Oklahoma Department of Agriculture, Food, and Forestry (ODAFF)

State Point of Contact: Jason Harvey

USDA AMS Agreement: SCBGP-OK-0019

Final Report

December 26, 2018

Does Withdraw Of Cattle Grazing Prior To Harvest Improve The Food Safety Of Native Pecan?

PROJECT SUMMARY

Oklahoma ranks first in native pecan production in the US. Native pecan groves frequently include cattle grazing to increase diversity and profitability. For grazing practices, the produce safety rule under the Food Safety Modernization Act requires "an adequate waiting period between grazing and harvesting for covered produce". However, there is limited information on "waiting period" between grazing and harvesting native pecans. We conducted a comprehensive microbiological study on the impact of grazing cattle withdraw periods on foodborne human pathogen contamination of native pecan. Results showed that Shiga toxin-producing *E. coli* (STEC) prevalence in cattle-grazed orchards was higher (38%) in areas with cattle at harvest than in fenced areas where cattle were removed two (29%) and four (27%) months prior to harvest. Similarly, 30% of the samples taken in areas without fencing were contaminated by *Salmonella*; 22% were contaminated in areas with two months of cattle removal, and 30% were contaminated in areas with four months of cattle removal. However, there were no significant differences ($p \leq 0.05$) in contamination rates between waiting periods for either pathogen. Prevalence in non-grazed orchards was significantly lower for STEC (13%) and *Salmonella* (7%) compared to cattle-grazed orchards ($p \leq 0.05$). The application of two- and four-month waiting periods may not be an adequate time for reducing the occurrence of foodborne pathogens in native pecan production environments.

PROJECT APPROACH

The microbiological surveys included five grazed (cattle) orchards each with three designated areas (electrically-fenced) representing three withdrawing periods prior to pecan harvest: 0, 60, and 120 days. Five non-grazed orchards were also sampled at harvest for comparison. Soil, cattle feces, and pecan samples were collected at least three times per grazed orchard through the production period whereas soil and pecan samples were collected at harvest per non-grazed orchard. Detection and isolation of STEC and *Salmonella* from pecans and environmental samples were performed by enrichment, selective isolation, and multiplex PCR. Prevalence of the pathogens was analyzed by contingency tables with Pearson's chi-squared test using JMP® 13.0 software (SAS, Cary, N.C.). The treatments measured were soil, cattle feces and fallen in-shell pecans at 4, 2, or 0 months of waiting periods from cattle-grazed orchards, and occurrence of the pathogens in grazed and non-grazed orchards. All tests were considered significant when $p \leq 0.05$. DNA fingerprinting of selected isolates by pulsed field gel electrophoresis (PFGE) was performed to access the relatedness among these strains.

GOALS AND OUTCOMES ACHIEVED

The long-term goal of this research is to contribute to a comprehensive Good Agricultural Practices document for pecan and specialty crop production. Although our results have shown that none of the waiting periods (2 and 4 months) could be considered adequate to ensure food safety, extending the waiting period from 2 months to 4 months had little impact on these safety margins while reducing the land usage. As pathogen-positive samples were also detected in

these collected from non-grazed pecan orchards, although at much lower rates, it suggests that cattle is not the only source of such contamination (such as wildlife). There is a great diversity among the pathogen strains isolated from the orchard, highlighting the complexity of potential human pathogen contamination sources of at farm level.

BENEFICIARIES

The direct beneficiaries of this project are the native pecan growers in Oklahoma (2143 growers) and Texas (2885 growers). This project provides much needed scientific data to determine an "adequate" waiting period between grazing forage in the grove and harvesting of native pecans. Such data are limited although they are critical for overall risk assessment, GAP guideline and intervention strategies development for the industry. Withdraw period for the grazing cattle prior to harvesting are also an important economic issue not only to the native pecan growers in Oklahoma but also other states such as Texas, Louisiana, Missouri, and Kansas. From regulatory perspective, such data would serve as baseline for decision making instead of operating in a void. The general public would benefit for such a balanced approach to this food safety issue by ensuring the produce is safe and is as economical as possible since unneeded regulations leading to added production and processing costs could be avoided with well documented information. These impacts are significant because they will provide basic data for regulatory decisions related to food safety risk and insights into developing effective contamination control and mitigation strategies.

LESSONS LEARNED

Human pathogens can survive in the environment for long period of time AND things do not always go as planned. The CHEF Mapper XA system, used to perform DNA fingerprinting, was malfunction during the middle of the project. We had to perform the DNA fingerprinting on the remaining selected isolates using a CHEF Mapper XA system at other unit of OSU (very slow process) and whole genome sequencing.

CONTACT PERSON

Dr. Li Maria Ma 405-744-5290
li.ma@okstate.edu

Comparison of Conventional and Alternative Management Practices For Vegetable Production

Activities Performed:

Shortly after being notified by USDA of the projects approved under USDA-AMS agreement SCBGP-OK-0019 and before ODAFF could enter into a contractual agreement with Redlands Community College (RCC), ODAFF was notified by RCC that they would not be moving forward with the project.

Problems and Delays:

Due to budget shortfalls, RCC reduced several key members of their faculty responsible for this project therefore making it necessary to cancel.

Future Project Plans:

ODAFF will be submitting a change of scope request for a new project to utilize these funds.

Funding Expended to Date:

N/A

Increase Vegetable Production Utilizing Movable Hoop Houses

PROJECT SUMMARY

With the extreme weather cycles in the Southern Great Plains, protected agriculture is receiving more attention. Hoop houses (high tunnels) have been utilized as a way to protect crops from early freezes and enable producers to establish crops earlier in the season allowing them to have harvestable crops earlier for the spring market season. The cost of a hoop house is substantially lower than a greenhouse and with the hoop houses using captured heat from the sunlight there is virtually no cost of operation. The biggest problem utilizing hoop houses has been that the structures are tied up with the crop for the length of time that the crop is growing. When it is time to establish an additional crop the first crop has to be terminated to allow the following crop to be established. Therefore, the growing season of the crops are typically shortened. Therefore, this project was developed to evaluate the potential of using a movable structure that would allow a producer to utilize the hoop house to protect their early crop then easily move it to another location to establish an additional crop. We also compared the difference between using the two different structures compared to the traditional in ground system that is typically used in vegetable production.

PROJECT APPROACH

Utilization of hoop houses have been reported to extend the growing season of fruits and vegetables into the cooler growing months, lengthening the growing season of several crops. This allows growers to take advantage of potentially higher prices when demands are high and supplies are limited. With the unpredictable weather in Oklahoma, the use of hoop houses allows a grower to establish summer crops earlier in the spring and maintain crops longer into the winter months. Growers growing winter crops can take advantage of the heat captured inside the structure to promote crop growth during the cooler winter months without additional cost of heating. The idea of a movable hoop house that would allow a grower to take full advantage of the structure to establish multiple crops within a year is a growing trend in the industry. Most of

these structures are only movable along rails constructed in the field or by disassembling the structure to move to another site. The concept that this study will evaluate is a movable structure that has been developed in Oklahoma utilizing wheels that allows the growers to easily move the structure throughout the field with minimal effort. The structures will be used to establish an early crop in the spring and then moved to another area of the field to establish a winter crop that will allow a grower to fully utilize the entire growing season of that particular crop. Unlike a permanent structure where a grower may have to terminate a crop early to allow another crop to be established. The production of this system will be compared to a permanent structure along with the traditional field production. Therefore, the objectives of this project are:

1. Evaluate the movable hoop house production compared to permanent hoop house production.
2. Evaluate the movable hoop house production compared to traditional field production.

GOALS AND OUTCOMES ACHIEVED

Utilization of the movable hoop houses will allow a producer to add one additional crop per year. In the study we established a spring crop (tomato and pepper), summer crop (cucumber and squash) and a fall crop (lettuce and kale) in all plots. In the movable hoop house structures and outside we were able to plant an additional winter crop (Cauliflower and Broccoli). Because of the length of growing season for the fall crop inside the permanent hoop house structures we were not able to plant the winter crop inside the permanent structures. The movable hoop houses allow us to move the structures off the crops and establish the next crop without terminating the previous crop therefore we were able to harvest the crops longer in the movable structures than in the permanent structures

With the extreme weather conditions that we experienced during 2016 through the spring of 2018, the movable structures allowed us to protect the early spring crops compared to the traditional field production that was planted outside. In 2018 the tomato and pepper crops in the traditional production plots were replanted twice because of late freezes that occurred in mid and late April, while the plants that were established approximately 45 days earlier inside the hoop houses were protected.

Not all data has been analyzed yet. A Graduate student at Oklahoma State University will analyze the data as a class project in the spring of 2019. One of the highlights from the data shows that the movable structures significantly increased the total production per structure (plot) over the permanent and outside production both in yield and fruit count. Using either the movable or permanent structure dramatically increase production and fruit count with over 2.7 times more production and 2.85 times more fruit.

BENEFICIARIES

Over the 27 months that this project was being conducted there were over 40 tour groups with over 600 participants, that visited the study area and this project was discussed.

After the harvest data is analyzed the findings will be presented at the Oklahoma/Arkansas Horticulture Industry Show, as well as a written article in the Noble Research Institute's News & Views Newsletter.

Noble will continue to work with the movable hoop house structures in the Noble Protected Agriculture demonstration area. We will continue to perfect the movable structures along with investigating cheap technology that we can implement on the structures that would allow a producer to control the temperature inside the houses remotely.

LESSONS LEARNED

More time needs to be devoted to these movable structures to perfect the temperature control inside the structures especially during the late winter and early spring time. With the size of structures that we used (14 ft. x 28 ft.), the houses would warm up too quickly when the sun was out. In fact there were days that we would have to start venting the house before the outside temperatures reach 32 F.

The other problem with the current houses were the accordion style end walls that allowed the structures to be moved without damaging the crops growing inside the structure, were very cumbersome to raise. The device that was used in the design was a hand crank that raise the end walls. The hand crank would take a couple minutes to raise the end walls and would be difficult for someone that did not have good hand strength if many end walls needed to be raised. The other problem with the end walls were that they seemed to restrict airflow inside the structure unless they were raised all the way up. In venting the houses on cold days this made it difficult to regulate the temperature inside the houses.

CONTACT PERSON

Charles Rohla (580) 224-6451
ctrohla@noble.org

ADDITIONAL INFORMATION

Noble will continue to work on the movable hoop house structures and will demonstrate them in Noble Protected Agriculture Demonstration Area. We will continue to address the problems we had issues with working with the structure and work on ways to perfect them. We would also like to find a way to automate the venting remotely to allow producers that work off the farms to be able to vent the structures at the proper times.

Effect of Nutrient Solution on Hydroponically Grown Vegetables and Herbs under Oklahoma Greenhouse Conditions

PROJECT SUMMARY

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 12 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, soilless substrates, and nutrient solutions (Arancon et al., 2015). Hydroponic cultivation in substrates like perlite and rockwool have been widely adopted for hydroponic production. Traditional hydroponic methods use circulating water and nutrient solution to maximize oxygen and nutrient uptake. Crops grown by hydroponic methods include vegetables, herbs, fruit, and ornamentals. Hydroponically grown leafy greens, basils, and vining crops can be sold at farmer's markets, grocery stores, or high end restaurants. Single heads of lettuce fetch on average $1.50-2.00 and even as high as $4 a head at farmer's markets (Salinger, 2004). Some estimates put the number of commercial hydroponic greenhouses in the U.S. at 65,000, and the number of hobby greenhouses in the U.S. at over three million.

The conversion from traditional water wasting methods to more environmentally friendly recirculating systems is likely to become more common in the greenhouse industry (David et al., 2005). There will likely be increased use of soilless growing methods in greenhouse-based horticultural systems because of societal pressure to reduce nutrient loss to the environment instead favoring production that increases efficiencies in water and fertilizer use (Magan et al., 2003). Hydroponic production recirculates the nutrient solution and water providing an environmentally friendly production practice (Jung et al., 2004). Rouphael et al. (2004) reported that plants grown in a hydroponic system had increased growth, yield, and quality compared to plants grown in soil.

Grower problems with hydroponic production are primarily caused by nutrients (Larsen, 1982). Because of the recent popularity in hydroponics, hundreds of different nutrient solutions and formulations are available to growers without research showing which products work best for what type of crop. Some formulations require growers to use different products at different stage of growth, while others claim to provide complete nutrient as a single source throughout production. Information on what nutrient products produce the greatest yields for different vegetable and herb cultivars is need to expand hydroponic greenhouse production in Oklahoma.

PROJECT APPROACH

The purpose of this project is to evaluate eight different vegetable/herb species for hydroponic production in Oklahoma using different nutrient formulations to provide growth information as a means to diversify crop production under controlled environments. The scope of the project benefited solely vegetables, which are specialty crops. This project was set up as a thesis research project under the mentorship and guidance from Dr. Dunn. The graduate student, Hardeep Singh, was responsible for setting up the hydroponic systems, running experiments, collecting data, and writing-up the results for dissemination to the public.

Tasks performed:

Spring Experiment 1 (2016-2018)

Seeds of lettuce, Swiss chard, basil, and spinach (Table 1) were sown into rock wool cubes. The seed nursery was irrigated with the tap water, and fertilizer was applied twice. The 4-6 week old nursery was then transplanted into an NFT system that had to be built. The three fertilizers used were Jacks 5-12-26, Peter's 5-11-26, and Dyna Gro 7-9-5. Calcium nitrate was used with the Jacks and Peters, because they do not supply calcium in their composition. As our tanks were of 40 gallon capacity, 147.41 grams of Jacks and Peters and 97.52 grams of calcium nitrate were added initially according to recommended rates, while Dyna Gro 7-9-5 recommended 1-2 tsp per liter of water, so 80 tsp (5.63 cups) were added to the 40 gallon capacity tank. Spinach was grown only using Peters. The experiment was repeated three times. The pH and EC of all the fertilizer solutions was maintained at 5.5-6.5 and 1.5-2.5 mm/cm, respectively. When plants reached the harvesting stage, experiments were ended and plant quality was assessed by recording fresh weight, dry weight, and SPAD and atLEAF reading. Nutrient analysis was carried by the Soil Water and Forage Analysis Laboratory (SWAFL) at Oklahoma State University.

Significant Results:

- 'Dragoon', 'Mirlo' and 'Rubysky' are well suited for production in hydroponics based on growth and color. Thus, 'Mirlo' and 'Rubysky' can be recommended for Dyna Gro and Peters while Peters can only be recommended for 'Dragoon'.
- For basil, 'Largeleaf' and 'Sweet' can be recommended for Jacks, while 'Lemon' can be recommended for both Jacks and Peters in hydroponics.
- For Swiss chard, 'Fordhooke Giant' can be recommended for hydroponic cultivation based on greater growth.
- 'Dragoon' cultivar of lettuce and 'Lemon' cultivar of basil showed tip burn in Dyna Gro and it was not due to calcium deficiency.
- For spinach, 'Seaside' produced less leaves, but overall shoot and root dry weight was not significantly different than 'Harmony', 'Riverside', and 'Space'.
- We failed to get lavender and rosemary established from seed, despite multiple attempts. We established rooted cuttings of lavender in an NFT system, but because of problems with root rot and slow growth they would not be recommended for production in an NFT hydroponic system.

Table 1

Species	Received	Round	Sowing nursery	Transplanting	Data collection
Lettuce: a) Dragon (Romaine)	01/29/2016	First	02/15/2016	03/28/2016	05/03/2016

b) Mirlo (Butterhead) c) Panisse (Oak Leaf) d) Oscard e) Ruby Sky f) Rex		Second	05/16/2016	06/26/2016	08/02/2016
		Third	07/19/2016	08/24/2016	10/02/2016
Swiss Chard: a) Rainbow Chard b) Barese c) Fordhook Giant	01/29/2016	First	02/15/2016	03/28/2016	05/01/2016
		Second	05/16/2016	06/26/2016	08/02/2016
		Third	07/19/2016	08/24/2016	10/02/2016
Spinach: a) Seaside b) Riverside c) Harmony d) Space	04/22/2016	First	04/23/2018	05/25/2018	07/05/2018
Basil a) Large Leaf b) Lemon c) Sweet	02/04/2016	First	02/15/2016	03/28/2016	05/03/2016
		Second	05/16/2016	06/26/2016	08/02/2016
		Third	07/19/2016	08/24/2016	10/02/2016

Spring Experiment 2 (2016-17)

On 1 February 2016 seeds of eggplant and sweet pepper were sown into the rock wool cubes. The seed nursery was irrigated with the tap water and fertilizer was applied twice. The 5-7 week old nursery was transplanted into a Dutch Bucket system that had to be built. The three fertilizers used were Jacks 5-12-26, Peter's 5-11-26, and Dyna Gro 7-9-5. Calcium nitrate was used with the Jacks and Peters, because they do not supply the calcium in their composition. As our tanks were of 40 gallon capacity, 165.84 grams of Jacks and Peters and 109.71 grams of calcium nitrate were added initially according to recommended rates, while Dyna Gro 7-9-5 recommended 1-2 tsp per liter of water, so 90 tsp (5.63 cups) were added to the 40 gallon tank. The pH and EC of all the fertilizer solutions was maintained between 5.5-6.5 and 2.5-3.5 mm/cm, respectively. When plants reached harvesting stage, experiments were ended and plant

quality was assessed by recording fresh weight, dry weight, fruit weight, number of diseased fruit. Nutrient analysis was carried Soil Water and Forage Analysis Laboratory (SWAFL) at Oklahoma State University.

Significant Results-

- 'Orangella' in Jacks and Peters can be recommended for sweet pepper production in hydroponics on the basis of greater shoot weight and fruit yield.
- 'Jaylo' can be recommended cultivar for eggplant in hydroponics on the basis of yield while there was no fertilizer effect on eggplant yield.
- Eggplant fruits turned yellow in all three fertilizers after 3 months. Pruning plants back did not eliminate the yellowing of fruit but starting with new nutrient solution bimonthly may help to solve the problem.
- Sweet peppers in Dyna Gro produced undersized fruits and plants died after 2 months.
- We failed to get squash established, which may have been the result of damping-off due to having continuous water on the roots after transplanting

Table 2

Species	Received	Nursery sowing	Transplanting	First harvest
Pepper a) Bentley b) Orangella	01/25/2015	02/01/2016- 1st rep 02/15/2017- 2nd rep	03/21/2016- 1st rep 03/23/2017- 2nd rep	05/23/2016- 1st rep 06/01/2017- 2nd rep
Eggplant a) Jaylo b) Angela	01/25/2015	02/01/2016-1st rep 02/15/2017- 2nd rep	03/21/2016- 1st rep 03/23/2017- 2nd rep	06/06/2016- 1st rep 06/10/2017- 2nd rep

GOALS AND OUTCOMES ACHIEVED

The goal of this research was to help growers in selection of fertilizer and cultivar suitable for hydroponics cultivation, which was achieved. A factsheet on pH and EC management in hydroponics, building vertical hydroponic towers, and pruning in hydroponics was published which can be found on below links. Data from the experiments can be viewed in the next to last link which corresponds to Hardeep's thesis. Hardeep Singh presented results by giving an oral presentation and a poster at ASHS in September of 2017 (see last link). Two manuscripts have been submitted to journals for publication too. Long term goals would be to build upon this research to further hydroponic growers, which has happened as two more grants in the area of

hydroponics has been secured. Major successful outcomes include being able to recommend Jack's hydroponic fertilizer for both leaf greens and fruiting crops. In addition, crops such as lettuce, Swiss chard, basil, peppers, and eggplant can be easily grown in hydroponics to produce a high value, year-round crop.

http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-10397/HLA-6722web.pdf
http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-10747/HLA-6724web.pdf
http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-10803/HLA-6725web.pdf
https://shareok.org/handle/11244/300316
https://ashs.confex.com/ashs/2017/meetingapp.cgi/Person/28979

BENEFICIARIES

Already established hydroponic operations like Scissortail Farms, Artic Farm, and Sage Farm have benefited from the results of this study based on the recommendation of what crops, fertilizer, and production practices are best. Eleven potentially new growers benefited with site tours of the different systems. Lastly, the factsheets alone have been viewed nearly 200 times a month each, which represents an even larger impact within Oklahoma and the specialty crop industry in the U.S. In total, at least 6 greenhouse operations growing or well start producing vegetables in controlled environments benefited directly from the research and indirectly over 2,000 individuals seeking out hydroponic information

LESSONS LEARNED

- pH, EC, and tank water level should be checked every other day and an automated nutrient and pH controller would be recommended to save on labor. For commercial production a backup generator is needed in case of power failures and an automated
- For all of these crops, pumps ran 24 hours a day, thus it is worth it to purchase a warranty with the pump and several backups.
- Getting lavender and rosemary seeds to germinate is difficult, so growers should take cuttings from stock plants and root in oasis foam cubes instead of trying to germinate seed.
- Just because a fertilizer works well for one crops, leafy greens for example, do not expect that the same fertilizer will work on fruiting crops.
- To lower the pH, vinegar should not be used. Both lemon juice and pH down did not affect plant growth; however pH down is a true buffer and kept the pH more stable at a lower cost than lemon juice.
- Spinach seeds should be pre-germinated in a moist towel under cool conditions then transplanted in an oasis cube for uniform and successful germination.
- Either oasis cubes or rockwool cubes can be used for seed germination, though if not buying in bulk oasis cubes were generally cheaper.
- Cool season crops like lettuce can be grown in a greenhouse during the summer, as long as the water temperature if kept cool to prevent bolting.
- Bell peppers grown hydroponically in a greenhouse will need to be shaded in July and August to avoid sun scold damage on the fruits.

CONTACT PERSON

Dr. Bruce Dunn (405) 744-6462
bruce.dunn@okstate.edu

Monitoring Settlement Dynamics of Important Cucurbit Viruses in Oklahoma and Development of a Multiplex-Virus Detection Kit

PROJECT SUMMARY

Oklahoma is a well-known cucurbit producing state. However, the cultivated acreage of cucurbits in Oklahoma has decreased mainly to viral diseases. The cucurbits cultivated in Oklahoma are cantaloupe, cucumber, melon, pumpkin, squash and watermelon. Cucurbit cultivation occurs mainly during the spring, summer, and part of the fall, which make these crops subject to multiple virus infections by insect-vectors aimed by the extended cultivation time. These infections have been detected in a number of varieties, causing virus-infected cucurbits plants to be reproduced in quality and yields. A robust, accurate, and reliable detection and diagnostic methods were needed at the start of this project for early detection of these viruses, which may be in low titer in asymptomatic (early infection) and symptomatic plant tissue. The reliable and sensitive detection tools to be developed by this project are required by diagnostic labs, cucurbits breeders, and extension officers for improving timely pest management in Oklahoma.

Field surveys were conducted in cucurbit fields during 2016, 2017, and 2018 growing seasons. A total of 1344 samples were collected and tested serologically using dot-immunobinding assay (DIBA) for 10 viruses: *Cucumber mosaic virus* (CMV), *Cucurbit green mottle mosaic virus* (CGMMV), *Cucurbit aphid-borne yellows virus* (CABYV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Melon necrotic spot virus* (MNSV), *Papaya ringspot virus* (PRSV), *Squash mosaic virus* (SqMV), *Squash leaf curl virus* (SLCV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV). Seven out of 10 viruses were detected in cucurbit fields in Oklahoma. The highest average incidence was recorded for PRSV, followed by ZYMV, WMV, MNSV, SqMV, CABYV, and CGMMV. None of the samples was positive for CMV, CYSDV and SLCV by DIBA. However, CMV and CYSDV were detected by multiplex RT-PCR assay during 2016 and 2018 field samples. Three viruses (CGMMV, CABYV, and CYSDV) were reported for the first time in cucurbit fields of Oklahoma during 2016-2018 growing seasons.

This research project developed a detection method that combines two innovative technologies. The first is for rapid collection and processing of cucurbit tissue sample. The method consist in directly trapping viruses in the plastic interior wall of PCR tubes, making the analyte (the virus) rapidly available for the reaction avoiding the use of costly and time consuming kits. The second method consist of a simultaneous (multiplex) amplification of nine relevant virus targets in Oklahoma by reverse transcription quantitative polymerase chain reaction coupled to high resolution melting (RT-qPCR+HRM): Final results, protocols of these two methods including surveys from 2016 and 2018 and cost are provided. Multiplex PCR also detected CYSDV for the

first time and confirmed the presence of CGMMV and CABYV from fields samples collected in 2016-2018 growing seasons.

PROJECT APPROACH

1. Recruiting

Another Master science graduate research assistant (GRA) (Lizbeth Peña-Zuniga), was recruited at Oklahoma State University (OSU). This GRA started her research program during the spring of 2016.

A Master science graduate research Assistant (GRA) (Vivke Khanal) was recruited at the University of Tulsa. This GRA started his research program during the fall, of 2016.

2. Virus antisera

As shown in Table 1, antisera for 10 plant viruses that infect cucurbits were purchased commercially from Agdia, Inc and AC Diagnostics and used in dot-immunobinding assay (DIBA). All nine viruses have RNA genome while SLCV is a DNA virus.

Table 1. Cucurbit samples collected during surveys were tested against the antisera of the 10 plant viruses.

No.	Virus	Abbreviation
1	*Cucumber mosaic virus*	CMV
2	*Cucurbit aphid-borne yellows virus*	CABYV
3	*Cucurbit yellow stunting disorder virus*	CYSDV
4	*Cucumber green mottle mosaic virus*	CGMMV
5	*Melon necrotic spot virus*	MNSV
6	*Papaya ringspot virus*	PRSV
7	*Squash leaf curl virus*	SLCV
8	*Squash mosaic virus*	SqMV
9	*Watermelon mosaic virus*	WMV
10	*Zucchini yellow mosaic virus*	ZYMV

3. Molecular research

Sequences from the polymerase protein gene (RNA) from six virus genera were retrieved from NCBI and aligned using MEGA 6.

- Primer sets were designed from conserved regions with the assistance of software applications such as PrimerQuest®, Primer3, Primer-BLAST, and BLASTn.
- uMELT was used for prediction of High Resolution Melting (HRM) profiles of melting temperatures (Tm) of diagnostics PCR products was.
- The tendency to form secondary structure of each primer was tested using Oligonalyzer 3.1. and mFOLD analysing the thermodynamic characteristics of each primer.
- A Multiplex RT-PCR was developed to be coupled to High Resolution Melting (HRM) analysis for rapid detection of the targeted viruses.

- The obtained melting profiles allowed a clear discrimination among these viruses.

A second generation of primers for RT-qPCR+HRM analysis was also developed and assessed. This new method has potential for application in epidemiological studies of viral diseases in cucurbit crops, microbial forensics and biosecurity.

GOALS AND OUTCOMES ACHIEVED

This project hypothesized 'Multiple viral infections occurring in the field can be intercepted and monitored by implementing novel and rapid detection technologies such as Reverse Transcription Polymerase Chain Reaction (RT-PCR)'.
Objectives were:

Objective (1) To survey cucurbit fields in Oklahoma during two years to determine local virus settlement dynamics in cucurbits crops for a better understanding and implementation of integrated pest management practices tailored to Oklahoma.

Objective (2) To develop a rapid friendly sample processing method for CMV, CABYV, CYSDV, MNSV, PRSV, SqMV, WMV, ZYMV, CABYV, and CYSDV based on rapid plant cell lysis using alkali and filtration and collection using the EICD which allows taking an aliquot of the released virus(es) straight for RT-PCR amplification within 3 to 5 minutes.

Objective (3) To assess and analyze the new sampling and processing RT-PCR method in terms of (A) reproducibility, (B) sensitivity, (C) specificity, and (D) cost.

Objective (4) To identify local leaders (growers and scientists) and additional plant clinics to whom the technology will be transferred.

Objective (5) The project team will communicate to the OK cucurbit growers about viruses found, their distribution and potential effects on cucurbit crops. We will partnership with County Extension Educators and cucurbit growers the technology transfer and the organization of field days, which will take place by the end of year two of this project.

Objective (1) (<u>by PI and Graduate Research Assistant at TU, Tulsa</u>)

The PI and graduate research student Vivek Khanal visited cucurbits fields located in eight out of nine districts of Oklahoma (Fig. 1) and collected samples during the 2016-2018 growing seasons. Cucurbit fields in the respective counties were visited every year and samples were randomly collected mostly from plants showing virus-like symptoms and some asymptomatic plants during the three growing seasons. In total, 1344 samples were collected from five cucurbits hosts (cucumber, cantaloupe, pumpkin, squash and watermelon) and tested against the antisera of 10 viruses listed in Table 1.

Seven out of 10 viruses were detected in cucurbit fields in Oklahoma. The highest average incidence was recorded for PRSV (586%), followed by ZYMV (27.4%), WMV (20.5%), MNSV (5.6%), SqMV (1.4%), CABYV (1.2%), and CGMMV (1.1%). None of the samples was positive for CMV, CYSDV and SLCV by DIBA. However, multiplex RT-PCR assay detected the presence of CMV and CYSDV in samples collected during 2016 and 2018 .

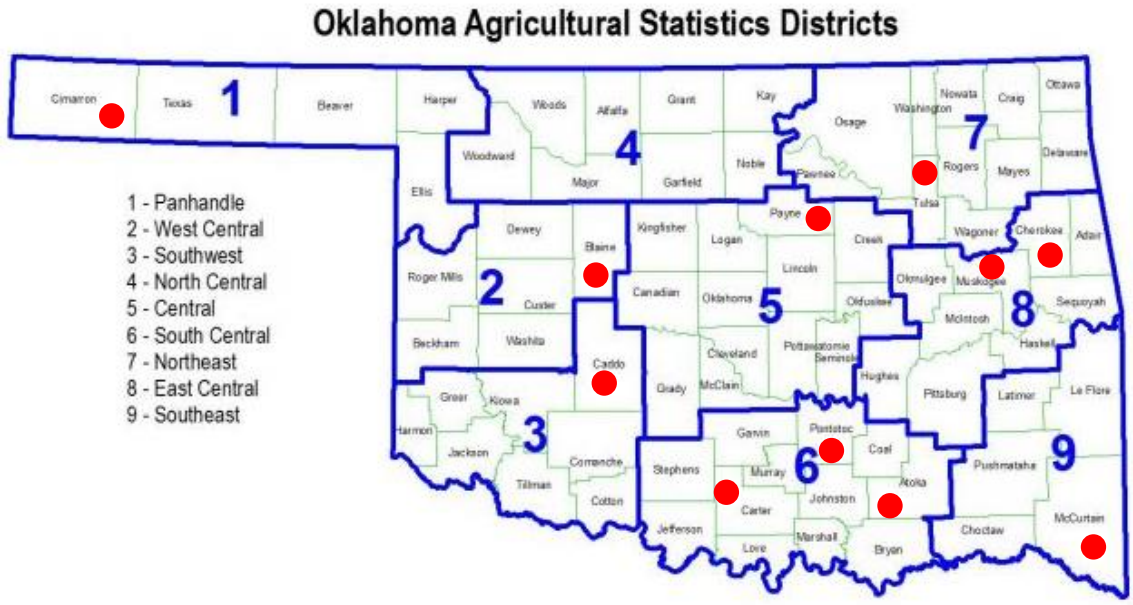


Fig. 1 Map of Oklahoma showing nine districts and counties. Counties with a red circle were surveyed multiple times and samples from cucurbit fields were collected during the 2016-2018 growing seasons.

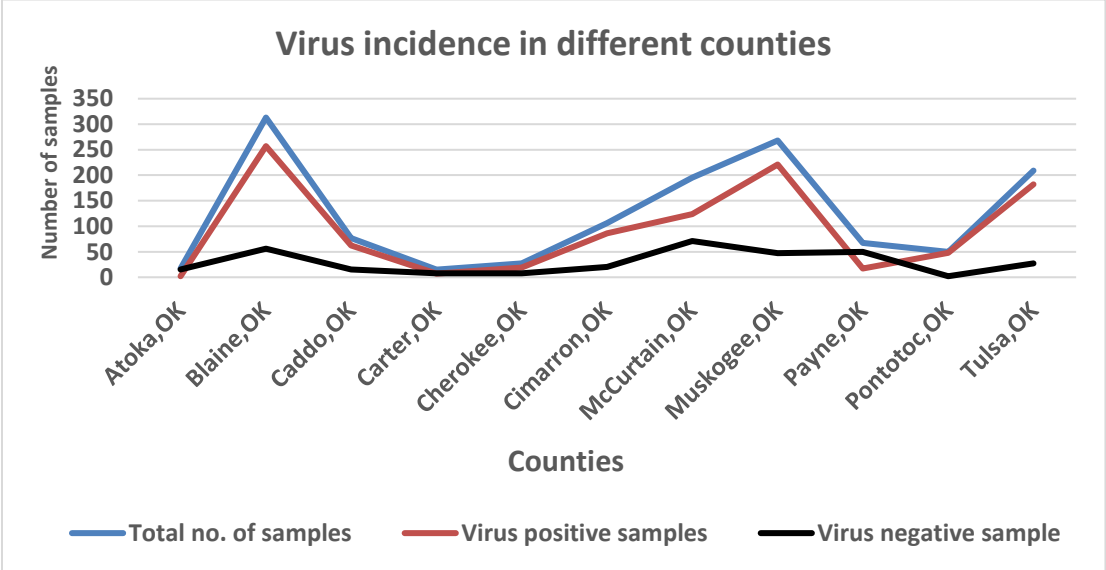


Fig. 2. Virus incidence in various counties. Samples were collected (2016-2018) and tested serologically in various counties. The graph shows a comparison of virus-infected and virus negative samples based on DIBA testing.

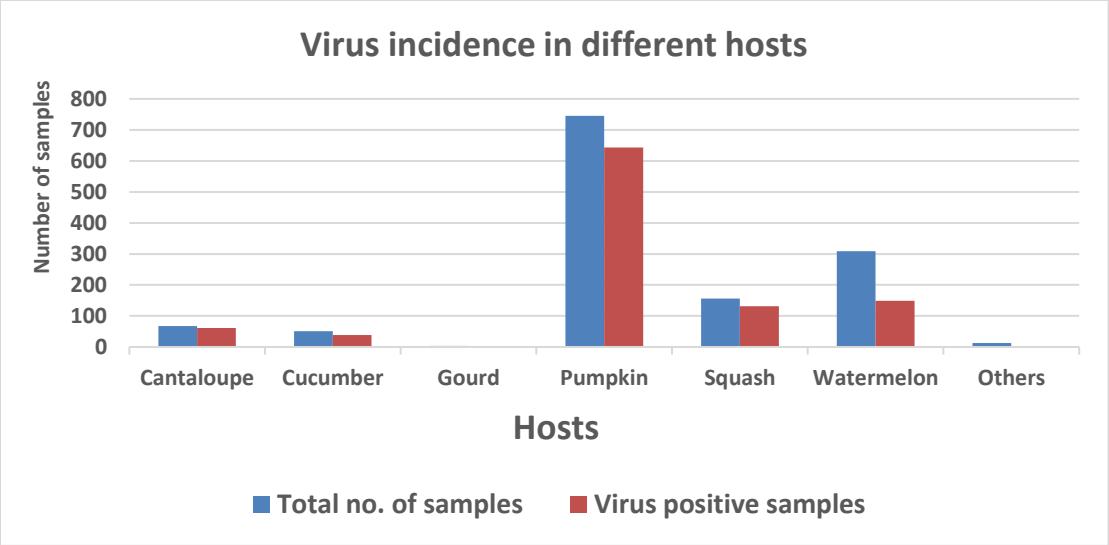


Fig. 3. Virus incidence in various hosts. The graph shows a compariosn of samples positive by DIBA collected from different hosts in 2016-2018 growing seasons.

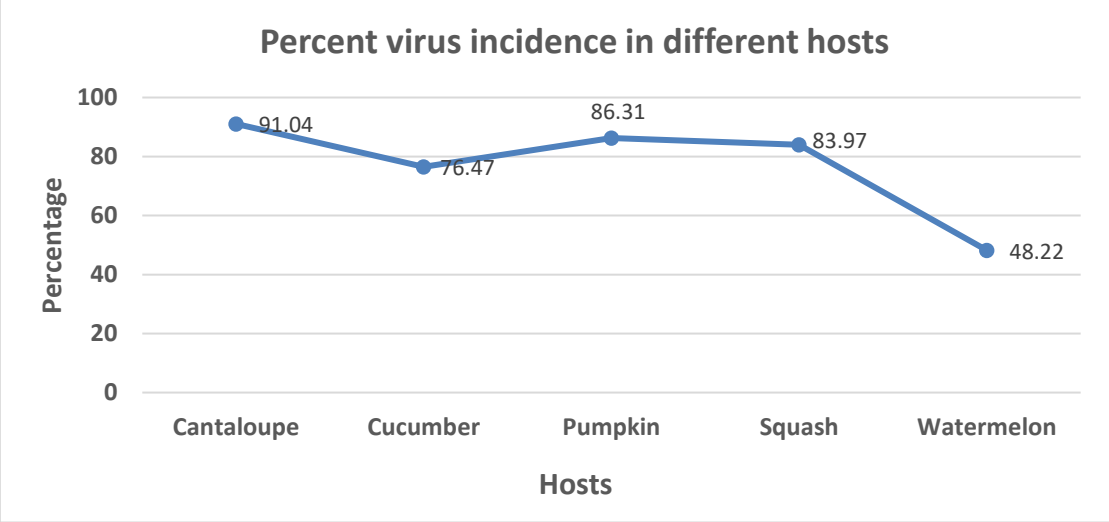


Fig. 4. Percent virus incidence in various cucubrit hosts from which samples were collected in 2016-2018 growing seaosns. Samples from all hosts except watermelon showed >70% virus infection.

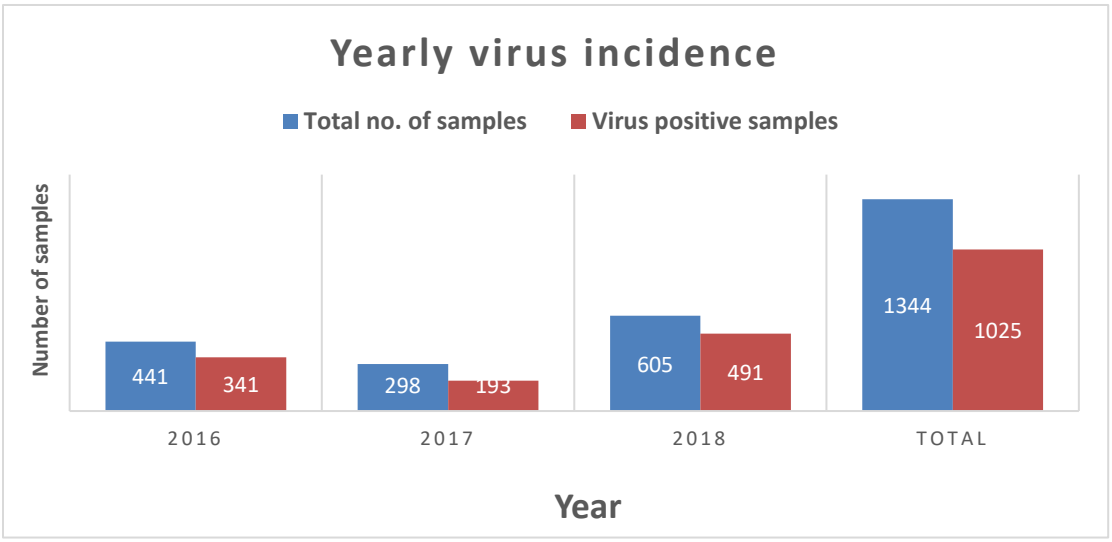


Fig. 5. Number of virus positive samples vs total number of samples collected in three different growing seasons

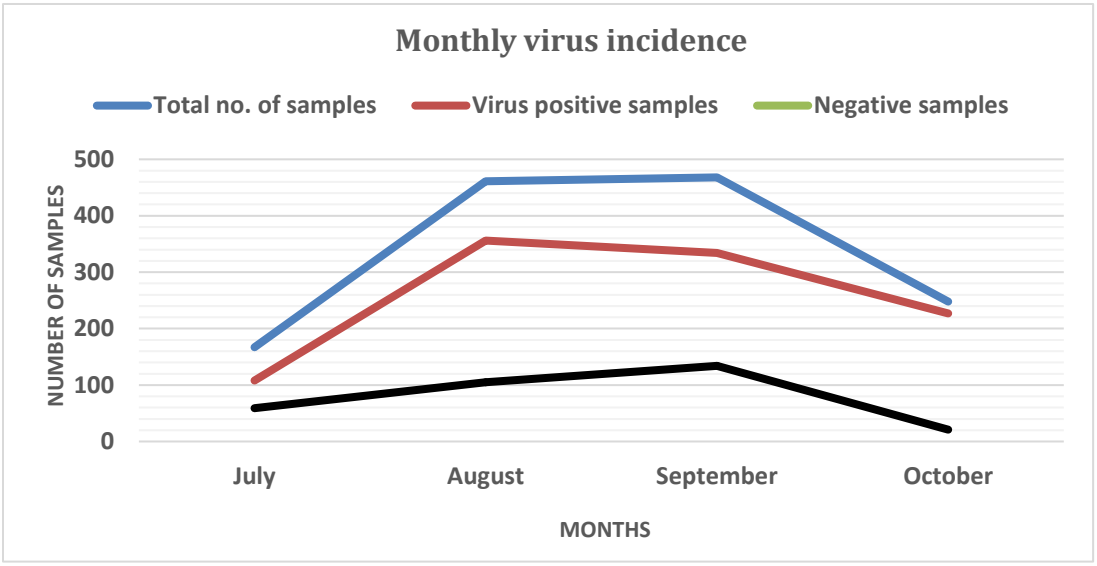


Fig. 6. Number of positive virus samples collected in each month during the 2016-2018 growing seasons

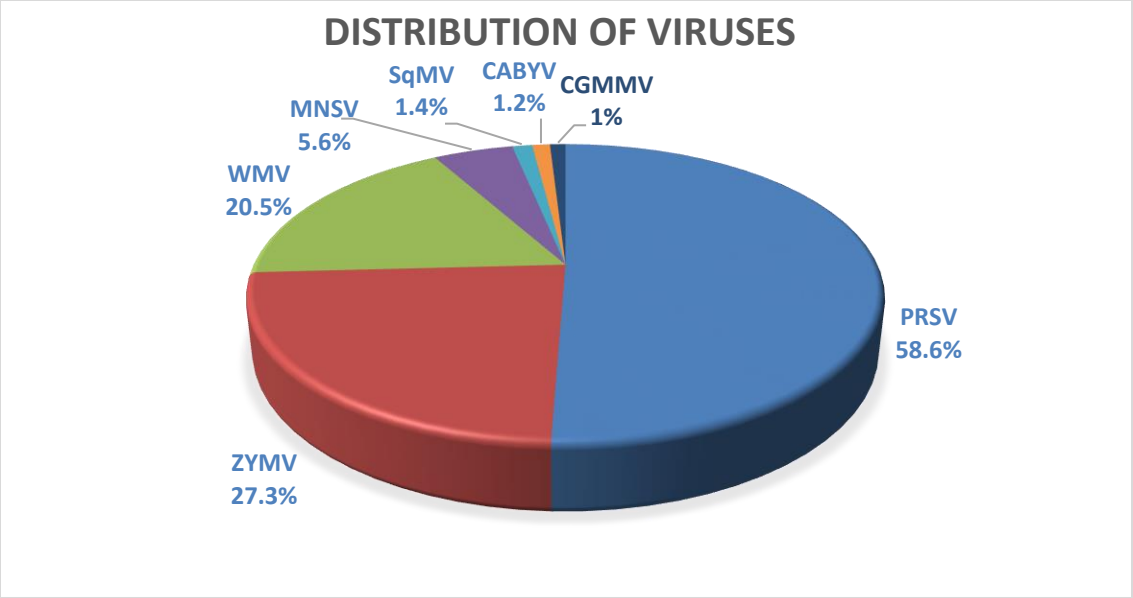


Fig 7. Percent distribution of seven viruses in cucurbit fields in Oklahoma as detected by DIBA.

Objective 2 & 3 (by Co-PI and Graduate Research Assistant at OSU, Stillwater)

Preliminary and detailed data about the progress of this molecular detection technology was provided in the 2016 and 2017 annual reports. This report compiles results only to minimize duplication.

Hands on research activities were timely performed by master graduate research student Lizbeth Peña-Zuñiga. A detection method that combines two innovative technologies, (1) for rapid collection and processing of cucurbit tissue samples, and (2) for simultaneous (multiplex) RT-PCR+HRM amplification of nine disease-causing viruses were developed. Targeted cucurbit infecting viruses are *Cucurbit green mottle mosaic virus* (CGMMV), *Cucumber mosaic virus* (CMV I and CMV II), *Cucurbit aphid-borne yellow virus* (CABYV), *Cucurbit yellow stunting disorder virus* (CYSDV), *Melon necrotic spot virus* (MNSV), *Papaya ringspot virus* (PRSV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV). Sequences from the RNA dependent RNA polymerase (RdRp) and capsid protein (CP) genes from nine targeted disease-causing viruses belonging to six plant virus genera were retrieved from NCBI and aligned using software. Primer sets were designed from conserved regions using the web interface software and the thermodynamic features of each primer sequence were further tested-analyzed *in silico* and *in vitro*. The main thermodynamic features of all designed primers are listed in Table 1.

(1) DIRECT VIRUS TRAPPING IN PLASTIC.
We developed a rapid collection and processing method for cucurbit tissue samples. Viruses are collected and trapped directly within the plastic PCR tube and no RNA extraction kit is needed. This procedure is cost and time saving and allows to streamline the lab work facilitating processing a larger number of samples per operator per day. The procedure is described in Annex 1.

(2) MULTIPLEX RT-qPCR+HRM

Once primers were designed, were tested in individual reactions and multiplex reactions. The obtained results allowed to arrange three multiplex assays based on differences among PCR products and their melting temperature (Tm). A five-virus (multiplex-A) and two three-virus (multiplex-B and multiplex C). The three developed assay combinations of PCR reactions are described in table 2.

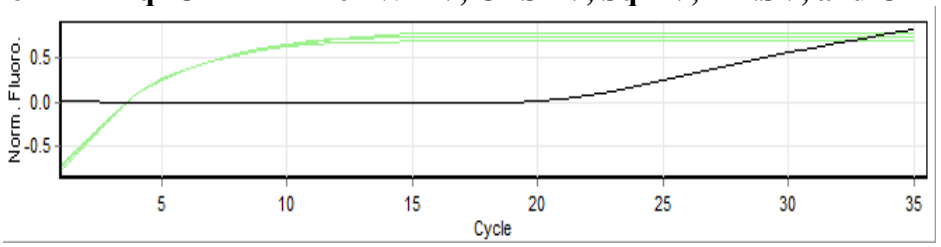
Table 2. Multiplex discriminatory RT-qPCR+HRM reactions arranged for detection of groups of five and three different viruses based in differences among virus product size and productTm.

TYPE OF REACTION	VIRUS and Tm (ºC)
Multiplex-A	WMV, CYSDV, SqMV, MNSV, CABYV 76.36 81.2 83.1 84.94 87.8
Multiplex-B	PRSV, CYSDV, CMV 77.76 81.2 84.24
Multiplex-C	CGMMV, ZYMV, CABYV 78.35 83.82 87.8

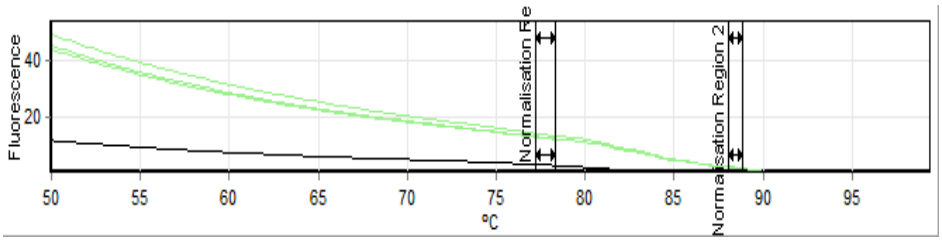
All positive, healthy and NTC controls were included and reacted as expected. A graphic representation of the multiplex assays, and controls is shown in Figure 1, 2 and 3.

Multiplex-A RT-qPCR+HRM for WMV, CYSDV, SqMV, MNSV, and CABYV

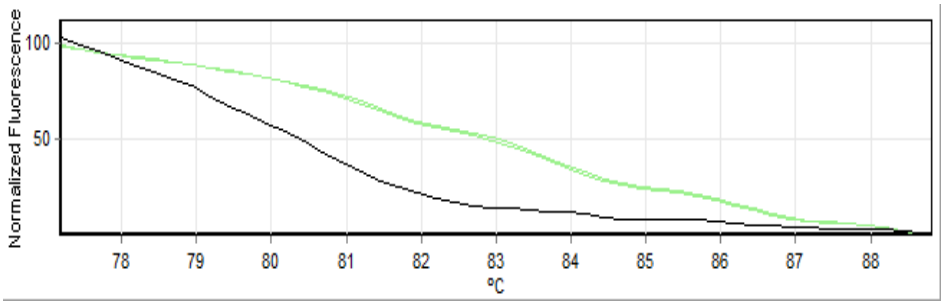
a.



b.



c.



d.

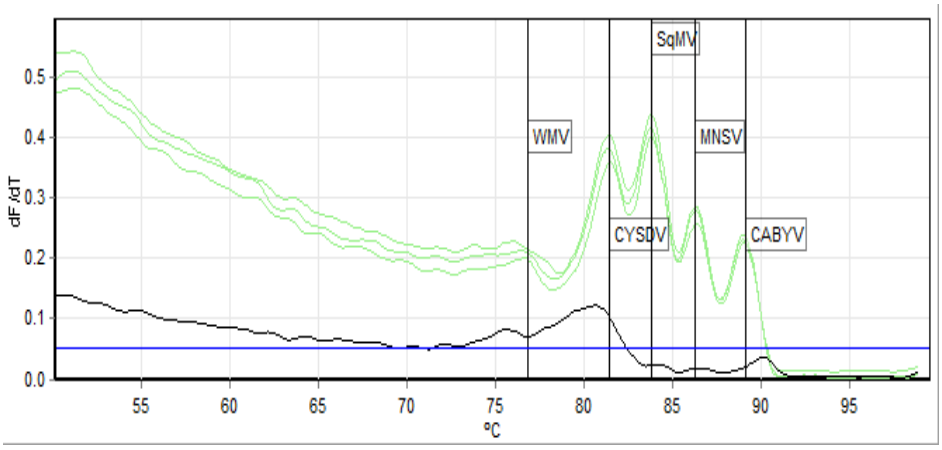
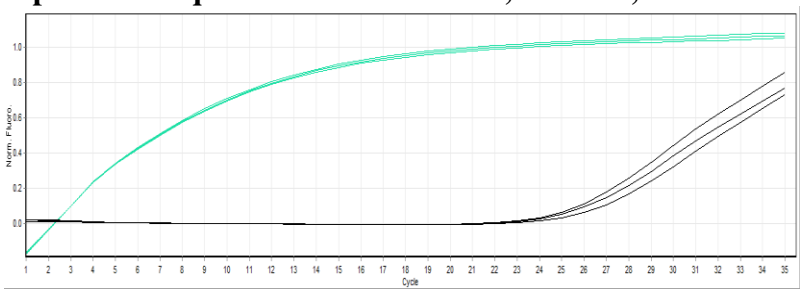


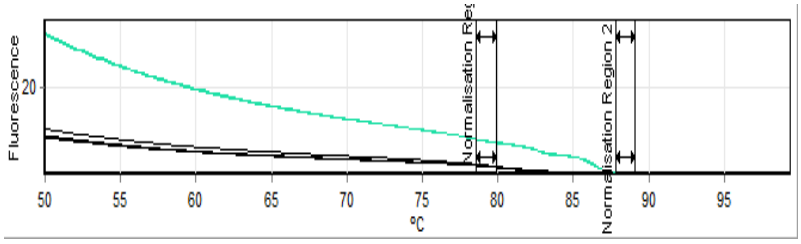
Figure 1. Virus reference controls WMV, CYSDV, SqMV, MNSV, and CABYV are shown in green and NTC in black **a.** RT-PCR from five reference positive control. **b.** Raw data showing the pre-melt, melt and post-melt regions. Notice the fluorescence variance and the positioning of the pre- and post-melt identification bars. **c.** Normalized fluorescence data derived from raw data plots showing the Tm breaking point of fluorescence of the viruses at Tm. **d.** Low Resolution Melt profile derivative plot (-dF/dT against T). The steepest slope is easily visualized as a melt peaks.

Multiplex-B RT-qPCR+HRM for PRSV, CYSDV, CMV

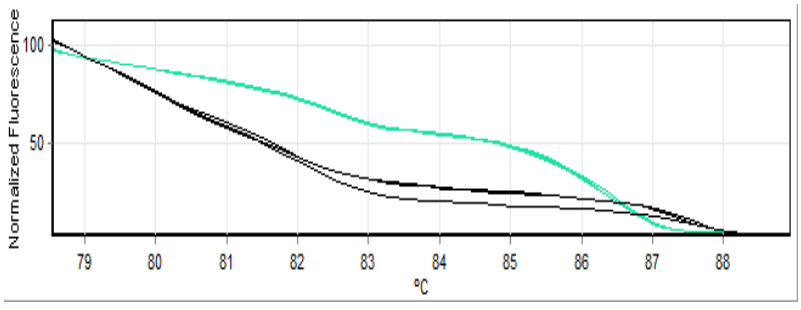
a.



b.



c.



d.

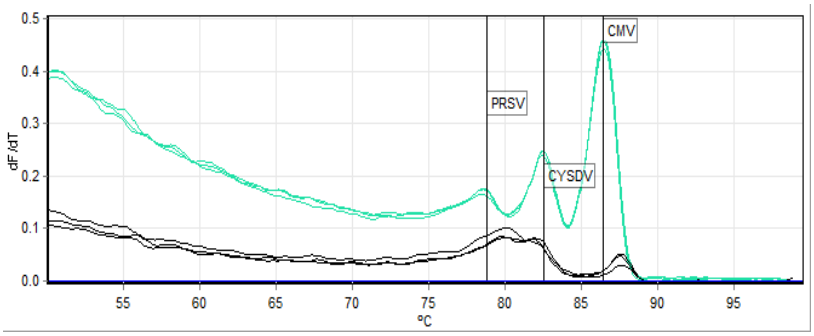
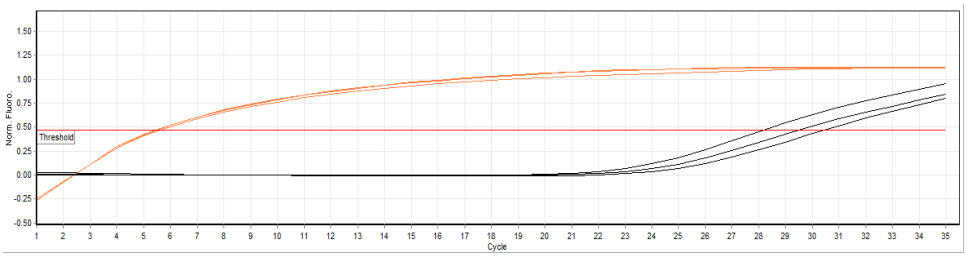
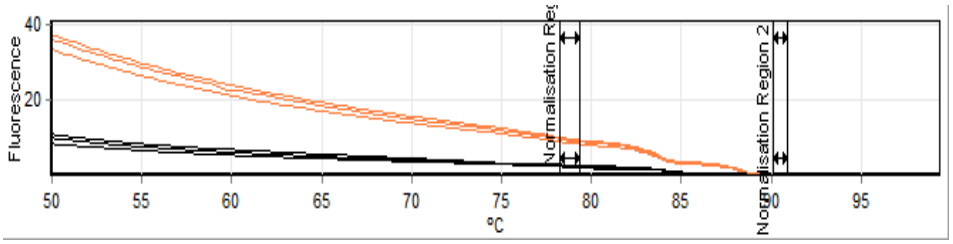


Figure 2. Virus reference controls PRSV, CYSDV, CMV are in green and NTC in black **a.** RT-PCR from three reference positive controls. **b.** Raw data showing the pre-melt, melt and post-melt regions. Notice the fluorescence variance and the positioning of the pre- and post-melt identification bars. **c.** Normalized fluorescence data derived from raw data plots showing the Tm breaking point of fluorescence of the viruses at Tm. **d.** Low Resolution Melt profile derivative plot (-dF/dT against T). The steepest slope is easily visualized as a melt peaks.

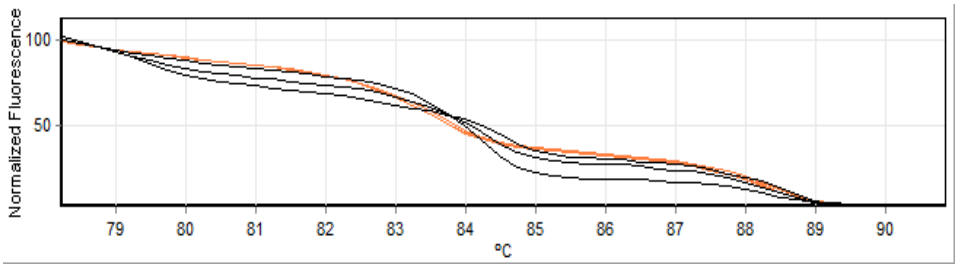
Multiplex-C RT-qPCR+HRM for CGMMV, ZYMV, CABYV



b.



c.



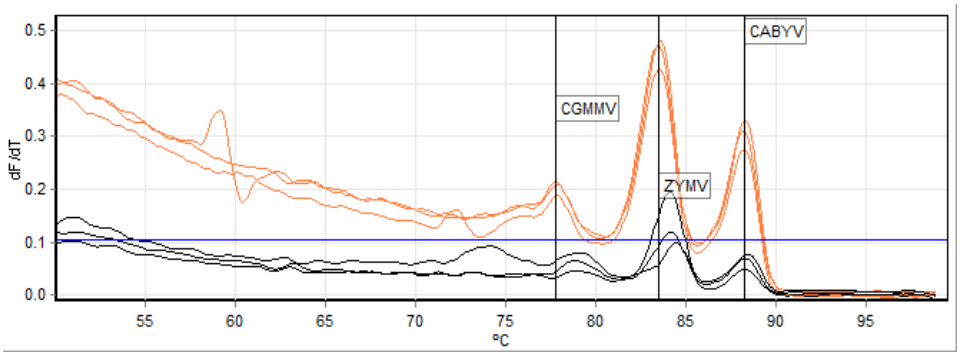
d. 

Figure 3. Virus reference controls CGMMV, ZYMV, CABYV are in orange and NTC in black **a.** RT-PCR from Three reference positive control. **b.** Raw data showing the pre-melt, melt and post-melt regions. Notice the fluorescence variance and the positioning of the pre- and post-melt identification bars. **c.** Normalized fluorescence data derived from raw data plots showing the Tm breaking point of fluorescence of the viruses at Tm. **d.** Low Resolution Melt profile derivative plot (-dF/dT against T). The steepest slope is easily visualized as a melt peaks.

General Multiplex RT-PCR High Resolution Melting (HRM) Procedure

All multiplex RT-PCR assays were performed in 10 µl reactions consisting of 5 µl HotStart 2X Master Mix (New England Biolabs), 1 µl of LCGreen® Plus melting dye (Biofire Defense, UT, USA), 0.5 µl of each forward and reverse primer (7.5 µM), 1 µl of cDNA template, and 2 µl nuclease-free water (Ambion, TX, USA). RT-qPCR+HRM was performed in an Rotor-Gene thermal cycler (QIAGEN, USA) with the following cycling: initial denaturation of 94∘C for 4 min followed by 40 cycles of denaturation at 94∘C for 20 s, annealing at 54∘C for 60 s, extension at 72∘C for 40 s, and final extension at 72∘C for 3 min. Once PCR is done, the 10 µl of amplified PCR product continue for HRM ramping the temperature from 50 to 99 ºC. Positive, non-template control (water) control, and healthy tissue were included in triplicates.

Screening of field samples by multiplex PCR

Samples were collected by PI and Vivek Khanal during the summers of 2016 (Muskogee) and 2018 (Tulsa) and were sent to OSU for screening using multiplex PCR. A RT-qPCR+HRM analysis of the samples is shown in Table 3.

Table 3. Summary of results from field samples collected from Muskogee County, OK, during the summer of 2016. CABYV, CGMMV, and CYSDV are reported in OK for the first time and are highlighted.

County	Year	Virus	Positive	Negative
Muskogee	Summer 2016	CABYV*	4	70
Muskogee	Summer 2016	CGMMV*	52	22
Muskogee	Summer 2016	CMV	59	15
Muskogee	Summer 2016	CYSDV*	5	69
Muskogee	Summer 2016	MNSV	33	41
Muskogee	Summer 2016	PRSV	9	65
Muskogee	Summer 2016	SqMV	43	31
Muskogee	Summer 2016	WMV	69	5

Muskogee	Summer 2016	ZYMV	70	4

* These three viruses were detected for the first time in Oklahoma. CABYV and CGMMV were detected by DIBA while all the three viruses were detected by multiplex RT-PCR assay.

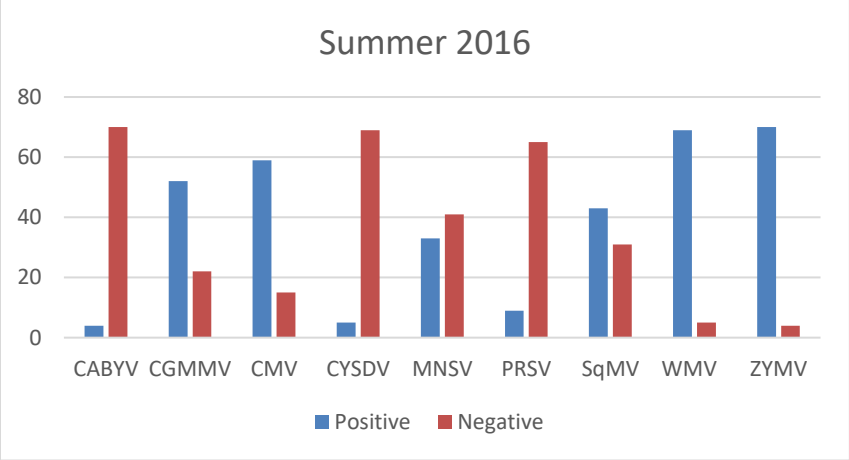


Figure 4. Comparative summary of results of positive and negative field samples collected in Muskogee County, OK.

Table 4. Summary of results from field samples collected in Tulsa County, OK, during the summer of 2018. CABYV, CGMMV, and CYSDV are reported in OK for the first time and are highlighted.

County	Year	Virus	Positive	Negative
Tulsa	Summer 2018	CABYV*	11	4
Tulsa	Summer 2018	CGMMV*	15	0
Tulsa	Summer 2018	CMV	15	0
Tulsa	Summer 2018	CYSDV*	6	9
Tulsa	Summer 2018	MNSV	0	15
Tulsa	Summer 2018	PRSV	10	5
Tulsa	Summer 2018	SqMV	1	14
Tulsa	Summer 2018	WMV	15	0
Tulsa	Summer 2018	ZYMV	10	5

* These three viruses were detected for the first time in Oklahoma. CABYV and CGMMV were detected by DIBA while all the three viruses were detected by multiplex RT-PCR assay.

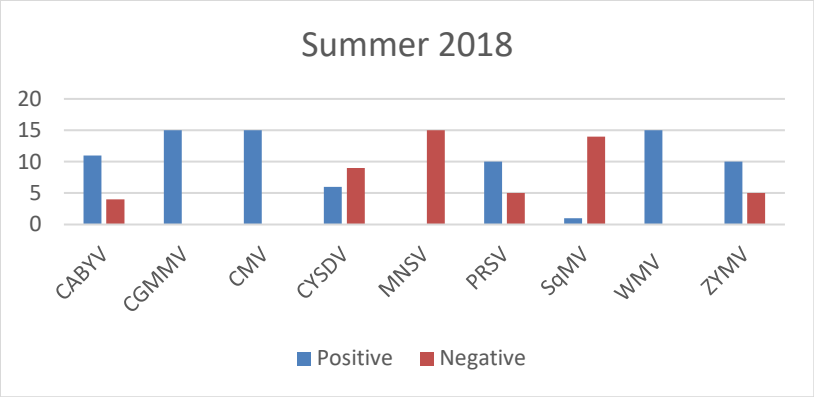


Figure 5. Comparative summary of results of positive and negative field samples collected in Tulsa County, OK.

Cost analysis

	Reagents	Cost of reagents ($)	Number of reactions	Cost of single rx ($)
	Positive controls (9)	144	36	4
	PBS Buffer (500 mL)	19.97	1000	0.01997
	Grider bags (100)	170	500	0.34
RNA trapping	0.2 mL tubes	174.21	1000	0.17421
	RNA sin	560	500	1.12
	Radom primers	93	1000	0.093
	dNTPs	59	1000	0.059
	DEPC Water (1L)	73	10000	0.0073
cDNA	RT(4000U)+Buffer	85	500	0.17
	Primer set (18)	162	10000	0.0162
	Hot Start	129	100	1.29
	DEPC Water (1L)	73	10000	0.0073
	LC Green	135	500	0.27
RT-qPCR + HRM	0.2 uL Rotor gene tubes	344	1000	0.344
			including nine positive controls	7.91098
			without positive controls	3.91098

Objective (4) (by PI and Co-PI)
The multiplex-RT-PCR assay developed in this project was transferred to the plant diagnostic clinic at the Oklahoma State University, the University of Tulsa, and some testers in the National Plant Diagnostic Network (NPDN). The staff at the OSU plant diagnostic clinic and two students from TU received training on the use of the multiplex-RT-PCR for the detection of cucurbit viruses.

Results of the multiplex RT-PCR were also presented to a broad scientific community attending the annual American Phytopathology Society (APS) meetings in 2016 and 2017. The APS meeting was held in Tampa Florida from August 5-9, 2016, and results from this project were presented in a poster. A large number of cucurbit plant pathologists from an audience of approximately 3 thousand participants, breeders, and industry professionals accessed this information and discussed the reliability of multiplex assay for cucurbit viruses.

Similarly, during 2017 at the APS meeting held in San Antonio, Texas, the results were presented orally through a seminar. Approximately 126 participants or more, including national and international scientists from academia, research (USDA, Texas A&M research, extension centers and other national and international institutes), industry, postdoctoral fellows, and both graduate and undergraduate students attended the talk. A healthy discussion followed the presentation as well as one to one discussion about the multiplex assay.

To further disseminate the results of the multiplex assay of cucurbit viruses, we are preparing a scientific article to be submitted to an international plant pathology journal for this technology being communicated worldwide.

Objective (5) (by PI and Co-PI)
During the last three years of extensive surveys of cucurbit crops, the PI visited several counties. Regular meeting were held with County Extension agents who guided the PI and team to major grower's fields. During the sampling, we had a chance to talk to growers and county extension agents on one to one basis or a group of growers in the same county. Both growers and County extension agents were interested and keen to learn about viruses in their cucurbits crops. During the discussion, we communicated research findings to growers and County Extension agents about cucurbit viruses. We also transferred the information about how to identify common symptoms of infecting viruses, their potential aphid vectors, and vector management of infected plants.

We have created a large database of cucurbit plants infected with viruses that were collected during the surveys organized by fields in specific counties. We will be able to transfer these as a pdf to all County Extension agents where cucurbits are grown to serve as a guide for them to help the growers in the identification of viruses early in the season. We will be willing to test their samples free of charge in the identification of viruses.

BENEFICIARIES

We have estimated approximately 495 beneficiaries recipients of information generated through this project's research accomplishments. These includes cucurbits growers, OSU county

Extension Agents, and a number of diagnosticians, cucurbits breeders, and plant pathologists (difficult to quantify) that attended two oral presentations delivered during the American Phytopathology Society meetings held in 2016 and 2017.

LESSONS LEARNED

We observed that only RNA viruses infect cucurbits in Oklahoma and there is no DNA virus was detected during the survey. Among the viruses, the three potyviruses (PRSV, WMV and ZYMV) are more prevalent in cucurbits fields of Oklahoma than the remaining other fours viruses which has less than 5% incidence. All the three potyviruses are transmitted in non-persistent manners by various aphids' species. Control of these aphids vector before the crop in the field and during the cropping system with insecticide spray is very important to effectively manage the spread of these viruses.

We learned from initial testing comparing Elution Independent Collection Device prototypes (EICD) and results obtained by directly capturing *Rose rosette virus* in PCR plastic tubes, that there was potential for adapting the last method for a larger number of viruses because all targeted cucurbit-infecting viruses have a similar capsid protein isoelectric point. Direct virus trapping in PCR plastic tubes was initially tested by Ochoa-Corona and Lee with *Citrus tristeza virus,* a Closterovirus, directly trapping in ELISA plates, and Binoy et al with *Rose rosette virus*, an *Emaravirus,* widely spreading in Oklahoma. Direct trapping turned to be an alternative because the rose tissue is rich in pigments and phenolic compounds that interfere with the PCR reaction. This was not a problem with cucurbits and rather facilitated the sampling processing. The adaptation of this method for cucurbit tissue processing has been useful and allowed a rapid progress of this research project.

CONTACT PERSON

Dr. Akhtar Ali (918) 631-2018
Akhtar-ali@utulsa.edu

ADDITIONAL INFORMATION

Two scientific articles have been published in scientific journals.

- Vivek Khanal and Akhtar Ali (2018). First Report of *Cucurbit aphid-borne yellows virus* Infecting *Cucurbita pepo* in Oklahoma. Plant Disease 102: (5) 1046. https://doi.org/10.1094/PDIS-10-17-1675-PDN
- Vivek Khanal and Akhtar Ali (2018). First complete genome sequence of Cucurbit aphid-borne yellows virus from Pumpkin in the United States. Microbiology Resources Announcement (In revision).

Two preliminary publications and presentation in national congress (America Phytopathological Society, 2016-2017) are listed. Two additional complete scientific articles for refereed journals are in preparation.

- L. Peña Zuniga, F. Ochoa-Corona, A. Ali. Multiplex RT-PCR detection of eight viruses infecting cucurbits and discrimination using High Resolution Melting analysis 2016. (Abstr.) *Phytopathology 106:S4.118.*
- Lizbeth Peña Zuñiga, Andrés Espíndola, Hassan Melouk, Akhtar Ali, Cardwell Kitty and Francisco M. Ochoa-Corona. 2017. Detection of cucurbit viruses in Oklahoma combining EDNA with Multiplex RT-PCR coupled with High Resolution Melting. (Abstr.) *Phytopathology 107:S5.36.*

Final remarks & Conclusion:
We surveyed cucurbits fields in Oklahoma during the three growing seasons and determined that nine viruses commonly infect cucurbit crops. We observed that among the nine viruses detected, three potyviruses are more frequently distributed all over the counties and infect all cucurbit hosts in the state.

We develop and validated with field samples eight sets of primers for detection and identification of nine viruses (CMV primers detect both CMV I and CMVII) in multiplex RT-qPCR combined to HRM that accurately detects nine important viruses associated with infectious disease in cucurbit production. The methods are sensitive down to femtograms. Detection and discrimination of nine virus-infecting cucurbits by Multiplex RT-qPCR-HRM allows an accurate viral detection and discriminatory diagnosis with high levels of confidence. Moreover, this multiplexing detection platform has potential to be applied in epidemiological studies of viral diseases in cucurbit crops, microbial forensics and biosecurity. All the objectives were completed.

Determining the Irrigation Requirements of Select Indigenous Crop Varieties for Oklahoma Specialty Crop Producers

PROJECT SUMMARY

Small holder producers in both disadvantaged and Native American communities strive to produce marketable legumes that receive higher economic value. There is great demand from schools, smaller grocery chains, local food focused restaurants, and Native American nations to buy locally produced food. These entities require a consistent, high quality product in order for them to buy from small holder producers. Small holder producers need to know how to grow and produce traditional heirloom varieties of legumes from Native American communities to have higher value products to sell to consumers. This project focused on using three promising varieties of legumes supplied from Sovereign Tribal Nations that are based in Oklahoma. The objective of this study was to understand the response of three different traditional heirloom legumes to differing levels of watering to provide information for Native American producers that are planning for irrigation systems to support market gardening production.

The specific issue being addressed was to develop recommendations for maximizing production on the main heirloom legume crops that are being grown by small holder producers from both disadvantaged communities and Native American nations. This was addressed through a cultural

practices study, irrigation study, and a crop variety evaluation. Another component of the study was to develop recommendations for efficient water use with these crops. This was completed with an irrigation study. To study irrigation efficiency, the approach was to set up several drip irrigation regimes (Main plot treatments) and grow the crops under each regime.

This project was important because these small holder producers could gain a competitive market edge by producing unique crops that have high nutritional and cultural value for a wide range of marketing products. Several of the larger Native American sovereign nations within Oklahoma have prioritized developing their traditional and indigenous food production like the Cherokee, Choctaw, Muscogee Creek, and Otoe-Missouri Nations. This project was also important because of the continued constraints upon water resources within Oklahoma. Small holder producers need to develop more efficient water use in production of vegetable and traditional crops. This project developed recommendations for helping small holder producers in Oklahoma to produce these traditional indigenous crops using the most efficient volume of water.

This project was timely because of the persistent droughts that Oklahoma frequently experiences. These droughts have helped producers and decision makers understand that if food is to be produced locally, water use and irrigation for small holder producers must be efficient.

PROJECT APPROACH

The study was designed as a randomized complete block design with three replications. Plots were organized so that irrigation treatment plots had each of the three legume varieties randomized within it. The first year's study had nine treatments and the second year's study included 8 treatments (Table 1). Seeding rates were four seeds per row-foot during both years. Plots were planted mid-May the first year and mid-June the second year.

Soil Preparation: The soil was tilled in order to have a clean planting area free of debris and weeds. A pre-emergence herbicide (Dual Magnum at 0.95 lbs. A.I.) was applied for weed control immediately following seeding both years. One weeding pass between rows was done mechanically with a tractor PTO driven tiller after the first month. All subsequent weeding was manually done using hand tools (hoes) commonly used by market gardeners.

Irrigation system set up: The irrigation system was built using drip irrigation to provide for different irrigation treatments. The system consisted of the T-1 irrigation line and a T-2 irrigation line in year 1 and T-1, T-2, and T-3 in year two. Each line was sourced from the Research Station water source. The source line was connected to an Orbit™ battery operated digital irrigation timer which provided water at the required intervals. A Dywer™ WM2-A-C-03 analog water meter allowed for accurate measurement of the irrigation water flow throughout the study. During times of heavy rainfall, the irrigation timers were delayed. The four water treatments were:

1) T-0 as a control with no irrigation
2) T-1 as critical point or mid-point irrigation. T-1 ran for 30 minutes every other day. Each T-1 treatment received a total of 540 gallons of irrigation water for the first season. On a per acre basis this was equivalent to 392,166 gallons.

3) T-2 as full irrigation. T-2 ran for 90 minutes every other day. Each T-2 treatment received a total of 953 gallons of irrigation water for the first season. On a per acre basis this was equivalent to 691,966 gallons.

T-3 as irrigation only to provide moisture for emergence for the second season (one irrigation event).

Harvesting: Due to the small size of the plots and the indeterminate growth habits of the Vigna species a decision was made to use multiple hand-harvesting for the plots the first year (Figure 1). First harvest began in August and concluded in October for the first year. Due to the constraints on labor required with hand harvesting a plot combine/thresher was used to harvest the plots the second year. Plots were harvested in late September the second year.

GOALS AND OUTCOMES ACHIEVED

Objective 1:
Results Year 1: Overall yields for Pottawatomie Pea did not vary significantly, but ranged from a low of 6193 for the no irrigation treatment to a high of 6752 lbs. per acre (non-shelled yield) for full irrigation (Table 3). Overall yield of Battered Buffalo Skull peas ranged from a low of 2788 lbs. per acre for the no irrigation treatment to 3161 and 3710 lbs. per acre for the T1 and T2 irrigation treatments, respectfully. This means that you should not expect to see a significant increase in yield if drip irrigation was provided to these species. These results were not expected. Overall yields for the Hidatsa bean did not vary significantly although they ranged from zero to 184 lbs. per acre. Pottawatomie and Battered Buffalo Skull as Cowpeas (Vigna sp.) have shown the ability to grow well in the summer. As with all Cowpeas these must be planted when soil temperatures are higher than 70o F.

Results Year 2: Overall yields for Pottawatomie Pea did not vary significantly, but ranged from a low of 905 lbs. per acre for the critical point irrigation treatment to a high of 1,389 lbs. per acre for emergence only irrigation (Table 4). Overall yield of Battered Buffalo Skull peas ranged from a low of 978 lbs. per acre for the no irrigation treatment to 1,321 and 1,704 lbs. per acre for the critical point and full irrigation treatments, respectfully. This means that a significant increase in yield should not be expected if drip irrigation was provided to these species. This was true for year one and again in year two.

Conclusions: This trial showed that Vigna species are robust and can adapt to high temperature and low moisture conditions and recover even when stressed by lack of rainfall. Under drought conditions both of these varieties would benefit from some supplemental irrigation, but during a normal rainfall year they would not benefit significantly from irrigation. The drawbacks to these varieties is that if produced on a large production scale then machine harvesting (combining) needs to be considered. Hidatsa Indian Red Beans were preserved by northern tribal nations and are more adapted to cool season growing. Therefore, they should be planted in early fall (late July-August) when temperatures begin dropping or earlier in the spring (April).

Table 1. Study treatments for 2016 and 2017.

Varieties	Study Year	Irrigation treatments			
		T-0	T-1	T-2	T-3
Pottawatomie Pea	2016	X	X	X	NA
Battered Buffalo Skull Pea	2016	X	X	X	NA
Hidatsa Indian Red Bush Bean	2016	X	X	X	NA
Pottawatomie Pea	2017	X	X	X	X
Battered Buffalo Skull Pea	2017	X	X	X	X
T-0 as a control with no irrigation T-1 as critical point or mid-point irrigation T-2 as full irrigation T-3 as emergence only irrigation					

Table 2. 2017 ODAFF Native American traditional legumes study, Crop water usage, Perkins, OK

Water treatment	Irrigation (in)	Rainfall (in)	Total water usage (irrigation + rainfall)
No irrigation	0	13.33	13.33
Critical point irrigation	6.72	13.33	20.05
Full irrigation	9.22	13.33	22.55
Emergence only	1.20	13.33	14.53

Table 3. 2016 ODAFF Native American traditional legumes study, Harvest data, Perkins, OK

	Unshelled weight pounds per acre		
Water treatment	Pottawatomie Pea	Hidatsa Indian Red Bean	Battered Buffalo Skull Pea
No irrigation	6193 a[z]	0 a	2788 a
Mid-point irrigation (T1)	6265 a	77 a	3161 a
Full irrigation (T2)	6752 a	184 a	3710 a

[z]Numbers in a column followed by the same letter exhibited no significant differences based on Duncan's Multiple Range Test where P=0.05.

Table 4. 2017 ODAFF Native American traditional legumes study, Harvest data, Perkins, OK

Pottawatomie Pea	Battered Buffalo Skull Pea

Water treatment	Weight shelled lbs./acre		% Moisture shelled peas		Weight shelled lbs./acre		% Moisture shelled peas	
No irrigation	1031	a[z]	7.9	a	978	a	8.6	a
Critical point irrigation	905	a	5.7	a	1321	a	8.3	a
Full irrigation	1360	a	12.3	a	1704	a	9.3	a
Emergence only	1389	a	11.6	a	1147	a	8.7	a

[z]Numbers in a column followed by the same letter exhibited no significant differences based on Duncan's Multiple Range Test where P=0.05.

Objectives 2:
The results of this work is made available to small holder producers, market gardeners, vegetable growers, Master Gardeners, and consumers on the Oklahoma State University Department of Horticulture website for the Extension Vegetable Program (http://www.hortla.okstate.edu/research-extension-youth/vegetables/vegetable-trial-reports) under the heading of "Vegetable Trial Reports". We have developed Oklahoma Cooperative Extension Service and Oklahoma State University Department of Horticulture and Landscape Architecture trial reports and they are listed under the 2016 and 2017 vegetable trial reports on the website and are available for download to producers and the public as a pdf file. In addition, information will be updated according to 2018 results and will be published in the 2018 vegetable trial report, published in early 2019. An Oklahoma Cooperative Extension Service current report is under review and will be distributed to the small-holder producers, Master Gardeners, and the general public in 2019.

Objective 3:
We presented the results of this work to approximately 42 stakeholders and small holder producers at both the 2017 and 2018 Oklahoma State University Department of Horticulture and Landscape Architecture Perkins Research Station Spring and Summer Field Days. Dissemination of project accomplishments and results were accomplished through distribution of pertinent information and the current report information at Master Gardener training workshops across Oklahoma for the 2018-2019 Master Gardener classes, the Horticulture Industries Show held in Oklahoma and Arkansas in 2018 and 2019, and to the public through the Oklahoma State University fact sheets web portal. Approximately 250 Master Gardeners were trained, and approximately 35 Horticulture Industries Show attendees viewed our poster presentation and handout summarizing the results of this project. In addition, a poster presentation was given in Tampa, FL at the Annual Agronomy Society of American and Crop Science Society of America Meetings in the Agronomy Extension session. Approximately 20 scientists, academics, and students attended and viewed the poster presentation and handouts at this event. These opportunities provided excellent venues in which information will be disseminated throughout the state, region, and United States so that the information could be used to benefit other small holder producers not directly involved with the project.

BENEFICIARIES

Through this project, a total of 347 beneficiaries were reached and affected by this project's accomplishments and results. This project directly benefited several key small holder producers from the following disadvantaged groups and Native American Nations: Muscogee Creek Nation, Ponca Nation, Choctaw Nation, Oklahoma African American population, and Oklahoma Hispanic population. The specialty crop beneficiaries of the project are disadvantaged small holder producers from African-American communities, Hispanic communities, and Native American small holder producers. These are producers who are expanding specialty crop production of indigenous edible legumes and herbs to supply demand among local food initiatives and Native American Nation food programs.

LESSONS LEARNED

Poor germination of the seeds due to delayed processing of beans required a replanting of the second season trials but that delay was remedied by a replanting. Native American seed savers and small-scale producers have concerns about use of inorganic chemical use. There are two trains of thought for Native American preferences for production that the researchers have encountered in the State. One group prefers organic production practices and specifically prefers not to use inorganic herbicides and pesticides. The other group is open to an integrated pest management approach (IPM) which encourages wise use of pesticides.

Determination of optimum production season for each variety: Hidatsa and Arikara beans are more adapted to cool season climates. These beans were preserved by northern tribal nations. Therefore, these should be planted in early fall when temperatures begin dropping or early spring. Pottawatomie and Battered Buffalo Skull are southern cow peas and are well adapted to warm climates. As with other southern cowpea varieties, these should be planted when soil temperatures reach at least 70F in the late spring/early summer. The challenge in growing both of these crops is proper timing for seeding and monitoring of soil and air temperatures throughout the season.

Extended Harvest requiring hand labor: It has been noted that hand harvesting of these indeterminate cowpea heirloom varieties is time consuming and that steps need to be taken to consider if manual labor is available in sufficient quantities in order for timely harvesting to take place. In comparison to current determinate varieties of improved cowpeas these two Native American heirloom cowpea varieties produce from early August till first frost (~Oct. 31 – Nov. 28). The improved determinate pea varieties produce peas that are upright, have minimal amounts of vegetation, and are easy to machine harvest. The negative aspect for the Native American heirloom cowpea varieties is that production is spread over several months and requires hand harvest four to six times during the 3 to 4 months of production. This is a problem for machine harvesting and would require spraying of a desiccant to kill the plant and enable machine harvesting. The positive benefit is that this variety would be an excellent variety for home gardens and for school gardens because it provides several cycles of flowering, pod set, and harvest for teaching and learning opportunities.

The researchers are also working on appropriate mechanical and hand processing methods for these different varieties. This will involve looking at the scale and financial resources of the

producers to see what is most appropriate. These varieties also appear to be good options for fresh harvest and selling at farmers' markets. Use of mechanical harvesting in year two means that producers will need recommendations about right sizing affordable but effective equipment. Die back during summer months for Hidatsa (90 – 120 day pinto bean variety): The Hidatsa variety died back during the summer months, even with full irrigation. This indicates that this variety cannot handle high air temperature and high humidity that is common during typical Oklahoma summers. When air temperature dropped in September, the Hidatsa variety began to recover, producer leaves, flower, and set viable pods. This indicates a need to move to early spring planting for the Hidatsa variety.

CONTACT PERSON

Justin Quetone Moss (405) 522.744-5729
justin.moss@okstate.edu

ADDITIONAL INFORMATION

Figure 1. Photographs of Battered Buffalo Skull Pea, Hidatsa Indian Red Bean, and Pottawattamie Pea grown in the field trials at Perkins, OK.

Battered Buffalo Skull Pea growth

Battered Buffalo Skull Pea pod-set

Hidatsa Indian Red Bean

Pottawattamie shelled pea

Battered Buffalo Skull Pea Flower

Pottawattamie vine with mature peas

"Fruits, Veggies, and Nuts, Oh My" Activity Booklet

PROJECT SUMMARY

The purpose for this project, "Fruits, Nuts, and Veggies, Oh My!" booklet, was sparked by the Oklahoma's 2014 state of the State's Health Report. It stated in 2013 12% of Oklahoma youth were obese and 15% were overweight. This report also stated Oklahoma has the 49th lowest rate of fruit consumption and the 44th lowest rate of vegetable consumption in the nation. Alarmingly, 44% of Oklahoma youth indicated they did not eat at least one piece of fruit each day and 40% reported not eating at least one vegetable every day.

The "Fruits, Nuts, and Veggies, Oh My!" booklet provides a plethora of recipes, games, crafts and science experiments for teachers to teach healthy food choices within their classrooms. This booklet provides teachers a concise resource to facilitate nutrition education on a daily, weekly or monthly basis.

Teachers in Oklahoma need ready to use resources in their classrooms to help address the issue of little fruit and vegetable consumption by their students. Having this easy to use resource will be more likely to teach lessons that include recipes and activities discussing the importance of local fruits and vegetables and their importance in maintaining a healthy diet and lifestyle.

Fruits, Nuts, and Veggies, Oh My is another tool in the Oklahoma Ag in the Classroom's Harvest of the Month program. This booklet expands on the initial posters' information about locally grown fruits and vegetables to including how to select and prepare these items. This booklet strengthens the knowledge of students and their parents who are the customer base of local farmers. All activities and recipes in the book promote specialty crops grown in Oklahoma which will help increase local sales for farmers. The booklet can be found on the Oklahoma AITC Website: https://www.agclassroom.org/ok/resources_classroom/fruits.php

PROJECT APPROACH

This booklet was printed and distributed to thousands of teachers and pre-service teachers across Oklahoma in a variety of settings such as our state summer conference, summer road trips and professional developments at local schools, and state-wide teacher events. A total of 4,500 books were printed using a total of $24,600.

The OAITC state coordinators chose recipes, crafts, poems, games, songs, and science experiments dealing with Oklahoma specialty crops to feature in this booklet. Coordinators developed questionnaire/survey for teachers to answer pre and post use of the booklet.

OAITC coordinators conducted workshops and professional development around the state, targeting the counties identified in the 2014 State of the State's Health Report as a highest priority for those workshops. OAITC coordinators featured the lessons and activities in the booklet and encouraged the teachers to participate in the pre and post surveys included with the booklet.

Getting teachers to complete the survey was the most difficult aspect of this project. Although we communicated with teachers in person and in email about the survey many teachers did not complete the survey despite using the booklet in their classrooms.

Specialty crops included in the booklet include: Watermelon, Apples, Blueberries, Pumpkin, Squash, Bell Peppers, Cauliflower, Okra, Sweet Potatoes, Honey, Tomato, Christmas Trees, Beets, Cabbage, Spinach, Lavender, Black-Eyed Peas, Cucumbers, Carrots, Pears, Blackberries, Pecans, Radish, Peaches, Broccoli, Zucchini, Strawberries, Lettuce, Turnips, Sweet Corn

GOALS AND OUTCOMES ACHIEVED

This resource was popular with teachers and a requested resource during professional development. Over 4,000 booklets have been distributed to date. After the first year and the small response to the survey about the booklet, OAITC staff added a link to the website for educators to respond about the booklet. Here is a link to the survey questions:
https://www.surveymonkey.com/r/FNV_Final_Survey
And here are a few of the pages, on the website, that had the link available:
https://agclassroom.org/ok/lessons/topic_cucurbits.php
https://agclassroom.org/ok/lessons/topic_pumpkins.php
https://agclassroom.org/ok/resources_classroom/fruits.php

OAITC staff conducted professional development about the booklet, and then followed up via email and newsletters about the booklet with those schools and teachers receiving the booklets. Of the teachers reporting of the survey, the trends continued the same as the first year. The recipes were reported by teachers as making the biggest impact on students. This is important because students are being exposed to a variety of healthy, nutritious foods. In the final analysis, students reported liking watermelon and peaches best out of all of the specialty crops. Over 90% of students reported they were eating more fruits and vegetables after completing the activities in the book.

Originally the goal was to have a 20% increase in the amount of fruits and vegetables being consumed by students in these classrooms. This goal was measured by creating an initial survey for teachers to use with students before using the Fruits, Nuts, Veggies, Oh My booklet and activities and then a final survey for the teachers to use with students after completing the activities. This survey was created on Survey Monkey. QR Codes to access both surveys were placed on the inside cover of the booklet. Teachers were shown the QR codes when they received the booklet and were asked to assist by completing the surveys. The survey link was also placed on the AITC website on pages containing the booklet and on pages with lessons relating to the booklet. The data that came in showed an increase in the fruit and vegetable consumption, according to the students: In the initial survey, 33% of classrooms reported 10-15 students ate fruit yesterday, 33% reported 15-20 students ate fruit yesterday, and 33% reported 25+ students ate fruit yesterday. In the initial survey, 100% of classrooms reported only 10-15 students ate vegetables yesterday. In the final survey, 50% of classrooms reported 10-15 students who stated they are now eating more fruit daily, 25% of classrooms reported 15-20 students stating they are eating more fruit daily, and 25% of classrooms reported 20-25 students stated they are now eating more fruit daily. In the final survey, 25% of classrooms reported 5-10 students who stated they are now eating more vegetable daily, 25% of classrooms reported 10-15 students who stated they are now eating more vegetable daily, 25% of classrooms reported 15-20 students who stated they are now eating more vegetable daily, 25% of classrooms reported 20-25 students who stated they are now eating more vegetable daily. When asked, 67% of students responded that they would like to eat more fruit on a daily basis, while only 50% said they would like to eat more vegetables. Prior to using the recipes and activities in the booklet, the majority of students surveyed said they liked to eat strawberries, followed by peaches, watermelon and tomatoes. On average, zero-five students in a class said they liked to eat radishes, cucumbers or spinach. After using the booklet in their classroom, teachers were asked to report their student's perceptions. Of those reporting, 100% of students said they were now eating more fruits and vegetables on a daily basis. Strawberries and watermelon were still the top choices, however the average changed and 15-20 students in a class, on average, reported liking cucumbers. Ten-fifteen students in a class, on average, reported liking radishes and spinach.

One of the biggest changes we noticed with this booklet is the number of specific visits to specialty crop lessons on our OAITC website. There were 3,532 unique page visits to those lessons related to the booklet in 2018 versus 3,155 unique visits to specialty crop lessons in 2017. The most significant change and success noticed by OAITC staff was in the number of specialty crop lessons downloaded. In 2018 there were 2,226 unique downloads of specialty crop lessons versus 661 unique downloads in 2017.

At the Ag in the Classroom State Summer Conference, 300 Participants were in attendance and received a copy of the booklet. There were 13 sessions, at this conference, focused specifically on specialty crops included in the booklet- Cucumbers, Berries, Melons, and Honey. The workshops featured AITC lessons to go with the activities in the booklet. 283 Participants at EngageOK 2017, also selected workshops focused on the booklet. EngageOK was held at 9 locations with 2 workshops, featuring the Fruits, Nuts, and Veggies, Oh My booklet, at each location. The AITC summer tour in 2017 had 50 participants who visited a pecan shelling facility, mushroom farm, and 2- you pick berry farms (blueberry and blackberry). During the summer of 2017 there were two other one day tours, with 100 total participants, who toured you

pick berry farms for blackberries & blueberries, and a Christmas tree farm, and created Farm to School recipes using specialty crops. The AITC summer tour in 2018 had 56 in attendance who visited a pecan shelling facility and mushroom farm.

BENEFICIARIES

The state's two largest agricultural organizations, Oklahoma Farm Bureau and American Farmers and Ranchers supported this OAITC project. Many of the members of these organization grow specialty crops. The Oklahoma Fruit and Vegetable Association and the Oklahoma Pecan Growers Association send members to the OAITC Advisory Council. They gave solid approval of the project, but they could not provide a quantitative figure on the impact this booklet had on Oklahoma specialty crop farmers.

Due to teachers traveling on the tours in 2017, five specialty crop venues in Oklahoma directly benefited from this project. 150 quarts of blackberries were purchased from two different you pick farms; 150 quarts of blueberries were purchased from two different you pick blueberry farms; Over 40 teachers purchased pecan products at the pecan shelling facility; and the mushroom farm educated 50 people on growing mushrooms. Due to teachers traveling on the tours in 2018, three specialty crop venues directly benefited from this project. Over 50 teachers purchased pecan products at the pecan shelling facility; and the mushroom farm educated 56 people on growing mushrooms.

Indirectly, the students of the 4,000 teachers who received the booklet, throughout the course of this project benefitted from more awareness and education about specialty crops.

LESSONS LEARNED

This was a successful project for OAITC that was well received by teachers and students. All of the activities in the booklet were new to OAITC. Teachers expressed their appreciation of new, fresh ideas.

OAITC staff was pleased by the increase of downloads and page visits to the website as a result of this project. That increase was larger than expected by staff. The downloads and page visits showed teachers were using the booklet and then other lessons and OAITC activities specifically about specialty crops.

One of the things that did not go as well as planned was being able to truly identify if students were eating more fruits and vegetables and if producers reaped any benefit from our booklet. The economic impact is hard to measure in a project like this. Students self-report on the increase of fruit and vegetable consumption to their teachers and then the teachers must report to us. This data might not be the most accurate. We will need to rethink how we collect data on future projects that involve food consumption.

CONTACT PERSON

Audery Harmon 405-740-0160
audrey.harmon@ag.ok.gov

Oklahoma Grown Farmers Market Promotion

PROJECT SUMMARY

In 2016, the Oklahoma Department of Agriculture awarded 24 Oklahoma Grown Farmers Markets with grants. Funding levels were determined by the number of vendors participating at the market on a weekly basis. The markets were selected by an application process with a panel of judges that scored the applicants. The grants were utilized to solely promote the consumption, purchase and production of specialty crops through signage, print and radio advertising and chef demonstrations. While consumers continue to become more health conscious and concerned about better eating habits, market managers continually look for new ways to reach the community. Out of the 24, all of the farmers markets awarded consist of small producers who rely on direct marketing to sell their products. Farmers Markets continue to be a major outlet for growers to sale their fruits and vegetables and the grants help impact growers and the sale of specialty crops across the state of Oklahoma.

As this project builds on previous work of the specialty crop grant program, reports show that markets across the state have seen an increase in consumer demand and specialty crop production. Over $2,869,088.83 was reported in specialty crop sales from the 24 Oklahoma Grown Markets that received grants. By offering grants to these Oklahoma Grown Farmers Markets, we have increased the demand and competitiveness for Oklahoma Grown specialty crops and attracted new customers and vendors to our markets.

PROJECT APPROACH

The Oklahoma Department of Agriculture awarded 24 Oklahoma Grown Farmers Markets with specialty crop grants. Grants were made available on a competitive basis to existing and start up 100% Oklahoma Grown Markets. Funding levels were determined by the number of vendors participating at the market on a weekly basis. Those markets with an average of 6-10 vendors weekly were eligible for up to $1,000, markets with 11-20 vendors could receive up to $2,000 and those markets with over 21 vendors weekly qualified for a maximum of $3,500 to promote specialty crops within their markets. The applications were reviewed by ODAFF staff and evaluated by an outside committee. Grants were distributed in two advancements with the market receiving their first advancement in advance and their second advancement after a progress report and receipts were turned in to ODAFF staff documenting how the first advancement was spent. After the grant was completely spent, the market sent a final report along with all receipts and examples of how the money was spent to promote specialty crops. Markets that were awarded used the grant funding on advertisements, newsletter publishing, signage and promotional ads to draw consumers to the market to purchase specialty crops. Staff also visited a number of markets that were awarded the grant to ensure the funds were used properly.

GOALS AND OUTCOMES ACHIEVED

The main goal with this project was to enhance the competitiveness of specialty crops while building consumer confidence in locally grown produce. The grant funds raised customer awareness and demand with over $2,869,088.83 in sales this year at Oklahoma Grown Farmers markets. With consumer demand and vendor participation continuing to grow, ODAFF feels that specialty crop advertising, signage, marketing promotion and newsletter publishing continue to be a good avenue to reach consumers in city and rural areas. Social media has also played a big role in advertising what specialty crops will be at the market each week as well as being very inexpensive compared to traditional print advertising. It has also allowed the markets to reach a new audience they might not have reached in the past.

BENEFICIARIES

The beneficiaries of these programs were current 100% Oklahoma Grown Farmers Markets, vendors, market managers, city officials and the buying customer. Reports show there are more than 850 growers that participate in Oklahoma Grown Farmers Markets with 145 new growers selling at these markets this year. Markets that receive grant funds continue to show an increase in sales and vendor participation at their market along with giving the consumer more options of fresh, locally grown produce. The grant funds raised customer awareness and demand with over $2,869,0883.83 in sales this year at Oklahoma Grown Farmers markets.

LESSONS LEARNED

The oil and gas decline has had a significant impact on the Oklahoma economy. Markets have seen a decline in attendance and have had to get creative and innovative about how to draw new customers to the market.

CONTACT PERSON

Ashley Bender (405) 522.4330
ashley.bender@ag.ok.gov

Evaluation of Strawberry Production in Oklahoma Utilizing Plasticulture

PROJECT SUMMARY

There is a great demand for fresh sweet fruit in Oklahoma. When it comes to growing fresh strawberries on plastic, Oklahoma is behind Arkansas and Missouri, but has the same potential.

For many years strawberries where grown in Oklahoma without plastic. Some of the issues with that is fruit is not as clean because fruit is laying on the ground or in grass or weeds. Fruit was also harder to pick. Plasticulture strawberries increased production, because of drip irrigation

and black plastic. The drip irrigation helps control the amount of inputs, such as fertilizer and water. It also reduces the amount of water and fertilizer used because it is all going into the root of the plant and not to areas of the field were water and fertilizer is not needed. Black plastic also aids in earlier production along with weed control.

With food safety being a big concern as we move forward in growing fruits and vegetables, it is important to show farmers how they can grow a cleaner produce on top of the plastic rather than on soil, grass or weeds. All strawberry varieties do not perform the same on black plastic mulch, therefore five varieties were selected for the study:

1. Camino Real
2. Festival
3. Albion
4. Sweet Charlie
5. Charlie

Time

Timing is critical when planting strawberries in plastic. The strawberries have to be planted in the Fall around September, so they can grow from September thru early December for crown development. The months leading up to September require a cover crop to be planted and tilled into the soil, along with soil test to determine the proper fertilization plan, ground worked and beds laid before the planting date.

In December if the temperature drops more than fifteen degrees in one day or we have extremely cold weather; row covers have to be installed in a timely manner. In early spring when plants start to bloom, they must also be covered if there is a chance of frost or you could lose the entire crop.

PROJECT APPROACH

In February 2016, staff members from ODAFF and the Noble Research Institute met with a group of growers who had previously participated in ODAFF's Plasticulture program and were interested in growing strawberries. The growers were selected due to their experience and success of growing various fruits and vegetables utilizing Plasticulture. During the meeting the timelines and responsibilities of the participants were discussed and we had some growers decide they could not participate.

To help build organic matter in the soil a cover crop of Sorghum Sudan (Hay Grazer) was planted on the six (6) participating locations during the early spring. The Sorghum Sudan was mowed a minimum of 2 times at each location and the residue was plowed into the ground to begin breaking down. Soil test were conducted at each location to determine the health of each plot and determine what amendments need to be added.

Participants were given a grid to follow when planting the different varieties. The beds were prepared with plastic and irrigation during September and the plugs were put in the ground

during the first part of October. Participants were also provided with rye grass to plant in between the rows to help build a good walking area at time of harvest.

The participants were asked to log the dates of harvest and weigh the berries as they were picked. At the end of the season the project reports were submitted to ODAFF and the information was entered into a spreadsheet in order to chart the differences between the varieties on first days of harvest, length of harvest season, and weight of berries being picked at each harvest.

GOALS AND OUTCOMES ACHIEVED

The goal of the project was to test strawberry varieties to determine which are best suited for Oklahoma growing conditions. Five varieties of strawberries were selected for the trial and three of those look to be promising for production in Oklahoma (Chandler, Sweet Charlie and Camino Real). One official field day was hosted with around 15 individuals attending. ODAFF and the Noble Research Institute received several calls and questions about the project inquiring which berries a grower should plant. A one year study is not enough to get a true scientific and statistical analysis so a second proposal was submitted in 2016 and a better understanding of which varieties are best suited for production in Oklahoma.

BENEFICIARIES

The direct beneficiaries are the 6 growers who participated in this project. Through their involvement they were able to test and determine what variety of berry grows best and is best suited for market with minimal financial risk. With this knowledge and an understanding of how to grow the strawberries on plastic will allow them to be at the forefront of resurrecting strawberry production in Oklahoma. In direct beneficiaries would be those growers interested in starting to grow strawberries or currently growing that can find out what varieties are best suited for their growing environment. During a field day at one of the participating farms we had 15 producers attend along with 40 people in attendance during a breakout session at the Oklahoma Arkansas Horticulture Industry Show.

LESSONS LEARNED

When planting a cover crop, Hay Grazer is a good option that will help build up your soils organic matter but you must have the appropriate time for it to be cut and tilled into the soil for it to break down properly. If that time is not available it may be better to plant cow peas as your cover crop.

The research gathered from the six producers showed that the Chandler variety was the best producing strawberry with Sweet Charlie and Camino Real close behind. Chandler and Sweet Charlie were the earliest producing berries and ended up producing just as long as the other berries in the trial. Even though Albion was the lowest performing berry in the trial, one producer said they would plant it again due to the size and flavor of the berry.

In Oklahoma; weather is always a limiting factor with late and early frost occurring along with periods of too little or too much rain. This year one producer lost his entire crop due to a severe

hail storm. When these types of occurrences happen there is not much that a producer can do to mitigate loss.

CONTACT PERSON

Jamey Allen (405) 522-4676
jamey.allen@ag.ok.gov

Oklahoma Wine Trails

PROJECT SUMMARY

The purpose of this project was to increase consumer awareness of the availability of wine made in Oklahoma, increase customer traffic at Oklahoma wineries and vineyards in order to educate consumers about wines made in the state and increase the production of grapes and other specialty crop fruit used to make wine by creating a greater demand for wine made in Oklahoma.

Even with all the data supporting the effectiveness of the original Oklahoma Wine Trail project including the addition of at least 5 new wineries and 2 new vineyards per year, the consumer research shows that there is still a large portion of the population that is unaware that vineyards and wineries exist in Oklahoma. According to research conducted by Oklahoma State University at an Oklahoma wine festival in 2017, 72% of attendees were not familiar with the Oklahoma Wine Trails and only 50% of attendees had visited an Oklahoma winery. Of the 47% of attendees that reported they drank wine on a weekly basis, only 37% of those indicated they occasionally drank wines made in Oklahoma. This data illustrates the need for a refreshment of the Oklahoma Wine Trails material requested in this proposal.

This project built on momentum created by the 2012 SCBG-funded Oklahoma Wine Trails project. The original concept was updated including new colors and design without compromising the logo and original branding. The web and print information was updated to include the new wineries and vineyards that have started business since the project's inception.

PROJECT APPROACH

Qualitative Work	Quantitative Work	Timeline
OK Agritourism staff updated the list of operating vineyards and wineries with tasting rooms. In addition, trail descriptions were updated to include content relevant to the added venues.	Wineries added since initial project : 20 Vineyards added since initial project: 6 Wineries defunct since initial project: 5 Vineyards defunct since initial project:3	February 2018

	Wineries and vineyards considering joining program but not ready yet: 6	
RFP was sent for design and social components. The Staplegun agency was chosen and began work on re-design of map and passport(companion piece). Decision was made to design stamps to coordinate with each trail and to be used by wineries on passport.		February-April 2018
Because OK Agritourism staff was aware of at least 6 new venues that were considering joining the program, the decision was made to print only the quantity of maps needed for one year to allow for revisions and reprint in 2019. This allowed in the budget an increase in printing of the passport. RFP for print components was sent, Paragon Press was awarded printing and Walker Companies was awarded stamp production.	Maps printed: 32,000 Passports printed: 20,000 Stamps made: 55	April-June 2018
OK Agritourism staff delivered maps to each winery and vineyard in the program as well as the Oklahoma Tourism and Recreation Warehouse for distribution through the online consumer portal.	Maps delivered to wineries: 10,000 Passports delivered to wineries: 8,000 Stamps delivered to wineries: 45 Maps delivered to OTRD: 18,000	July-September 2018
Social media campaign was initiated and monitored for effectiveness. Two static link ads, one carousel ad and one video were completed and launched. Statistics were evaluated using Facebook Insights and Google Analytics.	Totals for all 4 ads: Clicks – 16,673 Likes – 1,684 Comments – 125 Shares – 678 Reach – 298,931 New visitors to oklahomawinetrails.com directly from Facebook ads: 10,703	July – August 2018

Winery and vineyard producer response was evaluated through online and in-person survey. 18 online respondents, 5 in-person respondents.	78% say the Oklahoma Wine Trails material has increased traffic to the winery tasting room. 49% say the OWT material increased sales from their tasting room. 20% said they will plant more grapes and other fruit in order to keep up with demand	September 2018

GOALS AND OUTCOMES ACHIEVED

By distributing Oklahoma Wine Trails maps and promoting the Trails on Facebook, consumer awareness of wine made in Oklahoma has increased, therefore increasing demand of grapes and other fruits to be grown as evidenced by reports from the Oklahoma Tax Commission and the Facebook Insights and Google Analytics results below. (OTC fiscal year reporting ends in June of each year. FY19 June-September numbers were not available at the time of this report.)

- Expected Outcome: Increase in wine produced in Oklahoma
    - Performance measure – OTC reports
    - Benchmark – 370,471.509 liters in 2016
    - Target – 450,000 liters in 2018
    - Actual - 420,647.92 liters in 2018
    - Conclusion – wine produced in Oklahoma rose 13.5% during the timeline of the grant. However, as the bulk of the marketing of the grant project happened in the 1st quarter of FY19, it makes sense that the production of wine in the state would not see a large bump until the next harvest in July/August of 2019, therefore causing a lag in production numbers related to these reports.
    - Performance Monitoring – Reports are gathered by the Oklahoma Tax Commission on a monthly basis. Oklahoma Agritourism will share this data to specialty crop stakeholders through email databases and public meetings held by ODAFF, Oklahoma Cooperative Extension and Oklahoma Grape Industry Council.

- Expected Outcome: Increase in wholesale and retail sales of wine made in Oklahoma
    - Performance measure – OTC reports
    - Benchmark – $113,973.54 in 2016
    - Target – $150,000 in 2018

- Actual - $128,828.44 in 2018
- Conclusion – sales of wine produced in Oklahoma rose 13% during the timeline of the grant. However, the bulk of the marketing of the grant project happened in the 1st quarter of FY19 in which the Oklahoma Tax Commission has not numbers available from at the time of this report. Results are expected to increase in FY19.
- Performance Monitoring – Reports are gathered by the Oklahoma Tax Commission on a monthly basis. Oklahoma Agritourism will share this data to specialty crop stakeholders through email databases and public meetings held by ODAFF, Oklahoma Cooperative Extension and Oklahoma Grape Industry Council.

- Expected Outcome: Increase consumer engagement in the form of likes, comments and shares on social media posts and visits to website.
  - Performance measure – Facebook Insights and Google Analytics
    - Benchmark for website, www.OklahomaWineTrails.com
      - 2014 – 3,644 page views
      - 2015 – 10,686 page views
      - 2016 – 10,404 page views
      - 2017 – 16,357 page views
    - Target for website – 2018 – 20,000 page views
    - Actual 2018 – 20,090 as of Nov. 30
    - Conclusion – Social media advertising made a big difference in the increase in page views during the grant period. December is historically has several thousand additional page views so the total 2018 number is expected to be higher.
  - Performance monitoring – Google Analytics allows for up to the minute statistics at any time. Oklahoma Agritourism will share this data to specialty crop stakeholders through email databases and public meetings held by ODAFF, Oklahoma Cooperative Extension and Oklahoma Grape Industry Council.
    - Benchmark for social, www.Facebook.com/OKAgritourism
      - 2014-2016 – no targeted marketing for Oklahoma Wine Trails
      - 2017 – Three rotating ads distributed with targeted demographics, running mid-August to mid-November
        - Total number of impressions: 267,060
        - Total number of clicks: 7,204
        - Total number of comments, shares and likes: 1,237
      - Target – 2018 – combination of rotating ads and videos with targeted demographics

        - Total number of impressions: 500,000
        - Total number of clicks: 20,000
        - Total number of comments, shares and likes: 4,000
    - Actual – 2018 – combination of rotating ads and videos with targeted demographics
        - Total number of impressions: 1,080,000
        - Total number of clicks: 16,673
        - Total number of comments, shares and likes: 2,487
        - Conclusion: Although the ads did not quite meet all of our targets, every statistic more than doubled rendering the campaign a success.
  - Performance monitoring – Facebook Insights allows for up to the minute statistics at any time. Oklahoma Agritourism will share this data to specialty crop stakeholders through email databases and public meetings held by ODAFF, Oklahoma Cooperative Extension and Oklahoma Grape Industry Council.

- Expected Outcome: Increased traffic in tasting rooms at Oklahoma Wineries.
  - Performance measure – Survey
    - No previous data available. Survey will include year comparison questions
  - Benchmark – no previous data available
  - Target – 20% increase in traffic
  - Survey Results:
    - 52 online surveys sent, 20 responses gathered
    - 73% of respondents say they have seen an increase in traffic to their wineries tasting room as a result of the Oklahoma Wine Trails map and social media promotion
    - 42% of respondents say sales have increased from their tasting room as a result of the Oklahoma Wine Trails map and social media promotion
    - 21% of respondents say the success of the Oklahoma Wine Trails promotion has inspired them plant more grapes and other fruit in order to keep up with demand
  - Performance monitoring – Will send email surveys to wineries with tasting rooms and vineyards in the Oklahoma Agritourism program. Oklahoma Agritourism will share this data to specialty crop stakeholders through email databases and public meetings held by ODAFF, Oklahoma Cooperative Extension and Oklahoma Grape Industry Council.

BENEFICIARIES

Oklahoma wineries and vineyards are the main beneficiaries of the Oklahoma Wine Trails as well as the communities where the businesses are located. The 2018 version of the Oklahoma Wine Trails printed map and passport featured 45 wineries and 7 separate vineyards. The venues are located in two metropolitan areas, two towns with populations around 20,000 and 25 towns and rural communities with populations less than 5,000. Tourism traffic created by visitors using the Oklahoma Wine Trail materials will have an economic impact however that data is not available at this time.

LESSONS LEARNED

The biggest challenge with this project results from the biggest asset. The wine industry in Oklahoma is growing so fast, that new wineries are opening up every several months. Staff is able to keep up with the changes by updating the online database frequently, but this makes the printed version of the map obsolete very quickly. In order to combat this, staff tries to inform the public as much as possible that they need to check the website before planning trips.

Another challenge that has arisen is the availability of winery owners to be open enough days and hours to satisfy the demand in visitors. With a burgeoning business, the owners often are pulled away to festivals and farmers markets in order to sell their products on what would be the busiest visitor day of the week. Staff tries to caution venue owners of this dilemma so they are prepared to either hire extra staff, or understand their losses.
A surprising development came when visitors started asking staff and winery owners about other things to do in the area such as overnight accommodations, food establishments and other tourist oriented businesses. This is hopefully a sign of increased tourism to the communities surrounding the wineries with a positive economic impact.

The quantitative data set by the original grant proposal seems to have been a bit optimistic as some number goals were not met. In some areas, production of wine was hindered by crop failure due to weather conditions. Several large producers also closed their business due to personal reasons, thereby skewing the production numbers down. Overall, the project saw initial success, but number goals might take several years to materialize. The printed material is expected to stay in circulation and will be updated using state funds so as to ensure fresh content gets into consumers hands as soon as possible.

CONTACT PERSON

Meriruth Cohenour (405) 522-5652
Meriruth.cohenour@ag.ok.gov

ADDITIONAL INFORMATION

www.OklahomaWineTrails.com

Photo: Map, Passport and several examples of stamps used by wineries to mark visitors passports after a visit


Wine Trails
Oklahoma
Vineyards to Visit
How to Read a Wine Label
Centennial
PASSPORT
PASSPORT
Oklahoma Wine Trails
Est. 2014
Oklahoma Agritourism