conducted on parsed alignments of metagenomes to compare specific E-probe hit scores with negative-decoy e-probes. P-values lower than 0.05 are considered positive, and those higher than 0.05 suspect or negative (Espindola et al., 2018).

Limit of detection experiments *In-vitro*

To validate the limit of detection of EDNA-MiFi®it is necessary to control the concentration of the targeted pathogen. A synthetic sequence of the ArMV-RNA2 was artificially synthesized by GenScript®. The ArMV-RNA2 consisted of 3820 nucleotides and was inserted into a plasmid (pUC57) (Fig. 3). The plasmid carrying the ArMV-RNA2 was subsequently cloned into TOPO-TA E. coli cloning competent cells (Thermo Fisher®, Waltham, MA). The transformation into E. coli competent cells were by heat-shock (42 °C), followed by incubation at 37 °C for 8h in Ampicillin enriched LB media as indicated by the manufacturer (Fig.4).

The isolated colonies were enzyme digested to verify the presence of the RNA2 genome of ArMV. Twenty nanograms of plasmid were digested using SpeI as indicated by the manufacturer's protocol (New England Biolabs, Ipswich, MA). The digested products were loaded in 1% agarose gel and electrophoresed at 90V for 40 minutes.

Sensitivity of *in-vitro* detection using EDNA-MiFi®
The limit of detection in-vitro of each serially diluted constructs carrying the ArMV-RNA2 genome was calculated by EDNA MiFi®. Unassembled metagenomes were uploaded to the platform and queried against the ArMV 20 and 60 nt E-probes. EDNA MiFi® MiDetect was used with default parameters (percent identity and query coverage of 100%) to assess the 28 unassembled metagenomes of previously described dilution ratios (Table 1). The obtained metagenomes were parsed at four optimal e-values (5, 1, 1e-1, and 1e-2) which were empirically selected for viruses for analysis of sensitivity and specificity. The recommended number of 250 hits for quantification was selected. E-probes hit frequencies of raw reads were recorded for each dilution sample. The significant hit frequency was determined at P-value ≤ 0.05. The sum of scoring results for each detection determined the total number of hits for the ArMV E-probe set. This data was correlated to the concentration of the virus in the host (serially diluted plant virus) to calculate the limit of detection.

Verification of virus presence in libraries with qPCR
To verify ArMV-RNA2 presence of the serially diluted plant:virus ratios (*Vitis vinifera* L. genomic DNA: ArMV-RNA2) in libraries a qPCR was developed. A set of primers were designed to target a region of 164 nucleotides in the RNA2 of ArMV, sense primer ArMV-RNA2s: 5' TCATAGGGTCACTTCCAATACA -3' and anti-sense primer ArMV-RNA2as: 5'- TGTCAGCAGCACCAAGATAC -3'. Ten µl reactions consisting of 5 µl of PowerUp™ SYBR™ green master mix (Applied Biosystems™, Foster City, CA), 1 µl of each sense and antisense primers (5 µM), 2 µl of the library DNA template, and 1 µl nuclease-free water were amplified in a Rotor-Gene thermal cycler (QIAGEN, USA). The cycling parameters were: initial denaturation 94∘C for 2 min followed by 35 cycles of denaturation at 94∘C for 30 s, annealing at 54∘C for 30 s, extension at 72∘C for 30 s, and a final extension at 72∘C for 2 min, and High Resolution Melting ranging from 65 to 99 ∘C. Positive, healthy negative and non-template control were included (Fig. 5).

Minimum number E-probes needed for pathogen detection
E-probes were randomly selected from the original set of 54 in groups of 5 to 54. A pairwise T-test was performed, and mean E-probe scores and decoy scores were compared. P-values ≤ 0.05 were considered positive. The metagenomes tested were the triplicates belonging to the highest and lower virus titer.

Results from EDNA-Rose MiFi® and RT-qPCR+HRM of the 15 rose varieties demonstrated multiple virus infections are common in rose germplasm in Oklahoma (Table 2). The selected rose varieties were found to be infected with multiple virus combinations from two to five viruses. Ten out of 15 rose varieties were infected with three to five viruses and seven out of 15 with four to five viruses. This result offers a preview of the epidemiological complexity of the virus mix-infections in roses and the relevance of having developed a detection diagnostic method like EDNA Rose which will play a role in the virus-cleaning of rose varieties with economic value.

RESULTS.
Part 1 EDNA-Rose MiFi®

Multiple virus infections were detected in 15 rose varieties from Oklahoma. Table 2 shows the viruses detected infecting each of the rose specimen simultaneously. Table 2 shows detection either by EDNA-Rose MiFi® and/or EDNA-Rose i MiFi® and RT-qPCR+HRM. The high throughput sequencing (HTS) was made using a portable HTS technology platform MinION® from Oxford Nanopore, and by Reverse Transcription quantitative Polymerase Chain Reaction coupled to High Resolution Melting (RT-qPCR+HRM) (Fig. 1). The RT-qPCR+HRM assays were developed in our lab for validation of EDNA-Rose MiFi® *in-vitro*.

Table 2. EDNA-Rose MiFi® metagenomics results of 15 sequenced rose varieties from Oklahoma. Left column list the rose varieties tested. Top row list the 24 virus targeted, and the far right column list the number of viruses detected in each rose variety. Validation of EDNA-Rose MiFi® was confirmed by RT-PCR with two viruses, RRV and TSV (pink column). Boxes show the number of e-probes hits detected per virus.

ROSE VARIETY	ACLSV*	ApMV	ASGV*	ArMV	BCRV	INSV	IYSV	PDV	PNRSV	RpRSV*	RMCV	RoLCuV	RLRaV	RRV	RSDaV	RoYMV*	RoYLV	RoYVV*	SLRSV*	TRSV*	TSV	ToRSV*	TSWV	TYRV	Virus detected by EDNA	Total Virus detected by EDNA & RT-PCR
Kiss me Rose																					X		X	26	1	3
Pink surprise						X			X					64 X										2	2	4
5-13 hybrid														47 X							X	18	15	26	4	5
Como Park					12								1	48 X							X			27	4	5
Champlain														37 X							17 X			29	3	3
Caroline Hunt														41 X							17 X				2	2
Dulchen														110 X							X				1	2
5-21 hybrid														132 X							X			28	2	3
Lemon Splash				13	15	X								409 X						13	X			27	5	7
Top Gun														27 X									11	15	3	3
Rosa Seitigera								3						159 X					25					14	4	4
Apricot drift														2 X							X		X		1	3

mRT-qPCR+HRM is available for highlighted virus names

Virus detected positive by EDNA

Virus detected positive by EDNA and validated by RT-PCR

X virus tested RT-PCR positive after mRT-qPCR assays

* virus with Poly A tail

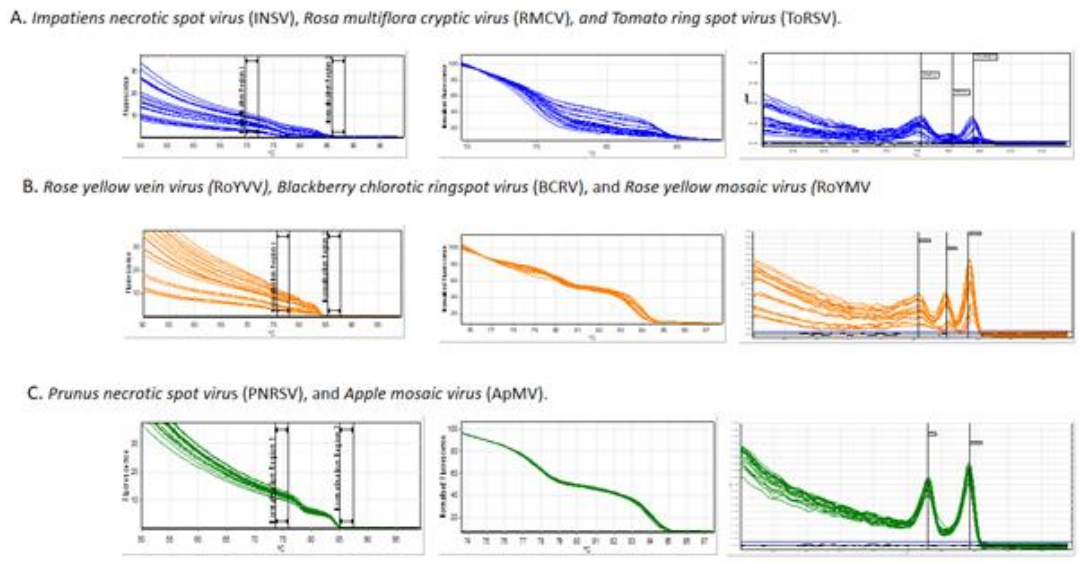

Fig. 2. mRTqPCR combined with High Resolution Melting (HRM) of PCR products. The three developed mRTqPCR+HRM methods consist of three sets of primers for detection of 13 rose infecting viruses. Graphs A, B, and C show the non-normalize (left), and normalized (center) loss of fluorescence of the obtained RT-PCR products. The multiplex Melt profile derivative plot (-dF/dT against T) (right) of common viral pathogens infecting roses. Sensitivity was determined from 1ng to 1 fg. Assay A detects INSV, RMCV, and ToRSV; Assay B. RoYVV, BCRV, RoYMV. Assay C. PNRSV and ApMV. Assay D is a single virus RT-PCR that detects RRV according to Dobhal et al 2015.

Part 2. Limit of detection of EDNA-Rose MiFi®

E-probes design for detection

Highly specific E-probes sets were designed for the ArMV RNA2. E-probe sets targeted specific sequences with uniform coverage in all the ArMV-RNA2 genome and having an even distribution (Figure 2). Two sets of E-probes were designed one consists of seven E-probes of 60 nt long, and the second are 54 E-probes 20 nt long.

***In-vitro* limit of detection**

The limit of detection was determined using twenty-eight metagenomes queried against the two ArMV-RNA2 E-probe sets of 20 and 60nt. The EDNA MiFi® MiDetect analysis was performed at four E-values (Table 4). As expected, the higher number of hits was registered in the metagenomes with high virus ratio concentrations between 55 and 27 ng respectively at all tested E-values (Table 4). The lower ratios of virus titer generated a lower number of quality scores even when reporting hits in the summary of the result of EDNA MiFi®. The quantified limit of detection for E-probes of 20 nt was 1.418 million virus copies at e-value 5, 14.1 copies of the virus at E-values 1, 1e-1, and 1e-2 (Fig. 3). E-probes of 60 nt generated negative results because of the small e-probe sample size and lack of variance in the e-probe scores, which limits the statistical analysis when comparing either too high or too low viral titers with the decoy control. This led to further research to determine the minimum number of E-probes needed to detect lower concentrations of the pathogen. A heatmap showing the distribution of hits of the 20 and 60 nt E-probes correlated to each analyzed value was done with R.

Minimum number of E-probes

The minimum number of 20 nt long E-probes required to detect the lowest virus concentration (14.1 copies) is 25 E-probes (Fig. 7). For a higher virus concentration at least 10 E-probes are required for a positive report. As expected, the frequency of hits tends to follow a normal distribution when an E-probe set contains at least 40 E-probes (Fig. 7. Right).

qPCR amplification

The two primers developed for verification of ArMV-RNA2 presence in libraries by qPCR amplified the expected product of 164 nt. The primers thermodymics calculated by Primer3 are: ArMV-RNA2 sense Tm 57.1, GC% 40.91, the self-complementarity score ANY taken as a measure of its tendency to anneal to itself or form secondary structure is 3, and the 3' termini self-complementarity of the sense primer taken as a measure of its tendency to form a primer-dimer with itself is 0.00. ArMV-RNA2 anti-sense Tm 57.4, GC% 50.00, the self-complementarity score ANY taken as a measure of its tendency to anneal to itself or form secondary structure is 3, and the 3' termini self-complementarity of the anti-sense primer taken as a measure of its tendency to form a primer-dimer with itself is 0.00.The specificity of the primers was tested in silico by BLASTn and Primer-Blast and only ArMV hits were retrieved. The obtained Ct values are reported in Table 2 and correlate with the number of hits detected by EDNA MiFi®. The qPCR was able to detect the targeted virus down to 1.41 copies. The melting temperature of the amplified product was 84.9 oC (Fig. 5).

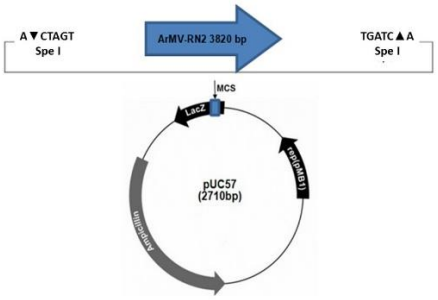

Fig. 3. Cloned pUC57 carrying the insertion of the arabis mosaic virus RNA2 genome. The location of the ArMV-RNA 2 insert (3820bp) is shown in the Multiple Cloning Site (MCS) at the LacZ expression promotor of the plasmid flanked by Spe I restriction sites.

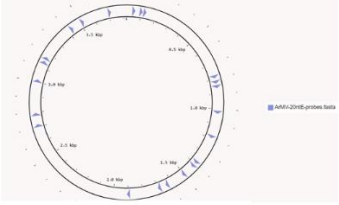

Fig. 4. Graphic representation of the linear genome of arabis mosaic virus RNA2, purple arrows represents the distribution of E-probes designed to the target sequence.

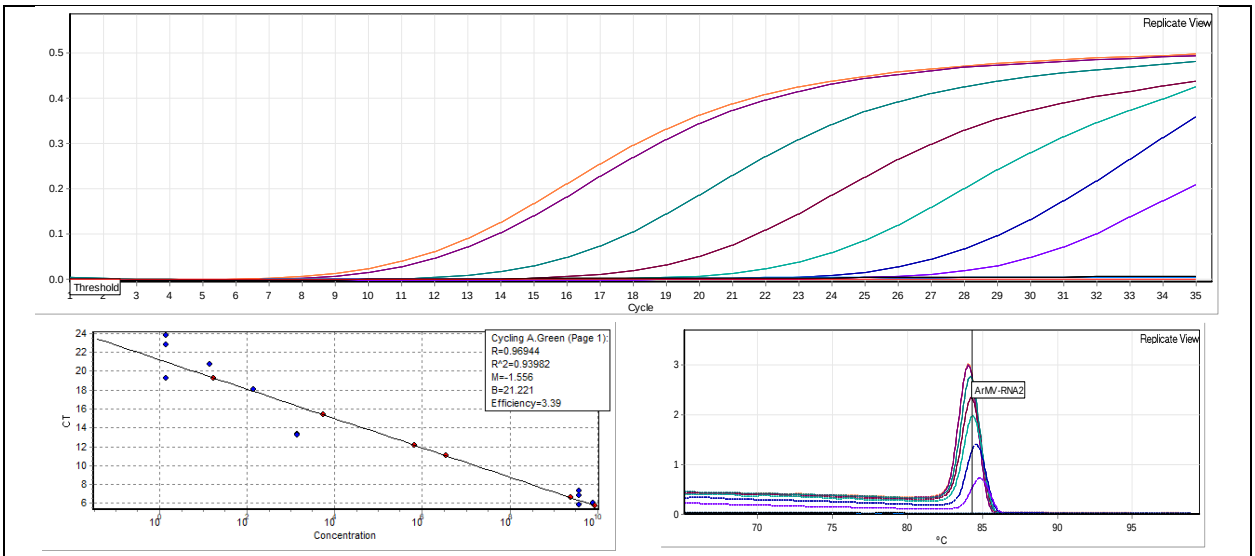

Fig. 5. Top: qPCR amplification of the ArMV-RNA2 construct serially diluted in Grapevine DNA down to 1.41 copies. Bottom left: logistical regression of the amplification. Bottom right: High Resolution Melting of the PCR amplified product. The Low-Resolution Melting profile derivative plot (-dF/dT against T) shows the Tm of the product at 84.9 ^{o}C. The steepest slope of the melting peak corresponds to the highest and lower virus concentration.

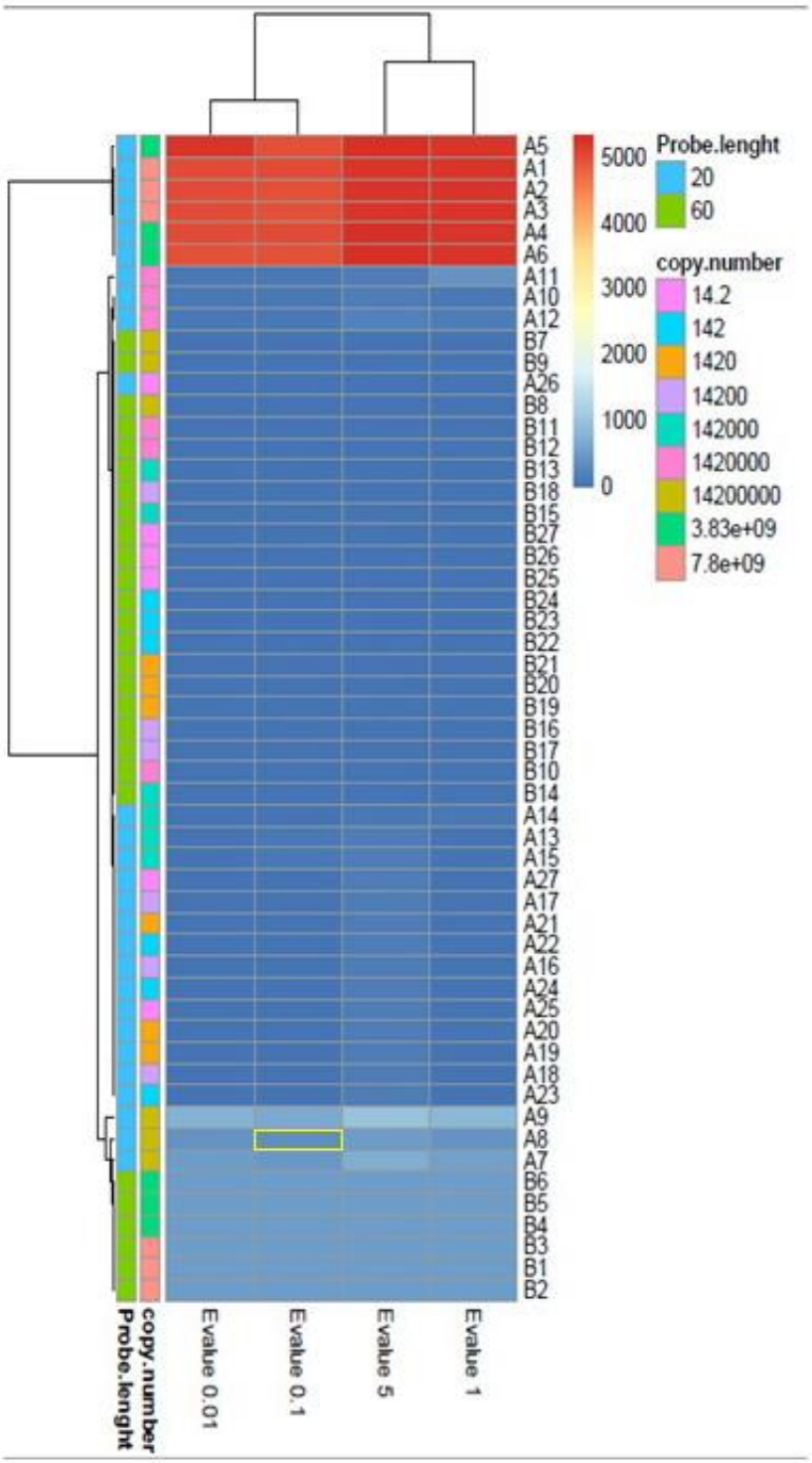

Fig. 6. Heatmap showing the distribution of hits of E-probes 20 and 60 nt long. Note the 20 and 60 nt E-probes are clustering intermixed because the two sets of E-probes share common nucleotide sequences. The lower limit of detection is shown at the intersection of E-value 0.1 and ArMV-RNA2 14.2 copies of the target.

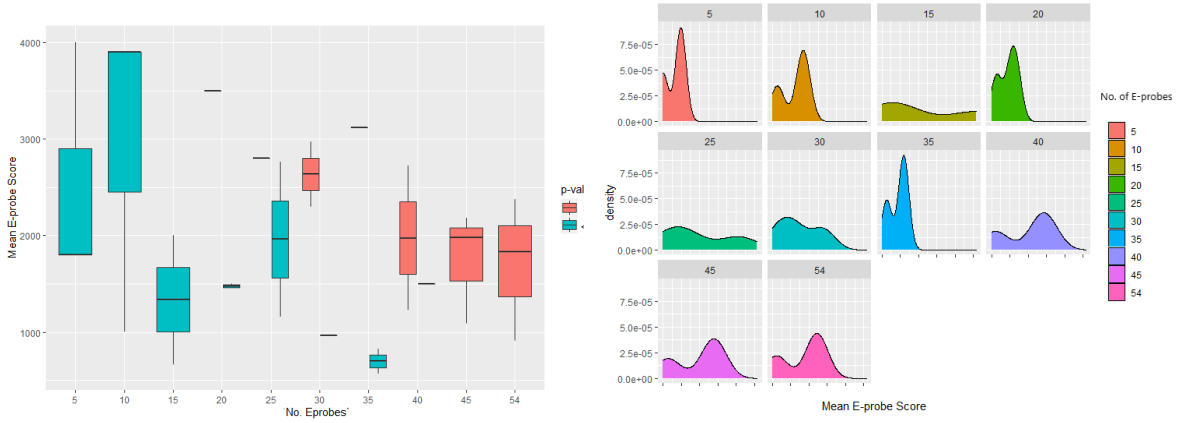

Fig. 7. Left: Minimum acceptable number of E-probes in the low serially diluted plant:virus ratio detected with EDNA ® at E-value 1e-1 after a T-test p-value ≤ 0.05. Right: Density representation of the distribution of hits based on the number of E-probes.

Table 3. DNA concentration of each plant:virus ratio simulating gradients of virus concentration occurring during infection. Grapevine DNA was used as the host background and healthy control. Each plant:virus ratio was sequenced by triplicate.

ArMV-RNA2 DNA construct (ng/µL)	**ArMV-RNA2 Copy number**	**Grapevine DNA (ng/µL)**	**Total nucleic acid concentrations (ng)**
55 ng	$7.803x10^{9}$	0	412.5
27 ng	$3.831x10^{9}$	27	405
100 pg	$1.418x10^{7}$	54	405.75
10 pg	$1.418x10^{6}$	54	405.075
1 pg	$1.418x10^{5}$	54	405.0075
100 fg	$1.418x10^{4}$	54	405.075
10 fg	$1.418x10^{3}$	54	405.0075
1 fg	$1.418x10^{2}$	54	405.00075
100 ag	142	54	405.000075

Table 4. Summary of comparative output detections of unassembled metagenomes of ArMV-RNA2 detected with EDNA® after HTS of serially diluted plant:virus ratios. The limit of detection of 14.1 copies at E-value 1e-1 is highlighted.

		E-value 5				E-value 1				E-value 1e-1				E-value 1e-2			
Copy number	C_t	20 hits	Mi Fi	60 hits	Mi Fi	20 hits	Mi Fi	60 hits	Mi Fi	20 hits	Mi Fi	60 hits	Mi Fi	20 hits	Mi Fi	60 hits	Mi Fi
7.80E+09		52 50	+	500	-	525 0	+	50 0	-	501 7	+	500	-	500 9	+	500	-
7.80E+09		52 50	+	500	-	525 0	+	50 0	-	501 1	+	500	-	500 4	+	500	-
7.80E+09	6.05	52 51	+	500	-	525 0	+	50 0	-	501 1	+	500	-	500 4	+	500	-
3.83E+09		53 25	+	500	-	526 2	+	50 0	-	500 8	+	500	-	500 8	-	500	-
3.83E+09		53 19	+	500	-	525 9	+	50 0	-	525 3	+	500	-	500 0	+	500	-
3.83E+09	6.67	53 17	+	500	-	525 5	+	50 0	-	500 1	+	500	-	500 1	+	500	-
1.42E+07		69 7	+	35	-	548	+	35	-	503	+	35	-	466	+	35	-
1.42E+07		51 7	+	14	-	403	+	14	-	374	+	14	-	348	+	14	-
1.42E+07		97 2	+	27	-	813	+	27	-	747	+	27	-	688	+	27	-
1.42E+06		15 9	+	2	-	74	+	2	-	59	+	2	-	55	+	2	-
1.42E+06		15 7	+	1	-	403	+	14	-	57	+	1	-	56	+	1	-
1.42E+06		21 2	+	0	-	115	+	4	-	86	+	4	-	74	+	4	-
1.42E+05		13 8	-	0	-	24	+	3	-	11	+	3	-	74	+	4	-
1.42E+05		10 4	-	0	-	71	+	1	-	6	+	0	-	9	+	3	-
1.42E+05		13 7	-	0	-	32	+	0	-	17	+	0	-	56	-	1	-
1.42E+04		15 6	-	0	-	15	+	0	-	1	-	0	-	1	-	0	-
1.42E+04		14 2	-	0	-	26	+	0	-	3	-	0	-	3	-	0	-
1.42E+04	13.3	12 1	-	1	-	21	+	1	-	7	+	1	-	6	+	0	-
1.42E+03		11 3	-	0	-	24	+	0	-	12	+	0	-	9	-	0	-
1.42E+03		13 1	-	0	-	22	+	0	-	7	-	0	-	7	-	0	-
1.42E+03	13.1	13 7	-	0	-	20	+	0	-	2	-	0	-	2	-	0	-

141.8		142	-	0	-	20	+	0	-	2	-	0	-	2	-	0	-
141.8		121	-	0	-	20	+	0	-	2	-	0	-	2	-	0	-
141.8	18.09	149	-	0	-	19	+	0	-	5	+	0	-	3	-	0	-
14.1		154	-	0	-	25	+	0	-	5	+	0	-	4	-	0	-
14.1		148	-	0	-	24	+	0	-	10	+	0	-	9	+	0	-
14.1	20.69	148	-	0	-	30	+	0	-	13	+	0	-	11	+	0	-
0		78	-	0	-	1	-	0	-	0	-	0	-	0	-	0	-

Table 5. The Minimum acceptable number of E-probes at the low serially diluted plant virus ratio detected with EDNA MiFi[T®] at E-value 1e-1 after a Pairwise T-test p-value ≤ 0.05 (random E-probe selection).

Copies of the ArMV-RNA2: 14.1

		E-value 1e-1						
Repetition	**No. Eprobes**	**T**	**df**	**p-value**	**Diagnosis**	**95%confidence interval**	**Mean E-probe Score**	**Mean Decoy Score**
1	5	t.test.default (score1, score2) : data are essentially constant						
	10	1	9	0.3434	NEGATIVE	-1262.157 3262.157	1001	1
	15	1	14	0.3343	NEGATIVE	-763.1911 2096.5245	667.6667	1
	20	1.8311	19	0.08281	NEGATIVE	-214.5593 3214.5593	1501	1
	25	1.8066	24	0.08338	NEGATIVE	-165.1879 2485.1879	1161	1
	30	1.7927	29	0.08346	NEGATIVE	-136.1774 2069.5107	967.6667	1
	35	1.4355	34	0.1603	NEGATIVE	-237.5563 1380.4135	572.4286	1
	40	2.3581	39	0.02348	POSITIVE	174.2597 2275.7403	1226	1
	45	2.343	44	0.02371	POSITIVE	152.2696 2025.5082	1089.889	1
	54	2.3234	53	0.02403	POSITIVE	124.0649 1690.7499	908.4074	1
2	5	1	4	0.3739	NEGATIVE	-3197.601 6797.601	1801	1

	10	1.7865	9	0.1077	NEGATIVE	-1038.3248838.324	3901	1
	15	1	14	0.3343	NEGATIVE	-1526.3824193.049	1334.333	1
	20	1.8285	19	0.08321	NEGATIVE	-209.72423109.7242	1451	1
	25	2.0472	24	0.05174	NEGATIVE	-22.514945542.51494	2761	1
	30	2.5084	29	0.01797	POSITIVE	547.74515385.5883	2967.667	1
	35	1.783	34	0.08351	NEGATIVE	-115.80451772.9473	829.5714	1
	40	1.964	39	0.05669	NEGATIVE	-44.855713044.85571	1501	1
	45	2.4362	44	0.01896	POSITIVE	341.62743613.9282	1978.778	1
	54	2.6217	53	0.01139	POSITIVE	430.75363235.9131	1834.333	1
3	5	1.633	4	0.1778	NEGATIVE	-2800.87410800.874	4001	1
	10	1.7865	9	0.1077	NEGATIVE	-1038.3248838.324	3901	1
	15	1.8708	14	0.08242	NEGATIVE	-292.87344292.8734	2001	1
	20	2.3333	19	0.03077	POSITIVE	360.46396639.5361	3501	1
	25	2.5849	24	0.01625	POSITIVE	564.37455035.6255	2801	1
	30	2.5242	29	0.01733	POSITIVE	436.44914163.5509	2301	1
	35	3.1797	34	0.003139	POSITIVE	1123.8485104.723	3115.286	1
	40	3.1279	39	0.003324	POSITIVE	962.86784487.1322	2726	1
	45	3.1464	44	0.002963	POSITIVE	782.82943572.7262	2178.778	1

		5	0.001	POSITI	988.4413		
54	3.4404	3	14	VE	3752.2994	2371.37	1

Discussion and conclusions

Part 1. EDNA-Rose MiFi®

In this study, results demonstrated EDNA-Rose MiFi® can be used for accurate detection of the reported virome infecting roses (22 in total). EDNA was conceived as a pathogen detection tool (Stobbe, et.al., 2013) and this research aims to present a new way of plant virus detection using HTS based on the application of the EDNA fundamental concept. Previous attempts portrayed EDNA as a tool designed for single pathogen detection (Espindola, et.al, 2015; Blagden, et.al, 2016). In this study, EDNA is presented as a prototype for broad reliable detection screening of germplasm able to successfully detect viruses. In this case, infecting rose varieties.

The current use of High Throughput Sequencing (HTS) has become extensively applied as a newly DNA-based pathogen detection technology applied to metagenomic samples (Massart, et.al, 2017). This study describes the screening of twelve rose varieties quantifying hit frequencies obtained for each rose variety queried against the E-probes hits. To make a detection, the number of hits of the specific E-probe sets was compared to the hits resulting from the decoy E-probes. The significant p-value for the positive results in metagenomes queried against RRV and TYRSV ($p \leq 0.05$) demonstrated that both viruses are present in most of the analyzed rose cultivars.

To present common bioinformatic pipelines are computer-intensive approaches for virus detection and discovery. Similar results among taxonomic classifiers and EDNA-Rose MiFi® were obtained, only cultivar *Lemon Splash* showed high virus titter for *Rose rosette virus*. This is because the virus is in high concentration. After all, this variety is highly susceptible to RRV infection, and the collected specimen showed characteristic symptoms of RRV infection. *Rosa seitigera* was the only rose spp. reported resistant to RRV. However, after the EDNA-Rose MiFi® analysis *Rosa seitigera* was the second variety with a high number of hits, this may be because of either resistance or a recent RRV event overcoming resistance. All other cultivars are reported susceptible after correlating with the hit frequency of the virus among the cultivars.

Di Bello et al., (2015) demonstrated that Rose Rosette Disease is the result of the presence of only RRV infection in a cultivar. This study shows there is a synergy during mixed infection in the studied cultivars among other viruses in addition to RRV. For example, the cultivar with a higher RRV titer, *Lemon splash*, showed was infected with four additional viruses besides RRV (409 hits). The hit frequency detected by EDNA-Rose MyDetectTM of ArMV (13hits), BCRM (15 hits), TRSV (13 hits), and TYRSV (27 hits), suggests RRV would be masking the symptomatology of other co-infecting viruses and probably lowering their replication rate. RRV and the genus Emaravirus have been determined as a complex evolutionary virus group (DiBello et al, 2015). The co-evolution of RRV with the host was determined by the complex genome plasticity of the Emaravirus genus by reassortment and duplication of pathogenicity

proteins (Tatineni et al., 2011) similarly this may lead to overcoming resistance in *Rosa seitigera* spp.

This study demonstrates multiple virus infections in the HTS obtained metagenomes. Besides cultivars *Kiss me Rose* and *Apricot drift*, the metagenomes of other 10 varieties showed infections with two or more rose reported viruses. The rose susceptible cultivars may host more than one virus. Rose infecting viruses showed different accumulation of hits among resistant and susceptible cultivars. *Rosa seitigera* was reported as a resistant species against RRV (Byrne et al., 2018), however, EDNA-Rose MiFi® demonstrated that this variety was infected by four viruses (PDV, RRV, SLRSV, and TYRSV), and accumulated a high RRV titer of 159 hits in Oklahoma.

The deepness of the sequencing platform used for viral detection is important (Pecman et.al., 2017).

The Oxford Nanopore platform was selected for this research because its field-deployable potential, and also because it generates long reads. Long reads facilitate full virus genome sequencing. Plant virus genomes are small if compared with the host rose genome. Therefore, if comparing the complete rose genome (88781.76 Mb) with a single virus genome, for instance, RRV (17.8 Kb) EDNA was able to find reads from this virus. Therefore, there is no need for deep sequencing platforms to detect the rose virome. Another alternative to find more reads of viral particles within a metagenome is to deplete the host RNA. Noteworthy, total RNA was extracted in this study, therefore most of the mRNA of the host was amplified in a higher ratio than the genome of the targeted virus. Poly A tail termination of some rose virus is also found within the analyzed metagenomes. The selected amplification method was ds cDNA before library preparation that will selectively amplify sequences with a Poly A, terminus. Further research has to use random hexamers to equally enrich all virus presence.

Even though traditional molecular diagnostics require previous knowledge of a small region of the target pathogen. EDNA database is built upon specific electronic probes generated from the complete reported pathogen genome sourced by the NCBI GeneBank or alternative repository. It is also demonstrated the usefulness and further applicability of Multiplex RT-qPCR –HRM which was applied in this research as a validation checkpoint before sequencing. This validation step determined the accuracy of EDNA Rose MiFi® during detection using HTS. The multiplex RT-PCR developed in this study detects and discriminates the most common virus reported in the US.

Although true positive hits were found in EDNA-Rose MiFi® analysis when compared with Multiplex RT-qPCR –HRM, some of them correlate between both detection methods. However, since the Multiplex RT-qPCR –HRM targets a small region (~90-350bp) some of the expected viruses were not detected by HTS. This is suggesting, a ribosomal depletion method should be applied before sequencing must be executing prior HTS (Kim, et.al, 2012).

Rose is a multispecies complex and is vulnerable to pathogen infection, even more in cultivated systems. Modern rose cultivars are a product of interspecific hybridization. Most of the garden roses are derived from hybrids with a genetic association of wild rose parentages.

Breeding selection and hybridization resulting in cultivars of 2x to 8x chromosomal cultivars focus on disease resistance (Horst & Cloyd, 2007). Achieving high levels of disease protection is the cornerstone and the main target in rose breeding programs. Once a viral infection establishes in a plant there is no remediation method available rather than eradication of the infected plant. Breeding programs focus on cloning rose genes with putative functions for disease resistance to avoid the infection of the virus to cultivated areas similar to the molecular marker-assisted breeding and host-virus interaction (Debener & Byrne, 2014). The Rose rosette disease trial at Oklahoma State University in Payne county (Perkins) is one of the nationwide research replicates of newly released rose cultivars with continuous disease monitoring. The sampled cultivars were varieties that are now in the market as well as two hybrids. All of the samples besides *Kiss me Rose* have a high concentration of RRV. The hybrids 5-13 and 5-21 showed 47 and 132 hits against RRV showing virus accumulation and less RRV characteristic symptomatology. As expected, the use of HTS as diagnostic tools combined with bioinformatics will benefit and hasten outright reported viral disease screening.

EDNA-Rose MiFi® provides a new framework for entire plant virus detection. Due to the multiple pathogen detection capabilities, EDNA has strengthened since its creation as a diagnosis method and has the potential to be applied widely at the border by biosecurity systems, quarantine laboratories, greenhouses, and plant diagnostic clinics. Future direction and application of this technology will be used for germplasm evaluation and in this way ensuring rose virus-free cultivars. Even more, shortly, a database that includes reported pathogens infecting a host will provide a complete and integrated diagnosis. Also, deep sequencing of the vector *Phyllocoptes fructiphilus* may give some answers to the scientific community of the host-virus-vector interaction into the studied pathosystem.

From a biosecurity perspective, the developed technology (EDNA-Rose MiFi®) has described capabilities for monitoring, control, and avoid foreign virus introduction to an economically horticultural crop (Mumford, et.al, 2016). This study aims to accelerate plant diagnosis with cut edge technologies needed for risk assessment and agricultural biosecurity.

Part 2. EDNA-MiFi® Limit of detection

The pipeline EDNA-MiFi® MiDetect overcomes the time-consuming traditional bioinformatic workflows applied during microbial discovery research and enables accurate results for pathogen detection (Stobbe et al., 2013; Stobbe 2014; Blagden et al., 2016, Espindola et al., 2015; Espindola et al., 2018).

This study addressed a method to approach the analytical limit of detection of EDNA-MiFi® not previously described for this pipeline. The use of highly specific E-probes designed for detection of the virus, in this case, ArMV-RNA2 adds a technical advantage to EDNA-MiFi® because allows an efficient computational effort for pathogen detection.

The validation of the limit of detection using controlled virus concentrations in a standard *in-vitro* system using the described plasmid pUC57 harboring the RNA2 ArMV demonstrated to be a robust attempt to determine the *in-vitro* the limit of detection for EDNA-MiFi®. The limit of detection of EDNA-MiFi® may vary depending on the probe length (nt) and the number of E-probes generated for the target sequence. These two parameters are dependent on the size of

the targeted virus genome and/or the number of targeted genomes (virome), including the near neighbor genomes. A precise limit of detection will require to determine the optimal probe number and size for each targeted virus genome to be screened.

In this study, the different number of E-probes generated to the ArMV-RNA2 template by EDNA-MiFi® MiProbe is different for E-probes of 20 and 60 nucleotides. For probes of 20 nt 54 E-probes were designed while only seven E-probes 60 nt long were generated. The 20 nt E-probes length was selected because ArMV generates subgenomic RNAs during replication in host cells (Ding & Voinnet, 2007) and a more effective coverage was expected. Also, the E-probe length (20nt) and parameter selection influence the validation of the limit of detection depending on the selection of the E-value. EDNA-MiFi® MiDetect offers four E-value options (5, 1, 1e-1, 1e-2). These options allow different levels of stringency and are to be selected by the EDNA-MiFi® operator. This study demonstrates that high E-values increase the positive hits in targets at low concentrations, and the negative control with 100% identity of the hit sequence, which is not desirable since these are false positives. Therefore, if the user selects higher E-values the probability of false-positive increases. The results obtained out of the 28 metagenomes showed the recommended E-value after validation is 1e-1 because allowed target detection down to 14.1 copies with no false positives in the screened metagenomes and the negative control.

Regarding the long E-probes, although true hits occur with the 60 nt E-probes (seven E-probes), these are reported by EDNA-MiFi® as negative, because the mean E-probe scores and mean decoy scores have no variance, causing the Pairwise T-test not to be computed. As a consequence, additional statistical modeling is needed to address the optimal statistical analysis of the large and low number of E-probes as well as samples having highly concentrated virus titer.

The Pairwise T-test performed on the twenty-eight metagenomes shows the range of E-probes needed for detection. Slightly 10 E-probes are required for positive detection of the targeted segment of ArMV-RNA2 genome at high titer, and 45 E-probes for positive detection at low titer.

Initially, EDNA research focused on single pathogen detection and was later improved to detect transcripts expression (Stobbe et al., 2014; Blagden et al., 2016; Espindola et al, 2018), at the light of the obtained results and because currently EDNA-MiFi® may screen numerous virus targets simultaneously the limit of detection may vary among targeted viruses.

EDNA-MiFi® is a sensitive and valuable tool for the detection and identification of infectious plant virus from HTS directly sequenced from plant tissue. The experimental data presented in this study contributes knowledge needed to make informed decisions regarding the number and length of E-probes, and the deepness and sequence coverage needed to accurately detect a plant virus within unassembled metagenomes queried using the pipeline EDNA MiFi®.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$17,584.00	$18,644.82
Fringe Benefits	$946.00	$1,769.20
Travel	$2,200.00	$919.75
Equipment	$0.00	$0.00
Supplies	$7,598.00	$7,010.76
Contractual	$24,672.00	$24654.47
Other	$0.00	$0.00
Direct Costs Sub-Total	$53,000.00	52,999.00
Indirect Costs	$0.00	$0.00
Total Federal Costs	$53,000.00	52,999.00

PROGRAM INCOME (IF APPLICABLE)

N/A

ADDITIONAL INFORMATION

N/A

Project Title	Oklahoma Farmers' Market Annual Conference			
Recipient Organization Name:	Oklahoma Nutrition Information and Education Project (ONIE)			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	9/30/2018
Recipient's Project Contact				
Name:	Dr. Karla Finnell			
Phone:	405-271-2017 x 4 6757			
Email:	Karla-Finnell@ouhsc.edu			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

The Oklahoma Nutrition Information and Education Project (ONIE) led the organization, implementation, and evaluation of the fourth annual Oklahoma Farmers' Market Conference (OKFMC). The conference provided education, outreach opportunities and resources to specialty crop producers, farmers' market managers, community stakeholders, and Agritourism managers from across the state. Conference topics included marketing and social media, accepting SNAP and Senior Farmers' Market Nutrition Program benefits, growing-season planning, establishing and sustaining farmers' market and Agritourism sites, compliance with the Food Safety Modernization Act, hydroponics and aquaponics, building stronger collaborative networks among community organizations and specialty crop producers, and networking opportunities. Specialty crop growers collaborated and acquired new knowledge and ideas, as indicated by conference evaluation materials. Interest in the conference has continued to grow, and a record number registered for the event (505). Attendance was hampered by ice storm hitting the entire state the evening prior to the conference, which led to impassable roads and hazardous travel conditions. Of the 505 registrants, 220 registrants attended, which is less than half, but an exceptional turnout given the weather conditions. Rescheduling the conference was not financially plausible given the sunk costs associated with catering, equipment, and room rentals. We recorded and live-streamed the opening session, four breakout sessions, and the keynote lunch speaker on Facebook, extending remote access. These videos were well-received and altogether viewed over 3,000 times. The recorded sessions are available to the public and may be found at the OK Farmers Market Facebook page and the ONIE Project YouTube Channel under the 2018 Oklahoma Farmers Market and Agritourism Playlist.
Video Recording Locations:
Facebook: facebook.com/pg/OKFarmersMarkets/videos
YouTube 2018 OKFMAC Playlist :https://www.youtube.com/playlist?list=PLtyTuPgds-9SHGW5sjiItCAKGJTOoZ_Ym&playnext=1&index=1

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*
1	Strengthen and diversify the network of specialty crop producers	x	
2	Increase access to Oklahoma specialty crops and enhance nutrition education and consumption	x	
3	Increase collaboration between specialty crop producers and local farmers' markets	x	
4	Develop and improve marketing channels for specialty crops through new or expanded farmers' markets and development of new revenue streams such as SNAP and SFMNP	x	

5	Develop capacity and knowledge that enhances the resources of conference participants	x	

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ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	Registered 505 participants representing potential and active specialty crop producers, farmers' market managers, Agritourism site managers, and community organizations	Objective 1, Objective 3
2	Delivered 16 unique education sessions and 2 featured speaker sessions covering new information and resources	Objective 5
3	Developed OK Farmers' Markets Facebook and Instagram for Farmers' Markets and Producers with 1,911 Facebook followers and 1,016 Instagram followers. 62 Facebook posts and 41 Instagram posts were posted, sharing resources and ideas made throughout the year. Facebook- Oklahoma Farmers Markets: facebook.com/OKFarmersMarkets/ Instagram- okfarmersmarkets: instagram.com/okfarmersmarkets/	Objective 1, Objective 3
4	27 unique exhibitors offered resources tailored to the priority audience	Objective 5
5	Number of farmers' markets registered with ODAFF increased from 82 in 2017 to 89 (+7%) in 2018	Objective 2, Objective 3, Objective 4
6	Increased the number of EBT-, SNAP- and SFMNP-eligible farmers' markets from 28 in 2017 to 40 (+42.85%) in 2018	Objective 2,

CHALLENGES AND DEVELOPMENTS

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#	Challenge or Development	Corrective Action or Project Change
1	Severe winter storm affected travel for entire state of Oklahoma	Streamed sessions on Oklahoma Farmers' Markets Facebook page with limited equipment available (cellphones, cameras, tripods). We are planning to integrate this strategy into future conferences for

#	Challenge or Development	Corrective Action or Project Change
		specialty crop growers because of its success.
2	Statewide SNAP redemptions increased from $137,731.05 to 173,655.35 (25.9%) from 2016 to 2017 but decreased 11.6% from 2017 to 2018, eradicating some of the earlier gains. This loss is attributable to crop failures, malfunctioning EBT machines at large markets (2), and loss of EBT machine operator volunteers, reduction in the number of market days, and a reduction in Double Up Oklahoma marketing. The loss does not reflect any loss of interest in specialty crop production.	Worked with community organizations and stakeholders to continue support of programing that enhances the awareness of SNAP at FM.

LESSONS LEARNED

Based on feedback from previous conferences it was determined that February is the best time to host the event since it is before the start of growing season and specialty crop producers can implement knowledge learned at the conference in their growing practices. The 2018 conference planning committee consisted of members from the ONIE team, Oklahoma Department of Agriculture, Food and Forestry along with identified stakeholders including farmers market managers, specialty crop producers, and members of community support organizations. The planning committee met monthly October through December of 2017, and then met bi-weekly During January and February 2018. Having a broad inclusive group that met regularly ensured that sessions topics were relevant and of peak interest. In addition, soliciting feedback from participants allowed us to schedule the conference at a time that farmers could attend.

The conference had 16 unique sessions (session descriptions in attached program) that focused on a variety of topics related to specialty crops. Conference sessions were selected based on feedback from 2017 Oklahoma Farmers Market Conference attendees and input from conference planning committee stakeholders. The selected sessions were chosen to ensure there would be a representative and beneficial session for each of the target audience groups during every conference breakout block. Speakers were chosen based on recommendations of the planning committee members. The planning group preferred speakers primarily who were working in the field, not academics, as well as those that could share innovative practices that were affordable for small growers and could be implemented in rural farmers markets. Speakers were primarily local, and a few attended from out-of-state.

Resources to increase access, collaboration, and knowledge were fostered by hosting an Expo area with exhibits, breakout sessions, and key notes sessions. An Expo featured accessible resources beneficial for specialty crop growers and market managers. To increase traffic to the exhibitor area, food and beverages for all breaks and lunch were launched from the Expo. Additionally, a market vendor recruiting station was set up in the Expo where market

managers were invited to display vendor applications for their respective markets that many specialty crop vendors utilize, connecting markets and specialty crop growers. To encourage networking at the conference, 15-minute breaks followed each session to provide an opportunity for participations to linger, reflect upon, and discuss knowledge garnered in the session.

In addition, several sessions were organized to create networking opportunities. Tables were dedicated to a particular topic. Participants would then assign themselves to a table or topic area wherein they were invited to share their perspective. Having a structured networking opportunity provides an opportunity for participants to meet each other and identify areas of common interest. Participants were also invited to join the Oklahoma Farmers Markets Facebook Group that served as a hub to continue the dialogue of topics presented at the conference. Oklahoma Farmers Markets Group Facebook Link: https://www.facebook.com/groups/175425532963332/.

Snow and ice are uncommon in Oklahoma, but January and February are the height of the winter weather season. Nevertheless, February is the best time to host the conference because it does not interrupt the growing season and specialty crop growers are able to immediately implement lessons learned in the next growing season. We learned that recorded sessions were a desired and highly utilized feature for those unable to attend in-person. For the 2019 conference, we plan to professionally record select sessions and the keynote address. We have also found how valuable social media can be in advertising and maintaining community with the priority population.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

The conference continues to gain support from stakeholders within the state and nationwide. Through social media, we continue to serve as a source of information and networking for past conference participants (https://www.facebook.com/OKFarmersMarkets/). ONIE team members also continue to attend relevant farmers' market meetings and conferences to identify potential speakers and sponsors for future conferences. It is likely that this conference will remain free to allow socially disadvantaged and beginning farmers to attend, and to prevent additional costs for specialty crop producers from across the state who incur travel costs to attend the conference.

BENEFICIARIES

Number of project beneficiaries: 3,558

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

OUTCOME MEASURE(S)

☐ **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)

#	Outcome and Indicator	Quantifiable Results
1	Outcome 3, Indicator 3. a	Ten new farmers' markets or direct marketing farmers will expand access or improve offerings to specialty crops by accepting SNAP benefits
2	Outcome 8, Indicator 5	SNAP sales at Oklahoma farmers' markets will increase from $137,731 to $158,391 and by 15% as a result of project activities

DATA COLLECTION

Evaluations were administrated at the end of each session and at the end of the conference. Results were analyzed by ONIE staff using SPSS statistical software. Conference evaluations were completed by 78 participants, and the resulting data were disseminated to conference committee members in a report (Appendix E). When asked "How likely are you to implement a new idea you learned?" 98.5% of respondents said somewhat to very likely, indicating that participants successfully learned new information that could be used to further the production and marketing of specialty crops.

Gross sales data for specialty crops in Oklahoma is currently not available. However, SNAP and SFMNP benefit redemptions at farmers' markets is an excellent proxy measure for specialty crops, as benefits can only be used on food items. ONIE holds a data-sharing agreement with OKDHS and receives weekly reports of all SNAP transactions at markets across the state.

The overwhelming majority of Oklahoma's SNAP-accepting farmers markets do not report overall vendor sells, including sales from specialty crop. Moreover, they are highly resistant to reporting this information. The only available, consistent, and quality sales data is from SNAP benefit redemptions and the Senior Farmers Market Nutrition Program (SFMNP).

SNAP benefit redemptions reflect specialty crops purchases, but also include purchases of meat, dairy, eggs, baked goods, and value-added items. The amount of SFMNP benefits redeemed augments this data. Unlike, SNAP, SFMNP restricts purchases primarily to specialty crops, including locally grown fruits, vegetables, fresh cut herbs. The only non-specialty crop benefits is unprocessed honey, a small value purchase. However, a limitation of interpreting this data to as a measure of increased specialty crops sales is that there caps on the state benefit allocation, as well as the award to individual is limited to $50.00 per market season. The amount of SNAP redemptions is one the best available measure of specialty crop sales growth.

Although there was a decline from 2017, as explained in the challenges section, there was still a marked increase in 2018 SNAP sales compared to 2016, and a 42.85% increase in the number of EBT-eligible farmers' markets from 2017, providing expanded opportunities to access specialty crop produce for low-income Oklahomans. The number of state-registered farmers' markets increased 7% from 2017 to 2018 (www.Okgrown.com). These are all positive trends. Newly registered farmers' markets and EBT-eligible farmers' markets provide additional opportunities for specialty crop growers to sell their produce and create more revenue for the state.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$10,510.00	$8,458.22
Fringe Benefits	$3,992.00	$2,936.05
Travel	$282.00	$146.50
Equipment	$0.00	$0.00
Supplies	$1,266.00	$767.09
Contractual	$0.00	$0.00
Other	$13,950.00	$9,696.05
Direct Costs Sub-Total	$30,000.00	$22,003.86
Indirect Costs	$0.00	$0.00
Total Federal Costs	$30,000.00	** 22,003.86

**- Due to cost savings not all of the money budgeted for this project was expended. The balance of funds will be redistributed to other projects such as the Specialty Cropopoly Junior Board game.

ADDITIONAL INFORMATION

2018 OKFMC Sessions and Speakers:

- **Welcoming Address**: *Secretary Jim Reese* - An update on Agriculture & Specialty Crops in Oklahoma.
- **Lunch Keynote Speaker**: *Dr. Jim Barham* How Agriculture Economics impacts the specialty crops and ideas on how to expand sales.
- **Evolution of Agritourism** - *Cathie Green, Wild Things Farm*
- **Starting & Sustaining a Successful Farmers Market**-*Tandy Kidd, ODAFF Market Coordinator & Trista Milliman, Nowata CAN Garden Market*
- **New Food Safety Rules Introduced** -*Justin Mcconaghy, ODAFF Produce Safety Program Coordinator & Kenda Woodburn, OSU Ex. Horticulture Educator*
- **Farm to School**-*Cheri Long, ODAFF Ag in the Classroom Coordinator*
- **Getting the Most From Your Land & Soil**- *Blane Stacy, OK Conservation Commission Soil Health Educator & Saleh Taghvaeian, OSU Extension Irrigation Specialist*
- **Overnight Guests**- *Meriruth Cohenour, ODAFF Agritourism Coordinator*
- **Extending the Growing Season**- *Mary Katherine Collins, Phocus Farms & Steve Upson, Noble Research Institute Senior Consultant*
- **Social Media 101**-*Alex Taylor, VI Marketing & Branding Social Media & Marla Saeger, Tahlequah Farmers Market Board President*
- **Accepting SNAP, Senior FM Benefits & Double Up**-*Cari Crittenden, OKDHS SNAP-Ed & SFMNP Coordinator, Rita Scott, OK Farm & Food Alliance Director of Outreach & Education & Tom Pennington, OKDHS Electronic Payment Systems Director*
- **Can I? Agriculture Regulations** - *Meriruth Cohenour, ODAFF Agritourism Coordinator*
- **Hydroponics & Aquaponics**-*Kaben Smallwood, Symbiotic Aquaponics & Daniel Wilson, The 365 Farm Owner*
- **Small Town Farmers Market Advantages**- *Cindy McDonald, Sapulpa Main Street & Market Manager, Robyn Franklin, Okmulgee Farmers Market Manager, Bobby Howard & Jennifer Avery, Okmulgee County TSET*
- **Advanced Social Media**- *Rachel Merritt, VI Marketing & Branding SM Director*
- **Feral Swine: Solutions & Actions**-*Scott Alls, Oklahoma Dept of Wildlife Conservation*
- **Farm but No Table?**-*Dr. Stacy Tomas, OSU Professor*
- **Successful Vegetable Varieties for Market Gardeners**-*Julia Laughlin, Oklahoma County Horticulture Educator*
- **Getting on Google**- *Jade Owen, ONIE Project Community Outreach Specialist & Jenna Moore, ONIE Project Analyst*
- **Making your Market Accessible**-*Valencia Stiggers, OK Disability Concerns Program Specialist & Doug MacMillian, OK Disability Concerns Agency Director*

Examples of 2018 OKFMC Vendors:

- Canadian County Extension Master Gardeners
- Central Oklahoma Young Farmers Coalition
- Choctaw Nation of Oklahoma Agricultural Outreach
- Community Food Bank of Eastern Oklahoma

- Farm FreshWeb.com
- Federal Reserve Bank of Kansas City
- Food & Agricultural Products Center (FAPC)
- Freestanding Aeroponic Gardens
- FRESHPOINT OKLAHOMA
- MazePlay
- Noble Research Institute, LLC
- Office of Disability Concerns
- OK Department of Human Services
- Oklahoma Black Historical Research Project, Inc.
- Oklahoma Farm Bureau
- Oklahoma Farmers & Ranchers Association
- Oklahoma Farmers Market Vendor Application Station
- Oklahoma Horticulture Society
- ONIE Project
- OSU Extension Community Nutrition Ed. Program (CNEP)
- OSU Oklahoma Small Business Development Center
- OSU Women in Ag & Farm Management
- Regional Food Bank of Oklahoma
- Southwest Oklahoma Community Action
- Symbiotic Aquaponic
- USDA Agricultural Marketing Service
- USDA Food & Nutrition Service

All vendors were required to provide resources to attendees that would improve their production, promotion, crop yield, and/or sales of specialty crops. An example is the OSU Extension's FAPC booth, discussed how to turn extra yield of specialty crops into value added packaged foods to last beyond growing season.

See also: Appendix #1

Project Title	U-Pick Education Website Integration			
Recipient Organization Name:	Oklahoma Department of Agriculture, Food and Forestry			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	9/30/2019
Recipient's Project Contact				
Name:	Micaela Danker			
Phone:	405-740-0794			
Email:	Micaela.danker@ag.ok.gov			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

The Oklahoma Agritourism program undertook a project to help producers educate consumers about proper u-pick practices, food safety and handling, nutritional value and value-added possibilities of specialty crops. The goals were accomplished by adding web content to the program's existing site and social media components that include picking tips, handling and storing guidelines, nutrition facts and recipes. The project also provides producers with tools to inform consumers about the existence of the web content.

Fresh, local produce is in high demand. Consumers are interested in connecting with the producer and learning about how the crops are grown. In addition, families and educational groups are seeking educational opportunities relating to the local food movement. Because of this, U-pick farms have increased significantly over the past few years and the Oklahoma Agritourism program has been successful in connecting the consumers with producers to a degree, however, with a growing urban population leading to a growing disconnect with agriculture, the need for increased education and awareness grows each year.

With this project we hired a marketing firm to create multiple educational materials for consumers. For our first step, we added content specifically for the Oklahoma Jelly-Making Trails on the Oklahoma Agritourism website. Content included a jelly making guide with information about produce availability, picking tips and storage tips specifically for strawberries, blueberries, blackberries and peaches. The website also included a map with producer information where consumers could access each specialty crop. The second step included a series of social media campaigns. The social media campaign included three recipe videos which showed consumers how to prepare blackberries, peaches or blueberries once they visited the Oklahoma Jelly Making Trails to buy/pick those local ingredients. The ad featuring grapes encouraged consumers to pick grapes from a local vineyard and also linked back to the website which featured content about basic kitchen utensils needed and steps for making your own jelly. For the third and final step, we had 15,000 push cards created and printed. The information included on the push cards educated consumers about season availability and picking tips for strawberries, blueberries, peaches and blackberries. The push cards also shared jelly making tips and included the web address leading to more recipes and resources.

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*
1	Educate consumers about proper u-pick practices and food safety of specialty crops.	XX	

2	Educate consumers about the nutritional value and value-added possibilities of specialty crops.	XX	
3	Educate consumers about the locations of u-pick specialty crop farms in Oklahoma.	XX	

ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	10,000 of the 15,000 printed push cards were distributed to 23 u-pick farms, 12 tradeshows and through each purchase at the Made in Oklahoma store at the 2018 Oklahoma State Fair. The push cards shared brief information about preparing specialty crops and food safety and also included a link to more information with recipes located on the corresponding website.	Objective 1, 2, 3 Outcome 3 Indicator 1
2	The video ad featuring blackberries ran for 30 days on Facebook in 2018 and 2019. Total numbers included: Reach: 154,812 Clicks: 20,003 Reactions, shares and comments: 4,317	Objective 2, 3 Outcome 3 Indicator 1
3	The video ad featuring blueberries ran for 30 days on Facebook in 2018 and 2019. Total numbers included: Reach: 124,989 Clicks: 10,186 Reactions, shares and comments: 346	Objective 2, 3 Outcome 3 Indicator 1
4	The link ad featuring grapes ran for 30 days on Facebook in 2018 and 2019. Total numbers included: Reach: 175,438 Link clicks: 7,928 Reactions, shares and comments: 1,118	Objective 2, 3 Outcome 3 Indicator 1
5	The video ad featuring peaches ran for 30 days on Facebook in 2019. Total numbers included: Reach: 98,070 Link clicks: 6,226 Reactions, shares and comments: 833	Objective 2, 3 Outcome 3 Indicator 1
6	During the period of time that the ads were running, OKJellyMaking.com received	Objective 2, 3 Outcome 3 Indicator 1

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
	23,285 new visitors coming directly from the ad links on Facebook.	

CHALLENGES AND DEVELOPMENTS

#	Challenge or Development	Corrective Action or Project Change
1	The blackberry cobbler video was the least innovative recipe we have used for any type of promotion, but it far performed any other recipe we have used terms of social engagement and traffic filtered to the educational website	Recipes will be evaluated in the future for emotional credibility and not just innovation.
2	A late freeze in April 2018 proved devastating to the state's peach crop. Promotion of peaches using social media usually accounts for approximately 30% of our total specialty crop web and social traffic for the year. We chose to run a grape ad instead of peach and although it performed well, many of our normal grape producers sold out early leading to some customer frustrations.	We plan to recruit more grape growers to the Jelly-Making Trails and hope for the best in regards to spring freezes and crop loss of peaches. The peach ad ran in 2019, but not 2018.

LESSONS LEARNED

For the social videos we ran them for 30 days in 2018 and 2019. After receiving the reports for both years, the ads reached far more people and had far more click links, reactions, shares, and comments in 2019 than 2018. This proves to show that quality content can be reused and still reach desired results or even exceed them.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

We still have 5,000 push cards left of the 15,000. We will pass those out at tradeshows and u-pick farms to continue promoting the Jelly-Making Trails campaign.

BENEFICIARIES

Number of project beneficiaries: 67

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

OUTCOME MEASURE(S)

☐ **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)

#	Outcome and Indicator	Quantifiable Results
1	Outcome 3, Indicator 1	During the u-pick season, social media ads informing people about the farms located on the Jelly-Making trails had a reach of 553,309 with 6,614 unique reactions (likes, comments, shares.) In addition, 23,285 individual people visited the Jelly-Making Trails website during the running time of the ads. The social media ads encouraged buying specialty crops from local producers while also learning different recipes to prepare them. The social media ads also linked back to the website which educated the consumers on all the different access points in our state.
2	Outcome 3, Indicator 1	10,000 push cards leading people to the educational information on the website were distributed to consumers on farms and tradeshows as well as the Oklahoma State Fair. The website included recipes, picking tips, storage tips, season availability and other resources.

DATA COLLECTION

Data was collected by contracted marketing firm through social media analytics. Once each advertisement ran, we received a social report detailing impressions and website clicks. The reports even went a step further to collect data on our audience age and gender.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$0.00	$0.00
Fringe Benefits	$0.00	$0.00
Travel	$0.00	$0.00
Equipment	$0.00	$0.00
Supplies	$0.00	$0.00
Contractual	$14,077.50	$15,815.00
Other	$2,713.76	$975.60
Direct Costs Sub-Total	$16,791.26	$16,790.00
Indirect Costs	$0.00	$0.00
Total Federal Costs	$16,791.26	$16,790.00

PROGRAM INCOME (IF APPLICABLE)

N/A

ADDITIONAL INFORMATION

www.okjellymaking.com

Project Title	Specialty Cropopoly Junior Board game			
Recipient Organization Name:	Oklahoma Department of Agriculture, Food & Forestry			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	12/2/2019
Recipient's Project Contact				
Name:	Audrey Harmon			

Phone:	405-740-0160
Email:	audrey.harmon@ag.ok.gov

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

The "Specialty Cropopoly Junior Board Game" is a resource used in Early Childhood Education classrooms, across Oklahoma, to help teachers and students learn about Specialty Crops. This educational tool provides the means for teachers to encourage students to learn about Specialty Crops with a game that is not only fun, but also a learning experience using math and reading connections. One of the goals of this resource was to provide information about each specialty crop, including nutritional information about each crop. The goal was for this resource to be disseminated to 2,800+ classrooms across the state. After using this resource, teachers and students should be able to identify up to 16 specialty crops in Oklahoma. Students can make personal connections to each crop and the nutrition it offers for healthier choices in eating. This game is played just like regular Monopoly Junior; however, it is specific to the Specialty Crops grown in Oklahoma. This project is important and timely because the health and nutritional choices of Oklahoma students and teachers has reached a critical stage and must be addressed. It is important for adults and children to know where their food comes. The specific issue is teachers and students in Oklahoma do not realize what specialty crops are grown in the state and their nutritional value. As a result, students are not being educated about healthy food choices therefore they are not making healthy food choices. This resource is used to teach this valuable information and includes nutrition information for fruits, vegetables, and pecans available in season in Oklahoma. This resource only deals with specialty crops specific to Oklahoma. The "property spaces" are specialty crops and the cards that accompany the property spaces have the specialty crop information, along with the nutrition information of each crop. The "chance" cards also include only information about the specialty crops

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*
1	At least 90% of students will report that playing the game is a fun way to learn about specialty crops grown in Oklahoma.	XX	
2	At least 45% of respondents will be able to name at least 1-2 specialty crops; at least 30% will be able to name 3-4 specialty crops; at least 25% of respondents will be able to name 5 or more specialty crops. These numbers may seem low, but for students in grades prekindergarten through third, they are age appropriate.	XX	
3	At least 75% of respondents will report trying or eating more fruits and vegetables in their diet.	XX	

4	At least 75% of respondents will report fun facts that they learned from playing the game.	XX	

ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	2,800 games were printed and delivered May of 2018. The staff rolled the new game out during their summer workshops and annual conference in order for teachers to have them for the beginning of upcoming school year. 625 games were distributed by Dec. 31, 2018. In 2019, the AITC staff distributed 1,317 games. This left 858 games to distribute in 2020. 658 of these games were distributed to schools in 2020 and through April of 2021. Due to limited school workshops in 2020 (because of COVID closures and lockdowns) all games were not distributed in 2020, so we are continuing to give games out in 2021. Only 171 games remain and these will continue to be distributed in 2021. It is expected these will be distributed by August of 2021.	Objective 1,2,3 4

CHALLENGES AND DEVELOPMENTS

#	Challenge or Development	Corrective Action or Project Change
1	QR Code survey on game wasn't used to report impact on students	A new survey was created and emailed directly to educators to complete in 2018 and again in 2019. The new link was also shared on Social Media. As a result of these efforts, teachers did take the survey to provide data showing the games are being used and the impact they are having.
2	The original survey that was sent out asked how many students could name 0-5, 6-10, 11-15, and 16-20 specialty crops, but the original objective was to find out how many could name 1-2, 3-4, and 5+ specialty crops.	Before the 2019 survey was sent out, the numbers were adjusted to match the original objective.
3	Very few teachers completed the survey in 2018.	In 2019, teachers were encouraged to participate in the survey by entering all

#	Challenge or Development	Corrective Action or Project Change
		participants into a drawing to receive a free book for their classroom. This strategy worked and the number of surveys completed increased drastically.

LESSONS LEARNED

This game is an excellent way to teach students about specialty crops grown in Oklahoma. The only difficulty has been getting teachers to submit data for the report. Adding the incentive to possibly win a book for their classroom helped with that problem. When reviewing the results of the surveys, objectives 2 and 3 didn't have exactly the results that were anticipated. However, the numbers are very close to the goal and the project is still considered a success.

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

The Ag in the Classroom staff still has 858 games to distribute. This is less than what the staff gave out in 2019, therefore they are confident they can distribute the games by the end of 2020. After that point, the requests for the game will be analyzed. If the game is still in demand, the game will be reprinted with funds/donations from Oklahoma commodity groups

BENEFICIARIES

Number of project beneficiaries: 1,942

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

OUTCOME MEASURE(S)

- ☐ **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)

#	Outcome and Indicator	Quantifiable Results
1	Outcome 2, Indicator 1. a.	Of the student surveys completed in 2019 after playing the Specialty Cropopoly Jr Board game, 98% of students reported they gained more knowledge about specialty crops (the goal was 75%, so this was 23% better than expected); 65% reported an intention to eat more specialty crops (the goal was 75%, so this was 10% less than expected); and 70% reported they have tried more specialty crops (the goal was 75%, so this was 5% less than expected).

DATA COLLECTION

A survey was created on Survey Monkey and emailed to all 1,942 educators who received the game. The survey was also shared on the AITC Social Media pages to ensure the teachers received it. The data from the survey was collected on Survey Monkey for AITC staff to evaluate. The survey was used to evaluate the impact the game made on students. Teacher emails were collected when games are distributed in order to connect with them. The survey asked teachers to submit data for each of the objectives the staff had submitted.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$0.00	$0.00
Fringe Benefits	$0.00	$0.00
Travel	$0.00	$0.00
Equipment	$0.00	$0.00
Supplies	$0.00	$0.00
Contractual	$32,000.00	$32,700.00
Other	$0.00	$0.00
Direct Costs Sub-Total	$32,000.00	$32,700.00
Indirect Costs	$0.00	$0.00
Total Federal Costs	$32,000.00	**$32,700.00

PROGRAM INCOME (IF APPLICABLE)

N/A

ADDITIONAL INFORMATION

** - To take advantage of price breaks from the production company it was decided to print more games. There is an overage of $700 which will be covered from money not expended in other projects.

Project Title	Educating Consumers About Christmas Trees and Tree Care At Oklahoma Agritourism Christmas Tree Farms			
Recipient Organization Name:	Oklahoma Department of Agriculture, Food & Forestry			
Period of Performance:	**Start Date:**	9/30/2017	**End Date:**	12/2/2019
Recipient's Project Contact				
Name:	Micaela Danker			
Phone:	405-740-0794			
Email:	Micaela.danker@ag.ok.gov			

PERFORMANCE NARRATIVE

PROJECT BACKGROUND

Christmas tree farms have been providing holiday fun for families for many years. As new and old consumers continue to enjoy their family traditions on Oklahoma Agritourism Christmas tree farms, there is a lack of education materials provided to these consumers. This project provided the tools to provide an educational experience as well. Artificial trees contain non-biodegradable plastics and possible metal toxins such as lead and end up in landfills at the end of their life. Most fake trees (85%) are imported from China, according to the U.S. Commerce Department. This project educated consumers on the benefits of buying a real tree. Real, fresh trees are thirsty, and a customer, specifically new customers, may not always know how to make their tree last through holiday festivities. An educational handout with tree care directions and reminders will encourage daily watering as well as safety tips to ensure a positive experience with using a real Christmas tree.

Oklahoma Agritourism distributed a set of five educational signs (12"x18") to each existing u-cut Christmas tree farm. These signs were placed throughout each farm for the consumers to read as they visit the farm. The signs included these facts:

- Real Christmas trees are biodegradable, which means they can be easily reused or recycled for mulch and other purposes.
- For every real Christmas tree harvested, 1 to 3 seedlings are planted the following year.
- For Oklahoma, the average growing time for a tree is 4-6 years.
- In 1990, the Oklahoma Department of Agriculture, Food and Forestry (ODAFF) in cooperation with the Oklahoma Christmas Tree Association (OCTA) developed the Oklahoma Improved Virginia Pine – the top tree variety for Oklahoma soil.
- 1 Acre of Christmas trees provides the daily oxygen requirements of 18 people.

Oklahoma Agritourism also distributed 20,000 educational handouts to each u-cut Christmas tree farm. The number of handouts depended on how many trees each farm planned to sell. We have farms that will sell anywhere from 75 trees in a season to 10,000 trees. Each educational handout included a QR code that links to a video that has already been produced and it included proper tree care at home. The educational video was created in 2018 by Oklahoma Agritourism – no state or federal funds were used. This video resulted from a survey to Christmas tree producers asking about solutions to their needs. One of the most asked questions from consumers to producers was how to maintain a fresh cut Christmas tree. An educational video highlighting tree care instructions was created and is available for producers to use on their social media platforms. This video was posted on the Oklahoma Agritourism Facebook page, where we will be able to track number of views and collect data from surveyed consumers. The purpose of this project was not a marketing effort. The goal was to educate consumers who are already visiting the farm, and to provide them with a positive experience so they continue to return as life-long customers. One way we can assist with providing a positive experience is by sharing educational tips about maintaining a fresh-cut tree throughout the holidays.

ACTIVITIES PERFORMED

OBJECTIVES

#	Objective	Completed?	
		Yes	No*
1	Educate consumers about environmental benefits of real Christmas trees.	XX	
2	Educate consumers about how to properly and safely maintain their real Christmas trees.	XX	

ACCOMPLISHMENTS

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
1	Educational signs and handouts designed	Objective 1 & 2
2	Educational signs and handouts passed out to Christmas tree farms	Objective 1 & 2

#	Accomplishment or Impact	Relevance to Objective, Outcome, and/or Indicator
3	Educational handout with video QR code passed out to consumers	Objective 1 & 2

CHALLENGES AND DEVELOPMENTS

ODAFF submitted this project to utilize unused funds from other grant projects approved in the FY 17 State Plan and only had 3 months to get it completed. Materials were printed and distributed to Christmas tree farms for their 2020 marketing season. Due to the timing we did not have previous years data to use to show quantifiable results. The final reports were due to ODAFF by December 1st and consumers were just starting to purchase their Christmas trees at this time and were just receiving the educational materials therefore we were not able to provide quantifiable data.

When putting the budget together for the proposal staff was given an incorrect price for the production and printing of the educational handout which was almost twice what it ended up costing, plus cost savings received for the number of pieces printed. Due to storage space limitations and wanting to be financially responsible ODAFF chose to print what could reasonably be used in a 2-3 year period and did not utilize the entire budgeted amount.

LESSONS LEARNED

Oklahoma Agritourism worked hand-in-hand with the Oklahoma Christmas Tree Association to determine which educational facts would be displayed on the signs. The OCTA producers are consistently interacting with consumers and know what those frequently asked questions are. By working with OCTA producers, they were able to guide this project in the right direction and to ensure the project reached its full potential

CONTINUATION AND DISSEMINATION OF RESULTS (IF APPLICABLE)

N/A

BENEFICIARIES

Number of project beneficiaries: 25

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

OUTCOME MEASURE(S)

- ☐ **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)

#	Outcome and Indicator	Quantifiable Results
1	Outcome 3, Indicator 1	The results for this outcome are not available yet. (See Challenges/Developments)

DATA COLLECTION

The educational handout has a QR code which will then lead consumers to a video link on the Oklahoma Agritourism Facebook page. We will be able to track how many consumers viewed the educational video about preparing/preserving a Christmas tree and a post-video survey will be linked to measure how many consumers gained knowledge. This data won't be collected until the end of Christmas tree season.

FEDERAL PROJECT EXPENDITURES

EXPENDITURES

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Personnel	$0.00	$0.00
Fringe Benefits	$0.00	$0.00
Travel	$0.00	$0.00
Equipment	$0.00	$0.00
Supplies	$0.00	$0.00
Contractual	$4,510.00	$4,910.00
Other	$18,448.50	$6,593.32
Direct Costs Sub-Total	$22,958.50	$11,503.32
Indirect Costs	$0.00	$0.00

Cost Category	Amount Approved in Budget	Actual Federal Expenditures (Federal Funds ONLY)
Total Federal Costs	$22,958.50	$11,503.32

PROGRAM INCOME (IF APPLICABLE)

N/A

ADDITIONAL INFORMATION