North Carolina Department and Agriculture & Consumer Services

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2015 Specialty Crop Block Grant Program

15SCBGPNC0003

Final Report

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Table of Contents:

Seasonal High Tunnel Outreach and Education* 3

Improving Value Propositions- NC Nursery Crop* 12

Increase Competitiveness of NC Potatoes* 19

Enhancing Online Outreach for NC Strawberries* 24

Branding NC Sweet Potatoes* 28

Wild-simulated Ginseng Cultivation & Marketing Initiative* 32

Recordkeeping and Crop Insurance Eligibility* 38

Evaluating Christmas Tree Postharvest Practices 42

Integrated Control of Cucurbit Downy Mildew* 65

Managing Black Rot in Sweet Potatoes* 73

Managing Phytophthora Blight of Pepper* 79

Somatic Embryogenesis of Fraser Fir 88

GAP Assistance to NC Growers 121

*Final Report previously accepted in 2nd Annual Report

PROJECT TITLE Seasonal High Tunnel Outreach and Education*

PROJECT SUMMARY

Project Purpose

Specialty crop producers can increase their competitiveness in the local produce market by utilizing seasonal high tunnels for specialty crop production. The main advantage of high tunnels is that they allow producers to extend their growing season; producing crops earlier in the spring and later in the fall. The ability to sell crops from a high tunnel when field grown crops are not available enables growers to get a premium for their high tunnel crops. Moreover, being the only one at market with those crops helps build customer loyalty. However, there are many challenges with growing specialty crops in high tunnels including variety selection, soil fertility management, irrigation, and disease and pest management. This project addressed these challenges by developing an integrated, multi-faceted solution that included three of the NCDA Specialty Crop Block's focus areas: 1) improving efficiency and reducing costs of production; 2) documenting organic and sustainable production practices; and 3) conducting specialty crop research.

Federal cost share programs for adopting conservation farming practices, such as the Natural Resources Conservation Service Environmental Quality Incentive Program (NRCS EQIP), provide opportunities for North Carolina specialty crop producers to increase their competitiveness in the local food market. In 2010, the NRCS started a Seasonal High Tunnel Initiative to provide cost share assistance for the construction of high tunnels to provide conservation services that improve plant, soil, water, and air quality, reduce nutrient and pesticide transport, and reduce energy use through local production of fruits and vegetables for local consumption. While no longer an independent initiative, NC NRCS continues to offer financial and technical assistance through the conservation practice, Seasonal High Tunnel System for Crops (798).

Since 2010, NC NRCS has awarded contracts for Seasonal High Tunnels to 581 farmers. Many of these contracts are to "new and beginning" and "historically underserved" farmers. Contract recipients are required to develop production plans that address irrigation, nutrient, and pest management, propose crop rotations plans, and a means of diversion of runoff from the structure. However, many contract recipients lack the production knowledge required to be successful and identify micro irrigation management as an additional obstacle. As a result, some Seasonal High Tunnel recipients have had contracts terminated and many more are in similar danger.

In a 2015 survey that asked about preferences for variety trial efforts by CFSA, a majority (64%) of Farm Services program participants said that high tunnel tomato variety trials would provide the greatest benefit. Tomatoes are a high value crop that is commonly grown in high tunnels. Producers can extend their season in the high tunnel by getting earlier harvests in the summer and later harvests in the fall. Foliar disease pressure is greater in field grown tomatoes than in high tunnel tomatoes. Less disease pressure results in higher yields and fruit quality. Many highly-skilled farmers are grafting tomatoes on to resistant root stock for use in high tunnels, particularly heirloom varieties. Heirlooms are highly

susceptible to soil-borne pathogens, and use of a disease-resistant root stock can significantly reduce infection by pathogens and boost yield. However, it remains unclear whether or not grafted heirloom varieties will outperform non-grafted varieties in a high tunnel when pathogen pressure is minimal.

In 2014, CFSA began overseeing the Elma C. Lomax Incubator Farm located in Concord, NC. Lomax Farm is the only certified organic incubator farm in the Southeast and is a training-ground where beginning farmers can gain access to land and equipment to start their own farm business. Research was conducted in the high tunnel at Lomax Farm in 2016 and 2017 to document best management practices and determine if the increased costs associated with grafted tomato production in high tunnels will be recovered/exceeded through higher yields. We compared yield from three grafted heirloom tomato varieties with the same non-grafted varieties. While there has been some research conducted in NC evaluating the performance of grafted heirloom varieties versus non-grafted, those studies evaluated heirloom performance using Maxifort as the root stock and the results of that work is not readily available to producers. Moreover, since then, new commercially available root stock varieties have entered the market. We evaluated the performance of heirloom varieties grafted on DRO141TX rootstock, developed to maintain high levels of production during long growing seasons with high temperatures, both of which occur when growing in high tunnels. We hosted a grafting workshop and field day at Lomax Farm in 2016 and 2017.

Importance and Timeliness

This project is important and timely because the North Carolina NRCS continues to get applications for Seasonal High Tunnel cost share assistance but does not have the staff capacity to provide technical assistance for neither prior nor new contracts. In order to provide a more successful high tunnel production experience for recipients and to reduce the incidence of contract termination, CFSA provided educational opportunities, technical assistance, and guidance documents to high tunnel producers.

PROJECT APPROACH

Activities Performed

In order to improve the competitiveness of NC specialty crop producers in the local produce market by utilizing seasonal high tunnels for specialty crop production we provided on-farm high tunnel production training to specialty crop producers, determined if the increased costs associated with grafted tomato production in high tunnels is recoverable through higher yields, and published two resources to help specialty crop producers growing in seasonal high tunnels. Specifically we:

- Conducted 12 workshops for 276 program participants;
- Provided direct consulting services (site visit, ongoing assistance) to 20 specialty crop producers;
- Provided technical information to 11 specialty crop producers;
- Conducted two years of high tunnel research comparing grafted versus non-grafted heirloom tomatoes;
- Published the *NC Organic Seasonal High Tunnel Tomato Production Guide* and the *NC High Tunnel Micro Irrigation Guide*; and
- Wrote five expert tip articles on organic production.

Benefits to Non-Specialty Crop Producers
All direct consulting program participants were specialty crop producers, workshop topics only covered specialty crop production in high tunnels, and the resource guides only include information on specialty crops. Therefore, the project did not benefit commodity producers.

Significant Contributions from Project Partners
The success of this project relied on significant contributions from project partners who helped host workshops and promote both workshops and our direct consulting services. Significant project partners included: 1) the NC NRCS StrikeForce Initiative; 2) the Smokey Mountain High Tunnel Initiative; 3) NC Cooperative Extension; and 4) North Carolina Growing Together. These partners, along with other extension agents, NRCS staff, and partner organizations helped promote our workshops and consulting services. Additional partners reviewed online resources, including NRCS and extension staff, and farmers.

GOALS AND OUTCOMES ACHIEVED

GOAL 1: Increase the success of NRCS Seasonal High Tunnel recipients by providing on-farm high tunnel production training to 170 specialty crop producers, twenty of whom will receive one-on-one training.

Promoted CFSA's seasonal high tunnel assistance program through direct emails, follow up emails with producers that previously expressed interest, at tabling and speaking events, and through our monthly electronic newsletter, including five expert tip articles (Table 1). Provided technical information to 296 specialty crop producers, of those producers 20 received one-on-one consultation (site visit, ongoing assistance) in order to increase their success growing in seasonal high tunnels (Table 2). Based on survey results, one-on-one consulting program participants participated sought high tunnel consulting services to learn about high tunnel planning (65%), purchasing and constructing (18%), irrigation (24%), and assistance addressing specific issues (35%). All program participants said they gained sufficient information enabling them to incorporate recommendations and 88% of them rated the consulting services as excellent or very good. Forty-three percent said they sold less than $2,500 from their high tunnel production, 36% sold $2,500 to $5,000 and 21% sold more than $5,000. Of those 21%, sales ranged from $12,000 to $40,000. Program participants identified tomatoes, cucumbers, peppers, greens, and herbs as their most profitable crops.

Table 1: Expert tip articles in CFSA's electronic newsletter from Jan. 1, 2015 to Jan. 31, 2016.

Expert Tip	Unique Views
Irrigation in High Tunnels	191
When to Plant in YOUR High Tunnel	255
Reducing Disease in Field Tomatoes	283
Successful Transplant Production	69
Grafting Heirloom Tomatoes	229

Table 2: Program participants who received direct consulting, which included a site visit and ongoing technical assistance through the Seasonal High Tunnel Outreach and Education program.

Antlion Acres, Wilmington	New Ground Farm, Pembroke
Brothers On Farm, Hayesville	Peacehaven Farm, Whitsett
Carolina Flowers, Marshall	Red Hawk Farm, Pittsboro
Corn Hill Farms, Kittrell	RominDale Farm, Clemmons
Crews Farm, Henderson	Rowlands Row Family Farm, Gold Hill
Dinner Bell Farm, Snow Camp	Spring Forth Farm, Hurdle Mills
Elam Gardens, Greensboro	Spring Haven Farm, Chapel Hill
Farmsland, Parkton	Stonehouse Farm and Produce, Whitakers
Lyons & Wade Family Farm, Raeford	Sunset Market Gardens, Reidsville
Maters and More, Mt Pleasant	Thankful Fork Farm, Lenoir

Conducted 12 workshops on seasonal high tunnel production to 276 specialty crop producers, extension agents, and government agriculture agency representatives. Based on pre- and post-test questionnaires 61% of workshop attendees increased their knowledge of high tunnel management and 72% gained confidence determining what to grow in a high tunnel based on their participation in the workshops. Workshops included:

- **When to Plant in a High Tunnel, Cherokee County, NC on Feb 18, 2016 (30 attendees).** Topics included 10 Best Warm Season Crops, 10 Best Cool Season Crop, and When to Plant in you High Tunnel.
- **Tomato Grafting, Concord, NC on Feb 25, 2016 (7 attendees).** Topics included how to graft, traits of heirloom tomatoes, and high tunnel production.
- **Season Extension Technology in Wholesale Production, Mount Olive, NC on March 7, 2016 (38 attendees).** Topics included using high tunnels and other technology to achieve season extension.
- **High Tunnel Production and Harvesting, Cherokee County, NC on July 12, 2016 (13 attendees).** Topics Included high tunnel production and marketing and GAP.
- **Cool Season Crops and High Tunnels, Cherokee County, NC on Aug 23, 2016 (8 attendees).** Topics included cool season crop planning and irrigation.
- **Using Season Extension Tunnels for Year-Round Production, Durham, NC on November 3, 2016 (43 attendees).** Topics included high tunnel construction, crop planning, connecting and connecting with buyers.
- **Farm Services Expo, Murphy, NC on Dec 6, 2016 (30 attendees).** Topics included crop planning for seasonal high tunnels.
- **High Tunnel Production and Management, Jacksonville, NC on Feb. 8, 2017 (41 attendees).** Topics included introduction to high tunnel production and financial planning.
- **High Tunnel Construction Demonstration, Cary, NC on February 11, 2017 (10 attendees).** Topics included high tunnel construction and crop planning.
- **Grafted Tomato Production in High Tunnels, Mount Olive, NC on March 6, 2017 (18 attendees).** Topics included how to graft, traits of heirloom tomatoes, and high tunnel production and preliminary study results.

- **Grafted Tomatoes in High Tunnels, Concord, NC on March 27, 2017 (18 attendees**). Topics included how to graft, traits of heirloom tomatoes, and high tunnel production and preliminary study results.
- **Grafted Tomatoes in High Tunnels, Durham, NC on November 4, 2017 (20 attendees).** Topics included how to graft, traits of heirloom tomatoes, and high tunnel production and study results.

GOAL 2: Determine if the increased costs associated with grafted tomato production in high tunnels will be recovered/exceeded through higher yields by conducting trials at the Lomax Farm comparing the yields from three grafted heirloom tomato varieties with the same non-grafted varieties under organic management.

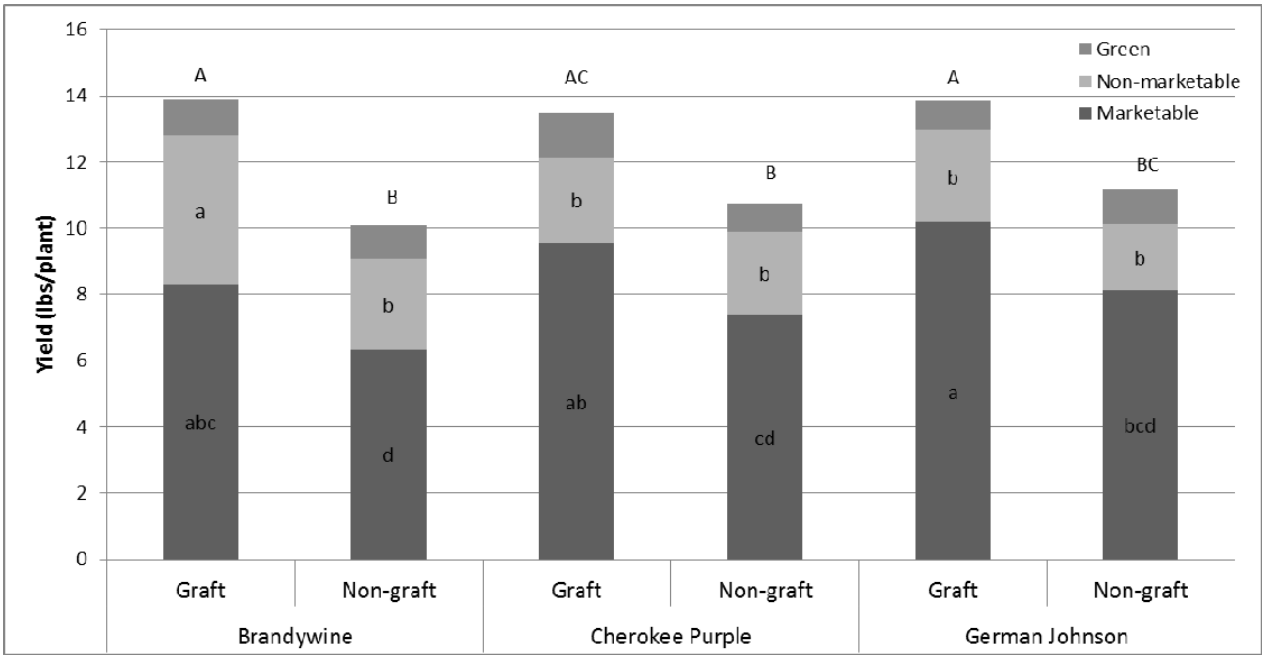
Research was conducted in 2016 and 2017 in a high tunnel at the Elma C. Lomax Farm in Concord, NC. 'German Johnson', 'Cherokee Purple', and 'Brandywine' tomato varieties were grafted onto DRO141TX on February 29 and March 1, 2016 and on February 28, 2017. Seeds for non-grafted treatments were started in the greenhouse 26/January 2016 and 27/January 2017 'German Johnson', 'Cherokee Purple', and 'Brandywine' tomato varieties were transplanted into plastic mulch on March 22, 2016 and March 24th, 2017. Plants were pruned once at the first flower cluster on 28/March 2016 and 31/March 2017 and staked and tied according to the Florida method. Plants were irrigated with a drip irrigation system daily.

A randomized split plot design was used for the experiment with six treatments replicated six times. Each treatment row was 4.6 meters long with 10 plants spaced 0.6 meters apart. Treatments included 1) Brandywine/DRO141TX, 2) German Johnson/DRO141TX, 3) Cherokee Purple/DRO141TX, 4) Brandywine, 5) German Johnson, 6) Cherokee Purple.

Weekly harvests of ripe fruit were conducted from 18, May to 22/July 2016 and 26/May to 28/July 2017. Tomatoes were sorted as marketable, unmarketable (diseased, cracked, or zippered) or green and then counted and weighed (Figure 1). Grafted treatments yielded similar marketable harvest amounts showing no statistical difference among varieties. Grafted German Johnson yielded a statistically greater marketable harvest as compared to all un-grafted treatments. Grafted Cherokee Purple, grafted Brandywine and un-grafted German Johnson showed similar marketable harvests and all statically greater then un-grafted Cherokee Purple and in-grafted Brandywine. Grafted Brandywine yielded a higher unmarketable harvest as compared to all other treatments. Green harvests amounts were statistically similar among all grafted plants showing great yields as compared to un-grafted treatments with the exception of similarity between grafted Cherokee Purple and un-grafted German Johnson.

Yield data were analyzed in R version 3.4.2 (R Core Team, 2017); differences among treatments were tested using Linear Mixed EffectsModel ANOVA (package *nlme*), with year as a random effect and grafting and variety as fixed effects, and means were separated using Least-Squares Means (package *lsmeans*).

Figure1. Tomato fruit yields (lbs per plant) of marketable, non-marketable and green fruit between grafted and non-grafted plants of three varieties (Brandywine, Cherokee Purple, German Johnson. Different lowercase letters indicate significant differences ($p<0.05$) across combined treatments for each class. Different uppercase letters indicate significant differences ($p<0.05$) across combined treatments for total yields (marketable + non-marketable + green).



Disease and insect pest pressure was monitored by visual observation weekly from 22/March to 24/July 2016 and 24/March to 28/July 2017. There was very little disease pressure, which is to be expected as disease management one reason for growing tomatoes in high tunnels.

Plants showed signs of stress, including foliar discoloration and lesions, in May 2016. Diagnostic testing submitted on 28/May 2016 confirmed foliar damage caused by high soil salt content, a common issue with high tunnel production. Following harvest of tomato plants on July 2016 sunflowers were planted and irrigated to reduce salt content in the high tunnel and included discussions on it in workshops and at the 2017 Lomax Field Day.

Plants also showed signs of stress, foliar discoloration and plant death in June 2017. Diagnostic testing submitted on 7/July 2017 confirm root knot nematode damage on un-grafted plants. Following total plant harvest in July 2017 high tunnel soil was solarized to reduce nematode damage in future crops and also included discussions on it in workshops.

The amount of time and inputs were recorded during both growing seasons in order to determine if the increased costs associated with grafted tomato production in high tunnels would be recovered through higher yields (Table 3).

Table 3. Estimated Economic Analysis for Grafted Versus Un-grafted Tomato Production in a 30' by 96' High Tunnel.[1]

ITEM	GRAFTED	UN-GRAFTED
Transplant Production		
Labor[2]	$505	$70
Supplies	$130	$45
Subtotal	*$635*	*$115*
In-Season Production		
Labor	$1,540	$1,540
Supplies	$615	$615
Subtotal	*$2,155*	*$2,155*
PRODUCTION TOTAL	**$2,790**	**$2,270**
Return		
Marketable Fruit	1,880	1,545
Revenue[3]	$4880	$4,017
NET PROFIT	**$2,098**	**$1,747**

[1]Based on 180 plants per 270 row feet in a 30' by 96' high tunnel.
[2]Calculated at a rate of $15 per hour based on time valued by local farmers.
[3]Calculated on a rate of $2.60 per pound based on average 2017 organic tomato prices in the Southeast (USDA Market News, 2017).

The use of grafted German Johnson, Brandywine and Cherokee Purple tomato plants in high tunnels can be an effective production method for farmers in North Carolina. Heirloom tomato plants are sought after for their flavor in many markets, however due to disease susceptibility and low harvest amounts many famers avoid growing such plants. While not a new technology, grafting tomatoes has not been widely adopted by tomato growers in NC. We believe this is due to a lack of comfort level grafting tomatoes, and an unawareness of the benefits grafted tomatoes offer, specifically disease management. This project helped fill that knowledge gap by providing specialty crop farmers with information on proper grafting techniques and high tunnel tomato production in order to increase a farmer's success.

In additional to a knowledge gap, uncertainty of the economic viability of grafted tomato production can also be a barrier for many farmers. This research indicated that while Input and labor cost for grafting transplants was greater than transplant production of un-grafted plants, higher marketable yields from the grafted plants compensated for the added cost of grafting.

The results of the research where shared during the annual Lomax Field Days on May 3, 2016 (42 attendees) and May 2, 2017 (20 attendees), at high tunnel workshops in year two, and at CFSA's Sustainable Agriculture Conference on Nov 3, 2017 (20 attendees). We did not publish an Annual Regional Variety Trial Report in 2016 and 2017 due to lack of funding to support that report, however, an economic analysis of grafted versus un-grafted tomato production in high tunnels is included in the *NC Organic Seasonal High Tunnel Tomato Production Guide.*

GOAL 3: Develop and publish the *NC Organic Seasonal High Tunnel Tomato Production* and *NC High Tunnel Micro Irrigation Guides.*

The *NC High Tunnel Micro Irrigation Guide* was published on CFSA's website in December 2016. Topic include: 1) basic soil science; 2) soil wetness; 3) how to determine plant water needs; 4) microclimate considerations; 5) types of micro-irrigation; 6) irrigation capabilities, 7) fertilizer injection; and 8) how to calculate irrigation amounts. The document was reviewed eternally by Joshua Spencer, Water Quality Specialist, NC USDA/NRCS John Beck, Extension Associate, North Carolina A&T University and Joseph Moore, owner/Operator, Mill Creek Farm.

The *NC Organic Seasonal High Tunnel Tomato Production* guide was published on CFSA's website in January 2018. Topics include: 1) variety Selection; 2) assessing the microclimate; 3) creating a production plan; 4) bed preparation; 5) trellising methods; 6) transplant to harvest; and 7) economic and potential profits. The document was reviewed externally by Gordon Mikell, Agronomist, SC USDA/NRCS and John Ivey, Owner/Operator, Jive Farm. It was promoted in our electronic newsletter and has received 176 unique views.

PROJECT ACTIVITY	STATUS
Conduct twenty one-on-one on-farm high tunnel trainings.	Conducted 20
Conduct tomato grafting workshop at Lomax Farm.	Completed 2
Conduct six on-farm workshops on high tunnel production.	Conducted 11
Conduct field days on high tunnel tomato production at Lomax Farm.	Conducted 2
Publish the *NC Organic Seasonal High Tunnel Tomato Production* and the *NC High Tunnel Micro Irrigation Guide* on CFSA's website.	Completed
Survey program participants to document annual sales from high tunnel production.	Completed

BENEFICIARIES

Direct beneficiaries of this project were specialty crop producers who received a Conservation Activity Plan Supporting Organic Transition (CAP 138), received direct technical assistance transitioning to certified organic production, or attending a workshop of organic certification and production. Beneficiaries served include:

TYPE of INFORMATION DISSEMINATION	NUMBER OF BENEFICIARIES
Viewed Online Resources	587[1]
Attended a Workshop	276
Received One-on-One High Tunnel Consultation	20
Received technical assistance producing high tunnels	11

LESSONS LEARNED

[1]Number of views of online resources contains all unique views, not solely those from NC. We are unable to differentiate views by state.

Based on pre- and post-workshop test responses, most of the workshop attendees had very little knowledge or confidence growing in high tunnels. On a scale of 1 to 10, with 10 being the highest, 84% indicated knowledge of management of high tunnels at a 5 or below, and 34% ranked their knowledge level at a one. Confidence determining what to grow in a high tunnel was also very low, 96% of workshop attendees ranked their confidence level at a five or below, and 47% ranked their confidence level as a one. Workshop attendees identified general management (30%), determining planting dates (30%), and soil fertility management (34%) as areas most challenging to them regarding high tunnel production. These three challenges indicate that growers lack some of the basic skills necessary for growing in high tunnels. Moreover, 56% of workshop attendees did not yet have a high tunnel. This indicates a strong need for continued technical assistance on high tunnel production for specialty crop producers to ensure their success growing in high tunnels in order to increase their competitiveness in the local produce market.

The research component of this project provided opportunities for local farmers and the general public to learn about high tunnel tomato production through volunteering. Volunteer hours on this project, for both years, totaled 170 hours. All volunteers were educated on the project and trained to a specific management task(s) regarding high tunnel tomato production including but not limited to: grafting, pruning, suckering, irrigation, and bed preparation. Volunteers included local farmers, gardeners, and community members. The amount of volunteer hours spent on the project indicates the need and interest in hands on learning opportunities regarding high tunnel production.

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ADDITIONAL INFORMATION

Seasonal High Tunnel Consulting landing page provides information about the consulting program.
NC Organic Seasonal High Tunnel Tomato Production provides information on proper high tunnel tomato production to help increase yields and crop success.
NC High Tunnel Micro Irrigation Guide provides a practical process to establish irrigation practices for various crops to be grown in a high tunnel.
Four articles on seasonal high tunnel production

- Irrigation in High Tunnels
- When to Plant in YOUR High Tunnel
- Reducing Disease in Field Tomatoes
- Successful Transplant Production
- Grafting Heirloom Tomatoes

PROJECT TITLE Improving Value Proposition – NC Nursery Crops*

PROJECT SUMMARY

Despite nearly $2 billion in total NC farmgate growth across all commodities during the five-year period between the 2007 and 2012 census, "Nursery, Floriculture, Greenhouse and Sod" production remained statistically flat and comprised less than 5% of the state's total farmgate for the first time since 1997.

According to the latest publicly available data regarding NC nursery stock and floriculture farmgate (USDA Census of Agriculture), combined horticulture and floriculture sales from North Carolina farms was approximately $521 million in 2012. Adjusted for inflation, NC "Nursery Stock Crop" farmgate fell by more than $44 million between the 2007 and 2012 USDA Census. Over that same period, sales of NC floriculture crops fell by approximately $2 million. Using 2007 as a baseline, combined NC nursery/floriculture sales would need to exceed $580 million to return pre-recessionary levels this year, an increase of $59 million (10.2%) from actual 2012 sales.

The demand for NC nursery crops has started to increase as new home and commercial construction began trending upward. Over the last two years, demand seems to be outpacing available supply given the industry's inherent lag time in production. In no small part, marketing programs funded by the SCBG program played an important role in increasing awareness and demand for NC nursery crops from 2011 – present.

Despite this forward momentum, further improvements to the industry's existing marketing platforms will address specific issues and hold the potential to create sustainable growth. For example, NCNLA's current "BuyNCPlants.com" website is a valuable tool that allows wholesale buyers to search through thousands of the nursery stock varieties grown by NC growers that provide listings. More than 25,000 unique visitors used BuyNCPlants.com last year.

Unfortunately, back-end management of BuyNCPlants.com is not user-friendly and precludes many interested nursery growers from wanting to utilize the site for marketing purposes. Only 80 of North Carolina's nearly 1,400 nursery stock producers listed products on BuyNCPlants.com. Further, the current site cannot integrate with the tools that growers use to manage crop inventory at their operation, making inventory management on BuyNCPlants.com impossible at present. Finally, BuyNCPlants.com is not responsive and cannot capture information from potential wholesale purchasers regarding products/quantities in which they are interested at present or in future years. An overhaul of the site is required, and these issues must be addressed in order to increase the marketability and usability of BuyNCPlants.com.

Another issue addressed is the need to further increase the consumer's perceived value of a well-designed and maintained landscape and the value of locally-grown plants. Few homeowners realize that, when properly positioned and maintained, trees and landscape plants can offer substantial savings on home heating and cooling costs while simultaneously providing significant environmental benefits to themselves and their surrounding community. A well-designed and maintained landscape improves home values, appreciating in value along with the property value of the land surrounding a home as the structure itself depreciates.

Even fewer homeowners understand the value of locally-grown plants, trees and shrubs that are tested and have endured in North Carolina nurseries under similar biotic and abiotic stresses as the products will experience in their home landscape. Redevelopment of NCNLA's "TarheelGardening.com" website, as well as the development of a new website to better promote and monetize these benefits, will increase consumer awareness of the value that NC trees and landscape plants can offer.

The next generation of consumers relies on the internet to research product quality and cost before purchasing materials either at a physical location or online. In a recent survey conducted by marketing firm GfK, nearly 1/3rd of consumers shopped using an omni-channel method, combining internet research with a physical visit to purchase lawn and garden materials. Unfortunately, these younger consumers are often put off by the industry's tendency to focus on the term "gardening," which connotes a hobby for someone with a significant amount of leisure time, and on more scientific functions of plants and terminology that they may perceive as intimidating.

The development of a new consumer-facing website that employs marketing methods used by other products (e.g., cars, wine, etc.) for the next generation of plant consumers would alleviate many of the real and perceived concerns of the next generation of plant consumers. By focusing more on images, brief descriptions, and basic plant characteristics (height, color, and appearance), younger consumers could select from among their desired characteristics to narrow their choices. In turn, simplifying the research process and connecting young consumers with retailers and landscape design firms that source NC nursery plants would most certainly increase consumption.

The final issue that the project addresses concerns both business and consumer recognition of NC nursery stock quality. In general, NC's nursery crop growers need to improve product quality while augmenting their business and marketing practices. While most current industry certifications and licensures in the state are focused on individuals, few (if any) focus on general growing and/or operating practices for a business entity.

The development of a business certification/branding initiative that recognizes improved agricultural, handling and manufacturing processes through the implementation of best management practices would have the dual impact of improving the quality of plants produced in North Carolina while permitting growers to benefit from the implementation of BMPs under a new brand that recognizes these proficiencies for marketing purposes.

Nursery growers have been forced to transition from a period of unsustainable growth (2000 – 2007) to a time of severe business contraction (2008 – 2012). Farm income from nursery crops is an important part of the economy across the state and replaced a significant amount of the state's total farmgate lost from declining tobacco production over the 10 years from 1997 - 2007.

The industry is beginning to recover. However, important issues have been identified that must be addressed to continue profitable and sustainable growth. To insure NC growers are competitive with growers from other states they need more efficient marketing tools to communicate with buyers.

The Census estimates continued strong population growth for the state of North Carolina. From 2013 to 2014, NC's population grew by more than 95,000 individuals, with a net migration increase of 57,445 people. As residential construction continues to increase across North Carolina, prospective homeowners across the state represent the potential for substantial sales growth if the nursery industry

can better demonstrate the value and benefits of locally grown plants and well-designed and maintained landscapes.

The green industry's leading ag economist, Dr. Charles Hall of Texas A&M, has conducted extensive research on this issue as documented in "Economic, Environmental, and Health/Well-Being Benefits Associated with Green Industry Products and Services." Nursery growers across the state must have the mediums available to improve upon demonstrating the value of its products and services to capitalize on emerging positive trends. This project is directed at improve the tools necessary to deliver that message.

Beyond its own borders, North Carolina's nursery industry remains very favorably positioned, both geographically and in terms of supporting resources. Despite recent setbacks, North Carolina remained among the country's largest nursery and floriculture production states, with combined nursery/floriculture sales in excess of $521 million. With enhanced marketing capacity and a more targeted focus, the opportunity for growth is considerable.

The state's diverse climatology and soil composition afford the production of trees and landscape plants that are viable through growing zones across the mid-Atlantic and northeastern U.S., extending up into Canada. Among the states that eclipse NC's nursery/floriculture production (California, Florida, Texas, Oregon, Michigan), none are as climatologically diverse or as strategically positioned geographically to meet the demand for plants where the majority of U.S. residents reside.

A window of opportunity exits as the economy and construction improves. In order to take full advantage of potential sales for NC nursery crops, the industry needs to address these important issues. This project plays a critical role in meeting these needs and help in recovering the more than $60 million lost in annual sales from 2008 – 2012.

The 2011 The SCBG project, "Market Recovery Initiative for the North Carolina Nursery and Greenhouse Industry" and the Cycle 6 SCBG project "Marketing Support for the North Carolina Nursery Industry" have made important progress impacting awareness and demand for nursery plants over the past three years. Our consumer web site, TarheelGardening.com, has increased from 27,000 visits in 2010 to 180,000 in 2014. Our website for wholesale buyers, BuyNCPlants.com, has increased 75%, from 12,000 to 21,000 visits.

PROJECT APPROACH

Task: Survey both NCNLA member and non-member growers, collect NCDA&CS data from Ag. Statistics division, collect web site traffic data for 2015 to establish benchmark data.

In September 2016, the North Carolina Nursery and Landscape Association (NCNLA), Raleigh, NC, hired Stratton Media Strategies to conduct a member/nonmember insight study. The study was designed to provide research and analysis to help NCNLA evaluate its products, its position in the market, and its credibility, and identify unmet needs and opportunities, among other areas. NCNLA represents approximately 1,200 nurseries, wholesalers, and other producers in North Carolina.

Responses break down as follows:
Total emailed.....4,057

—Undeliverable/bad address/duplicates 482
Net effective emailing 3,575
Total responses 812
RATE OF RESPONSE (812 ÷ 3,575) 22.7%

Task: **Meet with web designer to determine content of new website design. Review progress and provide input on design.**

In 2016, NCNLA hired MMI Publications to develop content and strategize implementation of the New Consumer website, NCGreenprints.com

Task: Meet with marketing consultant to review available marketing research and set up focus groups. Conduct proprietary market research analysis on consumer trends/sentiment. Review proposed marketing messages and results of focus groups to determine content for advertising.

NCNLA's contractor, MMI, researched and analyzed market data about consumer trends. The marketing messages implemented focused on the results of the data. The data identified that each generation segment of consumers had different priorities for their money. Each segment showed a difference in value of the landscape in their home. The task at hand was to identify a marketing strategy that spoke to each generation segment.

Task: Meet with NCNLA marketing task group to determine standards and requirements for new "Carolina Brand" certification for best management practices.

NCNLA ran into complications implementing this task effectively. This is planned to continue after this program is over when NCNLA can efficiently and effectively develop and promote the project.

Task: Launch new Carolina brand program to growers and promote participation.

NCNLA ran into complications implementing this task effectively. This is planned to continue after this program is over when NCNLA can efficiently and effectively develop and promote the project.

Task: Launch new BuyNCPlants website and advertise to wholesale buyers.

New website was launched in July of 2016. NCNLA marketed to North Carolina nurseries and suppliers to advertise in the platform.

There are 437 companies that list their plant material on this website and NCNLA is marketing the new site across the state and eastern half of the United States. NCNLA attended four national trades shows over the course of the project to advertise the new site. After the project is complete NCNLA intends to continue to make improvements and updates to the site.

NCNLA has successfully reached 6,040 user sessions from October 2016 to December 2017 and 89.1% of these users are new to the site.

Task: Launch updated TarheelGardening website and new website and advertise to end consumers.

New “Consumer” website was launched in September 2016. The new marketing concept will generate a new website targeting consumers. This new site is named NCGreenPrints.com. This website hosts “Greenprints” designed as blueprints to help homeowners develop horticulture projects for their home.

The North Carolina Nursery and Landscape Association (NCNLA), Raleigh, NC hired MMI Public Relations & Horticulture expert, Bryce Lane to develop content and messaging for the website and advertising campaigns. These campaigns have been launched and have reached over 9,357,000 impressions from April 4, 2017 thru December 31, 2017.

Campaign	Impressions
Custom Audience Modeling & RTG	7,039,378
Facebook News Feed	1,543,037
YouTube TruView	752,074
Retargeting	22,746
Grand Total	**9,357,235**

Task: Promote new marketing message and “Carolina Brand” to wholesale buyers and consumers.

This message was developed into the NCGreenprints website and promoted through the consumer site.

Task: Make appropriate adjustments to programs and advertising/marketing strategies to ensure growth and sustainability beyond grant term length.

NCNLA is continuing to work with contractors to implement updates and strategies to ensure growth and sustainability beyond the project term.

Task: Promote BuyNCPlants.com to buyers at NCNLA trade shows and various industry trade show throughout the mid-Atlantic and northeastern U.S. Promote “Carolina Brand” at NCNLA trade shows to domestic and international wholesale buyers.

NCNLA attended four trade shows throughout the project term. Cultivate is a national convention out of Columbus, OH. Cultivate had roughly 10,000 industry professionals that attended over the course of the four days each year. NCNLA attended Cultivate 2016 & July 2017. New England Grows is a national trade show in Baltimore, MD. New England Grows had roughly 750 attendees over the course of the 2 days each year.

The overall scope of the project’s benefitted commodities did not change.

The North Carolina Nursery & Landscape Association was the only contributors to this grant project. NCNLA performed the tasks to complete the project such as meetings, content development, and education to learn the new platforms in order to effectively promote them to the end user.

GOALS AND OUTCOMES ACHIEVED

NCNLA developed concepts and marketing tools to implement throughout the course of the grant.

Methods of development included research and collaboration with leading industry professionals.

1. Measurable Outcome: Increase Farm Income
The overall **goal** of this project is to increase the value and demand for NC nursery crops.

Performance measure: Change in nursery farm income
Target: Increase sales by 5% by late 2016 and increase sales an additional 5% by late 2017.
Benchmark: NCDA&CS agricultural statistics division annual farm income, NCNLA annual grower survey data at the end of 2015 and 2016 and an analysis of the 2017 Census of Agriculture.
Progress Achieved: The 2017 Census of Agriculture is not available when final report was due.

2. **Measurable Outcome: Increase Wholesale Buyer Engagement**
Our **goal** is to provide a more efficient marketing tool through BuyNCPlants.com and increase the use of this site among wholesale buyers connecting with NC growers.

Performance measure: Buyers that visit our website, BuyNCPlants.com, to locate and source plants from NC nurseries.
Target: Increase buyer use of this website by 5% - 10% in 2016 and an additional 5% in 2017 compared to the 2015 visits.
Benchmark: 2015 visits: BuyNCPlants website: 6,100 visits
Progress Achieved: NCNLA has successfully reached 20,454 user sessions from October 2016 to December 31, 2017 and 94.1% (18,384) of these users are new to the site.

3. Measurable Outcome: Increase Consumer Awareness
Our **goal** is to increase consumer awareness of NC grown plants, the benefits of landscaping, availability of new plant varieties from NC nurseries, benefits of professional certifications and to assist consumers in locating sources of NC plants. NCNLA currently maintains a consumer website and blog, TarHeelGardening.com, that provides such information. SCBG funds would be used to augment TarheelGardening.com and to develop a new site that is more user-friendly to younger consumers.

Performance measure: Number of people that visit our consumer website and blog, BuyNCPlants.com, to locate sources of plants and landscaping information.
Target: Number of people that visit our consumer website and blog, BuyNCPlants.com, to locate sources of plants and landscaping information as well as the new site. For the new, unnamed site, our target would be to establish a baseline of 25,000 unique visitors in 2016 and a 100% increase from that benchmark in 2017.
Benchmark: Total visits for 2015.
Progress Achieved: The baseline of 25,000 unique visitors was achieved in 2016 and the second phase of the ad campaigns were launched and have reached over 9,357,000 impressions from April 4, 2017 thru December 31, 2017. Increasing the unique visitors to NCGreenprints.com 20,484.

Campaign	Impressions
Custom Audience Modeling & RTG	7,039,378
Facebook News Feed	1,543,037
YouTube TruView	752,074
Retargeting	22,746
Grand Total	**9,357,235**

BENEFICIARIES

The beneficiaries of the project will be the approximately 1,400 nursery growers across North Carolina. Beneficiaries also include approximately 30,000 people employed by the nursery industry and in related rural green industry businesses. In addition, over 1 million consumers and 20,000 wholesale buyers will benefit from the information they receive about NC plants and landscaping.

The nursery industry is at a critical point in its recovery. Marketing experts recognize that in order to reach the full benefits of a marketing strategy, it must be consistent and continuous over several years. This project will build on the progress made with previous SCBG marketing projects by addressing key marketing issues over the next two years and will provide the maximum economic impact from the funds invested.

This project will provide a positive economic impact on the nursery industry through increased farm income and on local communities through increased employment. The potential to increase annual nursery farm income by $100 million and increase nursery employment by 30% back to pre-recession levels is possible over the next 3 years. Currently, there is approximately 30% under-utilized production capacity in NC nurseries. As nursery plant sales increase, growers will be able to increase employment and expand production. This project will play an important role in this growth.

LESSONS LEARNED

There were no delays or unexpected outcomes during the project.
All goals were achieved.

CONTACT PERSON

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PROJECT TITLE Increase Competitiveness of NC Potatoes*

PROJECT SUMMARY

The main goal of this project was to continue to enhance our approach to potato marketing and to provide funding to increase the competitiveness of North Carolina potatoes. We believe that this will increase the NC Potato Association's (NCPA) ability to market/promote potatoes produced in the State so that NC potato growers can effectively compete for market share with potatoes from other growing areas. The project enabled us to support and enhance our potato marketing efforts to effectively compete with potatoes from other growing areas that are available to the market during the same time frames. During recent years, the NCPA's ability to fund these projects through their grower assessments has been reduced due to increase in marketing expenses; such as increase in trade show costs, produce publications ads, etc.; and this has limited our ability to maintain and/or expand into new markets. This project planned to use the grant funds to continue and build on our current potato marketing efforts to promote North Carolina potatoes to benefit our industry. These efforts include state, national and international potato promotions.

To maintain and expand markets we must actively and aggressively market our crop. The potato markets in the eastern U.S. are highly competitive and market conditions continue to change. Over the course of the last twelve years potato acreage in NC has fluctuated from approximately 21,500 acres in 2002 to 14,500 in 2008, 17,000 in 2011, 16,500 in 2012, and to 13,500 acres in 2013. Current acreage is approx. 14,000 acres. Continual potato marketing efforts are needed as they benefit our growers, and they help to stabilize markets, enhance competitiveness, and improve profits and the long-term sustainability of NC potato farms. NC's potato industry supplies potatoes during a key market window when supplies of stored potatoes from other regions in the U.S. and Canada are in short supply and/or of reduced quality. Our farmers serve the potato industry at a critical time by providing a high-quality supply of fresh potatoes for the chip and table markets. Competition for market share is very high and our growers/industry must be creative and aggressive in maintaining current markets and acquiring additional markets.

The previous awarded grant from the NCDA-SCBGP in 2012 was used to successfully establish marketing projects for the NC potato farmers/industry. Similar marketing projects were continued in the 2015 grant. Both grants enabled NCPA to exhibit at national and international trade shows attended by corporate buyers. This enabled the NC potato industry to establish and enhance relationships with these national and international buyers, significantly enhancing awareness of the NC potato industry to the corporate buying industry. Funds were used in 2012 grant to redesign the NCPA website and in 2015 grant to update the NCPA website and to make videos to place on the site. This significantly enhanced the NC potato industry to promote their industry to buyers and consumers. A grower directory was placed on the site, showing varieties, each grower produces, location of the farms, and packing capabilities of the farms. Funds from both grants (2012 and 2015) allowed North Carolina State University(NCSU) potato researcher Mr. Mark Clough to travel to Potato Expos and meet/collaborate with fellow potato researchers from states such as Maine, New York, Florida, New Jersey, Michigan, Wisconsin and USDA potato research leaders. Mr. Clough works with some of these states and USDA research programs in selecting potato varieties to use in the NCSU research variety trails, enhancing NCSU's ability to breed NC varieties for the NC growers/industry use in chip and table markets. He collaborates with these states and USDA in his research efforts.

PROJECT APPROACH

1)NC Potato Association exhibited at the 2016 Potato Expo trade show held at The Mirage in Las Vegas, January 12 14 and 2017 Potato Expo held in San Francisco January 4-6. This is a potato specific event attended by US and Canada potato industry reps and buyers. Exhibiting at the Potato Expo creates and enhances awareness of the NC potato industry and presented very positive opportunities for meetings with buyers.

2) Ads were purchased and ran in the National Potato Council(NPC)2016 and 2017 Stat Book. This is a potato industry wide publication and ads bring awareness of NC potatoes to the national industry. NC is not a major potato producing region, but timing of harvest during June and July creates significant marketing opportunities for NC potatoes as the major potato producing regions in the US and Canada are storage states and their potatoes in storage are ending with quantities and quality. Ads: hard copy and digital, were also placed in Produce News and The Packer produce publications during May and June of 2016. Ads: hard copy and digital, were also placed in The Packer during June 2017and digital ads in Southeast Produce News Weekly(SEPNW) produce publications during May and June of 2017 and October 2017. SEPNW publisher Chip Carter conducted interviews/videos of NC growers and buyers during the NCPA annual meeting and featured the videos and include articles about the NC potato industry during May, June during NC potato harvest season and October before the Produce Marketing Association convention. These ads bring NC potatoes great visibility to retail and wholesale buyers who subscribe to these publications.

3)Bus was leased for tour of NC potato farms, NCSU potato variety research plots, and potato facilities during the 2016 and 2017 NCPA annual meeting held in May. Potato buyers attending the annual meeting participate on the bus tour. This gives them firsthand experience in seeing varieties grown in NC and learn how they will function in the market place.

4) exhibit booth space was purchased for 2016 PMA trade show held in Orlando, Fl. and 2017 PMA trade show held in New Orleans, La. PMA Fresh Summit is the largest produce trade show held in the US and attended by approx. 20,000 people that includes major retail, wholesale buyers from the US, Canada and other countries.

5) Harvest Creations Design made updates to the NCPA website that included the NC growers directory and created video of NC potato farms and growers that was placed on NCPA website. This gives the NC potato industry a great marketing tool for awareness to both buyers and consumers.
6) NCPA attended the Canadian produce Marketing Association(CPMA) convention during April 2016 in Calgary and April 2017 held in Toronto. Meetings were held with Canadian buyers to discuss NC potato crop conditions and conditions of Canadian potato crop held in storage and possibilities for NC crop to be exported into Canadian markets. CPMA is the largest produce trade show held in Canada, giving the NC potato industry great exposure to the largest export market for NC produce crops.

NCPA was the project partner. To be a member of NCPA, you must be a potato grower in NC and pay NCPA assessments. The NCPA has a long-standing commitment to improving opportunities for NC potato growers. We have been in existence for close to 90 years and consist of farmers who are interested in ensuring the long-term stability of NC potato farmers and the NC potato industry. With overall direction

of the NCPA board of directors, funds from this grant were used to support enhanced potato marketing efforts to increase market share for our growers.

GOALS AND OUTCOMES ACHIEVED

With the help of funding by this grant NCPA has been able to continue and enhance their approach to potato marketing by attending & exhibiting at Potato Expos (potato industry specific event), exhibiting at PMA trade shows, bus tours showcasing NCSU research varieties for NC at NCPA annual meetings, placing ads in NPC Stat Books(potato industry wide publication) and The Packer, Produce News, and SEPNW produce publications, attend the CPMA, and create additions and make updates to the NCPA website.

By attending/exhibiting at the 2016 and 2017 PMA, contacts were made with buyers at the trade shows that lead to significant sales relationships for the growers. Contacts with potato buyers were also made at the CPMA. Feedback from the growers and buyers determine that these relationships remain in place and should continue with multiple trailer load shipments to these buying companies in the future. Many of the same contacts were made at the Potato Expos and the annual meeting tours that further solidify grower/buyer relationships.

The main goal of this project was to continue to enhance our approach to potato marketing and to increase the competitiveness of North Carolina potatoes. This will increase the NCPA's ability to market/promote potatoes produced in the State so that NC potato growers can effectively compete for market share with potatoes from other growing areas. This was accomplished by completing the planned marketing activities of the grant proposal.

Multiple retail, foodservice, produce brokers were met at PMA and CPMA trade shows. Some of these contacts were existing customers and these contacts help to solidify relationships. Many of the buyers asked questions about the upcoming NC potato crop and let us know that there would be a good demand for the NC crop, due to storage crop conditions in northern states and eastern Canadian provinces. According to feedback from a major chip potato buying company in Ontario, Canada, they would be contracting with NC growers to import approx. 100 tractor trailer loads to potato chip facilities in Eastern Canada during the 2017 potato season. Exact number of loads sold to the other buyer contacts are difficult to establish but multiple loads were expected to be sold. Exhibiting at Potato Expo trade shows, ads in NPC Stat Book and The Packer, The Produce News, SEPNW publication, and website additions/updates significantly increases visibility and awareness of the NC potato industry. Approximately 25 buyers attending the NCPA annual meeting each year and participating on the bus tours and visit the NCSU research variety trails could see the new varieties that are being developed. Feedback from these buyers tells us that the bus tours of the research trails are a much looked forward to event.

The NC potato industry received great exposure to all segments of the produce industry by using the Specialty Crops Grant funding to participate in the Potato Expo that is attended by over 2,000 people involved in the potato industry; and PMA trade shows that is the largest produce trade show in the US attended by approximately 20,000 people that includes growers, buyers, other industry reps; place ads in NPC Stat book that's is distributed to over 14,000 in the potato industry, placed ads in The Packer produce print and online version of the publication that has over 50,000 produce industry readers,

Produce News online and print version that reaches 16,000 subscribers, and SEPNW, updating NCPA website that will give great exposure to buyers and consumers. Website statistics show a major increase in website visits when the ads were ruin in the Packer and produce News during May. Grower/buyer contacts were made at the PMA, CPMA, Potato Expo that led to significant sales of NC potatoes. Exact loads are difficult to determine but feedback from both grower and buyers establish that multiple loads were sold and these relationships remain in place and should continue into future years. A major chip potato buyer from Ontario, Canada that we spoke with at Potato Expo 2017 and attended the 2017 NCPA annual meeting imported approx. 100 tractor trailer loads of chip potatoes to Eastern Canada to supply potato chip facilities.

After surveys with growers and discussions with buyers, we learned that additional market for purple skin/purple flesh potatoes was achieved for an NC grower to grow and supply this variety of potatoes for the major supplier for "Terra Chips" brand. The major supplier is a grower in Maine, and they need a supplier from NC to complete a 12-month supply for Terra Chips. We met with this company in Maine during Potato Expo 2016. Market for chip potatoes to supply Frito Lay's eastern Canada chip plants was increased during 2017. We met with WD Potato during Potato Expo 2017 in January. They are a major supplier for Frito Lay in eastern Canada. NC growers planted for this market and shipped multiple loads to this market during June/July 2017. During CPMA 2017 we met with Vanco Farms of PEI. They are interested in sourcing organic potatoes from NC during June/July. They followed up and attended 2017 NC Potato Association annual meeting to meet growers to discuss this opportunity. We met with Heartland Farms at 2017 Potato Expo, they sourced NC potatoes for chipping during June/July 2017.

Buyer and broker contacts made during the course of this project:

- 2016 Potato Expo: La Joie Farms of Maine, WD Potato, EK Bare & Sons, Utz Potato Chips
- 2016 CPMA: Mid Isle Farms, Groenwegen & Sons, Cavendish Farms, Loblaw Companies, Rolo Bay Holdings
- 2016 NC Potato Association annual meeting: Herrs Foods, Wada Farms, Utz Potato Chips, WD Potato, Masser Farms, EK Bare & Sons, Metzler Systems, Snyders/Lance, Groenwegen & Sons, Cavendish Farms, Island Potato Consulting, L&M Companies, Mid Isle Farms, Loblaw Companies, Atlantic Potato Distributors
- 2016 PMA: Lidl, Harris Teeter, Mid isle Farms, Kroger, Lowes, Streef Produce, Publix
- 2017 Potato Expo: Hearland Farms, WD Potato, EK Bare & Sons, Utz Potato Chips
- 2017 CPMA: Mid Isle Farms, Groenwegen & Sons, Cavendish Farms, Loblaw Companies, Rolo Bay Holdings
- 2017 NC Potato Association annual meeting: Herrs Foods, Utz Potato Chips, WD Potato, Masser Farms, EK Bare & Sons, Metzler Systems, Snyders/Lance, Groenwegen & Sons, Cavendish Farms, Island Potato Consulting, L&M Companies, Mid Isle Farms, Heartland Farms,
- 2017 PMA: Loblaws, Kroeger, Publix, Piggly Wiggly, Lowes, Bushmans

BENEFICIARIES

Benefiting from this project were NC potato farmers; small, medium and large producers of table and chip potatoes. The project's accomplishments enhanced awareness of potatoes sold at roadside markets, potatoes for retail grocery chains, and potatoes grown for potato chips.

Benefiting from this project are approx. 80 NC potato farmers that produce over 5 acres of potatoes annually and small to medium-sized farmers who operate roadside markets, who number in the 300's, also sell potatoes that are either produced in small plots or purchased in bulk from larger potato farmers in NC. Overall economic impact is hard to determine, but from feedback received from buyers and growers, we are confident to say this project generated positive business for NC potato growers.

LESSONS LEARNED

This project shows that marketing is a very important aspect of potato farming in NC. As stated before, potato farming is a very competitive business and marketing plays a major role in the business. We have heard potato marketers/growers say that almost anyone can grow a crop, but you must be able to market/sell it. We feel the funding from this grant played a major role in NCPA's ability to participate in the activities and achieve the marketing goals to enhance competitiveness, and improve profits and the long-term sustainability of NC potato farms.

CONTACT PERSON

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ADDITIONAL INFORMATION

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http://ncpotatoes.org/your-summer-potato/
http://ncpotatoes.org/grower-shipper-directory/

PROJECT TITLE Enhancing Online Outreach for NC Strawberries*

PROJECT SUMMARY

We completed the project initiation and finalized our contractor for the website rebuilding project, which is Chariot Creative based in Raleigh, NC. We spent time delineating our goals for the new website with said contractor, as well as reviewing site plans and mock up designs for the new website. After completing this feedback and discussion portion, we were able to approve the new design and new features, and the website went live in July 2016. We promoted the new website to our members via email in July 2017 and invited members to participate in managing their listings in the online farm locator. This is a critical piece of our work plan, as it will help towards our goal of increasing traffic to the farm locator feature of our website. Since the launch of our website, we have spent a lot of time getting acclimated with all the new features available. We formed a website committee to review new site so we can make sure we have the most up to date information available and easily accessible. We are continuing to make changes to the new website to help make it easier to navigate through the pages.

We have completed the goal of initiating the project, finalizing our contractors (Chariot Creative) and launching our new website within the time periods provided media specialist contractor in our work plan. In March 2017, we hired a media specialist contractor, Communicopia Marketing Service, to help us promote our new website. Communicopia developed a statewide media list. Communicopia interviewed NCSA President Doug Patterson and gathered information from Kristy Phillips (NCSA Executive Secretary) to publish a news release. On March 31, 2017, the press release was distributed it to the media list statewide. The objective was to create awareness among strawberry growers, consumers and the media of the new website www.ncstrawberry.com. Communicopia followed up with phone calls, emails and monitored the media pickup (list attached). We also ran a full page spread ad in the North State Journal promoting our new website and included a map of our farm locator which is also located on our website. Created NCSI Strawberry Investigations page for Teachers/Educators to access lesson plans on the website. Uploaded lesson plans. Created a login session tracker for Teachers/Educators to access the lesson plans; allows double signup and records and emails are sent to info@ncstrawberry.com each access occurrence. Created event for 2017 Expo. Updated menu items for the Expo page. Implemented a login/private status to the event signup form in order to force membership or login. Created a new blog for plant listings (plants for sale).

PROJECT APPROACH

- Developed a mobile-friendly website that is accessible across multiple platforms
- Enhanced consumer appeal by greater use of photographs and video
- Improve and integrate existing features such as the “Farm Locator” and recipe collection so that they are more useful to consumers
- Streamline ability of growers to add or change farm information on the farm locator
- Improved NCSA’s content management of the site and modernize program platform
- Staff was trained in using the new website and integrated social media
- Created an online process for growers to register for NCSA’s Strawberry Expo
- Developed a database of interested consumers, teachers, and media who have visited the site and wish to receive further information

- Improved the farm locator feature on the website to drive more consumers to pick your own operations

Kristy Philips and Grace Tuschak have both been in frequent communication with our contractor, Chariot Creative and provided feedback on the new website during every step of the process. Grace Tuschak started UNC in late August to pursue her master's degree. Since Grace is now a full-time student, she is no longer able to help with the new website. With this being said, we formed a committee to help with website review. Kristy Phillips has worked directly with Chariot Creative to make edits and adjustments to the new website. Kristy is hands-on daily making sure that the website continues to benefit everyone that visits it.

GOALS AND OUTCOMES ACHIEVED

The project has been very successful in developing resources for growers, consumers and educators that can continuously be updated and will provide benefit for many years. The website allows easy access to the latest information available to our growers. We were able to add a page for our educators so they can access (33) K-5 lesson plans (5-7 per grade), plus photos, diagrams, on-farm activity recommendations. Our promotional catalog and order form is posted online so our growers can see the full list of items available. We receive weekly emails from growers or consumers that use our "contact us" feature on our website. Website analytics for 2015, 2016 and past patterns of use are not available. Benchmark figures were established during the 2017 harvest season and will be analyzed over the course of this current 2018 season to compare traffic to our website. Our target is a 30% increase in traffic to the site during main harvest months of April and May. The building and launching our website was a process for which we were completely dependent on our contractor to complete. This piece of the project took longer than we anticipated so the website launched later that we projected. Our website went live in July 2016, so we missed the 2016 strawberry season that ended in June. Due to the later launch date of our website, we were not able to achieve the target of 30% increase in traffic to our website. Due to the above normal winter temperature and early-spring frost, we had a very unusual start to the strawberry season. In NC, the strawberry season generally begins in early-to mid-April and goes through mid-June. In 2016, some farm in the Piedmont and Coastal areas opened in March, which was 3 weeks earlier than normal. We had to ask for a one-time, no cost extension to have additional time to complete our project following the end of 2017 strawberry season. A revised work plan and timeline was submitted. Two social media workshops were held during our 2017 Southeast Strawberry Expo in Wilmington. A total of 53 growers attended both workshops (Thursday – 32 / Friday – 21). Participants were asked to complete surveys at the end of each workshop and 95% of participants responded that they had increased their knowledge in social media and/or business websites as a result of attending the workshops. The Farm Locator feature on our website helps consumers to identify the strawberry growers in their area. Surveys were sent out in June 2017 to establish a benchmark and we will send out an identical survey in June following the 2018 season. The target is a 10% increase in customers being directed to Pick Your Own Growers specifically from our website.

Updated online registration page; Redirect the "Register Now" link listed on homepage directly to Expo Registration; Lock out only the ticket purchase options (logged in members only); added verbiage that directs to pay for membership first, then will be able to purchase tickets; Added verbiage to Expo page: To purchase Expo tickets you must either already be NCSA Member (see login) or you must purchase a membership and Expo tickets; Updated online registration page; Replaced "Register Now" with "Online Registration has closed" you can still register by downloading form (PDF) and emailing or calling; Created a new event that has no online ticket purchase and with blurb and links at the bottom under

"Tickets"; Exported final Expo reports from online registration; Removed old blogs and replace with Promo catalog and added blog for Farm Locator to homepage; Updated new membership contact information on farm locator; setup a test event page to view link without being logged in as a member or admin it will take you to the member levels page where you purchase membership; removed Expo info on homepage and replaced the intro message with info about membership sign-up and renewal; fixed issue with the favicon icon and replaced it with a better quality image of a strawberry from the logo (tiny icon that appears in the browser tabs); Renewed the Paid Membership Pro Developers Kit (keeps some of the membership customizations intact and offers support from the platform creators in case there's an issue we can't resolve and allows useful things like Bulk Member or Bulk Level changes. Worked with developers to see if data variable can be included in emails coming from PayPal account; Confirmed with developers that we cannot modify, alter or customize the PayPal Email Notification; only option would be to use another payment process or diff membership platform or receive a 2nd email from website, not PayPal that contained the farm name (per transaction). Uploaded 2018 forms (membership, promo catalog and order forms) and added a page regarding the 2018 Scholarships available for students (including new application) and updated blog for promo items.

A Social Media workshop was held at our 2017 Expo in Wilmington. 27 growers attended the workshop. The goal was to help growers promote their operations via their websites and social media platforms (such as Facebook, Instagram, Twitter). These are valuable tools that have great potential to help growers market their strawberries. As a result, 85% of participants responded that the workshop increased their knowledge in social media and/or business websites.

We completed the goal of developing a mobile-friendly website that is accessible across multiple platforms. Our new website launched in July 2017. Since then, we have sent out a statewide press release, sent emails and ran a full page spread ad in the North State Journal to promote our new website. The objective was to create awareness among strawberry growers, consumers and the media directing them how to find a strawberry farm near them. We also created NCSI Strawberry Investigations page for Teachers/Educators to access lesson plans on the website. Uploaded lesson plans. Created a login session tracker for Teachers/Educators to access the lesson plans; allows double signup and records and emails are sent to info@ncstrawberry.com each access occurrence. Created event for 2017 Expo and created online registration. Implemented a login/private status to the event signup form in order to force membership or login. Creating new blogs is another benefit added to our website. This allows us to create multiple blogs on our homepage about special events, workshops, plants for sale, etc.

BENEFICIARIES

Growers and consumers greatly benefit from our new website. Consumers can locate a farm near them and this helps our growers/members sell their strawberries during their harvest time. Our website is also a great resource for teachers as they are able to access lesson plans to help educate students about growing strawberries.

North Carolina strawberry farmers (250 members) who have access to new tools to improve their on-farm educational programs and food safety on the farm, thus increasing their value of their crop. Members access our "member only" section of our website that requires a login and password. We have additional people such as consumers and educators who access our website, such as our farm locator to help them to find a farm near them. The website project increased awareness of NC strawberry farms among teachers, students, and consumers, making them more likely to visit the farm and pick their own strawberries. Secondary beneficiaries of the project are farmers, teachers, children,

and consumers in other states. The NC Strawberry Association makes it resources equally available to the approximately 50% of its grower membership who live outside of NC.

LESSONS LEARNED

The new website was a huge undertaking. It required a lot more time than we predicted. On a positive note, it was completed and we have a beautiful new up to date website that is much more user friendly. We are very pleased with the outcome.

There were no unexpected outcomes

The goal is to promote and market NC Strawberries directly to consumers, and to increase grower use of our website for educational purposes. Increasing grower participation in association activities can increase their production quality and value.

CONTACT PERSON

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ADDITIONAL INFORMATION

Website: www.ncstrawberry.com

PROJECT TITLE Branding North Carolina Sweet Potatoes*

PROJECT SUMMARY

There is currently no brand awareness of North Carolina sweet potatoes to differentiate them from sweet potatoes grown in other states or countries. There had not been any studies to determine if a trademarked branding program would be beneficial to sweet potato farmers and packers in North Carolina. Without supporting data, the Board of the NC SweetPotato Commission was unable to make an informed decision on whether to pursue the time consuming and costly process of trademarking a brand. Results from this study provided data to guide our next marketing steps and ultimately make the decision on whether or not to proceed with a branding campaign.

North Carolina grows nearly 60% of all US sweet potatoes, but currently there is no differentiation between sweet potatoes grown in our state and those grown outside our borders. In order to grow our share of the marketplace, North Carolina needs to determine how it can set our product apart from and above other options consumers have in the produce aisle.

Better understanding your target market, their needs, and plotting a directional course needs to be sooner rather than later, as a branding campaign once launched takes time to develop and even years to hone and fine tune. As farmers in other states and even other countries begin growing sweet potatoes, North Carolina needs to develop its brand as the premier product that consumers will continue to demand, leading to an overall increase in sales.

This project did not build on a previously funded project with the SCBGP.

PROJECT APPROACH

The North Carolina SweetPotato Commission (NCSPC) applied for and was accepted to participate in the UNC Kenan-Flagler Business Schools STAR (Student Teams Achieving Results) program. The team looked to answer the question “Will a targeted branding campaign increase demand for NC Sweet Potatoes and help command a premium price?”

The North Carolina SweetPotato Commission (NCSPC) applied for and was accepted to participate in the UNC Kenan-Flagler Business Schools STAR (Student Teams Achieving Results) program. Their task was to answer the question “Will a targeted branding campaign increase demand for NC Sweet Potatoes and help command a premium price?”

During this 4-month study, Students from the STAR program met with NCSPC staff, growers, packers and shippers and sweet potato researchers of North Carolina. The first phase of their study was to reach members of the NC Sweet Potato Commission, retailers and consumers. Interviews were held with 14 growers, a legal consultant and 4 retailers as well as NCSPC Executive Director and Executive Committee members. Additionally, 2 focus group surveys were conducted with over 500 respondents and six expert interviews.

Once interviews were completed, data was put together in a 146-page presentation and was presented to the NCSPC Promotions Committee in August 2016. STAR program students graduated

before a presentation could be made to the Board of Directors of the NCSPC. The STAR team findings were presented to the Board of Directors in April 2017.

The team determined that branding NC sweet potatoes is feasible. According to their final report, "Effectively branding, using story and health-centered messaging would increase demand and yield a price premium for growers."

GOALS AND OUTCOMES ACHIEVED

NCSPC contracted with the STAR program to conduct research to determine if developing NC sweet potatoes as a brand will increase consumer recognition and increase demand for NC sweet potatoes. We achieved our goal of completing a feasibility study.

Set targets were achieved and a feasibility report has been completed and presented to the Promotions Committee and NCSPC Board of Directors.

The sole purpose of this grant to was to conduct a feasibility study as to whether or not branding NC sweet potatoes would be of benefit to the membership of the NC SweetPotato Commission. The students of the STAR team conducted research, created a brand analysis including legal consult and preliminary retail research and concluded that a 25% increase in the price of sweet potatoes could be garnered through a branded program.

- GOAL: To determine if developing North Carolina sweet potatoes as a brand will increase consumer recognition and increase purchases of North Carolina sweet potatoes. To educate all 370 growers and 25 packers/shippers as to whether or not implementing a branding program with increase market recognition and demand for North Carolina sweet potatoes.
    - While the goal was to educate growers and shippers of the outcome; it was determined that after a branded program was created would be the best time to include the rest of the membership. Should the creation of a branded program not be pursued, there would be no need to convey branding information.
    - It was determined through the interviews with both retailers and consumers that having a branded program with detailed messaging would in fact educate and create trust with end consumers therefore increasing demand.

- PERFORMANCE MEASURE: Completion of one feasibility study. Send 400 newsletters with an update on this project and the result. Make one presentation to the Sweet Potato Commission Board of Directors and one presentation to the membership during the annual meeting.
    - The STAR team students were unavailable after graduation to be able to make presentations; however, Heather Barnes, NCDA Marketing Specialist, and co-author of the grant, presented STAR team findings to the Board of Directors in April 2017. As the NCSPC continues to work on branding efforts, they will convey information through varying ways, printed newsletter, e-news and presentations at their annual meeting.

- BENCHMARK: There is currently not a branding program for North Carolina sweet potatoes.

- TARGET: Completion of one feasibility study. There are zero people who know whether a branding program will result in an increase in demand for North Carolina sweet potatoes. As a result of this project we expect the 400 members of the NC Sweet Potato Commission to know, through education efforts, if a branding program will result in brand recognition and demand in the market place.
    - Once presented to the Board of Directors of the NCSPC, it was determined to implement a Branding Task Force with the intent of creating a **"branded and trademarked"** program for the NCSPC. It is our belief that this only occurred as the result of this grant project.

- PERFORMANCE MONITORING PLAN- to monitor progress toward achieving each GOAL will include monthly updates from UNC Kenan-Flagler Business School Project Managers/Consultants
    - Consultants will conduct detailed interviews with packers of North Carolina sweet potatoes. They will also conduct detailed interviews as needed to complete a broad cross section of members/packers.

Information and results of the project will be shared with the specialty crop growers and other interested specialty crop stakeholders by:

- Reports will be made to the Promotion Committee during regular meetings
  - Monthly conference calls between the STAR team faculty advisor and NCSPC office were held to update project status.
  - Reports were presented when available with a group presentation as well as 146 page presentation.
- Updated reports will be given as needed preferably quarterly to the NCSPC Board of Directors
  - A final report was presented to NCSPC Board of Directors, April 2017.
- Presentation to membership at their annual meeting. There are over 400 members of the Commission.
- Update all 400 members in print via the SweetPotato Speak Newsletter
  - Should the NCSPC continue to work on branding efforts, they will convey information through various ways, including printed newsletter, e-news and presentations at the annual meeting.

- Update all 400 members via Member Portal online
  - The STAR report has been made available through the member online portal.

BENEFICIARIES

The sweet potato farmers and packers of NC will benefit from this project as the results of this study will impact marketing programs both domestically and internationally.

Based on their report, branded NC sweet potatoes could be sold for up to a 25% premium per pound.

To date the number of beneficiaries only consists of those interviewed, however, once a completed branded program is in place the beneficiaries will include every sweet potato producer, processor and retailer in the state of North Carolina. That number changes from year to year, but currently is over 400.

LESSONS LEARNED

We would recommend the STAR team to any commodity association. They presented a thoroughly researched study.

- Working with the STAR team allowed us greater reach for research on consumer trends. We learned valuable lessons in brand awareness as well as accompanying price points.
- We did not fully understand the STAR team availability was limited to only the spring semester, when the project took place. Had we had a better understanding of their availability we could have better prepared for reporting and would have invited the NCSPC Board of Directors to the Promotions Committee Meeting where the STAR team presented their final report.
- Our membership meets once per year. The timing of the project conclusion fell at a different time, therefore not allowing access to the STAR team for full membership delivery of findings. We will still be able to present the STAR findings, but have less to offer during Q&A sessions since we did not conduct the research ourselves. Should we take on a program utilizing a group of this fashion we will be better prepared for outcomes and meeting plans.

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PROJECT TITLE Wild-simulated Ginseng Cultivation & Marketing Initiative*

PROJECT SUMMARY

The overall goal of this project is to increase cultivation of wild-simulated American ginseng in the High Country of western NC. Watauga County has ideal site and environmental conditions for growing ginseng and the county Extension office is receiving many inquiries from many landowners about how to cultivate ginseng in "wild-simulated" conditions to produce income from their forestlands. While 90% of wild harvested and wild-simulated ginseng is exported to Asian markets, there also is increasing domestic interest in its use in foodstuffs, medicinal applications, and added value products created from it. There is potential to integrate ginseng into the 'local food' arena as a specialty crop used in local craft-beer brewing, health & medicinal products, and retail sales in farmers markets among others.

It is widely known that wild populations of the plant are increasingly diminishing due to overharvesting and poaching. This project will have an additional benefit of reducing the pressure on native ginseng populations and helping to reestablish populations of "wild" ginseng. Cultivating ginseng to grow roots that are preferred in Asian markets is a tricky, labor-intensive endeavor. The plant takes between 5-10 years to mature and requires specific site and environmental conditions to produce the highest quality roots. Over the last 5 years, prices have risen from $500 to $1300 per dried pound. Due to increased interest from landowners in planting ginseng and public interest (due to television shows) seed prices have also increased from $60 per pound 5 years ago to up to $200 per pound.

PROJECT APPROACH

- 9 technical workshops and on-farm demonstrations were carried out and covered a) site selection, preparation, nutrient management, and planting techniques; b) pest and disease management; c) ways to reduce poaching; d) proper harvesting and post-harvest methods (ie. drying roots); e) seed production, collection, and stratification. 4 local and regional ginseng experts spoke at trainings. Over 120 landowners participated in workshops and over 1,400 lbs of ginseng seed were established on privately owned woodlands in the high country. A wild-simulated ginseng demonstration plot was established to provide an opportunity to carry out future hands-on cultivation and production training and to provide data for producers on germination, estimated production, costs, etc.
- Project Partner, Appalachian Sustainable Agriculture Project (ASAP) produced a final report that was distributed to growers & media and made available on their website and distributed regionally http://asapconnections.org/report/exploration-market-opportunities-western-north-carolina-grown-ginseng-root/. Project partner PHARMN marketed the program and served as fiscal agent.
- Watauga County Cooperative Extension served as the primary collaborator for the project. Dr. Jim Hamilton, Watauga County Extension Director, is the local Extension expert on ginseng production and forest farming. He organized and delivered the technical training workshops and demonstrations held during the project period. Watauga County Cooperative Extension hosted training meetings at the Watauga County Agricultural Conference Center and established one of the demonstration sites for ginseng training sites. Watauga County Cooperative Extension also managed the registration & evaluation of workshops, arranged outside specialists, and procured the ginseng seed and rootlets for the project and worked directly with the producers. Dr. Hamilton also organized the training program for county law enforcement personnel with NCDA's Plant Conservation Program.

- David Welch with the NCDA Plant Conservation Program assisted Dr. Hamilton with the training program with county law enforcement personnel after we received word that the project was funded in fall 2015. The Plant Conservation Program at NCDA provided written resources, including statutes and protection strategies, that were presented during the meeting with the Watauga County Sheriff's Department. Mr. Welch passed away unexpectedly during the first year of the project; therefore, this element of the project only occurred during year one as his position took some time to be reposted and filled.

GOALS AND OUTCOMES ACHIEVED

PRE-PROJECT BENCHMARKS: At project inception, there were 5 intensively cultivated acres of ginseng known in the High Country region. There were approximately 40 known forest landowners interested in gaining technical proficiency in establishing & cultivating wild-simulated ginseng in Watauga County alone (based on 2013 workshop participation). There were eight ginseng producers in Watauga County who collectively expressed interest in developing a grower cooperative/association.

PRE-PROJECT TARGETS: A 50% increase in trained ginseng producers. A 50% increase in cultivated ginseng plantations/acreage in the High Country. More accurate cultivation, management, and harvest cost data available to interested producers. An active ginseng grower cooperative of 15-20 members that can secure a direct buyer in 5-7 years from being established.

POST PROJECT OUTCOMES:
Small-scale cultivation: 123 landowners established over 200 lbs of ginseng seed on approximately 40 total acres of property in the High Country counties of western NC. Between Jan 2016 and October 2017, 9 technical workshops and on-farm demonstrations took place with over 130 total participants.

Seed harvest: 75 lbs of ginseng seed was harvested and properly post-harvest handled for reestablishment using supply items purchased with grant funding.

Commercial-scale cultivation: 10 producers established 1,200 lbs of seed on 24 total acres.

Other project results: A market study was produced that identified several potential local outlets for selling of ginseng.

Outcomes exceeded goals: Over 120 landowners participated in workshops and cultivated over 200 lbs of ginseng seed at low-density rates on an estimated 40+ acres of privately owned woodlands in the high country during the project period. Additionally, ten 'commercial scale' producers established 1,200 pounds of ginseng on an additional 24 acres during the project period.

Outcomes met goals: Project partner ASAP (Appalachian Sustainable Agriculture Project) carried out a market analysis of potential markets for locally produced ginseng. The report was distributed widely to ginseng producers and local herbal & medicinal plant industry & enthusiasts and is available on their website. The full report/analysis can be accessed here: http://asapconnections.org/report/exploration-market-opportunities-western-north-carolina-grown-ginseng-root/

Since the project concluded, two participating ginseng producers have sold ginseng to local breweries for small-batch beer runs and one producer is selling packaged ginseng leaf tea at 4 local retailers.

Outcomes met goal in Watauga County: In Fall, 2015, (after receiving word that project had been approved) NCDA Plant Conservation Program specialist and Watauga County Extension Director met with 14 members of the Watauga County Sheriff's department to review legal protection of ginseng, to inform law enforcement of economic potential of crop and conservation status, and provide education on ginseng theft and potential mitigation measures. Since this training, Watauga County Sheriff's department has made several arrests for ginseng theft:
https://www.hcpress.com/crime-reports/two-ginseng-poachers-arrested-near-boone.html

Plant Conservation Specialist with NCDA passed away unexpectedly during the first year of the project; therefore, formal presentation only took place with Watauga County Sheriff's department. Hamilton answered some law enforcement questions with Ashe County Sheriff's department via phone conversations in 2017.

PERFORMANCE MEASURES:

- Attendance at workshop series, pre and post surveys on knowledge of establishment & management, total number of participants trained, acreage planted, and reported plant germination percentage/success.
- By investigating local/regional markets for wild-simulated ginseng, an important performance measure of this project for goal 2 will be the development of direct sales strategies that will secure links to larger-scale buyer(s)/exporter(s) and regional buyers of ginseng to yield premium returns to producers and the eventual establishment of a formal cooperative.
- The number of law enforcement officers trained in the legalities and cultural aspects of ginseng poaching and potential # of arrests of poachers would be a performance measure for goal 3.

Workshop series evaluation instrument and results:

Wild Simulated Ginseng Production Course Evaluation Instrument (for first year series)

I attended: **BOTH** field & classroom sessions The Classroom Session Only

How much did you know about **growing** ginseng **before** attending the workshop?

Nothing some a lot

How did your knowledge about ginseng INCREASE as a result of the workshops?

A little bit some a lot

Was this workshop a good value? (was it worth the money you paid for the information and the seed?)

Yes No

Would you consider planting/establishing more ginseng as a result of the workshops?

Yes No

How much ginseng seed would you potentially be interested in planting in the future?

½ lb or less 1-5 lbs 5-10 lbs more than 10 lbs

Wild Simulated Ginseng Production Course Evaluation

Survey Analysis

Attendance

- Out of the total of 56 participants to fill out the survey, 43 or 76.8% attended **both** the field and classroom sessions.
- 8 of the 56 or 14.3% of the participants only attended the **classroom session**.
- 50% of the participants who only attended the classroom session reported to already know **some** about growing ginseng **before** attending the workshop.
- 5 of the 56 or 8.9% of the survey participants did not answer whether they attended both the field and classroom sessions or only the classroom sessions.

Knowledge about growing ginseng before attending the workshop

- 30 of the 56 or 53.6% of the survey participants reported to know **nothing** about growing ginseng before attending the workshop.
- 20 of the 56 or 35.7% of the survey participants reported knowing **some** about growing ginseng.
- 6 of 56 or 10.7% of participants reported knowing **a lot** about growing ginseng before attending the workshop; though 5 out of 6 participants report also learning a lot as a result of their attendance, with the remaining one participant reporting to only learn **some.**

Amount of increase in knowledge about ginseng as a result of workshop

- 0 participants of the survey report only **a little** increase in their knowledge about ginseng
- 3 of the 56 or 5.4% of the participants report only learning **some** about ginseng. 2 out of three of these participants also reported to already know some before attending the workshop, with the remaining participant reporting to know a lot prior to the workshop.
- 53 of the 56 or 94.6% of survey participants report their knowledge on ginseng increasing **a lot** as a result of their attendance of the workshop.

Workshop value

- 100% of the participants reported the workshop to be worth the money they paid for the money and the seed.

Consideration of planting more ginseng as a result of attendance of the workshop

- 100% of participants reported that **yes** they would consider establishing more ginseng as a result of the workshops

Amount of ginseng participants would potentially be interested in planting in the future

- 10 of the 56 or 17.9% of participants report they would be interested in planting **1/2 lb or less**
- 22 of 56 or 39.3% reported they would be interested in planting **1 to 5 lbs** of ginseng
- 17.9% reported a potential interest in planting **5 to 10 lbs** of ginseng in the future.
- 14 of 56 or 25% of participants were potentially interested in planting **10 lbs or more**
- There was no significant correlation between the amount a participant was interested in planting in the future and the rest of their answers to the survey.

BENEFICIARIES

- Over 130 private forest landowners have established ginseng on their properties providing potential future economic value in underutilized forestlands. Depending on success of planting/harvesting (ginseng is a long-term perennial crop), individual forest landowners could expect a $10,000-$40,000 return on their property from this project.
- The Watauga County Extension Office has become a primary source for ginseng cultivation expertise in the state. Their Extension Director was recognized as the Outstanding Cooperative Extension Educator in North Carolina due to his work promoting and providing technical expertise on wild-simulated ginseng production.
- The 10 commercial scale growers who began work with this project are actively collaborating and sharing ideas, but have not formally created a 'growers cooperative'.
- Over 1,400 lbs of ginseng seed have been cultivated during the project period. Ginseng is a long-term perennial crop. It will take approximately 4-8 more years to document final outcomes of this project effort. It is expected that seed & rootlets produced from plants established during the project period will be available & providing economic return from this project in 2-3 years.
- Potential economic impact of the project: 1,400 lbs of seed planted in Watauga County between 2016-2017: estimated yield: 10,000 – 15,000 lbs dried root in 8+ years with an economic value of $2,000,000 - $4,000,000 depending on price of ginseng.

LESSONS LEARNED

- Follow up with individual landowners on their germination/planting success was difficult due to the sheer number of participants and scheduling/contact difficulties. As of fall, 2017, follow-up visits took place with 43 total producers.
- Media interest in this project was high. Ginseng production and some elements of the project were highlighted in a piece by Foreign Policy Magazine and VICE News in fall of 2016. Links below.
- Due to longevity of crop and planting methods, results & data collection for this project on estimated yields, seed production volume, pest impacts will continue to be gathered over the next several years.
- Invited specialists were extremely optimistic on what had been achieved to date. Pest management strategies that were implemented were considered "best practices"; however, damage from voles in one established plot was surprising.
- Establishing a cooperative with a new, as of yet "unproven" crop, is not something that producers are currently interested in as only one of them who had previously planted this crop has reached a harvest level of production
- Interest continues to be high for small-scale landowners interested in establishing ginseng for conservation, novelty, and income-generating purposes on their underutilized forest land.

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ADDITIONAL INFORMATION

http://asapconnections.org/broadcasts/ginseng-bringing-mountain-tradition-market/
http://asapconnections.org/report/exploration-market-opportunities-western-north-carolina-grown-ginseng-root/
https://news.vice.com/story/the-hunt-for-wild-ginseng-in-appalachias-semilegal-and-highly-lucrative-market?cl=fp
https://foreignpolicy.com/2016/09/07/the-thrill-of-the-hunt-ginseng-smuggling-poaching-boone-north-carolina-china/

PROJECT TITLE Recordkeeping and Crop Eligibility*

PROJECT SUMMARY

Specialty crop farmers need to take advantage of new, tailored crop insurance policies available through the USDA and FSA specifically WFRP and NAP. Barriers to access this crop insurance include recordkeeping requirements such as yield and price measures. To increase access to these crop insurance policies, specialty growers need to keep records on yield and price to a standard that allows them to be eligible for crop insurance. Crop insurance can be a powerful resource especially in increasing credit worthiness of a farm operation, which can help farmers gain access to land or scale up their operation. North Carolina's specialty crop production that is uninsurable with a single-crop policy, measured in cash receipts, is worth $708,526,000. The NAP and WFRP insurance programs mean that these crops are now eligible for improved and less expensive crop insurance programs. Providing information to producers about how records should be kept and reported has the potential to impact this entire sector of uninsured crops. The timeline for this project was designed to educate and assist farmers to take advantage of NAP and WFRP in 2017 by getting the necessary information and resources to apply by the Feb 28th deadline.

This project builds off a previous SCBG funded project titled, "Expanding Specialty Crop Risk Management Opportunities" (awarded in 2013). This project assisted farmers in understanding how to better manage risk on their farms through crop insurance policies. Many specialty crop farmers were not aware of WFRP or NAP and did not know that they were eligible for the policy. The Recordkeeping and Crop Eligibility Workshop project enhances this previous project by continuing to educate farmers on how to effectively manage risk and keep records to be able to apply for specialty crop insurance policies that have been developed to suit their operations.

PROJECT APPROACH

The project involved a varied approach to education both in person and electronically through workshops, webinars, and online instructional videos. We developed a curriculum for our workshops including a "Guide to Recordkeeping Basics" which goes over recordkeeping for farm operations in a broad sense and addresses keeping proper records to acquire and maintain crop insurance.

The project evaluated approximately 20 recordkeeping curricula for use in the workshops and materials. We also drew heavily from USDA fact sheets and risk management outreach materials to assure consistency with program guidelines.

Workshops
The primary goal of this project was to provide education and training for producers to understand how to apply and keep records for federal crop insurance policies for specialty crop producers. Workshops were conducted between September 2016 and March 2017. Many of these workshops coincided with larger conferences that educated producers on a variety of topics.

A total of 5 workshops were conducted across several regions in the state:

Piedmont: Louisburg, NC hosted by the Franklin County Small Farmers Association

Northeast: Windsor, NC hosted by Avoca Inc for clary sage producers

Southeast: Mount Olive, NC at Carolina Farm Stewardship Association's Organic Commodities and Livestock Conference

Mountains: Asheville, NC at the Appalachian Sustainable Agriculture Project's Business of Farming Conference

Foothills: Newton, NC at the Agri-tunity Conference hosted by Catawba County Cooperative Extension

The total number of workshop attendants cumulatively was 58.

Survey results: 100% of survey respondents reported increased knowledge of crop insurance policies available and recordkeeping best practices for crop insurance following the workshop. Respondents reported that they were highly likely to apply for WFRP or NAP for the year 2018. 75% of respondents said that they would be changing their recordkeeping habits after participating in the RAFI workshop.

Videos
Through the project, we created and uploaded 5 videos on recordkeeping for farmers (as related to applying for and using crop insurance). We have received approximately 40 views thus far and will send the videos out to our farmer email list as well as post it online and conduct outreach via social media to reach additional farmers.

We also updated our crop insurance resources for farmers on our website and added the new videos to our social media (YouTube account) and website.

Specialty crops were solely benefitted within the scope of the project. Workshop attendees reported growing commodity crops other than those considered to be specialty crops but the crop insurance policies covered were only intended to support the production of specialty crops.

GOALS AND OUTCOMES ACHIEVED

Goal 1: Educate 250 producers through development of online educational materials made available on RAFI's resourceful farmer website.

Our original goal was to produce 5 educational videos and obtain 250 views over time from producers. All 5 videos have been uploaded, but due to delays in post-production of the videos we were unable to get all the videos uploaded until the early part of this year delaying outreach to very late in the sign-up period. However these videos are part of ongoing programming on risk management programs. We plan to distribute the videos throughout the year to our network of 3,000+ North Carolina farmers (on our email list) and through our social media accounts, which have a large following of North Carolina farmers, to more closely coincide with preparation for program participation next year. We have also created a survey for the risk management
resources page where visitors to the site can provide feedback on how informative the site and the videos were for them.

We have also made changes to our website to make the resources easier to access and links easier to follow. We hope that the videos and other resources on this page will provide long-term assistance for specialty crop growers in understanding their crop insurance options.

Goal 2: Educate 75 producers through educational workshops targeted in counties in each region of the state.

Five workshops were successfully scheduled and conducted in each region of the state. A total of 58 individuals attended the workshops.

Our goal of 75 producers educated was not met due to slightly lower than expected turnout at each workshop. However, many of the producers that were present have agreed to share information with other producers that they know. Other attendees at the workshops included FSA and USDA agents and others who provide technical assistance to farmers. These attendants reported having increased knowledge of recordkeeping requirements and crop insurance policies available for specialty crop farmers. They plan to take lessons learned and use it in their work with farmer clients.

Farmers continue to learn about federal crop insurance policies available for specialty crop and diversified farming operations such as WFRP and NAP. At several workshops connections are made between growers, FSA agents, crop insurance agents, and other individuals, which will result in a strengthened specialty crop sector.

BENEFICIARIES

Groups that benefitted from this project captured a wide variety of individuals engaged in specialty crop operations in several regions across the state of North Carolina. Farmers who attended our workshops included beginning farmers, small farmers, and established farm operations transitioning into new specialty crop farm enterprises.

Other agencies such as FSA, USDA, and other North Carolina non-profits reported benefitting from hearing the information in the workshops. Workshops were provided at conferences hosted by agricultural non-profits in the state such as Carolina Farm Stewardship Association and Appalachian Sustainable Agriculture Project.

In 2014, there were 3 AGR-Lite (predecessor of Whole Farm Revenue) crop insurance policies sold in North Carolina, for a total indemnity of $1,184,274. This project has been part of a series of activities to provide outreach on Whole Farm Revenue (WFR) crop insurance to specialty crop producers in North Carolina. To date, for 2017 USDA has recorded 100 WFR policies sold, for a total indemnity of $149,921,595, and we expect that number to increase before the end of recording. North Carolina is now the state with the 4th highest participation in WFR, by liability, 9th by number of policies sold.

With 58 producers attending workshops, and 525 views of the videos, the total number of direct beneficiaries is 583.

LESSONS LEARNED

Insights
Many farmers that participated did not have crop insurance previously and found the workshops immensely helpful in understanding how it works and the benefits of carrying crop insurance on the farm. There continues to be a lack of knowledge among specialty crop producers on existing risk management and disaster assistance programs, and the importance of recordkeeping for accessing these programs. We are seeing individual farmers have very important results from the education and participation in the programs, including increased access to credit, and improved outcomes following disasters. But communicating these outcomes is difficult.

Our partnership with agribusiness consultant, Clif Parker, proved to be very instrumental in doing effective workshops. His very practical knowledge of risk management programs and the specific challenges of effective participation in these programs was instrumental in both development of the curriculum and trainings.

Unexpected Outcomes
We ran into significant technical difficulties in getting videos finished and uploaded. We had significant delays due to challenges with equipment and other technical issues. This really slowed down our outreach.

Lessons Learned
It was very difficult to get the attendance numbers that we wanted in our workshops. It will be a challenge to engage producers to learn about crop insurance and to make workshops more inviting for attendants. We are having ongoing discussions amongst staff and project partners on how we can draw more attendants in to the workshops. Our best-attended workshops were held within the context of a broader meeting, not just at a conference. We are working on providing what people want, with what we see folks need.

We also lost time due to staff transitions late in the grant period. Replacing transitioning staff during a critical window for workshop planning hampered our outreach efforts, and it took incoming staff critical time to get up to speed. We were helped by the continuity provided by both senior staff and partners, but this was a significant challenge for the project.

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PROJECT TITLE Evaluating Christmas Tree Postharvest Practices

PROJECT SUMMARY

Wholesale Christmas tree growers in North Carolina go to great lengths during harvest to provide fresh Fraser fir Christmas trees to retailers and end consumers. However, the complexities of moving a large, bulky, perishable product from steep mountain farms to retailers across the country is a difficult and time-consuming task. To a great extent, logistical hurdles drive when and how Christmas trees must be cut, stored, and shipped. Several market trends have pushed many NC growers to harvest trees and greenery earlier in the fall than ever before. This is especially true for major producers who sell to chain stores. Early harvest that occurs before cold weather and associated tree dormancy increases the likelihood of postharvest quality problems or a failure to keep trees to fresh. In addition, new technologies such as the palletization of Christmas trees significantly alter established harvest practices. Palletization of Christmas trees is a practice only conducted by major producers, but with extensive re-wholesaling across the industry, almost any grower's trees could end up in a pallet. Postharvest concerns at the time this project was proposed included overheating inside pallets of Christmas trees, the shelf life of Christmas trees (issues of moisture content, needle retention, and fire safety), and the occurrence of cracks in the trunks of Christmas trees. Any one of these problems can make a cut tree unsalable or result in a negative experience for the consumer.

This project encompassed the following objectives to address these issues. Christmas tree growers were to: 1) improve their management of pallet storage with increased understanding of compaction and curing of trees, 2) achieve more reliable results from pallet storage by chilling pallets using forced air cooling, 3) improve the shelf life, product reliability, and fire safety of tree and greenery products, 4) improve their management of harvest, reduce the occurrence of freshness problems, and communicate with retailers to improve tree and greenery care. These objectives were developed to address current grower needs and concerns.

This project was very important and timely for the NC Christmas tree industry. In 2013, several of the large-scale growers who palletize trees experienced overheating issues that highlighted the potential risk that all growers who palletize face. In 2014, the NC Christmas Tree Association Research Committee funded a small grant to address postharvest issues including heat of respiration in pallets of trees and trunk cracks in general. Then in 2015, the entire harvest season was unusually warm and wet. Postharvest issues were widespread. Preliminary pallet research conducted during the 2015 season before the onset of this grant helped to explain some of what was occurring inside pallets when they overheated. Since the research was started before growers regularly began cutting trees, the 2015 study identified problems before they showed up in growers trees allowing extension agents to warn growers. The extent of the problem in 2015 generated broader concern and more questions. Postharvest research has been an educational topic at every NCCTA meeting in 2016, 2017, and the winter of 2018. Extension programming has involved a combination of educational presentations, one-on-one problem-solving, and applied research.

Postharvest practices are not currently uniform and growers justify their own approach from a variety of beliefs – some with scientific basis and others justified more from a need for expediency. Without applied research in the context of current NC harvest practices, it is difficult to justify specific postharvest recommendations. While there are excellent postharvest studies that have been done in the past, they

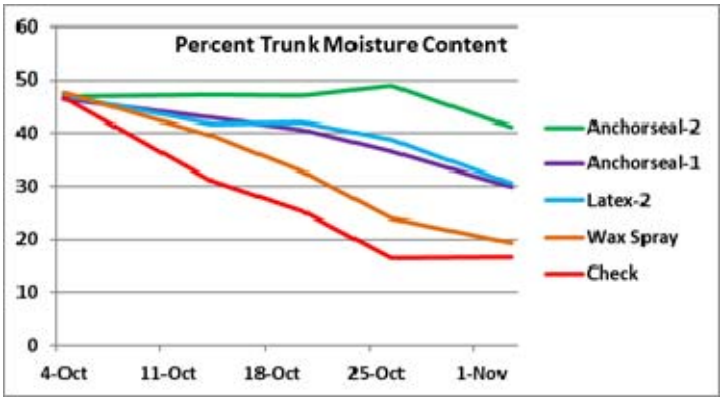
predated palletizing technology. A new series of studies was needed to bring the existing conceptual framework in line with current practices.

This project was new and not related to any other SCBGP or SCBGP-FB project.

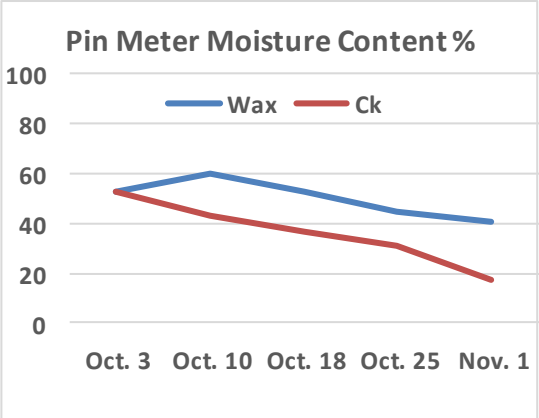
PROJECT APPROACH

The Christmas tree postharvest research studies in this project were conducted between October through December and January of the following year. In both 2016 and 2017, a series of Christmas tree studies were implemented that included harvest timing, trunk crack, heat of respiration, 1-MCP amendment, forced air cooling, and fire retardant studies. In addition, during the winter of 2017-18, branch studies tested Christmas tree preservatives, homemade water additive recipes, and a new method of measuring Christmas tree foliage moisture content.

o 2016 trunk crack study. Initiated on October 3, 2016 at Sexton Farms, this study included four paint and wax trunk treatments and an untreated check. Final data from the trunk crack study were collected on November 11. Thirty-three percent of trees in the study developed cracks with a majority of those occurring in the first 48 hours of the study. Extreme drought hastened rapid drying of study trees. No paint or wax treatments reduced the number of cracks which developed as compared to the untreated check. However, large differences in wood moisture content were observed. Some wax and paint treatments retained more than twice as much water as the untreated check at the cut end of the tree over the study period. Wood moisture content in the trunk of cut Christmas trees may influence the ability of a tree to re-hydrate when placed in a stand with water and therefore its fire-safety. While painting the trunk with paint or wax may not stop cracks from occurring under drought conditions, the treatment had other important benefits that merit further study.

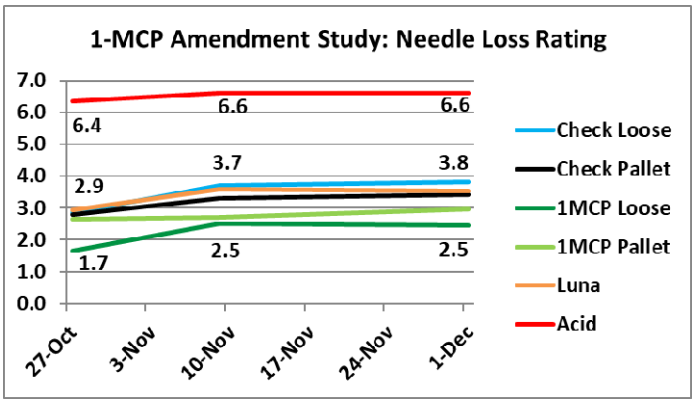


o 2017 trunk crack study. A different study design was used in 2017 to allow for destructive sampling and rehydration of trees in water-filled Christmas tree stands. Since no particular paint or wax treatment stood out in terms of trunk cracks in the 2016 study, only one wax sealing treatment was used. Seventy trees were treated with Anchorseal wax and seventy left untreated. Trees were cut on October 3. Randomly selected trees were destructively sampled for wood and branch moisture content at the beginning and throughout the duration of the study. Preliminary analysis of moisture content samples indicate that water moves both up and out in a tree and suggests that interior foliage toward the bottom of a tree is more likely to be dry. While there was a reduction in the number of small cracks at the cut end of the trunk in wax-treated trees, there was no difference in terms of the larger side cracks that actually make a tree unsalable. Three out of four trunk crack studies conducted since 2014 showed similar results. Once again, there was a large difference in the water content of the wood close to the cut end (30-50%). Wax-treated trees did take up more water than

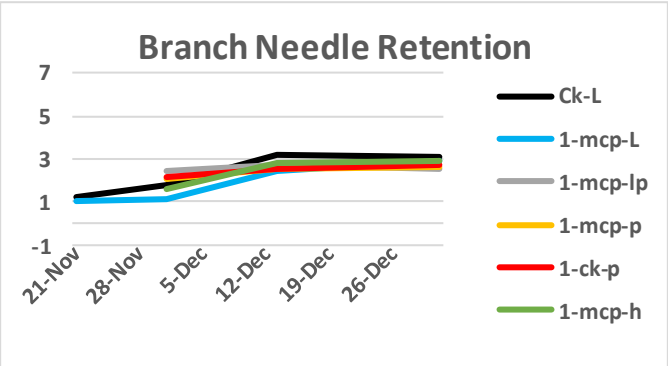


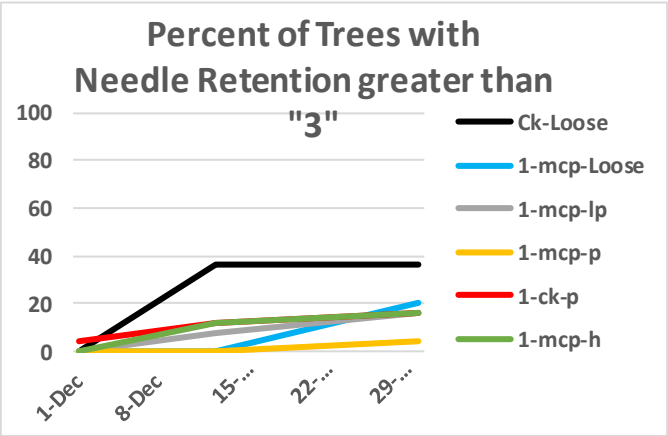
check trees later in the study when all trees where dryer. Trunk treatments do not appear to significantly reduce the occurrence of cracks in cut Fraser fir Christmas trees, but they do hold more of the finite water supply in a cut tree toward the stump. Further analysis of the moisture content of wood samples may reveal more about water distribution in dry trees and after they are rehydrated.

o 2016 1-MCP amendment study. Trees were treated with several different foliage treatments including Luna fungicide and propionic acid hay treatment applied in the field and 1-MCP gas applied after trees were cut. Trees were harvested on October 11, 2017. Data were collected from trees through December 1, 2016 at which time they were completely dried out. Treatment differences, particularly in needle retention, were observed. The propionic acid hay treatment dried out foliage, turned it brown, and induced severe needle loss. This treatment, borrowed from drying hay, does not need to be pursued further. The Luna fungicide treatment did not harm trees or alter needle retention. During such a dry season, potential benefit from a fungicide to reduce mold growth could not be ascertained. We did observe improved needle retention from both 1-MCP Smartfresh treatments. Loose baled trees treated with the 1-MCP gas exhibited better needle retention than untreated trees across three evaluation dates. Palletized trees given the same treatment also exhibited improved needle retention compared to the check but not as much as the loose trees. One can surmise that the gas treatment could circulate around loosely stored trees more easily than palletized trees. This treatment is of special interest for early-harvested trees destined for export markets where needle retention is a frequent issue. Even a slight improvement in needle retention could justify treatment cost in tropical markets.



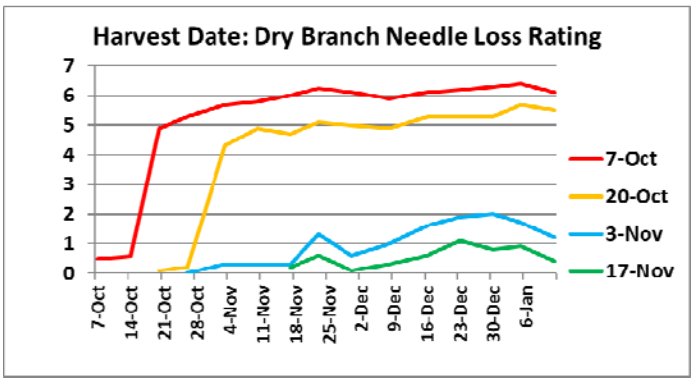
o 2017 1-MCP amendment study. The next year, the study focused on 1-MCP treatments to trees subjected to the normal range of harvest practices. Treatments were followed from the farm to a typical retail storage facility in South Carolina. Treatments included a field application of Harvista (a liquid formulation of 1- MCP), three gas treatments of 1-MCP (loose trees, loose trees later palletized, and palletized trees), and comparable loose and palletized check trees. Trees were cut on November 15, treated, and "farm" stored in a barn at Upper Mt. Research Station until November 21. At that point trees were transported to a retail storage facility at the Columbia, SC state farmers market. Trees were evaluated for moisture content, branch, whole tree, and internal needle retention through January 3. Average needle retention ratings and moisture content were not significantly different across treatments. However, when needle retention data were analyzed for the frequency of problematic trees (NR > 3), the

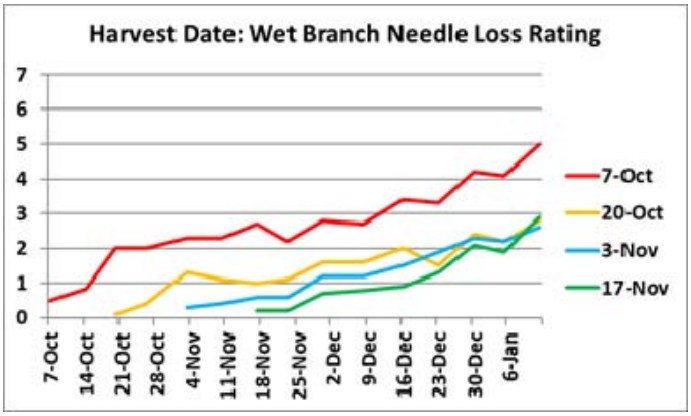




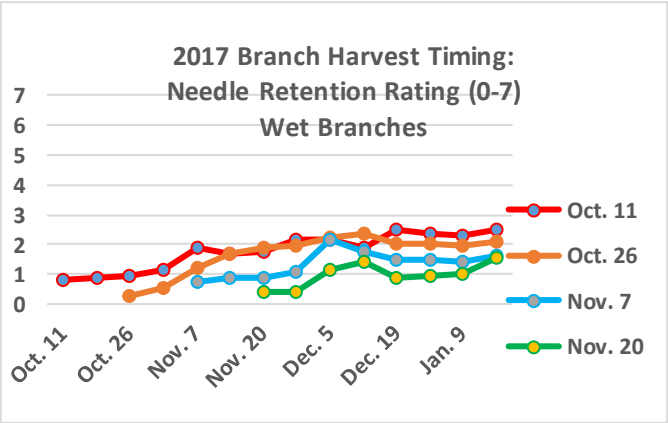
loose tree check treatment had nearly twice as many trees that were dropping an excessive number of needles than the loose-tree 1-MCP treatment (36% vs 20%). Even for trees which were harvested late and handled to minimize stress, the 1-MCP treatments appeared to reduce the frequency of moderate to severe needle loss. A similar treatment difference occurred between the loose tree check and palletized check suggesting that in a dry season like 2017, palletizing trees reduced stress and kept trees fresher than loose storage. Further, the spray-applied Harvista formulation appeared to work as well as the gas 1MCP which might make this treatment more expedient for growers.

o 2016 Harvest Timing Study. Started during the first week of October, 2016, the harvest timing study continued through the middle of January, 2017. Branch harvest dates were October 7 and 20 and November 3 and 17, 2016. Two branches from each of 30 trees were stored in a cool, dark barn at the UMRS, one dry and one in a jar of water. Very different needle retention results were observed between dry and water treatments. When stored dry, branches from both October harvest dates started losing a majority of needles within two weeks of being cut. Dry branches cut in November predominantly retained needles through the end of the study. Better needle retention was observed across all harvest dates when stored in water. However, average needle retention among branches harvested in early October and stored in water was worse than that observed for late October or November harvest dates. Both later harvest and the addition of water improved needle retention. While these results are not surprising and repeat results from previous years, the extent of October needle loss among dry branches was more extreme than previously observed. This could be explained by the comparative warmth of early October 2016. The goal of this study is to track the influence of seasonal variation on Fraser fir needle retention to better understand issues that growers face each harvest season.



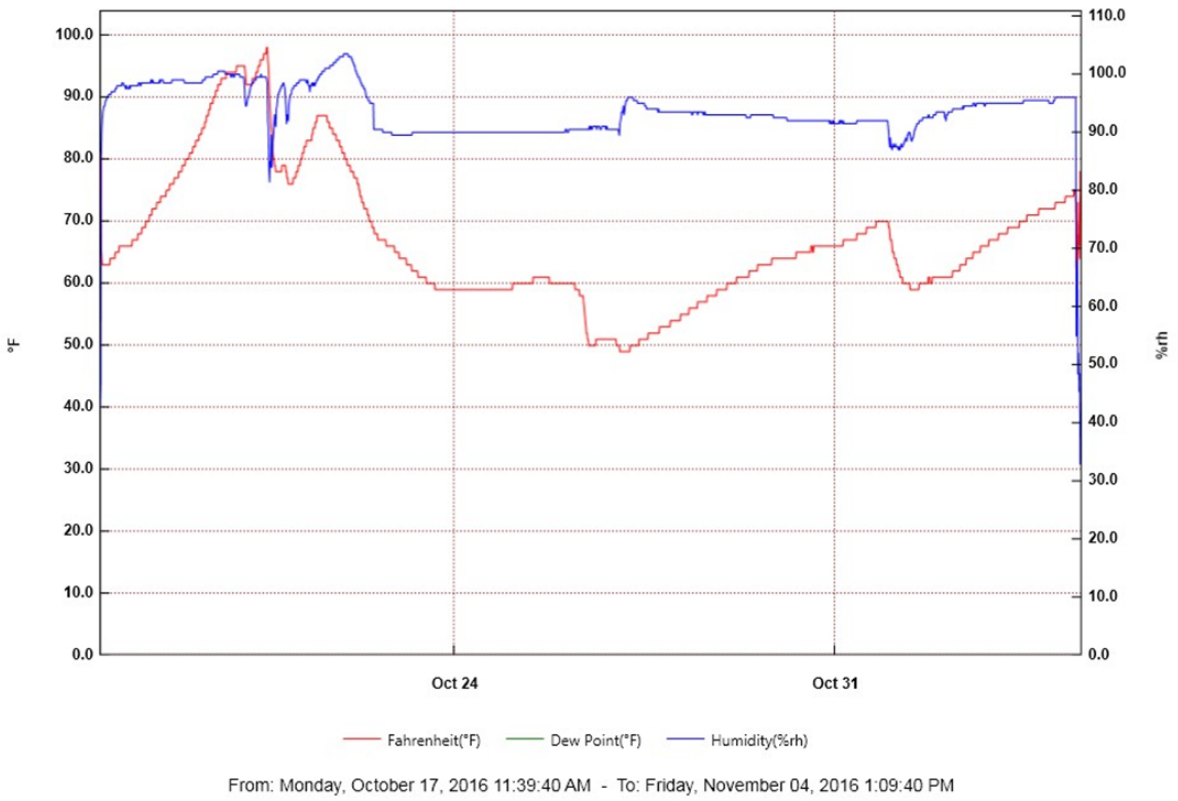


o 2017 Harvest Timing Study. The same study design used in 2016 was initiated on October 11, 2017 with branches harvested from the same section of the field at UMRS. With an early cool spell in October, results were less extreme in 2017 than the previous year. While dry branches harvested in early October did not retain needles as well as later dates, they held up much better than those harvested on similar dates in 2016. All the branches stored in water



including the early October harvest date retained needles at an acceptable level in 2017. The combination of at least partial dormancy and optimal storage moisture yielded more consistent results than previous years.

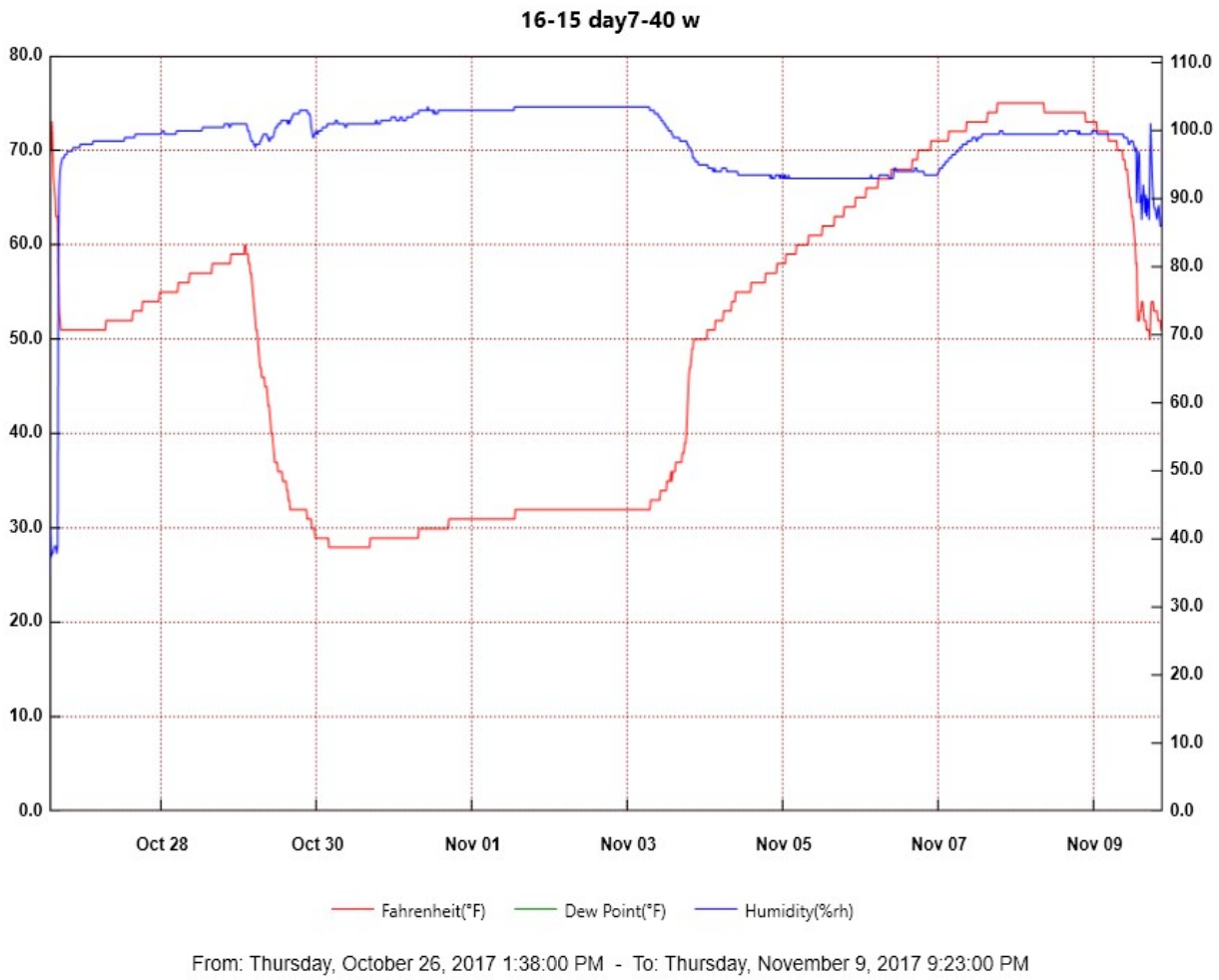
o 2016 Heat of Respiration Study. The 2016 heat of respiration (HoR) study involved construction of Christmas tree pallets at different intervals after the day of harvest to measure the impact of timing on overheating. Pallets were dismantled and trees evaluated on November 4, 2016 after the last pallet constructed had 10 days of storage. Overall, fewer trees were damaged in 2016 and to a lesser degree than in the heat of respiration study conducted in 2015. The worst scalding to trees in 2016 was associated with a 30 degree rise in temperature (shown in red in the chart below) that occurred in the pallet constructed the day trees were cut. Data loggers recorded expected HoR temperature patterns in



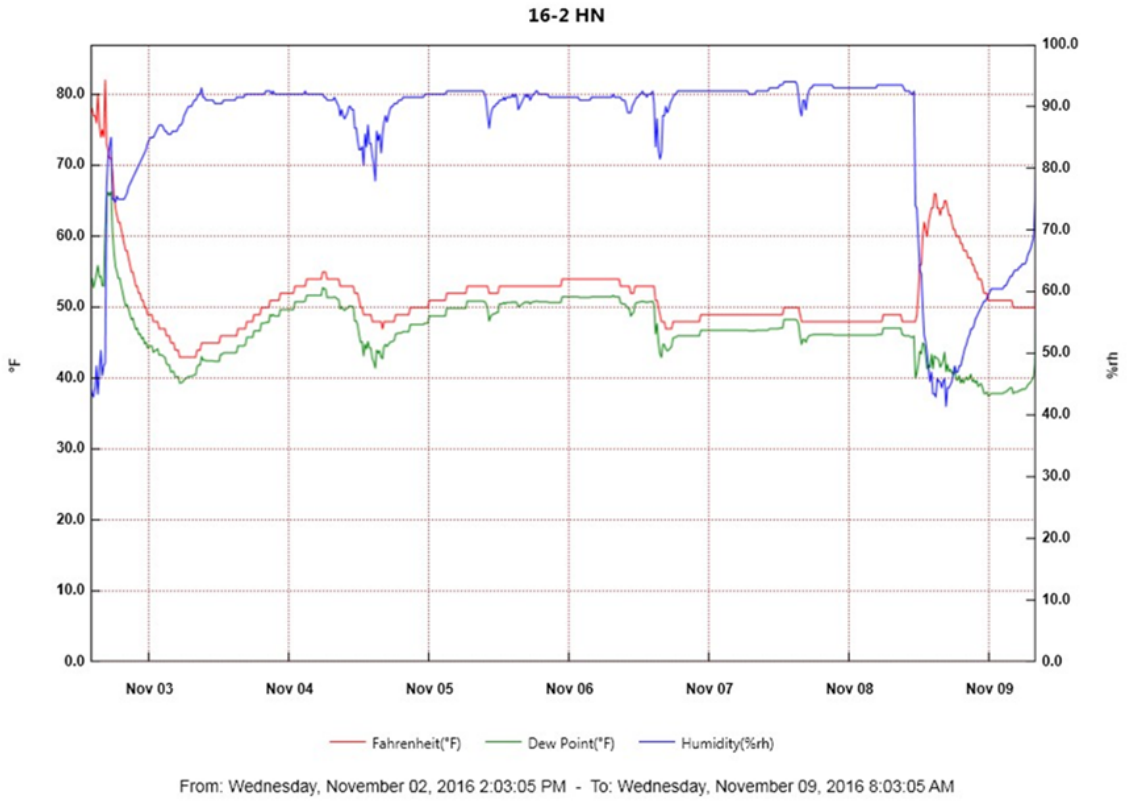
trees that were palletized soon after harvest (days 0, 1, and 2) but not in pallets of trees constructed 4 and 7 days after harvest. The pattern is consistent with what was observed in a 2015 HoR study. These results support the concept of curing trees before palletizing. More needle discoloration (possible mold or heat injury) was observed in both wet and dry pallets constructed on day 0 and the wet pallet constructed on day 7 than in other treatments. However, simply soaking cut trees prior to palletization did not appear to create the same overheating conditions observed in the 2015 study. With drought conditions in 2016, tree position in the pallet was significant with trees on top drying the most and trees located in the center of the pallet drying the least. Results from this study and the one conducted in 2015 help to contrast risks and appropriate management strategies suitable for wet and dry weather extremes represented by the two harvest seasons.

o 2017 Heat of Respiration Study. Rather than repeating four curing periods from the 2016 HoR timing study, the number of trees per pallet was the primary variable evaluated in 2017. Treatments

included 30, 35, and 40 trees per pallet in pallets built on the day of harvest (day 0) and day 7 after harvest. Two additional 40-tree pallets were added (one for each date) that contained trees that had been saturated with water immediately prior to pallet construction. Given the shortage of available trees in 2017, the 320 trees needed for this study were harvested from two farms and mixed across treatments. This HoR study ran from harvest on October 26 until evaluation on November 9. The loose check trees were the only treatment where moisture content dropped to marginal freshness levels (below MC%=100) by the end of the study. Simply storing trees in pallets was moisture conservative in a dry harvest season. More compacted pallets retained higher moisture levels than the more loosely packed pallets. Characteristic HoR "bumps" in temperature were not as strongly defined in 2017 than either of the previous years. After the early October cold snap, trees were at least partially dormant. Also, drought-stressed trees respire at lower rates. Both of these factors may have influenced results. However, every pallet in the 2017 study did exhibit rising temperatures about seven days after pallets were constructed (Red line in chart below). This heating pattern is likely associated with microbial activity on the surface of moist foliage. While rising temperatures from microbial activity were recorded, they did not reach the damaging levels that induce scald symptoms on trees. Highest observations of surface mold and highest needle loss ratings were observed in the two pallets containing water saturated trees. These results help us to understand two mechanisms which appear to be associated with Christmas tree pallet storage. When results from three HoR studies across three harvest seasons are considered, the worst problems either from HoR or microbial activity were observed with trees that had not experienced adequate cold and were palletized wet.

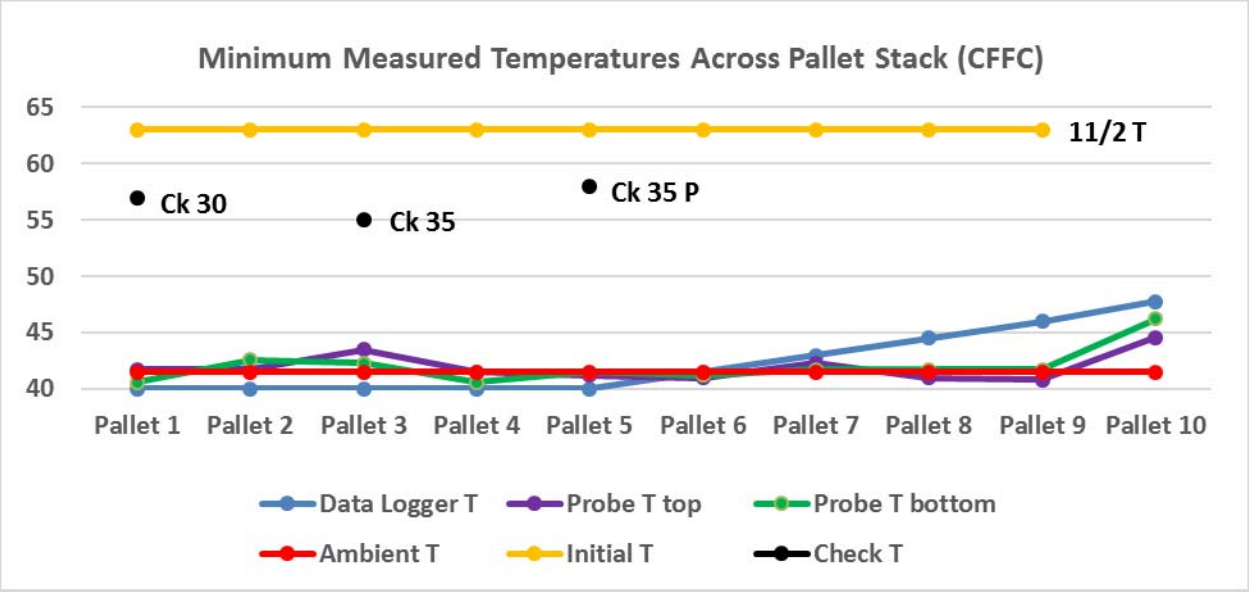


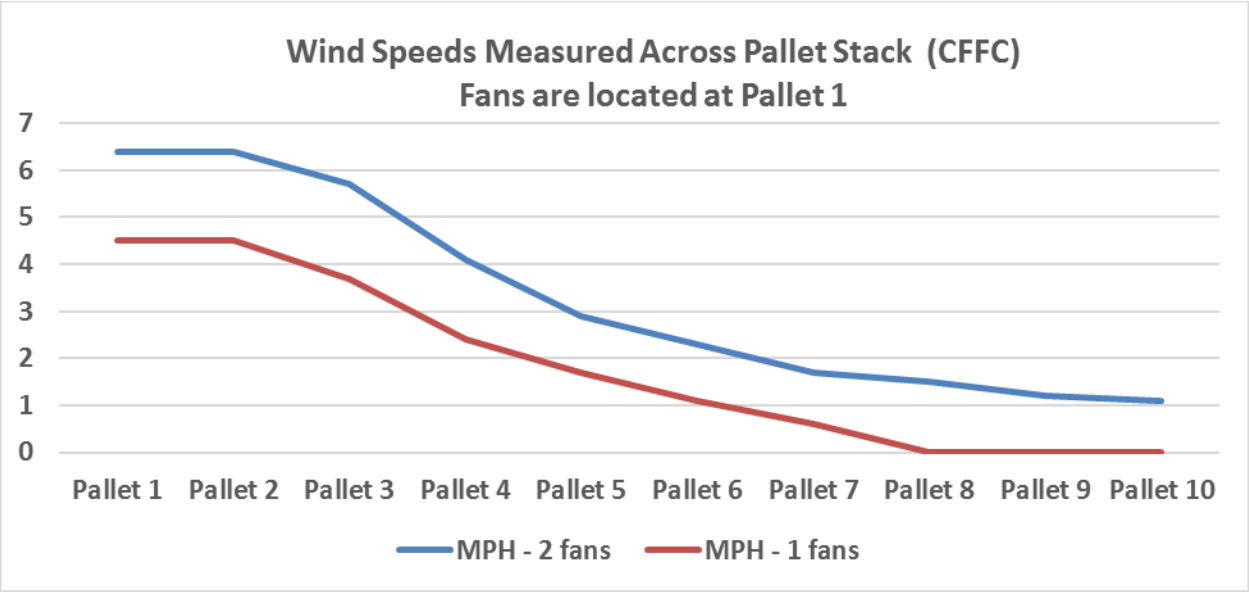
o Two forced air cooling studies were conducted in early November 2016. A study under ambient outdoor conditions was set up at Carolina Fraser Fir Company using 32 pallets of trees covered with tarps and two stacked 54-inch fans. Fans were used to pull cool air through the pallets during the night after pallets were constructed. The stack of pallets were immediately disassembled the next morning. Study pallets with data loggers were kept in the same shady storage area with untreated check pallets. Other pallets used to fill out the four by eight stack of pallets were released back to the grower's storage yard. While the check pallets did not go through damaging heats, "bumps" of increasing high temperatures characteristic of heat of respiration were recorded. In contrast, pallets treated with forced air cooling rapidly dropped in temperature while the fans ran and approached the ambient nighttime low of 42 degrees. Temperatures in the forced-air treated pallets did rise the following day, but never rose above the low fifties while data loggers remained in the pallets. One night of cooling reduced and kept temperatures low.



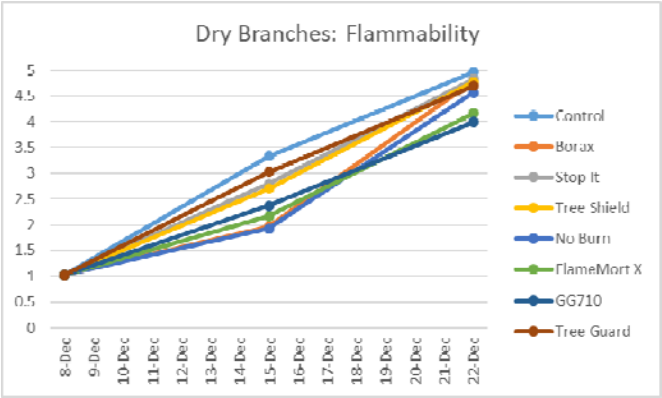
A similar study was also conducted the following week in the chilled warehouse at Kathy Shore Nursery. However, trees used in the study were exposed to freezing temperatures the night before harvest and remained cold. Without enough difference between tree temperatures and warehouse temperatures no benefit to using the fans or the chilled warehouse were observed. Kathy Shore delayed harvest until the onset of cooler weather – a smart move on her part, but problematic for our study. The combined effect of forced-air and refrigeration still merit further study under the right conditions.

o 2017 Heat of Respiration Studies. Both studies were repeated in 2017 with the same cooperators. Forced air was applied to forty pallets at each site in four rows of ten, double-stacked. Three check pallets were also monitored at each site. Temperatures were once again recorded using data loggers and a composting thermometer. Wind speed was measured at any gap along the stack of pallets. Graphs of temperature and wind speed provide a map of treatment effectiveness associated with proximity to the fan. At both sites, forced air cooling dropped pallet temperatures 20 degrees F. At CFFC, check pallet temperatures dropped 4-8 degrees. At KSN, two of three check pallets rose 6-8 degrees in temperature indicating the onset of heat of respiration. The fans did a better job of pulling in ambient cool air across the full array of pallets at Carolina Fraser Fir Company (CFFC results shown) than at Kathy Shore Nursery where the fans only chilled half the pallets. It may be possible to situate pallets too close together such that air flow is blocked. Powering these large fans was an issue that required a larger generator to avoid overheating fan motors. While results do show potential, the logistics of implementing forced air cooling may be a hurdle to adoption.


Minimum Measured Temperatures Across Pallet Stack (CFFC)
65
60
55
50
45
40
11/2 T
Ck 30
Ck 35
Ck 35 P
Pallet 1
Pallet 2
Pallet 3
Pallet 4
Pallet 5
Pallet 6
Pallet 7
Pallet 8
Pallet 9
Pallet 10
Data Logger T
Probe T top
Probe T bottom
Ambient T
Initial T
Check T


Wind Speeds Measured Across Pallet Stack (CFFC)
Fans are located at Pallet 1
7
6
5
4
3
2
1
0
Pallet 1
Pallet 2
Pallet 3
Pallet 4
Pallet 5
Pallet 6
Pallet 7
Pallet 8
Pallet 9
Pallet 10
MPH - 2 fans
MPH - 1 fans

o 2016 Fire Retardant Study. A fire retardant branch study was initiated in early December with seven different products. Fire retardants included Borax, Tree Shield, Tree Guard, Flamemort X, Stop It, No Burn, and GG710. After branches were treated according to product label directions, one was stored dry and the other "wet" (in a quart jar of water) both in a heated room. When several of the treatments in water rapidly started losing needles, possible contamination of the water with retardant became a concern. Therefore a second run of the water jar portion of the study was conducted. Branches were evaluated for moisture content, flammability, and needle retention. Several of the fire retardants appeared to induce needle loss in Fraser fir. In water, only Stop It and Borax retained needles as well as the untreated check. While on the average, all of these fire retardants reduce the flammability of dry Fraser fir foliage compared to the untreated check, some branches in every treatment continued to burn until doused in water (a rating of "5"). One must question whether the degree of control was adequate. The combination of increased needle loss and mixed flammability results made it impossible to identify a consistent, effective product better than the control.



o 2017 Fire Retardant Study. An additional fire retardant study was conducted in 2017 with a slightly different list of nine commercial products as compared to the 2016 study. Borax, Tree Shield, Tree Guard, Flamemort X, Stop It, No Burn, Stop It, Flamestop 1, and Tree Safe were tested. Once again dry and wet branches from 30 trees were evaluated for moisture content, needle retention, and flammability. This year, Borax and Tree Safe retained needles at approximately the same level as the control. All other products resulted higher levels of needle loss. Their ability to retard fire is negated if their use results in moderate to severe needle loss. At the four week mark, only watered branches of Flamestop 1 and Borax treatments resisted burning as well as the control. By the end of the six week study, all fire retardant treatments included more branches that burned until doused in water than branches that went out on their own. None of the materials tested provided an adequate level of safety. The addition of water is still the most important single practice a consumer can use to keep their tree safe.

o 2017 Christmas Tree Preservative Study. Six commercial Christmas tree preservatives (CTP's) were tested on Fraser fir branches in December 2017 through February 2018 at UMRS. This repeated research conducted in 2009 but with a different list of products. Study design is very similar to the fire retardant study except that products were added to the water rather than being sprayed on the foliage. Branches for the study were harvested on December 12, 2017. Two branches from 30 trees were stored in CTP-treated water for nine days. On December 21, half the branches were shifted to dry storage to simulate interrupted watering. The other half of branches remained in buckets treated with CTP's or the untreated check. The branches were evaluated for needle retention, flammability, and moisture content on a weekly basis through February 2. Branch samples from this project were still being dried at the time of the NC Christmas Tree Association winter meeting at the end of February. All data were collected and entered. Analysis remains to be completed.

o 2018 Homemade Water Additive Study. Thirteen different homemade water additives were tested on Fraser fir branches in January and February 2018. The Internet and media are rife with recommended recipes that may be harmful to cut Christmas trees. Conceptually, these recipes are

homemade versions of Christmas tree preservatives and in some cases may contain similar ingredients to commercial products. Branches for the study were harvested on January 4, 2018. Branches from 30 trees were stored in water treated with homemade additives or left untreated. The branches were evaluated for needle retention, flammability, and moisture content on a weekly basis through February 14. Branch samples from this project were still being dried at the time of the NC Christmas Tree Association winter meeting at the end of February. All data were collected and entered. As with the CTP study, analysis remains to be completed.

- 2018 Evaluation of Moisture Indicator Cards. Moisture content is a primary measurement made in Christmas tree postharvest research and is used as an indicator of freshness. However, methods of determining moisture content are awkward and time-consuming whether oven-dry weights or a pressure chamber is used. Neither method is suitable for a consumer to use. The idea of using humidity indicator (HI) cards was pursued as an alternative approach. HI cards were developed to track moisture exposure for electronics in storage or shipment. We tested different methods and durations of heating to moisture out of twigs. We used a microwave or submerged a sealed jar with a branch in it in boiling water. Branches at different stages of drying were tested. HI card results were compared to standard oven-dry weight moisture content determination. Analysis remains to be completed. Additional work with HI cards is planned in 2018.
- 2018 Internal Christmas Tree Pallet Pressure Measurements. Several ideas for measuring pressure within pallets were tested using trees cleared from a field at UMRS. A grower gave us access to her palletizing machine off season to test this. We experimented with a load cell film sensor and tire pillows filled with air, and then when that didn't work, with water. Using water in the air pillows, we did measure as much as 2,800 psi. Additional work is needed to build a device that is both rigid enough for multiple uses and representative of a tree inside the pallet. It also needs to be calibrated to the surface area in line with the primary force of the machine. This may never provide a level of accuracy desirable in research but it could demonstrate the compression value of different numbers of trees per pallet.
- Educational outreach. Results of postharvest studies have been presented at county meetings in Avery, Ashe, Mitchell, and Jackson Counties and at the North Carolina Christmas Tree Association 2017 and 2018 winter meetings. Our large forced air cooling fans were used to cool the building that housed the 2017 summer meeting of the NCCTA in Ashe County. While in use, they were displayed with a poster and a supporting educational handout. Postharvest study results were presented to regional sales reps of a major home improvement chain during the 2017 harvest. Other Christmas tree researchers learned about results from this project at the International Christmas Tree Research and Extension Conference held in Akureyri, Iceland in September 2017.

This project has focused specifically on postharvest issues of Christmas trees, a specialty crop. Principles and practices also apply to greenery products made from Christmas trees for display during the Christmas season. Branch studies designed to fit available space correlated especially well to postharvest quality of greenery. Even pallet studies can shed light on problems that have occurred in greenery storage as bins of greenery overheat under similar patterns and conditions as pallets of Christmas trees. No funds were directed toward other crops or commodities.

Project Partners:

- NC Cooperative Extension. County extension agents in Ashe, Avery, Mitchell, and Watauga Counties assisted in the planning, implementation, and evaluation of postharvest studies completed in this project. Whether a grower contact was needed, a strong back, or another set of eyes to evaluate trees coming out of a pallet treatment, Extension colleagues cheerfully pitched in to make things

happen. Brad Edwards, Christmas Tree IPM program assistant in Ashe County was a regular part of the team working independently or with the seasonal technician(s). Extension personnel might be paid state employees, but they volunteered to help with these postharvest studies -- often working in cold and miserable conditions. Together, they made valuable contributions to the direction of this research.

- Upper Mountain Research Station. Tracy Taylor, Superintendent, provided office space for the seasonal research technicians hired each harvest season for this project, one in 2016 and two in 2017. The UMRS provided Christmas trees for three of the 2016 studies including the 1-MCP amendment, harvest timing, and fire retardant studies. In 2017, the station provided trees for a trunk crack study, the 1-MCP amendment study, a harvest timing branch study, a fire retardant study, a Christmas tree preservative branch study, and a Christmas Tree homemade water additive study. Availability of trees from the UMRS was especially critical in 2017 when a shortage of trees made it more difficult for growers to cooperate on this research. Not only did the UMRS crew harvest the trees grown on the station, they also helped to harvest trees for the trunk crack study at Sexton Farms in 2016. UMRS staff constructed the wooden framework to transport and use the forced air cooling fans and helped to install those studies at both locations in 2016 and in 2017. One of the UMRS barns was used for tree storage and data collection. The UMRS staff has been a vital part of almost every aspect of this project from planning through implementation.

- Sexton Farms. Greg Sexton has worked with the project leader on postharvest research since 2012. In 2016, he provided 152 trees for the trunk crack study. Over the years Sexton farms has also provided trees for our first pallet studies, harvest timing studies, and retail pallet studies. They have been a valuable source of information and a key connection with several major chain store sales representatives.

- Kathy Shore Nursery. Kathy Shore provided trees for a 2015 heat of respiration study that provided a baseline of information for the pallet studies planned for this project. She provided 1,400 trees and cold storage for forced air cooling studies in 2016 and again in 2017. A cold front in 2016 naturally chilled study trees undermining expected results. By repeating the study in 2017, we were able to document treatment influence. By pulling chilled, humidified air through pallets of trees, field temperatures could be reduced to temperatures in the cooler in a matter of hours. Conducting this study in her warehouse in the midst of harvest and shipping was an imposition that the staff at Kathy Shore Nursery graciously accommodated. They also worked with us to test ways to measure pressure inside pallets.
- Carolina Fraser Fir Company (CFFC). Mike Shatley and his team cooperated on the first forced air cooling study conducted on Fraser fir in 2016 and again in 2017. Over the two years, CFFC provided 82

pallets of trees (2460 trees) at no charge. While none of the trees were damaged in the course of these short term studies and trees were released back to

CFFC to be sold, the cost was accommodating us during harvest. Conducting these studies in the midst of harvest added extra complexity to an already massive logistical operation. CFFC also harvested and transported half the trees (160 trees) for the 2017 heat of respiration study conducted at Shatley Farms. Those trees were not returned for sale and were paid for from grant funds. Given the shortage of trees in 2017 and the scale of harvest at CFFC, their participation in these studies was a measure of the value placed on this research.

- Shatley Farms, owned by Dan Shatley, was the host cooperator for the 2017 heat of respiration pallet study. They provided 160 Christmas trees (half of 320 trees needed), use of their Christmas tree palletizer (shown in the picture), and a storage location for the study. Trees were released to the farm and sold with no charge to the grant. This was the first postharvest project in which Shatley Farms cooperated.
- G & S Tree Farm. Matt Horney and Cale Smith are major wholesalers and palletizers in Avery County. They provided trees and labor for the 2016 heat of respiration study at one of their storage yards. The seven pallets in the study were evaluated in early November 2016. Cut prior to regular harvest, trees in this study were not sold. Funds from this project were used to compensate G & S Tree Farm for the trees.

- Severt's Tree Farm & Produce. In 2017, the Severt family provided a secure storage site near Columbia, SC at the regional farmers market for trees in the 1-MCP study. To simulate typical retail storage conditions in the South, a more representative climate than Laurel Springs, NC was needed. Severt's wholesale their mountain-grown Fraser fir Christmas trees at the farmers market and had enough space to accommodate our study. UMRS staff transported pallets and loose trees to the South Carolina farmers market. Mr. Severt and his staff moved pallets of trees as needed and disposed of trees when the study was completed.
- Mike Boyette is the postharvest extension specialist in Agricultural Engineering at NC State University. While not directly involved in this project, Dr. Boyette has been consulted at several stages of planning. At his recommendation, pallet weight was used as a substitute for measuring internal pallet pressures applied by palletizing machines.
- Fraser Knoll Christmas Supply Store. Tommy Wagoner at Fraser Knoll requested that Christmas tree preservative research (last conducted in 2008) be updated to include products that are currently sold by his store and others. Five of the six Christmas tree preservative products tested in 2017-18 were provided at his cost rather than full retail price.

GOALS AND OUTCOMES ACHIEVED

A primary goal of this project has been the generation of new knowledge in the area of Christmas tree postharvest quality. Work conducted over the 2016-17 harvest seasons advanced that goal in several areas. Forced-air cooling was applied successfully to pallets of Christmas trees for the first time. Heat of respiration studies furthered understanding of curing trees and the role of two different mechanisms (heat of respiration and microbial activity) in overheating in pallets. 1-MCP ethylene blockers were tested on loose and palletized trees. The ability of paint or wax to effectively stop trunk cracks under stress conditions was disproven. Harvest timing studies have documented differing needle retention patterns under very different seasons. Pallet storage of Fraser fir Christmas trees has been evaluated from farm storage to retail storage which provided insights into the strengths and weaknesses of the practice. Fire retardant studies underlined the importance of watering and the functional limits of fire retardant technology. Both commercial Christmas tree preservatives and home water additive recipes were tested for needle retention and flammability.

The only research question from the initial proposal that was not fully addressed, involved measuring pressures within pallets of Christmas trees. Several approaches were experimented with including the use of load cell films, measuring air pressure, and water pressure. While none of these techniques

worked adequately, there still might be ways to test this in the future. At Dr. Boyette's recommendation, pallet weight was used in 2017 as an indirect indicator of compaction.

A postharvest questionnaire was distributed to growers at the 2017 NCCTA summer meeting. It included questions pertaining to 2015 (prior to this project), 2016, and 2017 harvest seasons. Only eleven surveys were fully completed. From that small sample, seven (64%) reported some level of postharvest quality issue in either 2015 or 2016. They identified needle retention, moisture content / fire safety, and trunk cracks as top concerns in that order. Sun scald, string burn, and mold were less likely to be identified as top concerns. One major grower wrote in overheating of pallets as their number one issue. The survey provided some valuable information regarding grower practice. It did not provide a clear assessment of project outcomes.

While survey respondents did not adopt many new practices during the grant period, several did delay harvest by 4 to 7 days in 2016 compared to 2015. While this does not seem like much of a change, it was a very significant accommodation to an unusually warm harvest season. To shorten the harvest window, growers must plan for additional workers, additional trucks, trailers, and other equipment, and possibly even additional yard space. Growers have likened preparing for harvest to marshalling an army for an extended battle. A major grower might have to spend tens of thousands of dollars more to delay cutting just a few days. Once the course is set, it is very difficult to make changes. Several of the studies conducted in this project were delayed because cooperators were unwilling to start palletizing during hot weather. While it may have been a vexing complication to our research schedule, it was actually a positive demonstration of postharvest concepts being put to work. Whether those changes reflect solely the impact of research and education or a hard lesson of ruined trees in a previous season is a point that can be argued. Unfortunately, it is often the costly experience that best creates a "teachable moment."

Grower phone interviews were conducted during completion of the final grant report. Ten of the fifteen growers (or their managers) who regularly palletize trees were interviewed. While the number of growers interviewed were small, these major growers represented over 40% of the 2017 harvest. Only four questions were asked: Did they use NC State University and NC Extension information on postharvest tree care, did they adopt or change any practices, has it helped them to avoid any postharvest quality problems, and what dollar value would they put on that information.

This project has funded a two year period of ongoing postharvest quality research that began in 2010 and will continue with other sources of funding into the future. During the period of this grant, more than double the number of studies have been completed each harvest season compared to previous years. This increase was possible as a result of additional manpower provided by seasonal research technicians as well as an increased role of staff at Upper Mt. Research Station.

Each year, this work has focused on research questions that address grower needs, concerns, and problems. Studies represent a progression as one part of a problem is answered but leads to the next step. Where studies were duplicated, either a series of years was needed such as with harvest timing studies or more than one season was needed to substantiate results as with heat of respiration or forced air cooling.

Postharvest quality education based on this research has been an annual activity and will continue past this grant cycle. Annual educational presentations have been made at state and county extension meetings. Educational opportunities with Christmas tree growers include NC Christmas Tree Association

meetings and regular county extension Christmas tree meetings. Often these are time-limited and do not allow presentation of the full depth of information. A postharvest quality meeting is being planned for early fall 2018 with a separate session for major growers who palletize. Notebooks for major growers will be distributed prior to the 2018 build-up to harvest.

Results from the studies in this project will take time to integrate into Extension recommendations and grower practice. Practices like forced air cooling or use of 1-MCP amendments provide a potential advantage in product reliability but add cost and complexity to harvest. There will need more applied demonstration and grower involvement before anyone adopts them. 2018 research will move in that direction.

In the case of harvest timing studies which have been conducted in one form or another every year since 2012, repeated results with graphs of the previous season have been used to hammer home a simple message. Research clearly show why we recommend delaying harvest until after a week of cold weather. Late October is better than early October and November is better than October. Results and the seasonal weather summaries have helped to explain what made that year different and maybe why things went wrong. With understanding, growers have had greater control of seasonal variation. While a chilling model has not yet been developed, we may have enough years with different weather patterns to proceed.

Objective 1. Heat of respiration / curing pallet study	2016 & 2017 studies completed
Objective 1. Measurement of internal pallet pressures	Not completed, pallet weights measured in 2017, other methods of measuring pressure explored in 2018
Objective 2. Forced air cooling study	Two 2016 studies & two 2017 studies completed
Objective 3. Harvest date branch study	2016 & 2017 studies completed
Objective 3. 1-MCP amendment study	2016 & 2017 studies completed
Objective 3. Fire retardant study	2016 & 2017 studies completed Additional related 2017 studies on Christmas tree preservatives and homemade water additives
Objective 3. Trunk crack study	2016 study completed. 2017 study expanded to include more destructive sampling for moisture content
Objective 4. Grower education	County and state postharvest quality presentations completed in 2017 and 2018
Objective 4. Grower pretest survey	Survey distributed at 2017 NCCTA summer meeting. Grower interviews completed in 2018.

Two clearly defined targets were written under measurable outcomes in the proposal for this grant: an increase in postharvest care knowledge among growers and a positive estimated financial value assigned by growers to that knowledge. During the grant period, educational efforts consisted of one-

on-one sessions with growers and presentations at nearly all state and county meetings. One survey was distributed in 2017. Major grower interviews were conducted during completion of the final report in 2018.
The survey conducted in 2017 provided insights to grower practice. Most respondents identified using postharvest practices associated with proper management of field activities and farm storage (such as coordinated and rapid movement of trees from the field). Most also identified working with customers on delivery dates, frequency of deliveries, and postharvest education. Use of refrigerated storage or transportation was not widely reflected among respondents. To a great extent, the use of practices was determined by a respondent's scale of production rather than their adoption of new postharvest information.

The 2018 major grower interviews provided clear progress toward achieving set targets. Collectively, they represented more than 2 million trees sold in 2017 or 40% of NC sales. All ten respondents used NCSU or Extension information. Every respondent adopted curing practices prior to palletizing trees. All but one used composting thermometers to monitor pallet temperatures. Several mentioned various modifications they implemented. One grower rotated the pallet orientation in their machine to give trees six more inches of room and reduce compaction. A loss of two pallets per truck was balanced by less risk of overheating. One used corrugated 4 inch plastic pipe first tested in the forced air cooling study to cool fresh greenery that was overheating. Another operation is building a large shade structure to keep palletized trees out of the sun. By palletizing, one grower reduced yard crews from fifteen to seven people and reduced loading times from three hours to twenty-five minutes. While the exact costs associated with this example were not provided, a significant savings is apparent and likely shared by other growers who palletize. Interviewees used NCSU postharvest information to avoid problems that involved "1-2 truckloads of trees a year," to "hundreds of pallets." Hundreds of pallets represent a $60-$80,000 problem. Respondents placed value on this information from $7,000 to $150,000 a year with an average of $69,700 and a collective value of over $625,000.

Successful outcomes for postharvest quality can be difficult to quantify. Problems avoided are difficult to count. Also, most growers made major investments to keep cut trees fresh earlier in their business and are likely to only make incremental changes to the overall structure of harvest practices now. However, there are some ways that we can point to successful outcomes among growers.

One simple measure of the impact PHQ research and education are growers' use of composting thermometers to monitor the temperature of Christmas trees stored in pallets. Of the fifteen major growers who regularly palletize, at least twelve of them bought and use composting thermometers to check pallet temperatures. In doing so, they recognize the potential for overheating problems, manage what they can prior to palletizing trees, and monitor during storage. Using this information, growers have stopped or delayed palletizing until conditions are more appropriate. Many have found and opened up overheating pallets before trees are seriously damaged. While expensive, keeping a problem on the farm is ultimately less expensive than shipping replacement trees to a customer or losing a dissatisfied wholesale customer.

A 2017 NC Extension "success story" quantified successful outcomes associated with palletization of Christmas trees. Extension recommendations to palletizers to "cure" trees prior to palletization reduced incidence of overheating in palletized trees for an estimated savings of $180,000 based on saving 9,000 trees across six operations (1,500 trees per operation). Of course, "trees saved" from a potential postharvest problem can only be estimated, but in 2015, more than one grower had to open and re-

palletize 100 pallets that contained 3,500 trees. This success story was conservative in the number of growers who presently cure trees who avoided overheating problems, the number of pallets involved in overheating without curing, the number of trees potentially damaged per pallet, and the wholesale price of trees. As the 2018 major grower interviews demonstrated where 10 growers collectively valued educational information at over $625,000, a much larger benefit could have been claimed.

An important outcome of this project has been on-farm presence during harvest. At more than one major-grower farm, we were able to show visiting regional chain store buyers postharvest study results. With the harvest timing study, we didn't need a graph – we could simply show obvious needle retention differences by treatment date. Our discussion of negative effects of early harvest has led to increased flexibility on delivery dates at their stores. In years when cold weather comes late, these home improvement chain stores have become much more receptive to delaying delivery to maintain product quality. In an industry where dates were locked in from year to year, a one-week delay is a huge concession to keeping Christmas trees fresh. We have been invited to visit with chain store representatives annually to summarize postharvest research and answer their questions. This has been one of the few ways major retailers have been engaged in discussions with Extension personnel about Christmas tree care.

BENEFICIARIES

Three groups of Christmas tree growers benefited from the completion of this project:

Major growers. Research projects that involved pallets of Christmas trees (heat of respiration, forced-air cooling, & 1-MCP amendments) were most beneficial to those growers who palletize. In NC, there are fifteen growers who regularly palletize their trees for chain stores although several more growers own palletizing machines. These growers are all major growers that wholesale in excess of 100,000 trees annually. Several harvest 3-400,000 trees and one harvests in excess of 600,000 trees. Representing the second largest segment of production in NC, major growers buy and re-wholesale trees from many other growers. In this way, success of major growers helps to drive demand for other growers' trees. Because chain stores order cut Christmas trees as much as two weeks earlier than lower-volume customers, growers who sell to them must harvest trees earlier when weather and tree dormancy are less favorable.

NC Christmas tree growers. Several of the studies conducted in this project had the potential to benefit all 1,300 NC Christmas tree growers whether they wholesale palletized trees to chain stores, wholesale loose trees to anyone, or sell trees one-by-one from the farm. While market segments are broken into chain stores, independent retailers, grower operated retail lots, and choose & cut; most growers sell tree in several if not all categories. While some postharvest problems are worse for chain store suppliers because of sheer volume of trees, other problems cut across all segments. NC growers have identified needle retention, fire safety, and trunk cracks as key postharvest quality concerns. In this project, harvest timing, trunk crack, fire repellent, Christmas tree preservative, and home water additive studies specifically address these key concerns.

Greenery manufacturers. The greenery industry in NC is part of the overall Christmas tree industry and is operated predominantly by Christmas tree growers and their families. A majority of the labor is provided by migrant workers. The greenery industry consumes a majority of cull and many second quality

Christmas trees. Only a few growers plant fields of trees specifically for the greenery industry. Postharvest issues with greenery are aggravated beyond problems seen in Christmas trees. Wreaths and roping are made from branches that have more limited moisture reserves than a Christmas tree. While Christmas trees can be watered, greenery dries out and stays dry. Greenery harvest begins earlier than Christmas tree harvest on most farms – often two weeks earlier. Bough harvest can start as early as October 7 at the largest operations. Inadequate dormancy leads to poor needle retention. Refrigerated and humidified storage can hold product while in storage but not reproduce dormancy. Studies conducted in this project like harvest timing and the 1-MCP amendment study, have even greater implications for greenery manufacturers than they do for the Christmas tree side of the business.

The 2012 US Agricultural Census reported 1,370 Christmas tree operations in NC with more than 40,000 acres of trees. Assuming a 10-year rotation, an average of 1,500 trees harvested per acre, & 85% productive land use, approximately five million trees are being harvested annually in NC with a value of more than $90 million. Approximately half of the trees sold each year, or 2.5 million trees, are palletized to go to chain stores. To achieve early November delivery dates at least a quarter of chain store trees must be cut in late October. This puts at least 600,000 trees at risk of being harvested before dormancy can fully set the needles. Additional factors such as drought, excessive rainfall, or unusually warm weather increase the risk of problems that include trunk cracks, overheating, needle drop, and drying. The loss of freshness is a subtractive process as stress events add up across harvest and handling. Problems can show up in a few trees here and there but sometimes occur catastrophically. Reports of 100 pallets overheating on one yard or three truckloads (26 pallets each) being rejected by a wholesale customer do occur. Seventy-eight pallets of trees represent a value of more than $50,000 including transportation costs. Opening, sorting, replacing damaged trees, and re-palletizing 100 pallets could easily cost a grower more than $30,000. However, the grower would be “counting his blessings” as he absorbed the cost because the problem was identified before trees left the farm.

The greenery industry has been generating an estimated $25 million annually. More than 2,000 people are employed in western NC during the months of October and November to make wreaths, roping, centerpieces, kissing balls, and other greenery arrangements. Major producers have bought empty factory buildings and converted warehouses to refrigerated storage. Just the investment in refrigeration represents hundreds of thousands of dollars. Starting two to three weeks earlier than Christmas tree harvest, these perishable products are at greater risk of losing than are Christmas trees. After poor product performance in 2015, one chain store eliminated thousands of roles of Fraser fir roping that represented $1 million in sales as a special early season sale item. Current product performance determines the future reputation and growth potential of this industry.

LESSONS LEARNED

This project has provided many positive results that help us to better understand postharvest quality issues of Fraser fir Christmas trees:

- Harvest timing studies clearly showed the importance of delaying harvest until after several nights of cold temperatures. Good performance of branches stored in water jars demonstrated the value of minimizing drying stress to maintain good needle retention. The use of humidified and chilled warehouses for tree and greenery is the best example of putting this principle into practice but other storage facilities can also increase humidity in dry years.

- Heat of respiration studies identified two mechanisms for overheating in pallets that occur at different points during pallet storage: initial heat of respiration during the first four days after harvest and heat of microbial activity that started seven days after pallets were constructed. Increased pallet density and moisture were associated with a larger percentage of trees with needle retention and mold problems.
- County agents and growers took results from heat of respiration studies even as data were being collected. Based upon overheating observed in study pallets, growers bought and used composting thermometers to monitor temperatures in their own pallets. By catching problems before shipment the extent of damage to trees and impacts to customer relations was minimized.
- Treatments of 1-MCP did provide a measurable improvement in needle retention in 2016 when trees were harvested early and stored under hot, dry conditions. In 2017, when trees were harvested within the normal harvest season, no differences in average needle retention were observed. However, when the frequency of trees with moderate to severe needle loss was examined, the untreated check had nearly twice as many problems as any of the 1-MCP treatments. Initial applications of this treatment could be justified particularly for export trees that are harvested earlier than trees shipped domestically.
- Forced-air cooling studies were successful demonstrations of a postharvest practice first developed for fruits and vegetables. Pallet temperatures were rapidly reduced 20-30 degrees to within a degree of ambient nighttime or cooler temperatures. However with the expense and added complexity represented by this practice, the use of fans will have to be further developed and demonstrated before growers will consider adopting them.
- While our trunk crack studies did not support painting cut ends of trees with wax for the sake of reducing cracks, these studies did show a potential benefit of the treatment in holding moisture closer to the cut end of the tree and its ability to re-hydrate when eventually displayed. Extensive sampling for moisture content has provided data which may be used to map the pattern of moisture flow in cut trees as they dry out.
- The greatest lesson from fire retardant studies conducted in this project confirm the importance of regular watering. No product sprayed on foliage could overcome the flammability of dry foliage. Both years, borax performed as well on watered branches as any of the more expensive commercial formulations. Addition of water was the most important treatment. A consumer would be better served to pay attention to keeping a tree watered and away from sources of flame than to spend money on a fire retardant that might help if the tree isn't too dry.

Several unexpected outcomes occurred over the course of this study:

- Growers' adoption of the practice of curing trees prior to palletization was an unplanned but almost immediate outcome of the heat of respiration studies. Our studies were conducted ahead of regular tree harvest and palletization. When problems developed in study pallets, we were able to warn growers a few days before problems started occurring in their own pallets of trees. Curing trees allowed initial heat to dissipate and not get trapped in pallets of trees. However, rather than curing trees in shaded storage yards as traditional practice would suggest, several growers cured trees in the field where they are cut. While less labor-intensive, it put trees risk of drying out and getting scalded by the sun. First practiced in Denmark where the angle of winter sun is much lower, field-curing trees is a risky practice in a Southern state like NC where the angle of the sun is much higher during harvest. Additional education will be needed to promote shade-curing of baled trees at the loading yards.
- Documentation of microbial activity in palletized trees was unexpected. In the heat of

respiration studies, an initial period of heating was expected that would decline several days after harvest as respiration of cut trees slowed. A second cycle of overheating from microbial activity was not expected. Evaluating treatments in which trees were saturated with water and others using pallets with different tree densities helped to identify factors that contribute to microbial activity. The clumping of loose dead needles inside palletized trees (needles that did not drop normally in the field as they should have) is another contributing factor to microbial activity.

- In 2016, a second round of the fire retardant treatments in which branches were stored in water was necessary. Severe needle loss occurred to branches in water in the first study within a few days. Upon reflection, we recognized that we were submerging the bottom of branches that had been treated with fire retardants in water. Toxic levels of salts likely induced needle drop. For the second round, the bottoms of branches were not treated. Needle retention followed a much slower course with a different outcome across repellent treatments. Water contamination has implications for the general use of fire retardants both in terms of spray application and the flakiness of some products. It is quite possible to spray into a tree stand if the tree is already up. If branches are disturbed during decoration, flakes of retardant can fall into the water in the stand. The risk of imbibing salt could contribute to variability of needle retention associated with some fire retardant products.

Outcome measures not achieved and lessons learned:

- Trunk crack treatments with paint or wax did not significantly reduce the occurrence of side cracks on Fraser fir Christmas trees subjected to field drying in 2016 or 2017 studies. A study in 2014 provided positive results in which no painted trunks cracked, but those results have not been duplicated. Some growers still believe wax or paint treatment is beneficial. Both scenarios might be true. Trunk cracks occur at a low incidence normally. To increase the incidence of cracking, our research was conducted before the normal season under severe drying conditions that growers strive to avoid. While paint and wax treatments have failed under the extreme conditions selected for research, they might reduce cracks under low-stress conditions created in growers' storage yards.

  However, it is very difficult to study a problem that occurs at only 1 or 2 percent under normal conditions. A different study design is needed using grower's trees that are followed to their own retail lot. Rather than pulling trees out of commercial production, these trees would be sold. Trees could be monitored from the field to the retail lot up to the point when they are sold. This would better reflect industry practice than the current research approach. By following a larger number of trees, there still might be enough cracks to test treatments under normal conditions.
- Measuring pressures inside pallets of Christmas trees as they are being constructed never worked out during the duration of this project. We had the idea of using a home-made "bite-force sensor" similar to what zoologists use to measure the force of animals bites. In concept, it could be placed between two Christmas trees during pallet construction and possibly be pulled out without deconstructing the pallet. We never could get an agricultural engineer or load cell sales representative to help us develop such a tool. We tried several approaches ourselves including the use of less-expensive film load cells and hydraulic tire pillows. However, nothing we used could be pulled out of a compressed pallet. As an indirect indicator of compaction, we measured pallet weights instead. Unless variability of tree size is strictly controlled, the correlation of tree number to pallet weight is poor. As we look to the next harvest season, we plan to continue experimenting with hydraulic tire pillows and to

explore options to build a removable pallet pressure sensor.

- Forced-air cooling studies in this project were largely successful at reducing tree temperatures by as much as 30 degrees. However, in the 2017 studies, cooling at the far end from the fans was not as effective as it was nearer to the fans nor were measured wind speeds as high. There may be some adjustments to how the stacks of pallets are arranged that could improve air flow. For this fine-tuning work, wind speed is the important variable and a fairly rapid measurement to take. Several adjustments could be evaluated over the course of a few hours if temperature curves were not needed. Adjustments might include interior aisle width, filling gaps in pallets, and the number/length of pallets under the tarp. Fine-tuning this practice could make it more feasible for growers to adopt.

Additional Lessons Learned:

- In 2017 we hired two seasonal research technicians instead of the one hired in 2016. While all three technicians hired for this project were intelligent, hard-working people, there was an advantage to relying on a team rather than an individual. The additional manpower increased the independence and productivity of the position. Several tasks could be completed more than twice as fast by two people than by one person alone. As a result, we were able to conduct several additional studies during the winter of 2018 beyond what was planned the original proposal.
- In the proposal for this project, much of the evidence for success were based on pre- and posttest surveys. Response to the initial survey was not very good. Response to open-ended questions was superficial and not all multiple choice questions were filled in. Distributing surveys during an educational meeting often results in a rushed effort. A different distribution method could have been used. There is also a level of survey saturation that overwhelms growers. At one meeting, growers were given three surveys and some also participated in a grant-related focus group. If surveys are used in the future, they should be distributed as part of an educational session with opportunity for discussion rather than something handed out as participants prepare to leave.
- The use of grower interviews provided more immediate feedback than did the survey used in this study. Participants could be engaged in more dialogue and be encouraged to answer more deeply. When responding to the value of information, they could talk through an example and come up with an answer where the paper survey question would have been left blank. Further, growers were more willing to share ideas not framed by initial questions. Several ideas were suggested for additional research as well as their willingness to cooperate in the future. None of this would have been stimulated in the process of filling out a questionnaire.
- A big part of the budget for this project was devoted to purchasing trees from growers. Having money to buy trees made it possible to do destructive studies like the trunk crack and heat of respiration studies. It was especially important during the period of this grant as the industry moved from over supply to under supply of trees. Even with funds to buy trees, growers had to volunteer their limited inventory. Trees used for research were not available for customers. In some cases, the grade or size of tree provided to us was more variable than what would be sent to a wholesale customer – a factor which may have obscured some results. Despite this, we were thankful to have any trees at all to work with given the present market. All in all, the ability to buy trees for research was a critical asset for this project.
- Using UMRS facilities and trees increased the efficiency and productivity of this project. We relied on a mature block of trees for any of the projects that did not need a standardized "pallet tree" (narrow taper and lighter branch density). With close proximity to our technicians

and the added support of station staff, projects could be set up comparatively easily without disrupting growers' busy schedules. However, the UMRS trees we used were left over from an abandoned project. What we did not use were sold in 2017. At present, there are no more trees to use on the station. Steps are being taken to establish annual plantings at UMRS to support future projects, but that is a rotation away. Working together on this project has forged closer relationships between Extension and the UMRS. Long range plans are being developed that will insure a partnership on future Christmas tree research for decades to come.

- The rewards of working with UMRS staff have been great, but relying on that relationship can have an opportunity cost in regards to working with growers. While often more difficult to set up, to maintain, and to protect; on-farm studies do provide numerous benefits over time. Cooperation forges relationships with a grower and his staff. On-farm studies are more likely to involve the grower in the planning of a study. The grower doesn't just see its progress, he or she has ownership of the research. With that level of involvement, not only do they have better understanding – they become an advocate and spokesperson for the research they were part of. This project included a balance of research station and on-farm studies. Both parts were vital to getting as much done as we did.

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PROJECT TITLE Integrated Control of Cucurbit Downy Mildew*

PROJECT SUMMARY

Cucurbit downy mildew (CDM) is a highly destructive foliar disease of cucurbit vegetables, including cucumber, watermelon, cantaloupe, squash, pumpkin, zucchini, and gourd. Valued at over $1.5 billion in the United States (US) and over $81 million in NC, these crops can be rapidly defoliated by *Pseudoperonospora cubensis*, a fungal-like oomycete that causes CDM and can travel long distances through air currents. Until 2004, CDM was controlled in cucumber by the use of resistant cultivars and, in other cucurbits, with commonly used fungicides. Nonetheless, pathogen populations overcame host resistance in cucumber, and every year since, widespread crop failures have occurred throughout cucumber fields in the eastern half of the US. Fungicides are currently the only form of control available to commercial cucurbit growers for preventing and managing CDM. However, *P. cubensis* is well known for its ability to quickly develop resistance to fungicides. In NC, *P. cubensis* has been reported to be resistant to mefenoxam, strobilurins, and mandipropamid. In 2013, fungicide trials revealed poor control of CDM in NC using Presidio (a.i. fluopicolide), the leading product used for CDM control in the past few years. Fungicide trials during 2014 in NC indicated reduced efficacy of two other key fungicides: Previcur Flex (a.i. propamocarb) and Ranman (a.i. cyazofamid).

Only a handful of spray programs are now effective in controlling CDM, fortunately, new control options are in the horizon including new fungicides and tolerant varieties. While there is preliminary efficacy data for new fungicides generated by PI Quesada in NC, there wasno information regarding performance of new tolerant varieties as a standalone control measure and in combination with a spray program. As new fungicides and tolerant varieties become available for growers, it is imperative that they be used in an integrated program of tolerant varieties protected with fungicides that have different modes of action to ensure durability of both control strategies. If there are not enough different fungicides to use within a program, any new fungicide will likely be overused, resulting in an increased risk of DM developing resistance to it. The same is true for controlling disease by using host resistance as a standalone control method for a pathogen that can overcome varieties quickly, as it already happened in 2004. Understanding changes in DM populations as we monitor fungicide efficacy will explain how fungicide resistance becomes established in DM populations so as to prevent its development. Our two-pronged strategy will improve DM control and ensure that growers can reliably manage a disease outbreak.

PROJECT APPROACH

1. Obtain cucumber variety seed and fungicides for experiments, and set up fields (02/2016 – 06/2016)
Plots were single beds on 5-ft centers covered with white plastic mulch; 14-ft long with 5-ft fallow borders on each end and non-treated guard rows on each side. The previous year the field was planted with sweetpotato. Cucumber was direct seeded (2-ft in-row spacing, 2 seed/hill) in raised beds and thinned to one plant per hill after emergence (7 plants/plot). Three non-treated commercial cucumber varieties were included in the trial in addition to non-treated Vlaspik and Vlaspik treated with different fungicide applications. Irrigation and fertilization were applied via drip tape. Treatments were randomized into four complete blocks.

2. Conduct fungicide efficacy trials with single registered and unregistered products, and new cucumber varieties. Conduct field trials with spray programs in combination with resistant varieties. Sample pathogen populations for genetic analyses (06/2016 – 10/2016)

Fungicide treatments were applied using a CO_2-pressurized backpack sprayer equipped with hollow cone nozzles (TXVS-26) delivering 40 gal/A at 45 psi. The first three spray applications were made with a single-nozzle boom and the last one with a 2-nozzle boom (19-in. spacing). Applications were made weekly. Disease severity was assessed weekly as percent leaf area with necrosis per plot. Fruit were harvested the last three weeks of the trial. Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and the Waller-Duncan test to separate means.

Downy mildew was first detected at approximately 3% disease severity in the field and progressed throughout the course of the trial. Non-treated Peacemaker, Orondis Opti A and Non-treated Citadel controlled downy mildew well and produced the greatest weight of total marketable fruit. Ranman also managed *P. cubensis* when compared to the Non-treated Vlaspik. No other treatments provided commercially acceptable levels of disease control. No phytotoxicity was observed. In the table, treatments are sorted by disease severity.

		Disease Severity* (%)		
Treatment and rate of product per acre	**Application No.**	**1st rating**	**Final rating**	**Mkt Yield (lb/plot)**
Non-treated Peacemaker	N/A	9.8 h**	20.5 h	22.53 a
Orondis Opti A 0.83 SC 2 fl oz	1-4	14.5 g	26.5 h	13.24 b
Non-treated Citadel	N/A	16.3 fg	28.5 h	10.27 bc
Ranman 3.33 SC 2.75 fl oz	1-4			
Induce SL 0.25% V/V	1-4	16.5 fg	38.0 g	8.71 cd
Curzate 60 DF 5 oz	1-4	12.0 gh	41.0 fg	8.08 cde
Previcur Flex 6 F 19.2 fl oz	1-4	23.0 cde	43.0 efg	7.28 c-f
Gavel 75 WG 32 oz	1-4	23.0 cde	45.0 d-g	6.35 c-f
Presidio 4 SC 4 fl oz	1-4			
Induce SL 0.25% V/V	1-4	16.0 fg	46.5 c-f	8.19 cde
Manzate Pro Stick75 DG 48 oz	1-4	24.3 b-e	47.5 c-f	6.15 c-f
V-10208 4 SC 10 fl oz	1-4	20.3 ef	48.3 c-f	8.65 cd
Omega 500 F 24 fl oz	1-4	21.3 de	49.0 c-f	5.31 def
Zampro 4.33 SC 14 fl oz	1-4			
Induce SL 0.25% V/V	1-4	21.8 de	49.3 c-f	7.65 c-f
Revus 2.08 SC 8 fl oz	1-4	21.8 de	49.5 b-e	5.97 def
Tanos 50 WG 8 oz	1-4	22.3 de	50.8 b-e	7.31 c-f
Zing! 4.9 SC 36 fl oz	1-4	23.3 cde	51.8 bcd	6.59 c-f
Merivon 42.5 SC 6.7 fl oz	1-4	27.0 abc	52.0 bcd	6.02 def
Forum 43.5 SC 6 fl oz	1-4	23.5 cde	52.8 bcd	5.22 def
Bravo Weather Stik 6 SC 48 fl oz	1-4	23.3 cde	54.0 bc	8.73 cd
Ridomil Gold Bravo36.4 SC 40 fl oz	1-4	22.5 cde	54.8 bc	4.77 def
Cabrio 20 EG 12 oz	1-4	25.8 a-d	57.8 ab	5.79 def
Non-treated Expedition	N/A	28.5 ab	64.3 a	4.15 ef
Non-treated Vlaspik	N/A	29.5 a	64.3 a	3.71 f

* Disease rating scale based on percent necrotic foliage caused by *P. cubensis*.

** Treatments followed by the same letter(s) within a column are not statistically different (*P*=0.05, Waller-Duncan k=100).

3. Presentation of findings at pickle grower meeting, Year 1 (10/2016 – 10/2016)

Nine presentations were given to disseminate information about cucurbit downy mildew diagnostics and management:

Quesada-Ocampo L. M. Management of cucumber downy mildew using fungicides and host resistance. Pickle Packers International Annual Meeting. Charleston, SC, October 2016.

Quesada-Ocampo L. M. Diagnosis and management of cucurbit diseases. Cucurbit field day. Cleveland, NC, September 2016.

Wallace E. C. and Quesada-Ocampo L. M. *Pseudoperonospora cubensis* on commercial and non-commercial cucurbits in North Carolina: population structure determined by simple sequence repeats (SSRs). APS Meeting, Tampa, FL, August 2016.

Wallace E. C. and Quesada-Ocampo L. M. Genetic structure of *Pseudoperonospora cubensis* populations infecting commercial and non-commercial cucurbits in North Carolina. XIth Eucarpia Cucurbitaceae Meeting, Warsaw, Poland, July 2016.

Quesada-Ocampo L. M. Using every tool in the toolbox to improve vegetable disease control. Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI, June 2016.

Quesada-Ocampo L. M. Using genomics approaches for rapid development of species-specific diagnostics for cucurbit downy mildew. Canadian Phytopathological Society Annual Meeting, Biovigilance Symposium, Moncton, Canada, June 2016.

Quesada-Ocampo L. M. Cucurbit downy mildew management, diagnostics, and pathogen populations. Pickle Packers International Spring Meeting. Raleigh, NC, April 2016.

Quesada-Ocampo L. M. Downy mildew and Phytophthora control in cucurbits. NC Watermelon Convention. Wrightsville Beach, NC, March 2016.

Quesada-Ocampo L. M. Downy mildew updates for cucurbits. Southeast Regional Fruit and Vegetable Conference. Savannah, GA, January 2016.

Wallace E. C. and Quesada-Ocampo L. M. *Pseudoperonospora cubensis* on commercial and non-commercial cucurbits in North Carolina: population structure determined by simple sequence repeats (SSRs). APS Meeting, Tampa, FL, August 2016.

Wallace E. C. and Quesada-Ocampo L. M. Genetic structure of *Pseudoperonospora cubensis* populations infecting commercial and non-commercial cucurbits in North Carolina. XIth Eucarpia Cucurbitaceae Meeting, Warsaw, Poland, July 2016.

Quesada-Ocampo L. M. Using every tool in the toolbox to improve vegetable disease control. Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI, June 2016.

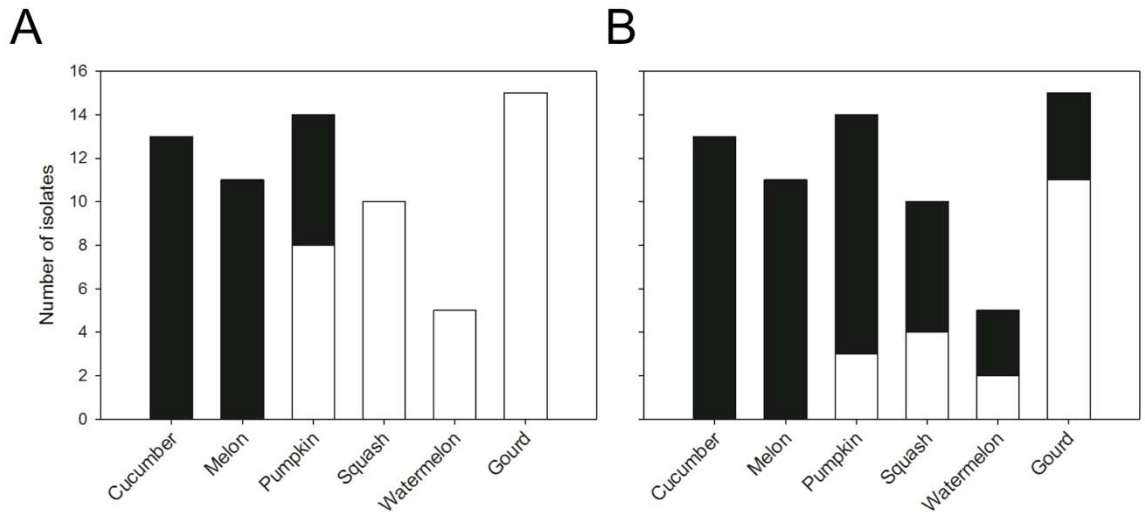
Quesada-Ocampo L. M. Using genomics approaches for rapid development of species-specific diagnostics for cucurbit downy mildew. Canadian Phytopathological Society Annual Meeting, Biovigilance Symposium, Moncton, Canada, June 2016.

Quesada-Ocampo L. M. Using every tool in the toolbox to keep North Carolina vegetables healthy. Department of Biochemistry Seminar, North Carolina State University, Raleigh, NC, February 2016.

Quesada-Ocampo L. M. Integrating field and genomic strategies to keep North Carolina vegetables healthy. AgBiome, Durham, NC, February 2016.

4. Analyze pathogen populations resistant to fungicides with genetic markers (11/2016 – 11/2016)

Fungicide resistance markers for both the cellulose synthase gene and the cytochrome b gene for FRAC (Fungicide Resistance Action Committee) codes 40 and 11 fungicides have been developed. Population analyses on both mutations has shown that *P. cubensis* isolates that are recovered from cucumber contain fungicide resistance mutations at a significantly higher rate than that of isolates from other cucurbit hosts like gourds.



Fungicide resistance in *P. cubensis* found on various cucurbit hosts collected throughout North Carolina. Solid bars denote resistant isolates that contain fungicide mutations associated with resistance, where clear bars denote sensitive isolates without the mutations. (A) Isolates that contained CesA3 gene mutations (G1105V/W). (B) Isolates that contained cyt B gene mutations (G143A).

5. Presentation of findings at vegetable grower meeting, Year 1 (12/2016 – 12/2016)

Quesada-Ocampo L. M. Cucurbit disease management. Commercial vegetable grower symposium. Henderson, NC, February 2017.

Quesada-Ocampo L. M. Disease identification on vegetables. Certified Crop Advisor Training. Smithfield, NC, December 2016.

Quesada-Ocampo L. M. Fungicides and host resistance for cucurbit downy mildew management. 31st Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, December 2016.

6. Obtain cucumber variety seed and fungicides for experiments, and set up fields (01/2017 – 06/2017)

Plots were single beds on 5-ft centers covered with white plastic mulch; 14-ft long with 5-ft fallow borders on each end and non-treated guard rows on each side. The previous year the field was planted with sweetpotato. Cucumber was direct seeded (2-ft in-row spacing, 2 seed/hill) in raised beds and thinned to one plant per hill after emergence (7 plants/plot). Three non-treated commercial cucumber varieties were included in the trial in addition to non-treated Vlaspik and Vlaspik treated with different fungicide applications. Irrigation and fertilization (4-0-8, N-P-K) were applied via drip tape. Treatments were randomized into four complete blocks.

7. Conduct fungicide efficacy trials with single registered and unregistered products, and new cucumber varieties. Conduct field trials with spray programs in combination with resistant varieties. Sample pathogen populations for genetic analyses (07/2017 – 10/2017)

Fungicide treatments were applied using a CO_2-pressurized backpack sprayer equipped with hollow cone nozzles (TXVS-26) delivering 40 gal/A at 45 psi. Applications were made weekly. Disease severity was assessed weekly as percent leaf area with necrosis per plot. Fruit were harvested the last four weeks of the trial. Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and Fisher's Protected LSD test to separate means. Downy mildew was first detected at approximately 2% disease severity in the field and progressed throughout the course of the

trial. Non-treated Peacemaker controlled downy mildew well and produced the greatest weight of total marketable fruit. Non-treated Citadel managed *P. cubensis* and yielded well when compared to the non-treated Expedition and Vlaspik. No other treatments provided commercially acceptable levels of disease control. No phytotoxicity was observed. In the table, treatments are sorted by disease severity.

Treatment and rate of product per acre	Application no.[y]	Disease severity[z] (%)		Mkt yield (lb/plot)
		5-Sep	19-Sep	
Non-treated Peacemaker	N/A	7.3 j[x]	40.1 j	31.48 a
Non-treated Citadel	N/A	10.3 ij	46.2 ij	27.23 ab
Previcur Flex 6 F 19.2 fl oz	1-4	15.0 f-i	51.9 hij	26.23 ab
Omega 500 F 24 fl oz	1-4	16.7 d-g	56.9 ghi	24.35 bc
Ranman 3.33 SC 2.75 fl oz	1-4			26.83 ab
Induce SL 0.25% V/V	1-4	10.0 ij	57.0 ghi	
Orondis Opti A 0.83 SC 2 fl oz	1-4	12.0 g-j	58.8 f-i	24.00 bc
Zampro 4.33 SC 14 fl oz	1-4			22.00 bcd
Induce SL 0.25% V/V	1-4	16.3 e-h	59.5 e-i	
V-10208 4 SC 10 fl oz	1-4	18.0 c-f	66.4 d-h	21.68 b-e
Ridomil Gold Bravo36.4 SC 40 fl oz	1-4	20.0 b-f	67.0 d-g	18.78 c-g
Gavel 75 WG 32 oz	1-4	19.7 b-f	70.3 c-g	21.83 b-e
Manzate Pro Stick75 DG 48 oz	1-4	21.7 a-e	72.2 c-f	17.83 d-h
Presidio 4 SC 4 fl oz	1-4			16.18 d-i
Induce SL 0.25% V/V	1-4	19.0 b-f	73.6 cde	
Curzate 60 DF 5 oz	1-4	10.7 hij	74.0 cd	18.80 c-g
Zing! 4.9 SC 36 fl oz	1-4	21.7 a-e	74.3 cd	19.75 c-f
Bravo Weather Stik 6 SC 48 fl oz	1-4	22.3 a-d	75.1 bcd	21.48 b-e
Tanos 50 WG 8 oz	1-4	18.3 b-f	76.2 bcd	16.85 d-e
Revus 2.08 SC 8 fl oz	1-4	20.0 b-f	80.7 abc	14.00 f-i
Forum 43.5 SC 6 fl oz	1-4	19.3 b-f	80.7 abc	16.10 e-i
Merivon 42.5 SC 6.7 fl oz	1-4	23.7 abc	81.5 abc	13.08 ghi
Non-treated Vlaspik	N/A	27.3 a	86.4 ab	12.18 hi
Cabrio 20 EG 12 oz	1-4	24.0 ab	88.9 a	14.98 f-i
Non-treated Expedition	N/A	22.0 a-e	90.0 a	11.58 i

[z] Disease rating scale based on percent necrotic foliage caused by *P. cubensis*.
[y] Application dates; 1=22 Aug, 2=29 Aug, 5 Sep and 4=14 Sep.
[x] Treatments followed by the same letter(s) within a column are not statistically different (*P*=0.05, Fisher's Protected LSD Test).

8. Presentation of results at pickle field day, Year 2 (10/2017 – 10/2017)

Quesada-Ocampo L. M. Cucurbit disease management. Commercial vegetable grower symposium. Henderson, NC, February 2017.

9. Analyze pathogen populations resistant to fungicides with genetic markers (11/2017 – 12/2017)

The results from analysis in year 2 confirm the observations from year 1. *P. cubensis* isolates that are recovered from cucumber contain fungicide resistance mutations at a significantly higher rate than that of isolates from other cucurbit hosts like gourds.

10. Presentation of findings at vegetable grower meeting, Year 2 (12/2017 – 12/2017)

D'Arcangelo K., Adams M., and Quesada-Ocampo L. M. Controlling downy mildew in cucumber. 32nd Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, December 2017.

11. Writing of reports and publications (12/2017 – 01/2018)

Four interim and one final report have been submitted.

The following abstract has been published:

Wallace E. C. and Quesada-Ocampo L. M. (2016) *Pseudoperonospora cubensis* on commercial and non-commercial cucurbits in North Carolina: population structure determine by simple sequence repeats (SSRs). Phytopathology 106: S4.12.

Fungicide recommendations for cucurbit downy mildew were updated in two production guides:

Quesada-Ocampo L. M., Meadows I., and Louws F. (2017) Disease control for commercial vegetables. North Carolina Agricultural and Chemicals Manual.

Quesada-Ocampo L. M. (2016) Disease control for commercial vegetables. North Carolina Agricultural and Chemicals Manual.

Results from seven fungicide efficacy trials have been published to provide growers with the latest information for NC:

Adams M. L. and Quesada-Ocampo L. M. (2017) Evaluation of fungicides for control of downy mildew on cucumber, Clayton 2016. Plant Disease Management Reports 11: V100.

Adams M. L. and Quesada-Ocampo L. M. (2017) Evaluation of fungicides for control of downy mildew on cucumber, Kinston II 2016. Plant Disease Management Reports 11: V096.

Adams M. L. and Quesada-Ocampo L. M. (2017) Evaluation of fungicides and cultivars for control of downy mildew on cucumber, Kinston 2016. Plant Disease Management Reports 11: V097.

Adams M. L. and Quesada-Ocampo L. M. (2017) Evaluation of cultivars and fungicides for control of downy mildew on cucumber, Clinton 2016. Plant Disease Management Reports 11: V098.

Adams M. L., Noel N. A., and Quesada-Ocampo L. M. (2016) Evaluation of fungicides for control of downy mildew on cucumber, Clayton 2015. PDMR 10: V084.

Adams M. L. and Quesada-Ocampo L. M. (2016) Evaluation of fungicides for control of downy mildew on cucumber, Cleveland 2015. PDMR 10: V085.

Adams M. L. and Quesada-Ocampo L. M. (2016) Evaluation of fungicides for control of downy mildew on cucumber, Kinston 2015. PDMR 10: V086.

Three disease alerts have been published:

Quesada-Ocampo L. M. and D'Arcangelo K. Cucumber downy mildew confirmed in North Carolina and South Carolina. Extension Plant Pathology Portal. June 6, 2017.

Quesada-Ocampo L. M. Watermelon downy mildew reported in North Carolina. Extension Plant Pathology Portal. June 17, 2016.

Quesada-Ocampo L. M. Cucumber downy mildew reported in North Carolina. Extension Plant Pathology Portal. June 1, 2016.

GOALS AND OUTCOMES ACHIEVED

Objective 1: Monitor efficacy of single registered and unregistered fungicides and new cucumber varieties for CDM control as standalone strategies.

Field trials were performed and included 18 fungicide treatments and 4 pickling cucumber lines with susceptible and tolerant phenotypes to cucurbit downy mildew.

Objective 2: Determine CDM population changes due to fungicide resistance to optimize spray programs and avoid loss of fungicide efficacy.
We have developed a PCR-based marker for fungicide resistance to two fungicides. During the trials, isolates were collected and later genotyped for fungicide resistance.

Objective 3: Establish which combinations of spray programs and cucumber varieties are optimal for control of CDM.
Field trials were performed and included 4 pickling cucumber lines with susceptible and tolerant phenotypes to cucurbit downy mildew in combination with three different spray programs and an untreated control.

Objective 4: Disseminate research results to stakeholders through web-based tools and presentations at grower meetings and field days.
Sixteen oral presentations, one abstract, two production guides, seven fungicide efficacy trials, and three disease alerts have been generated to disseminate information about cucurbit downy mildew diagnostics and management.

BENEFICIARIES

This project also aimed to provide training to experienced and new cucumber growers on best practices to prevent cucurbit downy mildew. The PI Lina Quesada, representing NCSU, established a collaboration with the NC Vegetable Growers Association to ensure that all research findings were translated into solutions that were adopted by NC cucumber growers to improve production. This project was performed in several counties of North Carolina including Wake (Raleigh), Johnston (Clayton), Lenoir (Kinston), Sampson (Clinton), Rowan (Salisbury), and Haywood (Waynesville).

LESSONS LEARNED

NC is one of the largest cucurbit producers in the US. This project had a direct impact on cucurbit growers in NC since CDM affects crops every year. Growers report economic losses as a result of the high cost of disease control and/or inadequate disease management resulting in plant death and decreased yields. As a result of CDM, growers of cucurbit crops face production costs that are no longer predictable. For instance, in 2012, the CDM outbreak occurred in early May and control costs quickly escalated. CDM costs growers and processors $6.4 million based on the processors paying $125/acre in fungicide costs to control disease ($4.8 million) and growers paying $42/acre machinery cost to apply six sprays ($1.6 million).

Growers now have science-based control recommendations that will ensure only effective fungicides and varieties are used and crop loss prevented. Changes in CDM pathogen populations and fungicide sensitivity were tracked at the field and genetic level so that durable chemical control strategies were used. Through the extension role of the PI, we translated our findings into solutions for growers.

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PROJECT TITLE Black Rot in Sweet Potato*

PROJECT SUMMARY

North Carolina (NC) is the number one producer of sweepotatoes in the United States (US), contributing over 50% of the total production at a value of more than $478 million. Ceratocystis fimbriata causes black rot in sweetpotato, and during 2014 it was especially devastating likely due to the heavy rainfall experienced the last two years throughout the growing season that favored infections. Black rot is a highly destructive disease of sweetpotato, an industry valued at over $200 million annually in North Carolina. Infection occurs via wounds that come into contact with infested soil, through planting of infected plant material, or by using infested tools. Black rot is mainly controlled in sweetpotato using cultural practices such as using certified pathogen-free seed, rotating infested fields and preventing penetration of the pathogen via wounds. Nonetheless, since the pathogen is found in infested soils and sweetpotato is a root crop, it can be difficult to guarantee no contact between soil and wounded plant tissue. Fungicides and tolerant varieties may provide an additional tool as part of an integrated management strategy that growers can combine with cultural practices to prevent black rot. However, there is no data to evaluate the efficacy of sweetpotato varieties or labeled fungicides in controlling black rot, thus growers are not using these management strategies. To prevent losses due to application of ineffective fungicides or planting susceptible varieties in infested fields, it is imperative to have a clear understanding of what products are effective in a spray program and which varieties are less susceptible to black rot. Effective management strategies for black rot in addition to cultural strategies need to be identified to maintain the competitiveness of the NC sweetpotato industry even when environmental conditions favor disease. To the best of our knowledge, management of black rot by using host resistance or chemical control has not been addressed by other researchers in NC or elsewhere, even though several fungicides are labeled to control this pathogen and new varieties have been released by Louisiana and North Carolina in the past few years that could be tolerant to black rot. The aim of this project was to identify integrated management strategies for black rot control that will make the sweetpotato industry in NC more competitive.

PROJECT APPROACH

1. Sampling of fields and packing facilities to obtain a diverse collection of isolates for experiments (02/2016 – 07/2016)

Sampling and isolation of *C. fimbriata* from infected sweetpotatoes: Sweetpotatoes with black rot symptoms were sampled from commercial packinghouses in North Carolina (NC). 83 isolates were collected from 10 commercial sweetpotato farms, covering 5 counties in eastern NC. Diseased sweetpotatoes were selected regardless of cultivar. Isolations were performed by cutting out a small triangular section from the edge of the lesion and placing onto potato dextrose agar (PDA) amended with 20 mg/L of ampicillin and 20 mg/L of rifampicin. Isolates were then single-spored and stored as mycelia plugs in a 1.5 mL tube with sterile water and a hemp seed at 4°C for long-term preservation.

Genotyping of *C. fimbriata* isolates: DNA was extracted from a set of 50 isolates. Total DNA was used to amplify the internal transcribed spacer (ITS) region with primers ITS1 and ITS4. The translation elongation factor (TEF) region was also amplified for all isolates using primers ef1 and ef2. PCR products were sequenced at the Genomic Sciences Laboratory, NC State University, using the forward primer that

was used to amplify the DNA. Sequences were compared against the National Center for Biotechnology Information (NCBI) GenBank database using BLAST and all isolated were confirmed as *C. fimbriata*.

Mating type determination: Total DNA was used to amplify a portion of the high mobility group (HMG) box of the *MAT-2* mating type gene using primers CFM2-1 and CFM2-2 in each of the fifty selected *C. fimbriata* isolates. Amplified samples were considered mating type 2 (MAT-2) whereas failure to amplify indicates mating type 1 (MAT-1). PCR products of the *MAT-2* samples were sequenced as described above for phylogenic analysis. All fifty tested isolates were verified as mating type 2 based on PCR amplification.

Phylogenetic analysis: Sequences were aligned using ClustalWS in the Jalview 2.9.0 software package. Alignments were visualized using Jalview 2.9.0 and the sequence ends were manually trimmed to remove low quality bases. The ITS, TEF, and *MAT-2* datasets were concatenated and haplotypes identified using the haplotype block workflow in the Mobyle SNAP Workbench with a minimum of a two base pair difference. Alignment of the 50 ITS, TEF, and *MAT-2* sequences was collapsed into a single haplotype in the Mobyle SNAP Workbench indicating no variation in the set of isolates at the sequenced loci, thus, no additional phylogenic analysis was conducted.

2. Experiments to evaluate sweetpotato varieties for resistance to black rot (08/2016 – 02/2017)
Fourteen sweetpotato cultivars and advanced breeding lines were evaluated for resistance to black rot in the storage roots. Sweetpotatoes were grown at the Horticultural Crops Research Station in Clinton, NC using standard production practices. Following harvest, roots were properly cured at 29°C and stored at approximately 15°C. Prior to inoculation, roots were rinsed with water and surfaced sterilized by soaking in 5% bleach for five minutes. Roots were rinsed once more and allowed to air dry in a biosafety cabinet.

Two *C. fimbriata* isolates (AS186 and AS236) were used for cultivar evaluation and were selected due to differences in isolation year and location. Eighteen roots of each sweetpotato variety were used for each experiment. Among them, six roots were used for control, six roots for inoculation with isolate AS186, and six roots for inoculation with isolate AS236. Roots were wounded by inserting a sterile toothpick 5 mm into the sweetpotato and inoculated by pipetting 20 µL of a *C. fimbriata* spore suspension into the wound. Spores were suspended in a 0.05% Tween solution and adjusted to 10^6 spores per mL. After inoculation, roots were placed in pairs into clear, 28 cm x 20 cm x 14 cm plastic containers, each with three sterile paper towels dampened with 15 mL of sterile water to maintain high humidity as previously described. Control roots were wounded but not inoculated. Roots were stored in the dark at approximately 23°C. Results were being recorded by measuring lesion diameter at 4, 7, 11, 14, 18, and 21 days post inoculation (dpi). The experiment was conducted three times. Area Under the Disease Progress Curve (AUDPC) values were calculated based on lesion diameter measurements for each root using the trapezoidal method and were subject to an analysis of variance (ANOVA). A post-hoc Bonferroni correction was applied to separate treatments.

Postharvest evaluations revealed no complete resistance among the tested cultivars and breeding lines. All sweetpotato cultivars produced firm, black, dry lesions around the point of inoculation and lesions did not extend below the vascular tissue. Characteristic perithecia of *C. fimbriata* were observed on the surface of infected tissue. Although all tested cultivars were susceptible, analysis of AUDPC values calculated over a three-week period revealed some cultivars were less susceptible than others (Fig. 1). Stokes Purple, a purple-fleshed sweetpotato, proved to be the least susceptible of all tested cultivars.

Covington was second to Stokes Purple and was the least susceptible orange-flesh cultivar. While Stokes Purple and Covington were not significantly different than most cultivars at the 95% confidence level, they were significantly different (P = 0.01) than the orange-fleshed cultivar Bellevue, which was the most susceptible cultivar in the current study. All other tested cultivars were not significantly different from any of the cultivars tested.

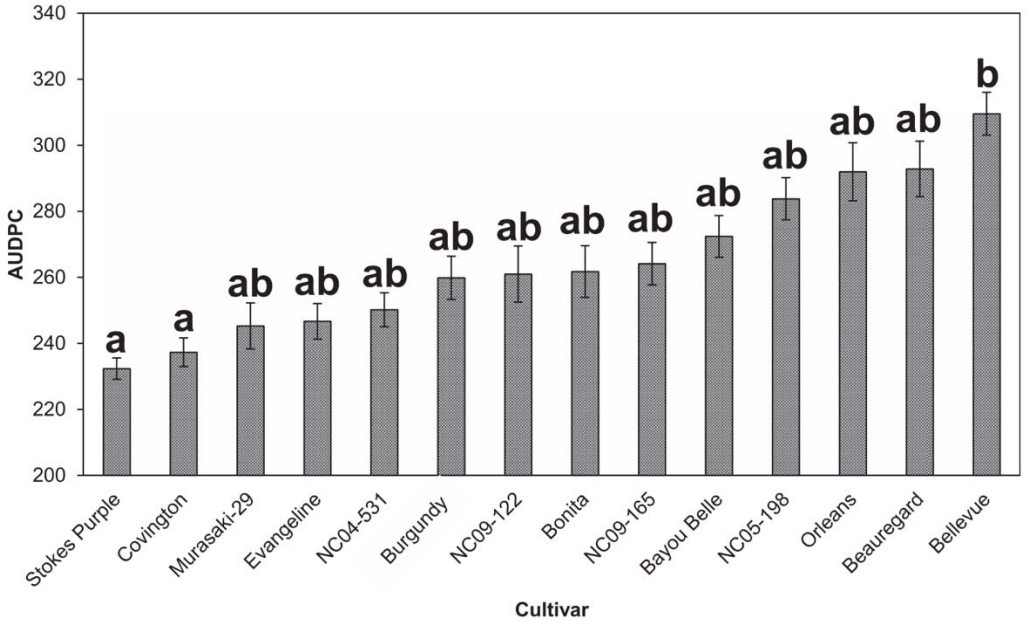

Fig. 1: Relative susceptibility of 14 common sweetpotato cultivars and advanced breeding cultivars to black rot disease based on area under the disease progress curve (AUDPC) values calculated over a three-week period. Cultivars marked with similar letters do not significantly differ at a = 0.05 under a Bonferroni correction.

3. Presentation of findings at sweetpotato field day, Year 1 (10/2016 – 10/2016)

The following presentations were given to growers and packers to disseminate information about black rot diagnostics and management:

Quesada-Ocampo L. M. Black rot update. Sweetpotato field day. Clinton, NC, September 2016.

4. Presentation of findings at sweetpotato grower meeting, Year 1 (01/2017 – 01/2017)

The following presentations were given to growers and packers to disseminate information about black rot diagnostics and management:

Quesada-Ocampo L. M. Emerging diseases in sweetpotato production. Regional Sweetpotato Meeting. Nashville, NC, February 2017.

Quesada-Ocampo L. M. Emerging diseases in sweetpotato production. NC Sweetpotato Commission Meeting. Clinton, NC, January 2017.

Quesada-Ocampo L. M. Sweetpotato diseases. NC Sweetpotato Commission Meeting. Clinton, NC, January 2017.

Quesada-Ocampo L. M. Managing sweetpotato black rot. NC Sweetpotato Commission. Smithfield, NC, February 2016.

Quesada-Ocampo L. M. Black rot: an emerging challenge in sweetpotato production. US Sweetpotato Council Convention. Chattanooga, TN, January 2016.
Quesada-Ocampo L. M. Sweetpotato black rot. NC Certified Sweetpotato Seed Growers. Benson, NC, January 2016.

5. Experiments to identify products for black rot control (03/2017 – 10/2017)

Sweetpotato roots used in the study were obtained from a commercial packing facility at the time of each inoculation and were rinsed in water prior to use. Roots were previously cured and were selected based upon similar size, shape, and disease free appearance. Sweetpotatoes were wounded by scraping a single side of each root with a handheld cheese grater. After wounding, seventy wounded sweetpotatoes were placed into a 121 L bin containing 45 L of water. Roots were inoculated by placing twelve black rot infected roots into the bin of water with the wounded roots, gently agitating to ensure uniformity throughout the bin, and letting them soak for sixty minutes. Following inoculation, roots were taken out of the water and allowed to air dry. Roots were then laid out with the wounded side up and fungicide treatments were applied at specific rates using a CO_2 powered backpack sprayer. After fungicide application, roots were placed into clear, plastic containers (40 by 50 by 17.9 cm) and stored at 15ºC and 95% relative humidity for 21 days. Four replications per treatment were included with ten roots per replication. The experiment was conducted twice. Roots were rated for disease incidence 7, 17, and 21 days after inoculation and for disease severity 21 days after inoculation. Data were analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and the Waller-Duncan test to separate means.

Black rot was first observed 14 days after inoculation in experiment 1 and 7 days after inoculation in experiment 2. Disease severity in the untreated control was moderate (62.5 and 38.8%), as estimated by percent infection of the wounded tissue. Omega 500F, Inspire, Scholar SC, Mertect 340F, and Stadium fungicides all consistently provided significant reductions in disease severity. Stadium provided the best control at 1.3 and 0.0% severity. No phytotoxity was observed in any treatments.

Experiment 1	Disease Incidence (%)*			Disease Severity (%)*
	7 days	17 days	21 days	21 days
Treatment and product rate				
Untreated Control	0.0 a	100.0 a	100.0 a	62.5 a
Botran 75W 1.2 g/L	0.0 a	95.0 a	100.0 a	31.3 b
Omega 500F 4.1 mL/L	0.0 a	85.0 a	90.0 a	17.5 c
Inspire 3.4 mL/L	0.0 a	20.0 b	30.0 b	5.0 d
Scholar SC 2.5 mL/L	0.0 a	5.0 b	15.0 bc	3.8 d
Mertect 340F 8.4 mL/L	0.0 a	20.0 b	30.0 b	5.0 d
Stadium 6.1 mL/L	0.0 a	5.0 b	5.0 c	1.3 d

Experiment 2	Disease Incidence (%)*			Disease Severity (%)*
	7 days	17 days	21 days	21 days
Treatment and product rate				
Untreated Control	42.5 a	80.0 a	85.0 a	38.8 b
Botran 75W 1.2 g/L	55.0 a	80.0 a	90.0 a	60.0 a
Omega 500F 4.1 mL/L	2.5 b	17.5 b	22.5 b	7.5 c
Inspire 3.4 mL/L	2.5 b	12.5 bc	12.5 bc	5.0 c

Scholar SC 2.5 mL/L	5.0 b	7.5 bc	15.0 bc	5.0 c
Mertect 340F 8.4 mL/L	0.0 b	0.0 c	0.0 c	0.0 c
Stadium 6.1 mL/L	0.0 b	0.0 c	0.0 c	0.0 c

*Treatments followed by the same letter(s) within a column are not statistically different (P=0.05, Waller-Duncan k=100).

6. Presentation of results at sweetpotato field days, Year 2 (10/2017 – 10/2017)

Parada C., Collins H., and Quesada-Ocampo L. M. Expanding the fungicide toolkit for control of black rot in sweetpotato. Sweetpotato field day. Clinton, NC, September 2017.

7. Writing of reports, black rot control fact sheet, and publications (11/2017 – 01/2018)
Four interim and one final report have been submitted.

A black rot of sweetpotato fact sheet has been generated: http://content.ces.ncsu.edu/black-rot-of-sweetpotato

Three disease alerts have been published:

Quesada-Ocampo L. M. Section 18 label renewed for Mertect to control black rot postharvest on sweetpotato in North Carolina. Extension Plant Pathology Portal. December 19, 2016.
Quesada-Ocampo L. M. Section 18 label approved for Mertect to control black rot postharvest on sweetpotato in North Carolina. Extension Plant Pathology Portal. August 16, 2016.
Quesada-Ocampo L. M. Emergency section 18 label approved for Mertect to control black rot postharvest on sweetpotato in North Carolina. Extension Plant Pathology Portal. January 11, 2016.

8. Presentation of findings at sweetpotato grower meeting, Year 2 (01/2018 – 01/2018)
Quesada-Ocampo L. M. Translational strategies to address emerging diseases in sweetpotato production. US Sweetpotato Council Convention. Wilmington, NC, January 2018.
Quesada-Ocampo L. M. Sweetpotato disease management. Southeast Regional Fruit and Vegetable Conference. Savannah, GA, January 2018.

GOALS AND OUTCOMES ACHIEVED

Objective 1: Evaluate select sweetpotato varieties for resistance to black rot
Pathogen isolates needed for the variety evaluations have been collected and characterized. Variety evaluations were completed according to the project work plan.

Objective 2: Evaluate the efficacy of labeled fungicides for control of black rot in sweetpotato
Pathogen isolates needed for the fungicide evaluations have been collected and characterized. Fungicide evaluations were completed according to the project work plan.

Objective 3: Disseminate findings to stakeholders by presenting results at grower meetings and field days, and by developing web-based and mobile-friendly educational resources

Thirteen oral presentations, one fact sheet, and three disease alerts have been generated to provide information to growers and packers about black rot control in sweetpotato. The fact sheet and disease alerts were published in mobile-friendly platforms.

BENEFICIARIES

This project also aimed to provide training to experienced and new sweetpotato growers on best practices to prevent black rot, since the industry is quickly expanding in the state. The PI Lina Quesada, representing NCSU, established a collaboration with the NC Sweetpotato Commission and the NC Certified Sweetpotato Seed Growers Association to ensure that all research findings were translated into solutions that were adopted by NC sweetpotato growers to improve production. This project was performed in Wake (Raleigh) county but samples of the pathogen were collected from several counties including to Sampson, Wilson, Nash, Johnston, and Duplin.

LESSONS LEARNED

NC produces approximately 51% of sweetpotatoes in the US and this proposal addressed integrated control of black rot, which is an urgent matter to the sweetpotato industry in NC. Fungal diseases cause severe losses to the sweetpotato industry every year and black rot was the number one problem during 2014. Approximately 26% of samples submitted for diagnostics to the NCSU Plant Disease and Insect Clinic were infected with black rot. In 2014, approximately 73,000 acres were planted to sweetpotatoes valued at over $354 million and production expanded to 85,000 acres in 2015. Sweetpotato acreage expansion is an exciting economic opportunity for NC but may also result in increased disease challenges for the industry due to less available land for rotation and reduced availability of clean seed to meet demand. Since this pathogen can persist in the soil it is very likely that similar disease outbreaks will occur in the near future, thus, it is imperative that growers have the information they need to effectively control this disease. This project provided host resistance and chemical control information that can be used by growers immediately to complement cultural practices and create a non-conducive environment for disease. Finally, through this proposal we trained stakeholders during grower meetings with attendance of 300-400 growers to ensure that findings of this research are adopted and black rot control is improved. Providing consumers consistently with high quality and pathogen free sweetpotatoes is of utmost importance for the long-term profitability of the fresh market, processing, and seed industries.

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PROJECT TITLE Managing Phytophthora blight of pepper*

PROJECT SUMMARY

Phytophthora blight of pepper is caused by *Phytophthora capsici,* a devastating oomycete plant pathogen. *P. capsici* is difficult to control due to its wide host range, which limits the efficacy of crop rotation, its ability to quickly become resistant to fungicides, the lack of resistant varieties, its ability to spread through infested irrigation water, and its ability to persist in soils for over 10 years once it has been introduced. *P. capsici* infects vegetable crops such as cucumber, melon, squash, pumpkin, tomato, pepper, eggplant, lima, snap and wax bean crops, and has recently been found to infect Fraser fir. All of these crops are grown in North Carolina and the state ranks among the top 10 in the USA for the production of most of these crops. *P. capsici* has the potential to become a threat to several crop industries in NC if effective management strategies are not identified for hosts, such as pepper, that are commonly infected by this pathogen in the state every year.

During 2013, Phytophthora blight was especially devastating due to the heavy rainfall throughout the growing season that favored disease. *P. capsici* is mainly controlled in pepper by protecting susceptible plants with fungicides; however, the pathogen is known for quickly developing resistance to commonly used products. To prevent losses due to application of ineffective fungicides, it is imperative to make growers aware of the presence of fungicide-resistant *P. capsici* isolates in their fields to determine what alternative products should be used. Though discouraging for growers, knowing if *P. capsici* isolates in their fields are resistant to a particular fungicide, could save them the time and money of applying ineffective fungicides. In addition, including host resistance or tolerance as part of an integrated management strategy would help in protecting the durability of crop protection products and reduce pesticide applications. However, there is no information regarding resistance or tolerance of pepper varieties to NC *P. capsici* populations.

P. capsici can produce over-wintering spores called oospores that are formed when both mating types (A1 and A2) of the pathogen are present in a field. Oospores can persist in soils for over 10 years, and if strategies such as crop rotation or fumigation are not implemented to reduce pathogen levels, fields may become too infested for vegetable production. However, if only one mating type of the pathogen is present in a particular field, it is likely that isolates will not survive a killing frost, thus, it is important to establish if one or two mating types are present in the *P. capsici* population infesting a given field.

The goal of this project was to develop and implement an integrated management strategy for *Phytophthora* that relies on multiple tools rather than only on intensive pesticide use.

PROJECT APPROACH

1. Obtain pepper variety seed and fungicides for experiments, and set up fields (02/2016 – 04/2016)
Fungicide efficacy trials: Plots were single beds on 5-ft centers covered with black plastic mulch; 25-ft long with 5-ft fallow borders on each end. The previous year the field was planted with watermelon followed by rye as a cover crop. A total of 6 different registered and unregistered fungicides were obtained from crop protection companies for evaluations. Seed from a susceptible pepper variety was also obtained from a seed company.

Variety trials: Plots were 10 ft long and spaced 2ft apart. The previous year the field was planted with bell peppers and Phytophthora blight was inoculated in the field. Seed for 22 cultivars and 10 lines was obtained from seed companies or purchased to conduct this trial.

2. Conduct fungicide efficacy trials with single registered and unregistered products, and conduct variety trials (05/2016 – 08/2016)

Fungicide efficacy trials: Peppers were transplanted (1-ft in-row spacing) in raised beds (25 plants/plot). Irrigation and fertilization were applied via drip tape. Treatments were randomized into four complete blocks. Plots were inoculated with 1g of *P. capsici*-infested millet seed at the base of each plant and covered with soil. Fungicide foliar treatments were applied using a CO2-pressurized backpack sprayer equipped with a single-nozzle, handheld boom with a hollow cone nozzle (TXVS-26) delivering 40 gal/A at 45 psi. A soil-directed drench application (100 mL) was applied at the base of each plant immediately after transplanting. Applications were made on 7-day intervals. Disease incidence was assessed weekly and analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and the Waller-Duncan test to separate means. Phytophthora blight was first observed at low levels (3% incidence). Disease progressed throughout the course of the trial reaching 70% in the non-treated plots. The combination treatment of Experimental + Ridomil Gold SL provided excellent control of Phytophthora blight. Treatments containing alternations of Presidio, V-10208, Revus, Ridomil Gold SL and Ridomil Gold Copper applied as either drench; foliar spray or drip application also controlled *P. capsici*. In Table 1, treatments are sorted by disease incidence on 16 Jul.

Table 1: Fungicide efficacy evaluations with single registered and unregistered products for Phytophthora blight control on pepper.

	Application	Disease Severity* (%)		
Treatment and rate of product per acre	**No.**	**1st rating**	**2nd rating**	**Final rating**
Experimental (Drench) 6.84 fl oz	1			
Ridomil Gold SL 480 SL (Drench) 4.28 fl oz	1	1.2 d** (5%)	1.3 e (5%)	2.0 c (8%)
Presidio 4SC (Drench) 4 fl oz	1			
V-10208 4SC (Drip) 8 fl oz	2,6			
Presidio 4SC (Drip) 4 fl oz	4			
Revus 2.08SC 8 fl oz	3,5,7	2.9 cd (12%)	6.0 cde (24%)	7.5 bc (30%)
V-10208 4SC (Drench) 8 fl oz	1			
Presidio 4SC (Drip) 4 fl oz	2,4,6			
V-10208 4SC (Drip) 8 fl oz	3,5,7	6.0 bc (26%)	7.8 cd (31%)	8.0 bc (32%)
Ridomil Gold SL 4SL (Drench) 1 pt	1			
Revus 2.08SC 8 fl oz	2,4,6			
Ridomil Gold Copper 65WP 2 lb	3,5,7	5.5 bc (23%)	8.0 cd (32%)	8.3 bc (33%)
Presidio 4SC (Drench) 4 fl oz	1			
V-10208 4SC 8 fl oz	2,4,6			
Presidio 4SC 4 fl oz	3,5,7	4.8 bc (23%)	7.3 cd (29%)	8.3 bc (33%)
Presidio 4SC (Drench) 4 fl oz	1			
V-10208 4SC (Drip) 8 fl oz	2,4,6			
Presidio 4SC (Drip) 4 fl oz	3,5,7	3.0 cd (13%)	5.5 de (22%)	8.3 bc (33%)
V-10208 4SC (Drench) 8 fl oz	1			
Presidio 4SC 4 fl oz	2,4,6			
V-10208 4SC 8 fl oz	3,5,7	5.4 bc (27%)	8.5 cd (34%)	9.5 b (38%)
Experimental (Drip) 6.84 fl oz	1			
Ridomil Gold SL 480SL (Drip) 4.28 fl oz	1			
Revus 2.08SC 8 fl oz	2,4,6			
Kocide 46.1DF 1 lb	2-7			
Induce 90SL 0.125 % v/v	2,4,6	8.3 b (34%)	11.5 bc (46%)	13.3 ab (53%)
Experimental (Drip) 6.84 fl oz	1	17.1 a (69%)	17.5 a (70%)	17.5 a (70%)

Ridomil Gold SL 480SL (Drip) 4.28 fl oz	1			
Non-treated	N/A	16.2 a (66%)	16.8 ab (67%)	17.5 a (70%)

*Disease incidence based on the average number of diseased plants per plot (25 plants/plot).
**Treatments followed by the same letter(s) within a column are not statistically different (P=0.05, Waller-Duncan k=100).

Variety trials: Thirty-two entries (22 cultivars and 10 lines) were sown in the greenhouse. Seedlings were left outside in a protected area to harden for 3 days, then transplanted (1 ft in row spacing) in raised beds (10 plants/plot) by a mechanical transplanter that also applied a starter fertilizer (17-17-17). Irrigation was applied using overhead sprinklers to promote disease. During the season, weeds in the plots were controlled by hand weeding while weeds between rows were cultivated. A completely randomized block design with four replications was used. Inoculation of the plants was achieved by carefully inserting 1 g of *P. capsici* infested millet seed directly into the soil adjacent to each plant crown, avoiding root or crown injury. Disease incidence was assessed weekly and analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and the Tukey's HSD test to separate means.

Of the cultivars planted in this trial, Marta-R, Fidel, and Meeting had the lowest plant death percentage and therefore the most resistance to the isolates of *P. capsici* used in this study (Table 2). The land race Fidel remained relatively symptomless throughout the experiment. Cultivars Red Knight, Plato, and Bastille had the highest plant death percentage. They were therefore considered highly susceptible. The cultivars Camelot, Quattro, Aristotle, Keystone, Karisma, EXP. 10, SV3782PP, PS 00941819, Archimedes, Revelation, EXP. 5, EXP. 2, EXP. 6, SV3198HJ, Fabuloso, EXP. 1, EXP. 8, EXP. 7, Declaration, and EXP.4 had plant death percentage not significantly different from Plato, Red Knight, and Bastille, and therefore are not likely to perform well in fields infested with *P. capsici*. The experimental lines 9 and 3 had significantly less plant death than the tolerant cultivar Paladin.

Table 2: Variety evaluations for resistance to Phytophthora blight of pepper.

Entry	Plant Death[z] (%)		
	1st rating	2nd rating	Final rating
Martha-R	7.5 f	7.5 h	7.5 h
Fidel	7.5 f	10 h	10 gh
Meeting	7.5 f	15 gh	22.5 fgh
EXP.9	17.5 ef	25 fgh	27.5 e-h
Ebano-R	25 c-f	40 d-h	42.5 d-h
EXP.3	20 def	37.5 e-h	42.5 d-h
Revolution	40 a-f	45 b-h	45 c-h
Paladin	20 def	42.5 c-h	47.5 b-h
Vanguard	32.5 b-f	47.5 a-h	47.5 b-h
EXP.4	42.5 a-f	55 a-h	55 a-h
Declaration	55 a-f	55 a-h	57.5 a-g
EXP.7	35 b-f	50 a-h	57.5 a-g
EXP.8	42.5 a-f	52.5 a-h	57.5 a-g
EXP.1	45 a-f	57.5 a-h	62.5 a-f
Fabuloso	32.5 b-f	57.5 a-h	65 a-f
SV3198HJ	47.5 a-f	60 a-g	65 a-f
EXP.6	60 a-e	67.5 a-f	75 a-e
EXP.2	62.5 a-e	72.5 a-f	77.5 a-d

EXP.5	62.5 a-e	72.5 a-f	77.5 a-d
Revelation	47.5 a-f	75 a-e	80 a-d
Archimedes	62.5 a-e	72.5 a-f	82.5 a-d
PS 09941819	57.5 a-f	75 a-e	82.5 a-d
SV3782PP	70 a-d	85 a-e	85 a-d
EXP.10	72.5 abc	85 a-e	87.5 a-d
Karisma	72.5 abc	82.5 a-e	87.5 a-d
Keystone	77.5 ab	87.5 a-d	87.5 a-d
Aristotle	55 a-f	87.5 a-d	90 a-d
Quattro	75 abc	87.5 a-d	92.5 abc
Camelot	75 abc	90 abc	95 ab
Bastille	90 a	92.5 ab	97.5 a
Plato	80 ab	95 a	97.5 a
Red Knight	72.5 abc	95 a	97.5 a

[z] Disease rating scale based on the percentage of dead plants caused by *P. capsici.*

3. Sample and analyze pathogen populations for mating type (09/2016 – 11/2016)

Phytophthora capsici isolates have been collected from pepper fields across North Carolina. Mating type determination indicated that 75% of isolates were A2 and 25% were A1.

4. Presentation of findings at vegetable grower meeting, Year 1 (12/2016 – 12/2016)

The following presentations have been given to disseminate information about Phytophthora blight diagnostics and management:

Parada-Rojas C. H. and Quesada-Ocampo L. M. Evaluation of commercial hot and bell pepper cultivars for resistance to *Phytophthora capsici*. APS Meeting, Tampa, FL, August 2016.

Quesada-Ocampo L. M. Using every tool in the toolbox to improve vegetable disease control. Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing, MI, June 2016.

Quesada-Ocampo L. M. Using every tool in the toolbox to keep North Carolina vegetables healthy. Department of Biochemistry Seminar, North Carolina State University, Raleigh, NC, February 2016.

Quesada-Ocampo L. M. Integrating field and genomic strategies to keep North Carolina vegetables healthy. AgBiome, Durham, NC, February 2016.

5. Obtain pepper variety seed and fungicides for experiments, and set up fields (01/2017 – 04/2017)

Fungicide efficacy trials: Plots were single beds on 5-ft centers covered with black plastic mulch; 20-ft long with 10-ft fallow borders on each end. The previous year the field was planted with rye. Pepper was transplanted (1-ft in-row spacing) in raised beds (20 plants/plot). Irrigation and fertilization were applied via drip tape. Treatments were randomized into four complete blocks. Plots were inoculated with 1g of *P. capsici*-infested millet seed at the base of each plant and covered with soil. Fungicide foliar treatments were applied using a CO_2-pressurized backpack sprayer equipped with a single-nozzle, handheld boom with a hollow cone nozzle (TXVS-26) delivering 40 gal/A at 45 psi. A soil-directed drench application (85 mL) was applied at the base of each plant immediately after transplanting. Applications were made weekly. Disease incidence was assessed weekly and analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and the Waller-Duncan test to separate means. Phytophthora blight was first observed at low levels (3% incidence) (Table 3). Disease progressed throughout the course of the trial reaching 59% disease incidence in the non-treated plots.

The combination treatment of Presidio, Ridomil Gold SL and Orondis Gold provided excellent control of Phytophthora blight. The treatment containing alternations of Presidio, Ridomil Gold SL and V-10208 controlled *P. capsici*. Orondis Gold, Ridomil Gold SL and Presidio also managed Phytophthora well.

Table 3: Fungicide efficacy evaluations with single registered and unregistered products for Phytophthora blight control on pepper.

Treatment and rate of product per acre	Application No.	Disease Incidence* (%) 1st rating	2nd rating	Final rating
Presidio 4SC (Drench) 4 fl oz	1	0.8 a** (4%)	1.3 b (7%)	2.3 d (12%)
Ridomil Gold 480SL (Drip) 1 pt	2,3,5,7			
Orondis Gold 200SC (Drip) 4.8 fl oz	3,5			
Presidio 4SC (Drip) 4 fl oz	4,6			
Presidio 4SC (Drench) 4 fl oz	1	2.8 a (14%)	3.3 ab (17%)	4.8 cd (24%)
Ridomil Gold 480SL (Drip) 1 pt	2,7			
V-10208 4SC (Drip) 8 fl oz	3,5			
Presidio 4SC (Drip) 4 fl oz	4,6			
Orondis Gold 200SC (Drench) 4.8 fl oz	1	4.3 a (22%)	4.8 ab (24%)	5.5 bcd (28%)
Ridomil Gold 480SL (Drench) 8 fl oz	1			
Orondis Gold 200SC (Drip) 4.8 fl oz	2,4,5			
Ridomil Gold 480SL (Drip) 1 pt	3,7			
Presidio 4SC (Drip) 4 fl oz	6			
Orondis Gold 200SC (Drench) 4.8 fl oz	1	1.5 a (8%)	3.8 ab (19%)	6.0 a-d (30%)
Ridomil Gold 480SL (Drench) 6 fl oz	1			
Revus 2.09SC 8 fl oz	2,4,6			
Kocide 3000 46.1DF 1 lb	2-7			
Induce 90L 0.125% V/V	2,4,6			
Orondis Gold 200SC (Drench) 2.4 fl oz	1	1.8 a (9%)	5.5 ab (28%)	7.5 a-d (38%)
Ridomil Gold 480SL (Drench) 3 fl oz	1			
Revus 2.09SC 8 fl oz	2,4,6			
Kocide 3000 46.1DF 1 lb	2-7			
Induce 90L 0.125% V/V	2,4,6			
Orondis Gold 200SC (Drip) 4.8 fl oz	1	2.5 a (13%)	6.0 ab (30%)	9.0 a-d (45%)
Ridomil Gold 480SL (Drip) 6 fl oz	1			
Revus 2.09SC 8 fl oz	2,4,6			
Kocide 3000 46.1DF 1 lb	2-7			
Induce 90L 0.125% V/V	2,4,6			
Orondis Gold 200SC (Drench) 4.8 fl oz	1	3.3 a (17%)	5.8 ab (29%)	9.3 abc (47%)
Ridomil Gold 480SL (Drench) 8 fl oz	1			
Presidio 4SC (Drip) 4 fl oz	2,4,6			
V-10208 4SC (Drip) 8 fl oz	3,5			
Ridomil Gold 480SL (Drip) 1 pt	7			
Presidio (Drench) 4 fl oz	1	2.0 a (10%)	5.5 ab (28%)	9.5 abc (48%)
Ridomil Gold 480SL (Drench) 6 fl oz	1			
Revus 2.09SC 8 fl oz	2,4,6			
Kocide 3000 46.1DF 1 lb	2-7			
Induce 90SL 0.125 % v/v	2,4,6			
Non-treated	N/A	3.8 a (19%)	9.8 a (49%)	11.8 ab (59%)
Orondis Gold 200SC (Drip) 2.4 fl oz	1	4.8 a (24%)	9.8 a (49%)	12.8 a (64%)
Ridomil Gold 480SL (Drip) 3 fl oz	1			
Revus 2.09SC 8 fl oz	2,4,6			
Kocide 3000 46.1DF 1 lb	2-7			
Induce 90L 0.125% V/V	2,4,6			

*Disease incidence based on the average number of diseased plants per plot (25 plants/plot).

**Treatments followed by the same letter(s) within a column are not statistically different (*P*=0.05, Waller-Duncan k=100).

Variety trials: The field site has history of Phytophthora blight and soil type is Sandy. Twenty-two cultivars and 10 experimental lines were selected for this experiment. Pepper seedlings were grown in the greenhouse under natural light. Seedlings were left outside in a protected area to harden for 3 days, then transplanted (1 ft in row spacing) in raised beds (10 plants/plot) by a mechanical transplanter that also applied a starter fertilizer (17-17-17). Plants were irrigated using overhead sprinklers to promote disease. Plots were 10 ft long and spaced 2ft apart. During the season, weeds in the plots were controlled by hand weeding while weeds between rows were cultivated. Plants were arranged in a completely randomized block design with four replications. Inoculation of the plants was achieved by carefully inserting 1 g of *P. capsici* infested millet seed directly into the soil adjacent to each plant crown, avoiding root or crown injury. Disease incidence was assessed weekly and analyzed in the software ARM (Gylling Data Management, Brookings, SD) using analysis of variance (AOV) and Tukey's HSD test to separate means (*P* = 0.05). Of the cultivars planted in this trial, Marta-R, Fidel, and Meeting had the lowest plant death percentage and therefore the most resistance to the isolates of *P. capsici* used in this study (Table 4). The land race Fidel remained relatively symptomless throughout the experiment. Cultivars SV3198HJ, Revelation, Revolution, Red Knight, Quattro, EXP.2, EXP.10, Ebano-R, and Bastille had the highest plant death percentage. They were therefore considered highly susceptible. The cultivars Vanguard, SV3782PP, PS 09941819, Plato, EXP.1, Declaration, Keystone, EXP.3 – 9, Karisma, Camelot, Aristotle, and Fabuloso had plant death percentage not significantly different from Ebano-R, Red Knight, and Bastille, and therefore are not likely to perform well in fields infested with *P. capsici*. Cultivars Paladin and Archimedes registered less than 60% plant death. These cultivars are considered tolerant and could be included along with integrated plant management strategies.

Table 4: Variety evaluations for resistance to Phytophthora blight of pepper.

Entry	Plant Death[z] (%)		
	1st rating	**2nd rating**	**Final rating**
Martha-R	2.5 e	2.5 c	2.5 e
Fidel	7.5 de	7.5 c	7.5 de
Meeting	12.5 cde	12.5 c	12.5 de
Paladin	17.5 b-e	20 bc	25 cde
Archimedes	47.5 a-e	50 abc	50 bcd
Fabuloso	57.5 a-d	65 ab	65 abc
Aristotle	65 abc	67.5 ab	67.5 abc
Camelot	75 a	75 a	75 ab
EXP.8	70 ab	72.5 a	82.5 ab
EXP.3	57.5 a-d	70 ab	85 ab
EXP.9	75 a	82.5 a	87.5 ab
EXP.4	80 a	90 a	90 ab
Karisma	82.5 a	87.5 a	90 ab
EXP.5	87.5 a	92.5 a	92.5 ab
EXP.6	87.5 a	90 a	92.5 ab
Keystone	90 a	92.5 a	92.5 ab
EXP.7	95 a	92.5 a	95 ab
Declaration	87.5 a	90 a	97.5 a
EXP.1	87.5 a	95 a	97.5 a

Plato	95 a	97.5 a	97.5 a
PS 09941819	85 a	87.5 a	97.5 a
SV3782PP	92.5 a	95 a	97.5 a
Vanguard	82.5 a	95 a	97.5 a
Bastille	100 a	100 a	100 a
Ebano-R	97.5 a	100 a	100 a
EXP.10	100 a	100 a	100 a
EXP.2	92.5 a	100 a	100 a
Quattro	95 a	97.5 a	100 a
Red Knight	95 a	100 a	100 a
Revelation	95 a	100 a	100 a
Revolution	95 a	100 a	100 a
SV3198HJ	92.5 a	97.5 a	100 a

[z] Disease rating scale based on the percentage of dead plants caused by *P. capsici.*

6. Sample and analyze pathogen populations for mating type (09/2017 – 11/2017)
Phytophthora capsici isolates have been collected from pepper fields across North Carolina. Mating type determination indicated that 80% of isolates were A2 and 20% were A1.

7. Presentation of findings at vegetable grower meeting, Year 2 (12/2017 – 12/2017)
Parada C., Adams M., and Quesada-Ocampo L. M. Phytophthora on pepper: cultural and chemical control options. 32nd Annual Southeast Vegetable and Fruit Expo. Myrtle Beach, NC, December 2017.

8. Writing of reports and publications (12/2017 – 01/2018)
Four interim and one final report have been submitted.

The following abstract has been published:

Parada-Rojas C. H. and Quesada-Ocampo L. M. (2016) Evaluation of commercial hot and bell pepper cultivars for resistance to *Phytophthora capsici*. Phytopathology 106: S4.96.

Fungicide recommendations for Phytohthora blight were updated in production guides:

Quesada-Ocampo L. M., Meadows I., and Louws F. (2017) Disease control for commercial vegetables. North Carolina Agricultural and Chemicals Manual.
Quesada-Ocampo L. M. (2016) Disease control for commercial vegetables. North Carolina Agricultural and Chemicals Manual.

The following fact sheet has been published:

Phytophthora blight of peppers: http://content.ces.ncsu.edu/phytophthora-blight-of-peppers

GOALS AND OUTCOMES ACHIEVED

Objective 1: Identify effective fungicide programs to control Phytophthora blight of pepper by testing registered and unregistered products, as well as different application methods.

Fungicide efficacy trials: The combination treatment of Experimental + Ridomil Gold SL provided excellent control of Phytophthora blight. Treatments containing alternations of Presidio, V-10208, Revus, Ridomil Gold SL and Ridomil Gold Copper applied as either drench; foliar spray or drip application also controlled *P. capsici*. The combination treatment of Presidio, Ridomil Gold SL and Orondis Gold provided excellent control of Phytophthora blight. The treatment containing alternations of Presidio, Ridomil Gold SL and V-10208 controlled *P. capsici*. Orondis Gold, Ridomil Gold SL and Presidio also managed Phytophthora well.
A total of 18 programs including 6 different registered and unregistered fungicides were evaluated.

Objective 2: Test commercial pepper varieties for resistance to Phytophthora blight.

Variety trials: Of the cultivars planted in this trial, Marta-R, Meeting, and S5035-B had the lowest plant death percentage and therefore the most resistance to the isolates of *P. capsici* used in this study. The land race Fidel remained relatively symptomless throughout the experiment. Cultivars Plato, Red Knight, and Bastille had the highest disease percentage. They were therefore considered highly susceptible. The cultivars Camelot, Quattro, PS00941819, Keystone, Aristotle, Karisma, X5675, Archimedes, HPX9767-B, Revelation, SV3782PP, H10T4706-A, HPX9769-A, SV3198HJ, Fabuloso, HPX9772-A, P2325-B, 3457-R, and Declaration had plant death percentage not significantly different from Plato, Red Knight, and Bastille. The lines H1653 and S5035-B had significantly less plant death than the tolerant cultivar Paladin. A total of 22 cultivars and 10 pepper lines were evaluated for Phytophthora blight resistance.

Objective 3: Determine the mating type of NC *P. capsici* isolates.
Phytophthora capsici isolates have been collected from pepper fields across North Carolina. Mating type determination indicated that 80% of isolates were A2 and 20% were A1.

Objective 4: Disseminate research results to stakeholders through web-based and mobile-friendly tools and presentations at grower meetings and field days.
Five oral presentations, one fact sheet, one abstract, and two production guide have been generated to provide information to growers about Phytophthora blight control in peppers. The fact sheet was published in mobile-friendly platforms.

BENEFICIARIES

This project also aimed to provide training to experienced and new pepper growers on best practices to prevent Phytophthora blight. The PI Lina Quesada, representing NCSU, established a collaboration with the NC Vegetable Growers Association to ensure that all research findings were translated into solutions that were adopted by NC pepper growers to improve production. This project was performed in Wake (Raleigh) and Moore (Jackson Springs) counties but pathogen isolates were sampled from several counties including limited to Johnston, Sampson, Duplin, Rowan, and Haywood.

LESSONS LEARNED

NC is the seventh pepper producer in the US, with 2,200 acres planted every year in approximately 300 farms and valued at $19.5 million. This project had a direct impact on pepper growers in NC since Phytophthora blight occurs frequently on this crop. For example, in the past six years over 35 samples

have been submitted to the NCSU Plant Disease and Insect Clinic with Phytophthora blight from counties such as Henderson, McDowell, Cleveland, Wayne, Sampson, Ashe, Madison, Alexander, Rowan, Brunswick, Duplin, Camden, Dade, Haywood, Buncombe, Currituck, Carteret, Edgecombe, Graham, and Scotland. During the Southeast Vegetable Expo in Myrtle Beach, over 40 growers attended the Phytophthora blight management session and several expressed interest in research to improve control strategies. Growers report economic losses of 70-100% when environmental conditions are favorable for disease, which limits efficacy of fungicides and increases costs of disease control. By combining crop protection with host resistance or tolerance, growers reduced pesticide applications and did not face unpredictable losses during a rainy growing season. As a result of this project, growers have updated fungicide and variety control recommendations that will prevent crop loss. The extension role of the PI allowed for effective dissemination of findings to pepper growers.

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PROJECT TITLE Somatic Embryogenesis of Fraser Fir

PROJECT SUMMARY

The Christmas Tree Genetics Program at North Carolina State University developed improvements to the somatic embryogenesis process for Fraser fir. Fraser fir Christmas trees are one of North Carolina's largest specialty crops, producing revenue of over $100 million annually. Our state has over 1,300 growers cultivating an estimated 50 million Fraser fir Christmas trees on over 25,000 acres. Somatic embryogenesis (SE) is a tissue culture technique that generates genetically identical individuals providing a pathway for large-scale clonal propagation. It is also an important biotechnological tool and a key step in the development of gene editing techniques that could enable rapid improvement of important Christmas tree characteristics. Our research improved initiation of SE lines from mature seeds, proliferation of cultures as liquid suspensions, germination of mature somatic embryos and cryostorage techniques. Additionally, we were the first to successfully apply SE technology to Trojan fir, an exotic species of interest to N.C. growers because of its resistance to Phytophthora root rot. SE seedlings (over 150 Fraser fir and 20 Trojan fir) are currently under greenhouse cultivation and SE lines (20 Fraser fir and 7 Trojan fir) have been cryogenically stored for future use. Results from this work were featured in a book chapter, two presentations at an international scientific conference, three poster/presentations in local undergraduate symposia and will be submitted in a manuscript to a refereed journal. Stakeholders were informed of these efforts via two presentations at N.C. Christmas Tree Association meetings and two articles in their trade journal, *Limbs & Needles*.

PROJECT APPROACH

Activities by Objective	Relevance
Objective 1 *Improve our knowledge of the laboratory steps of somatic embryogenesis (SE) for Fraser fir*	
<u>Initiation</u> Over 150 SE lines of Fraser fir from six elite open-pollinated families were initiated from immature seeds using published protocols. Additional lines of Fraser fir and Trojan fir were initiated from mature stored seed which is considerably more challenging. Larvae of a seed chalcid (*Megastimus specularis*) were identified in some immature seeds.	Published protocol for initiating Fraser fir SE lines are robust and transferable across labs and genetic material. Successful SE initiation from mature (as opposed to developing) seed allows initiation to occur throughout the year rather than during a two-week window in June/July. Our seed chalcid discovery led to additional research funded by the NC Christmas Tree Association to better understand chalcid biology, distribution in the NC Premium Fraser Fir Seed Coop's orchard, clonal variation to infestation, and management options.
<u>Proliferation</u> SE lines were captured and maintained using a published gelled medium. As reported in the literature, cultures were generally difficult to maintain long-term. While some	Additional research is required to improve long-term viability of captured Fraser fir SE lines. We improved proliferation of lines via suspension culture techniques. This offers several

contamination occurred over time, many cultures slowly declined in vigor. Nevertheless, about 20 moderately or vigorously growing Fraser fir and seven Trojan fir lines have been maintained. We improved methods to grow Fraser fir SE cultures as liquid suspension cultures. Maturation We induced somatic seedling maturation in two Fraser fir and seven Trojan fir lines. Germination We demonstrated that somatic seedling germination improved if, once they reach 6-8 mm in length on a published germination medium, they are transferred to a dark and cold (2-4°C) treatment for 28 days before exposure to a 16:8 photoperiod.	advantages over cultures growing on gelled medium including: 1) faster growth, 2) culture uniformity, 3) ease of use, 4) container reusability, and 5) rapid growth measurements when grown in nephelo flasks. Most commercial SE systems rely on liquid cultures. Somatic embryo maturation, assessed by the formation of cotyledons (embryonic needles), is a necessity step for a successful SE program. The conditions to successfully produce mature embryos for these lines have proven repeatable but a better understanding is needed to successfully mature more lines. Efforts toward this goal will continue post-funding. Successful germination (root growth) is important for an operational SE program. We demonstrated that the use of a cold treatment enhances germination (root growth) of mature somatic seedlings of Fraser and Trojan fir.
Objective *2* *Evaluate greenhouse practices for producing somatic seedlings of Fraser fir for field testing in the NC Mountains.* Over 150 Fraser and 20 Trojan fir somatic seedlings have been transferred from the lab. They are being cultured in a propagation house at the NCSU Horticultural Field Lab in which irrigation is provided via a computer-controlled boom system according to vapor pressure deficit (VPD). During the transfer, the height of each seedlings as well as quality of its shoot and root system were recorded. Seedling survival and growth will be closely monitored and additional seedlings will be transferred as available.	Greenhouse and field growth of somatic seedlings is a necessary step in the operational use of SE technology but often overlooked in research programs. After the funding period, we will work to improve greenhouse cultural practices for SE seedlings. We anticipate establishing trials of somatic and normal (zygotic) seedlings in the NC Mountains to evaluate their field survival and growth.
Objective *3 Cyrostore Fraser fir clones with high capacity to produce somatic embryos for use in the development of transformation technology.* We demonstrated that a published protocol for placing material into cryostorage was faster and	Cryogenic storage of SE lines allows long-term storage of important genetic material for future

more efficient than the published techniques for firs. Twenty Fraser fir lines from six families and seven Trojan fir lines from six families have been cryogenically stored. Two to 19 tubes of each line were stored for an average of nine tubes per line. Nine out of 10 lines tested exhibited re-growth after removal from cryostorage.	use. Among other advantages, this enables the propagation of select clones subsequent to assessing their value in field tests.

Dr. John Frampton was principal investigator and oversaw and coordinated the project. Dr. Lilian Matallana was co-investigator and helped supervise the day-to-day laboratory work and participate in critical steps of the propagation. Robert Thomas was lab technician and performed the day-to-day tasks such as media preparation, transferring cultures, and cleaning lab ware. Several undergraduate students worked on this project part-time. The grower-operated Premium North Carolina Fraser Fir Seed Cooperative granted permission to collect cones for this project and cooperated in subsequent chalcid studies that arose from this project. The North Carolina Christmas Tree Association helped dissemination of project results by publishing two articles in their trade journal, *Limbs & Needles*, and scheduling presentations at two association meetings.

GOALS AND OUTCOMES ACHIEVED

The ultimate goal of this effort is to improve the profitability and competitiveness of North Carolina's Christmas tree industry through clonal propagation and the development and use of gene-edited Fraser fir planting stock. The more immediate goal of this project is to improve somatic embryogenesis methods for Fraser fir thus, removing a major obstacle toward achieving clonal propagation and gene-editing techniques.

Short-term measurable outcomes include 1) scientific communication of results, 2) sharing of project results and goals with stakeholders and 3) Fraser (and Trojan) fir somatic seedlings in the greenhouse (described in the *Accomplishments* section).

Results from this work have been featured in a book chapter, two presentations at an international scientific conference, three poster/presentations in local undergraduate symposia and will be submitted in a manuscript to a refereed journal. Stakeholders were informed of these efforts via two presentations at N.C. Christmas Tree Association meetings and two articles in their trade journal, *Limbs & Needles*.

After the project period, the NCSU Christmas Tree Genetics Program and Forest Biotechnology Group have collaborated and co-funded efforts to continue this project. Gel cultures, liquid cultures, cryogenically stored cultures and somatic seedlings in the greenhouse are being maintained. Additionally, the hypotheses about inducing maturation from liquid cultures are being tested. Drs. Wang and Frampton have been notified of funding for a NCDA&CS Specialty Crops Block project entitled, *CRISPR-based Genome Editing in Christmas Trees*, to begin January 2019 that will continue development of the latter stages of somatic embryogenesis as well as initiate CRISPR research for firs. Additionally, they are working on a proposal to the Christmas Tree Promotion Board related to developing CRISPR technology for firs.

List of Publications and Communication of Project Results

- Pullman, G.S. and J. Frampton**.** 2018. Fraser fir (*Abies fraseri* [Pursh] Poir.). p33-47 in: Jain, S.M. and P.K. Gupta (eds.), Step Wise Protocols for Somatic Embryogenesis of Important Woody Plants, Forestry Sciences 84. Springer. https://doi.org/10.1007/978-3-319-89483-6_3
- Frampton, J., L. Matallana and R. Thomas. 2016. Somatic embryogenesis of Fraser fir. *Limbs & Needles* 43(1): 21-23.
- Frampton, J., L. Mattalana and A.M. Braham 2018. NCSU undergraduate students help advance Christmas tree research. *Limbs & Needles*. 45(2): 18-20.
- Frampton, J. L. Matallana, and R. Thomas. 2017. Somatic embryogenesis of Fraser fir. N.C. Christmas Tree Association Winter Meeting, 23 February. Blowing Rock, NC. (presentation & poster)
- Frampton, J. L. Matallana, and R. Thomas. 2018. Somatic embryogenesis of Fraser fir. N.C. Christmas Tree Association Winter Meeting, 2 March. Blowing Rock, NC. (presentation)
- Thomas, R., L. Matallana, and J. Frampton. 2017. Somatic embryogenesis in Fraser fir (*Abies fraseri*): optimizing levels of abscisic acid, polyethylene glycol and maltose for maturation. 13th International Christmas Tree Research and Extension Conference, 4-8 Sept. Akureyri, Iceland. (poster and presentation)
- Matallana, L. K. Goode, M. Bertone, R. Jetton and J. Frampton. 2017. Fantastic bugs and where to find them: the biology of chalcid seed predation. 13th International Christmas Tree Research and Extension Conference, 4-8 Sept. Akureyri, Iceland. (abstract and presentation)
- Bowen, A.L., R. Thomas and J. Frampton. 2018. *Growth and Maturation Rates for Embryogenic Trojan fir Proliferated on Gelled Medium or as Liquid Suspension Cultures.* Undergraduate Research and Creativity Symposium. 18 April. NCSU, Raleigh. (poster)
- Pavic, D., S. McNatton, R. Thomas, L. Matallana and J. Frampton. 2018. *From Flasks to Fields: The Challenge of true Fir Somatic Embryo Maturation*. Undergraduate Research and Creativity Symposium. 18 April. NCSU, Raleigh. (poster, awarded outstanding)
- Goode, K. L. Matallana, M. Bertone, R. Jetton, J. Frampton. 2017. *How the wasps stole Christmas (trees)*. State of North Carolina Undergraduate Research & Creative Symposium (SNCURCS). 15 November. Campbell University, Buies Creek. (presentation)

Our research improved initiation of SE lines from mature seeds, proliferation of cultures as liquid suspensions, germination of mature somatic embryos and cryostorage techniques. Additionally, we were the first to successfully apply SE technology to Trojan fir, an exotic species of interest to N.C. growers because of its resistance to Phytophthora root rot. SE seedlings (over 150 Fraser fir and 20 Trojan fir) are currently under greenhouse cultivation and SE lines (20 Fraser fir and 7 Trojan fir) have been cryogenically stored for future use. Additionally, we discovered a chalcid seed problem in a grower-operated Fraser fir seed orchard and provided guidance on the biology and management of this insect.

BENEFICIARIES

This long-term project will ultimately benefit North Carolina Fraser fir Christmas tree growers. Our state has over 1,300 growers cultivating an estimated 50 million Fraser fir Christmas trees on over 25, acres and generating an annual revenue exceeding $100 million.

LESSONS LEARNED

We assumed that cultures derived from liquid suspension cultures would respond similarly to published maturation treatments as cultures derived from gelled media. Future researchers should test for and/or plan for this apparent differential response.

We conducted a large experiment investigating the effect of three compounds commonly used in the literature to induce somatic embryo maturation [abscisic acid (ABA), maltose and polyethylene glycol (PEG)]. A range of concentrations (0-15 mg/l ABA, 0-60 mg/l maltose, 0-99 mg/l PEG) were applied to eight Fraser fir SE lines. These treatments bracketed the literature-recommended combination for Fraser fir. However, ***only one line (51-23) matured to develop distinct cotyledons*** and surprisingly, this line did not require ABA for maturation.

After re-visiting the fir SE literature, we subsequently, began evaluating the use of an auxin competitor (PCIB) as a pre- and/or co-treatment when applying ABA, maltose and PEG. Separately, we also tested the effect of a range of cytokinin (BAP) concentrations on maturation. In these experiments we discovered that one line (24-53) formed mature embryos when given a one-week pre-treatment on maintenance medium before transfer to the maturation treatments. We are testing this pre-treatment on other lines. We are also evaluating pulsing various ABA concentrations (0-15 mg/l) for 1, 2 or 3 weeks in comparison to a continuous application because we often see the early stages of maturation (dense embryonic head formation) for some treatments but subsequently development stops.

Research with SE maturation is continuing beyond the funding period to improve our understanding of the process and to allow us to induce maturation for additional SE lines.

In retrospect, our timeline to produce enough somatic seedlings to evaluate greenhouse practices was overly optimistic, in part due to the sequential nature of SE and difficulties in the maturation step. While we were successful in transferring well over our targeted 100 Fraser fir somatic seedlings to the greenhouse (plus 20 Trojan fir seedlings), there was not sufficient time to evaluate greenhouse practices for cultivating somatic seedlings. Nevertheless, this research is proceeding post-funding.

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Appendix

Fraser Fir (*Abies fraseri* [Pursh] Poir.)

Gerald S. Pullman and John Frampton

1 Introduction

Fraser fir (*Abies fraseri* [Pursh] Poir.) is a coniferous species native to the Southern Appalachian Mountains of the Eastern United States and occurs in disjunct high-elevation populations in Western North Carolina, Eastern Tennessee, and Southwest Virginia. It is commercially grown as a popular Christmas tree for the dense and fragrant foliage and excellent post-harvest needle retention and is a multimillion-dollar business in the Southern Appalachian Mountains. In North Carolina alone, revenues of over $100 million occur annually (NC Coop. Ext. 2017).

The balsam woolly adelgid (*Adelges piceae* Ratzeburg), an introduced insect pest, and air pollution have killed most old growth Fraser fir. Prospects for young wild trees are uncertain (McManamay et al. 2011). The adelgid often kills trees within 2–7 years of infestation in natural and commercial stands. Global warming also poses a problem for wild and commercial populations. To survive, populations are expected to retreat to higher elevations and colder temperatures. According to the International Union for the Conservation of Nature and Natural Resources (IUCN), Fraser fir is vulnerable to extinction. As temperatures rise, it will become increasingly important to conserve rare and valuable germplasm to maintain biodiversity (Fenning et al. 2008).

Seed production often begins at about 15 years of age with poor early seed crops that generally occur every other year. A vegetative propagation system is needed to

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produce trees with desirable growth characteristics, disease tolerance, and pest tolerance. Grafting and rooted cutting systems have been researched but are not commercially used on a large scale due to expense, low multiplication and undesirable maturation effects including plagiotropic growth (Hinesley and Frampton 2002; Hibbert-Frey et al. 2010, 2011; Rosier et al. 2004, 2005). Somatic embryogenesis (SE) technology may fill the clonal propagation need by (1) shortening the time to produce desired planting stock, (2) allowing control of genetic variation, (3) permitting commercial production of hybrids, and (4) facilitating genetic engineering efforts to add or combine desirable traits. From a conservation point of view, SE technology will facilitate long-term safekeeping through cryopreservation and relocation of the species if needed.

Conifer SE proceeds through four steps: initiation, multiplication, maturation and germination plus cryopreservation when storage of cultures is desired (Pullman et al. 2003a). Somatic embryogenesis in *Abies* was first reported by Schuller et al. (1989) for silver fir [*Abies alba* (Mill.)]. Other *Abies* species and hybrids have been shown to produce embryogenic tissue (ET) and mature embryos that continue to develop (Nørgaard and Krogstrup 1995; Salajova et al. 1996; Jasik et al. 1999; Salaj et al. 2005; Vondrakova et al. 2011; Korecky and Vitamvas 2011; Vookova and Kormutak 2014). Unlike many conifers, ET for *Abies* species is most often induced on medium with only cytokinins as phytohormones. Rajbhandari and Stomp (1997) started ET from one of 44 Fraser fir families using precotyledonary embryos; however, the one initiation that developed did not continue to grow. Guevin and Kirby (1997) reported a maximum induction frequency of 3.5% using isolated full-term Fraser fir embryos. Kim et al. (2009) reported 4.7 and 2.2% initiation for two Fraser fir families and 0% for three more sources using whole female gametophytes (FG) containing immature embryos at a proembryo developmental stage.

Improved protocols for ET initiation, culture capture and growth, somatic embryo development and maturation and cryogenic storage are presented based on the findings of Pullman et al. (2016).

2 Initiation of Embryogenic Tissue

2.1 Cone and Embryo Stage Collection

Fraser fir cones from conservation or breeding programs are used to initiate an ET culture. Open or cross-pollinated cones are collected in late June when immature embryo stages range from 2 to 4 (Pullman and Webb 1994, Fig. 1). Cones are transported or shipped on ice, received within 24–48 h and processed immediately or stored in plastic bags at 4–5 °C for several weeks. Note: to maximize initiation seed cone collection should target embryos at stage 3 (Fig. 2). Full term seeds may be used for ET initiation however; initiation percentages are often lower.

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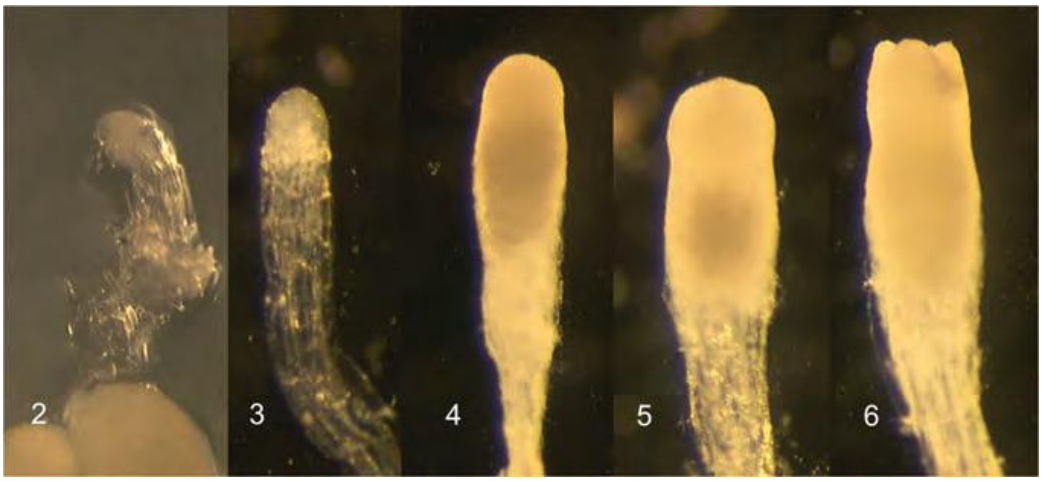

Fig. 1 Fraser fir zygotic embryos at stages 2–6 using the staging system of Pullman and Webb (1994). Adapted from Pullman et al. (2016)

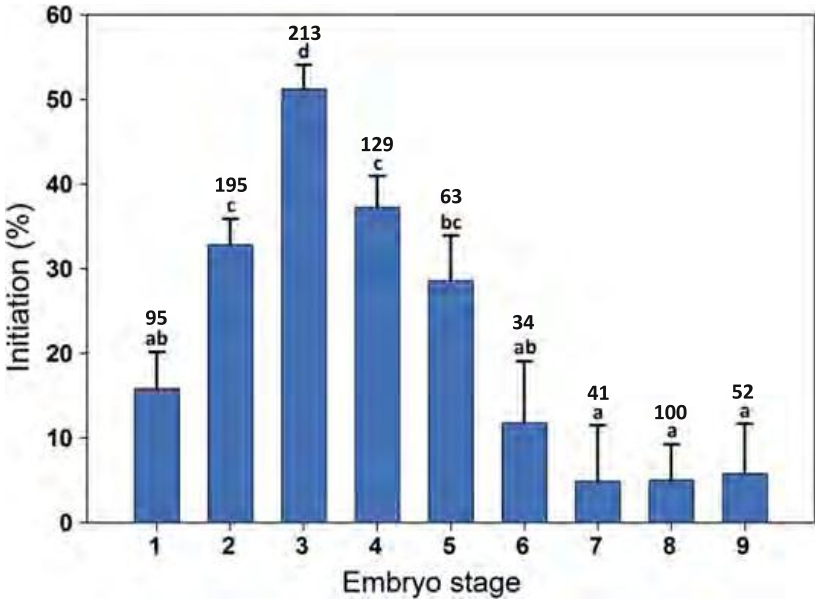

Fig. 2 Initiation percentages by embryo explant developmental stage using four families in 2009. Standard error bars are shown with a letter above the bar. Bars with the same letter are not statistically different by multiple range test at P = 0.05. The bar is based on the number of explants shown above the bar. Adapted from Pullman et al. (2016)

2.2 *Initiation Medium Preparation*

Medium FFI (Table 1) is prepared, and pH adjusted to 5.7 with KOH or HCl after addition of all ingredients except gelling agent and filter-sterilized materials. Medium is autoclaved at 121°C for 20 min. Filter-sterilized aqueous stock solutions of L-glutamine, brassinolide and ABA are added to medium cooled to about 55 °C.

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Paclobutrazol (95.8% active ingredients, purchased from Duchefa, Netherlands) stock solution slurries containing 1 mg/mL are vortexed and rapidly added to the medium prior to autoclaving. At a concentration of 35 mg L^{-1}, paclobutrazol will dissolve fully. Currently we use brassinosteroids that can be purchased from Sigma-Aldrich (St. Louis, MO), Duchefa (Haarlem, The Netherlands) or PhytoTechnology Laboratories (Showcase Mission, KS). Brassinolide stock solutions are prepared in absolute ethanol (Aaper Alcohol and Chemical Co.). Thus, media with 0.1 μM brassinolide also contained 48 μL/L ethanol, a level that, in prior tests with other coniferous species, did not result in statistically significant changes in ET growth.

2.3 Seed Sterilization and Explant Preparation

Cones are opened and separated into individual ovuliferous scales. A small square is cut from the bract containing the undamaged seed attached to the scale. Damage may occur to the thin seed coat when it is detached from the ovuliferous scale due to overexposure to sterilization fluids. Seeds with ovuliferous scale or full term seeds are washed in running cold tap water for 10 min, agitated for 10 min in 10% Liqui-Nox (detergent) containing 2 mL Tween 20/L, and rinsed for 30 min with running tap water. Mature or immature seeds are sterilized with procedures developed for loblolly pine (*Pinus taeda* L.) (Pullman et al. 2005a, 2016). Seeds are agitated aseptically in 20% H_2O_2 for 10 min and rinsed five times for 5 min with sterile deionized water.

2.4 Aseptic Dissection

For immature seed, the coat, integuments and nucelli are carefully removed from the female gametophyte (FG). FG is carefully cut open and the dominant embryo lifted out while attached to the FG and the exposed embryo and FG are placed cut surface down onto initiation medium FFI (Table 1). For mature seed, coat, integuments and nucelli are removed, the FG is split and the mature embryo is removed and placed onto FFI medium without the FG present. Wu et al. (2012) has shown that loblolly pine full-term FG contain *myo*-inositol hexakisphosphate that inhibits early-stage somatic embryo growth. When Pullman et al. (2016) compared initiation for full term embryos with or without the FG present, more initiation occurred for explants without the FG. Explants can be placed on 7 mL of medium in 60 × 15 mm Petri plates or for cost savings, 7 mL of medium per wells of a multi-well plate such as Costar no. 3506 6-Well Culture Cluster Plates. Plates are wrapped in two layers of Parafilm and incubated at 23–25 °C in the dark.

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Table 1 Fraser fir initiation, maintenance, germination and cryopreservation medium components

Components	Initiation	Capture	Maturation	Germination	Cryopreservation
mg/L	FFI	FFCP	FFM	397[b]	FFCR
KNO_3	–	–	–	1170	–
NH_4NO_3	–	–	–	206.3	–
K_2HPO_4	87.1	–	–	–	–
KH_2PO_4	–	340	340	85	340
$CaCl_2{\cdot}2H_2O$	–	–	–	220	–
$CaSO_4{\cdot}2H_2O$	37.8	37.8	37.8	–	37.8
$MgSO_4{\cdot}7H_2O$	394.2	394	394	185.5	394
KI	0.083	0.083	0.083	0.415	0.083
H_3BO_3	2.48	2.48	2.48	3.1	2.48
H_3PO_4	180.6	373	373	–	373
$MnSO_4{\cdot}H_2O$	18.6	18.6	18.6	8.45	18.6
$ZnSO_4{\cdot}7H_2O$	5.76	5.76	5.76	4.3	5.76
$Na_2MoO_4{\cdot}2H_2O$	0.103	0.103	0.103	0.125	0.103
$CuSO_4{\cdot}5H_2O$	3.75	3.75	3.75	0.25	3.75
$CoCl_2{\cdot}6H_2O$	0.012	0.012	0.012	0.125	0.012
$NiCl_2{\cdot}6H_2O$	1.188	1.188	1.188	–	1.188
$FeSO_4{\cdot}7H_2O$	16.68	16.8	16.8	13.93	16.8
Na_2EDTA	–	–	–	18.65	–
myo-Inositol	1000	1000	1000	100	1000
Sucrose	10,000	10,000	–	20,000	10,000
Maltose	–	–	40,000	–	–
Sorbitol	–	–	–	–	72,868
$C_{12}H_{10}Mg_3O_{14}{\cdot}9H_20$	266	266	266	–	266
L-Glutamine[a]	2000	2000	2000	–	2000
Thiamine·HCL	1	1	1	1.0	1
Pyridoxine·HCL	0.5	0.5	0.5	0.5	0.5
Nicotinic acid	0.5	0.5	0.5	0.5	0.5
Glycine	–	–	–	2.0	–
BAP	1.0	1.1	–	–	1.1
Brassinolide[a]	0.048	–	–	–	–
Paclobutrazol	0.33	–	–	–	–
Abscisic acid	1	–	5	–	–
$Na_2S_2O_3$	158.1	–	–	–	–
Acros PEG 8000	–	–	100,000	–	–
Activated charcoal	–	–	–	2500	–
Tissue culture agar	–	–	–	8000	–
Gelrite	3000	3000	3000	–	–
pH	5.7	5.7	5.7	5.7	5.7

[a]Filter-sterilized stock solution was added after autoclaving and cooling to 55–60 °C
[b]Medium 397 (Pullman et al. 2003a)

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2.5 Embryogenic Tissue Evaluation

Initiation is evaluated 11–13 weeks after dissection. Multiple points of ET initiation are often present on an explant. A typical sequence of initiation from immature zygotic embryos is shown in Fig. 3.

3 Embryogenic Tissue Capture and Maintenance

3.1 Embryogenic Tissue Capture

After 11–13 weeks ET from an initiation is transferred to capture medium FFCP (Table 1). Tissue weights are tracked over three three-week subcultures, and an initiation is considered "captured" when it reaches 200 mg. About half of the new initiations will continue growth to become captured. A target weight of 200 mg was selected based on observations in *P. taeda* where captured cultures reaching this mass tended to continue growth while cultures of less weight had a greater chance of growth decline (G. S. Pullman, personal observation). The remaining 50% do not grow even though ET formed during initiation or initially grew slowly and then stopped growth within several weeks to several months.

3.2 Embryogenic Tissue Maintenance

Once the culture is actively growing it can be maintained on gelled or liquid medium. Cultures grown on gelled medium (FFCP) are transferred to fresh medium after about three weeks with ET masses about 1 cm in diameter divided in two.

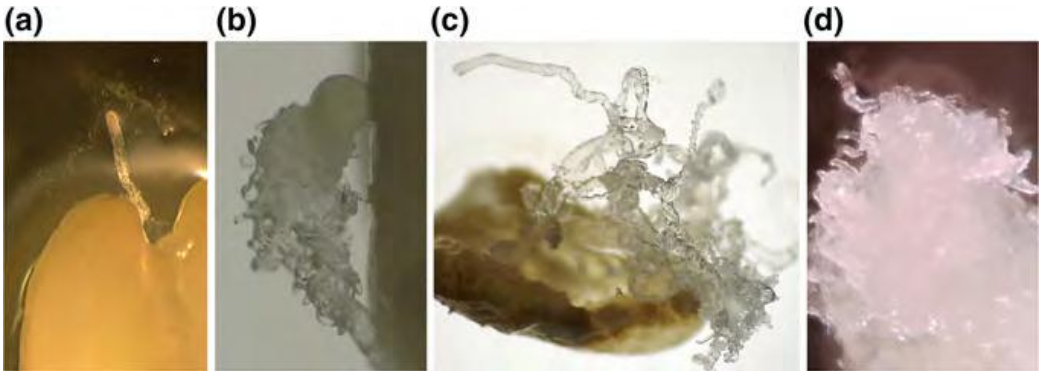

Fig. 3 Sequence of embryogenic tissue initiation in Fraser fir. **a** Female gametophyte and dominant zygotic embryo (stage 3) placed on medium. **b** Approximately one month after placement on initiation medium tissue from the dominant embryo is beginning to differentiate. **c** Embryogenic tissue is forming. **d** Proliferating embryogenic tissue

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Observations suggest that tissue grown on gelled medium may produce slightly more advanced embryos during the development and maturation step possibly due to maintained pre-conditioning materials.

3.3 Embryogenic Tissue Suspensions

Cultures containing early-staged embryos grown in liquid medium (FFCP minus gelling agent) (Fig. 4) often grow faster, have less contamination, are more amenable to automated transfer, allow improved observation of embryos and have reduced labor requirements for maintenance (Pullman and Skryabina 2007).

To establish suspension cultures, pour 20 ml of FFCP liquid medium (i.e., without Gelrite) into a 125 mL Erlenmeyer flask under sterile conditions and inoculate with 1.0 g fresh weight ET. The ET should be harvested from the surface of cultures grown on gelled FFCP medium avoiding the wet compact tissue under the surface layer where embryo health declines. The harvested ET should consist of small tufts of embryos rather than large masses that may not break up in suspension. Culture growth rates vary by genotype but attempt to harvest tissue when the culture reaches its maximum growth rate, usually 14–21 days from the previous sub-culture. If ET is harvested too soon, the full multiplication potential of the culture is compromised. If harvested too late, the ET embryo quality may have declined. It is better to err toward harvesting too soon.

Place flasks containing inoculated liquid medium onto a gyratory (orbital) shaker set at 100 rpm in dark at 23–25 °C. Assess growth of the culture after 24 h by measuring settled cell volume (SCV). To measure SCV: (1) pour the flask contents into a 100 ml graduated cylinder under sterile conditions, (2) allow the ET to settle for 15 min, (3) measure the height (mm) of the 100 ml column with a ruler,

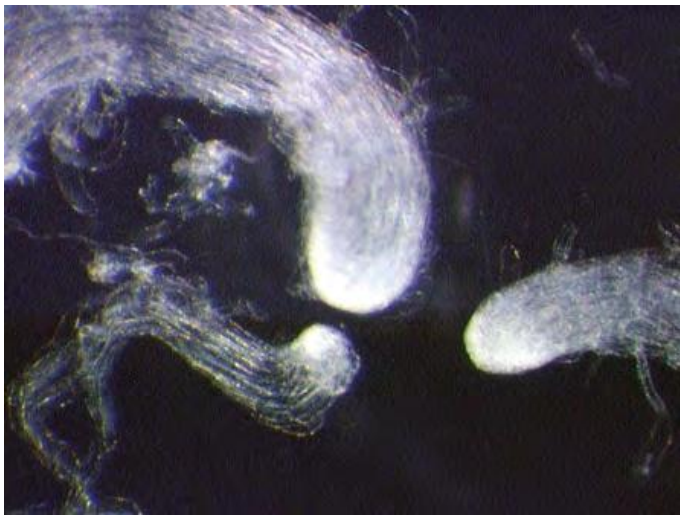

Fig. 4 Fraser fir somatic embryos grown in liquid culture suspension

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(4) measure the height (mm) of the settled tissue, and (5) calculate SCV using the following equation, SCV (%) = [ET height (mm)]/[Total content height (mm)] 100. Expect an initial SCV of approximately 20%. Pour the cylinder contents back into the flask and return to the shaker. Reassess SCV weekly from the initial inoculation day. If no SCV increase is detected after 21 days, the attempt to establish a suspension culture has likely failed.

Once an ET culture reaches about 50% SCV, remove the liquid portion above the settled ET from the graduated cylinder using a sterile pipette. Pour fresh FFCP liquid medium into the cylinder bringing total volume to 40 ml (for a 250 ml flask) or 80 ml (for a 500 ml flask). Transfer into a larger flask and assess SCV weekly. The culture is considered established once ET achieves about 80% SCV. At this point, continue to multiply the culture by pouring 10 ml of swirled suspension into each for four new 250 ml flasks and bringing their total volume to 40 ml with fresh FFCP medium. For larger flasks, pour 20 ml of swirled suspension into each of four new 500 ml flasks and bring total volume to 80 ml.

In general, cultures are difficult to maintain long-term. Some culture contamination may occur and often cultures slowly decline over time. Signs of decline include slowing or lack of ET growth and/or loss of ability to develop cotyledonary embryos. To stop culture decline we store cultures cryogenically as soon after capture as possible. (See section on ET cryogenic storage.)

4 Somatic Embryo Development and Maturation

ET grown on gelled or liquid FFCP medium can be plated onto maturation medium. ET (pieces 5–8 mm) from gelled FFCP is placed onto FFM (Table 1). Alternately, ET suspensions grown in liquid FFCP can be settled, excess liquid removed, and aliquots of suspension (0.5–1.0 ml) dispersed onto support—material placed on FFM. Plates are wrapped in Parafilm and incubated in darkness at about 25 °C. After two 6-wk transfers to fresh FFM, somatic embryos are observed (Fig. 5).

In work with *Pinus taeda* embryo suspensions, Pullman and Bucalo (2011), used monofilament support spun from nylon fabric (06400JP-72 with 162 × 162 fibers/in and 60 μm openings) purchased from Decotex Inc., Pawling, New York. In tests, using 6, 29 and 15 genotypes, Pullman et al. (2016) found average yields of cotyledonary embryos of 17.2 per plate, 0.7 to 58.7/g of ET with an average across genotypes of 24.5 cotyledonary embryos/g and 22–925/g with an average of 227 cotyledonary embryos/g ET. Across the three tests, 46% of the genotypes produced cotyledonary embryos on FFM.

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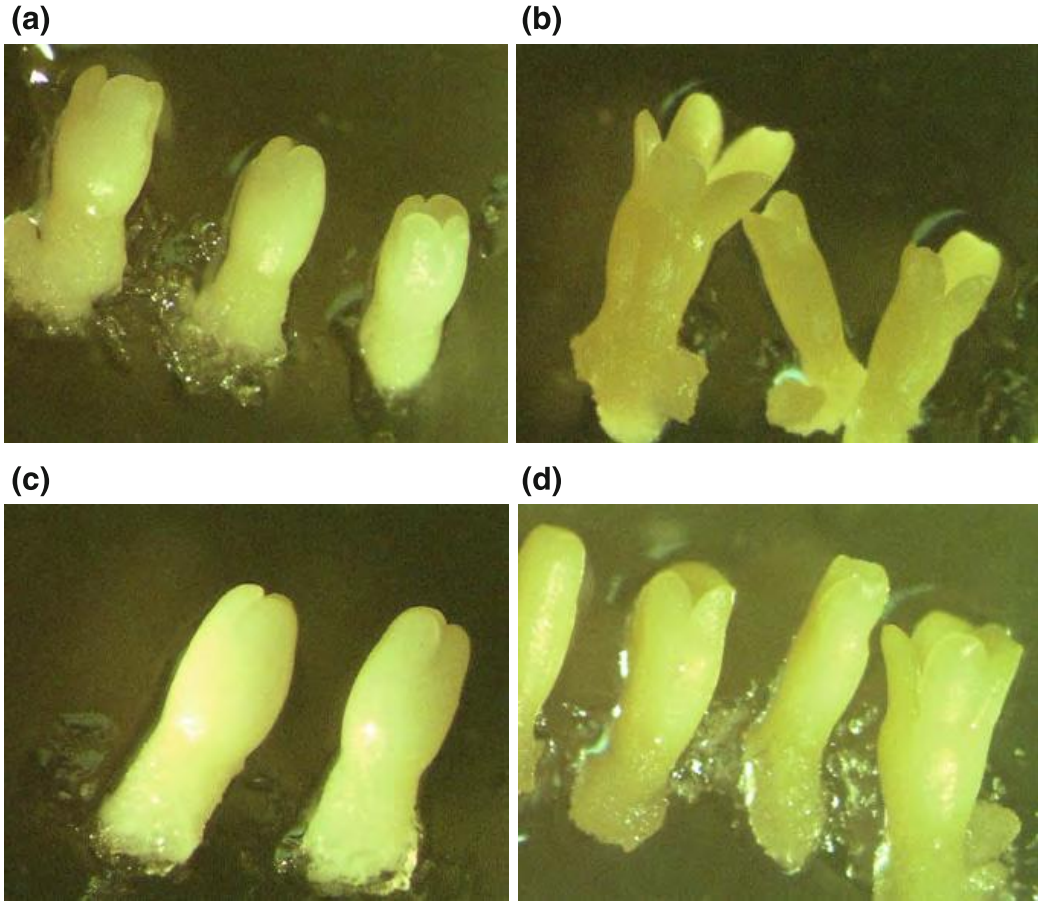

Fig. 5 Fraser fir cotyledonary embryos produced on maturation medium FFM. Four genotypes from two open-pollinated families are shown. Reproduced from Pullman et al. (2016) with permission from Springer

5 Somatic Embryo Germination

After three months on maturation medium, somatic embryos are selected that exhibit normal shape and maximal size. Embryos are placed horizontally on 20 mL of germination medium 397 (Table 1, Pullman et al. 2003a) in 100 × 20 mm Petri plates. Plates are wrapped with Parafilm and incubated at 25–26 °C for 7 days in the dark and then under lights with a 16/8 h (day/night) photoperiod from cool white fluorescent lamps at an intensity of ~30 mmol photons/m^{-2} s^{-1}. After 2 months, embryos are transferred to fresh medium and after eight weeks are observed for presence of roots and shoots. An embryo is considered germinated when it contains a root and a shoot. Within four weeks, hypocotyls expand followed by cotyledon expansion. At 4–8 weeks, apical primordia appear and slowly epicotyl and needles form and roots began to occasionally emerge (Fig. 6). Most germinated embryos grew slowly and set a tight apical bud that did not grow out. Two months of stratification on medium 397 at 4 °C increased germination

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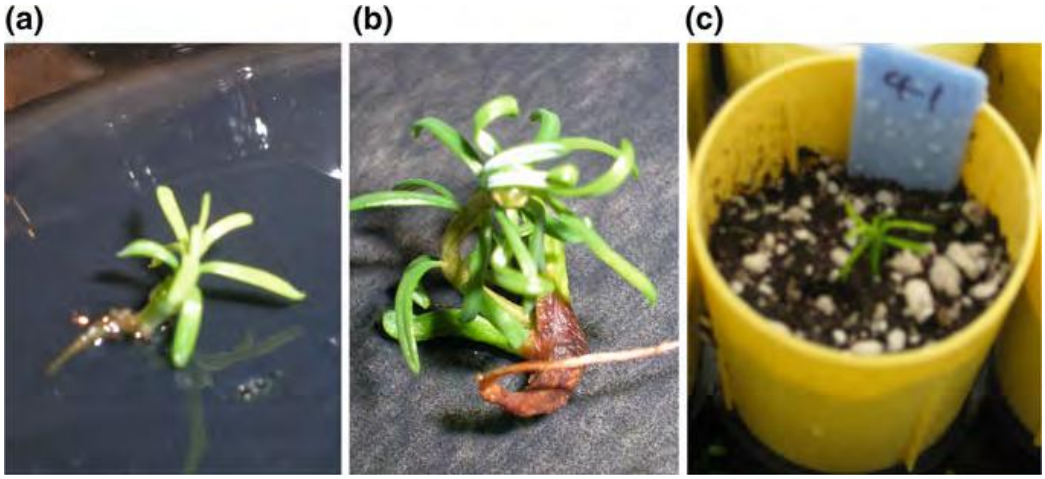

Fig. 6 Fraser fir somatic seedlings with root and epicotyl growth. Reproduced from Pullman et al. (2016) with permission from Springer

(Pullman et al. 2016), but somatic seedlings still grew slowly. Germination and seedling establishment is the weakest step in the Fraser fir protocol and requires further research.

6 Cryogenic Storage of Embryogenic Tissue

A modification of the method of Pullman et al. (2003a), described in detail by Pullman and Bucalo (2011) and Ma et al. (2012), can be used with ET grown in semisolid or liquid medium (Pullman et al. 2016). Cultures are grown on gelled or liquid FFCP medium (Table 2) with approximate monthly or bimonthly transfers, respectively. FFCR medium was developed from FFCP medium with added 0.4 M sorbitol. Cultures are incubated overnight on a shaker in liquid FFCR medium at a ratio of 1 g semisolid-grown tissue: 1.5 mL liquid medium or 1 part settled cells from liquid FFCR medium: 4 parts liquid FFCP medium. Filter-sterilized dimethyl sulfoxide (DMSO) is added slowly to cultures on ice to a final concentration of 5%. Then, 1.8 mL aliquots of the preparation are added to 2 mL Nalgene cryogenic vials. Vials are placed into a programmable freezer, cooled to −35 °C at a rate of 0.33 °C/min and then submerged in liquid nitrogen (LN). To retrieve cultures, vials are removed from LN, warmed in a 37 °C water bath for 1–2 min and contents are poured onto nylon mesh (Decotex Inc., Pawling, New York) resting on FFCP medium. After 1 h, and again 18 h later, the nylon mesh and cells are moved to fresh FFCP medium and maintained in the dark. After approximately six weeks viable tissue retrieved from LN will show signs of new ET growth. Once regrowth occurs, ET has been shown to grow normal cotyledonary embryos on FFM (Pullman et al. 2016).

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7 Discussion

Protocols are presented that can multiply and preserve Fraser fir: ET initiation and capture, embryo maturation and germination and cryopreservation of cultures in LN. Results far exceed those previously reported for the species.

Initiation. Several medium additives and environmental conditions have been shown to improve ET initiation from conifers (Pullman and Bucalo 2011, 2014; Pullman et al. 2015). A blend of AL salts with brassinolide, paclobutrazol, ABA and sodium thiosulfate resulted in high-frequency initiation from immature or full-term Fraser fir embryos (Pullman et al. 2016). ET initiation averaged 6–62% for seed from 11 high-value mother trees. Isolated immature embryos showed initiation frequencies ranging from 18 to 50% on the best medium (FFI, Table 2, Pullman et al. 2016).

AL salts developed for subalpine fir based on metal analyses of seed tissues are high in P, Cu, and Ni and low in Ca compared to other salt bases used for conifer SE (Kvaalen et al. 2005). Unique to this medium is citrate in place of EDTA as an iron chelator. Citrate has been shown to be the major iron ligand in xylem sap, it provides energy as part of the tricarboxylate cycle and it stimulates early stage somatic embryo growth for loblolly pine (De Silva et al. 2008; Silva et al. 2009). Brassinosteroids improved initiation in loblolly pine, Norway spruce, Douglas fir (Pullman et al. 2003c), Himalayan blue pine (*Pinus wallichiana* A. B. Jacks., Malabadi and Nataraja 2007) and Fraser fir (Pullman et al. 2016). Paclobutrazol, a gibberellin synthesis inhibitor, promoted initiation and early-stage somatic embryo growth for several coniferous species (Pullman et al. 2005b). Loblolly pine, slash pine (*Pinus elliottii* Engelm.), Douglas fir and Norway spruce all responded to 1.0, 1.0, 1.0, and 0.33 mg L^{-1} paclobutrazol with increased initiation by 17, 48, 40, and 25%, respectively. Based on analyses showing ABA in early-stage FG, ABA increased loblolly pine ET initiation (Kapik et al. 1995, Handley 1997, 1999; Pullman et al. 2003b). Aitken-Christie and Parkes (1996) also found ABA increased initiation in Monterey pine (*Pinus radiata* D. Don) using dissected pre-cotyledonary bullet stage embryos and media without auxins or cytokinins. High initiation frequency for Douglas fir occurred when media contained ABA and decreased significantly when ABA was removed (Pullman et al. 2009). Sodium thiosulfate or other reducing agents have improved initiation in several coniferous species including Fraser fir (Pullman et al. 2016).

Initiation from immature conifer embryos depends on the embryo stage rather than time of year. Use of a staging system is critical to help understand variation in zygotic embryo development due to mother tree location, time of year and genotype (Fig. 1, Pullman and Webb 1994). ET grew from immature zygotic embryos at stages 1–6 with a clear optimum at stage 3 (Fig. 2). Cold storage (4°) can assist in obtaining optimal stages by dissecting seed sources with optimal stages first and allowing cones with early-stages to slowly advance towards the target stage while in storage. Exposed dominant embryos placed next to the gametophyte increased

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initiation from immature seed when compared to leaving embryos in whole female gametophytes (Pullman et al. 2016).

Capture and maintenance. AL salts are the same as for initiation with the exception of potassium and phosphate which are increased 2.5 and 2.7 times. Brassinolide, paclobutrazol, ABA and sodium thiosulfate are removed and BAP is increased to 1.1 mg/L. Over six capture trials, Pullman et al. (2016) found about half of the initiations continued growth on FFCP medium. However, over time many cultures became contaminated, stopped growth, slowed growth or lost ability to produce cotyledonary embryos. To avoid significant culture decline, cultures were cryopreserved shortly after capture prior to the maturation step.

Significant culture decline has been reported for somatic embryogenesis in coniferous species (Nørgaard and Krogstrup 1995; Timmis 1998; Pullman et al. 2003b; Breton et al. 2006; Pullman and Bucalo 2014; Aurich et al. 2014). Recently, Aurich et al. (2014) reported that long-term culture of Caucasian fir [*Abies nordmanniana* (Steven) Spach] clones resulted in declining numbers of normally-developed mature embryos. Culture decline and lack of full embryo maturation are significant problems that impede commercial implementation of SE.

Maturation. FFM contains the same salts used for capture with the addition of 10% PEG 8000, 5 mg/L ABA and a change from 1% sucrose to 4% maltose. Pullman et al. (2016) reviewed development of this medium that is based on prior reports of *Abies* species maturation and experiences with embryo development for *Pinus taeda* and *Pseudotsuga menziesii*. In three tests, 23 of 50 genotypes produced cotyledonary embryos with yields ranging from 0.7 to 925/g ET.

Germination. Germination and conversion of *Abies* somatic embryos are often low and are reviewed by Pullman et al. (2016). Fraser fir had low germination for the few genotypes able to produce cotyledonary embryos. Partial drying did not improve germination, but two months of storage at 4 °C (stratification) did (Pullman et al. 2016). Long-term cold storage has been reported to improve germination and survival rates (Welty 2000; Liao and Juan 2015). Cotyledonary stage somatic *Abies* seedlings are known to go into dormancy under long day conditions and post acclimatization treatments at 5 °C or lower for at least six weeks will break this dormancy (Vookova and Kormutak 2014). Research on Fraser fir germination should focus on breaking dormancy induced during the conversion process. While our sequence of germination is slow and awkward compared to normal seed embryos, the ability to germinate somatic embryos from several genotypes produced on FFM medium provides a baseline for future improvements.

Cryopreservation. Cryostorage of seeds, somatic embryos or plant meristems is useful for storage of germplasm and can stop culture decline while in storage. Cryopreservation protocols based on slow-cooling and fast-thawing have been developed for Greek fir, Caucasian fir and hybrids of *A. cephalonica* and *A. alba* × *A. numidica* (Norgaard et al. 1993; Aronen et al. 1999; Misson et al. 2006; Salaj et al. 2010; Krajnakova et al. 2011). Early cryopreservation of Caucasian fir cultures stopped culture decline and allowed the production of stable quantities of somatic embryos after culture revival (Aurich et al. 2014). In our tests, 12 of 22 (55%) of the genotypes cryopreserved using FFCR medium survived, and

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several genotypes were shown to produce cotyledonary embryos capable of germination after LN storage.

A highly effective initiation protocol is presented using dominant immature Fraser fir embryos. With efficient initiation we developed capture, maturation, germination and cryopreservation steps although more effort is needed. Our protocol may be useful to improve initiation in other recalcitrant firs and coniferous species.

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Abstracts

Oral Presentations

Poster Presentations

Host: The Icelandic Forest Research Mógilsá

skógræktin

ICELAND 2017

Somatic Embryogenesis in Fraser fir (*Abies fraseri*): Optimizing Levels of Abscisic Acid, Polyethylene Glycol and Maltose for Maturation

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Somatic embryogenesis is a plant tissue culture technique used to make genetically identical copies of a single plant. This versatile technique has been applied to a variety of plant species, from foodstuffs such as corn to horticultural plants. In conifers, somatic embryogenesis is used to produce millions of seedlings per year from commercially valuable species such as loblolly pine, Douglas fir and Norway spruce. The development of a commercially viable somatic embryogenesis system could revolutionize the Christmas tree industry by 1) drastically shortening the time to produce planting stock, 2) allowing multiple desirable traits to be combined, 3) enabling control of genetic variation, *i.e.*, improving stand uniformity, 4) permitting commercial production of interspecific hybrids, 5) providing a necessary platform for genetic transformation, and 6) facilitating the development of synthetic seed technologies.

Once an embryogenic culture is established, it can proliferate into many millions of copies. In the proliferative stage, the somatic embryos (SE) are immature, lacking cotyledons and roots. In order to induce SE to mature into functional plants, chemicals such as abscisic acid (ABA), polyethylene glycol (PEG), and maltose are commonly added to the medium. However, the optimum amounts and combinations of these chemicals vary dramatically across conifer species, and even between different genotypes of the same species. In this report, we investigate the interactive effects of ABA, PEG and maltose on Fraser fir embryo maturation using a 4^3 factorial experimental design. The 64 treatment combinations were applied to eight clonal lines originating from seed of four open-pollinated families collected from the North Carolina Premium Fraser Fir Seed Cooperative's Big Springs Seed Orchard.

Abstract: Oral Presentation

Fantastic bugs and where to find them: the biology of chalcid seed predation

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Rapid changes in world's climate, globalization and current trade patterns are affecting forest pests and the damage they cause. These changes directly impact the development, survival, reproductive cycle and dissemination of pest species while also altering plant host susceptibility and changing the behavioral interactions among pests and other species such as natural enemies and parasitoids. Seed chalcids (*Megastigmus* spp.) are tiny wasps whose larvae destroy seeds of various plant species by consuming the megagametophyte (or endosperm) and embryo. Losses caused by *Megastigmus* have been reported in different conifers in the USA and Europe. We discovered insect larvae in immature and mature Fraser fir seeds and have been improving methods for rearing chalcid adults under lab conditions. Larval and female and male adult specimens of the insect were submitted to the NCSU Plant Disease and Insect Clinic and identified as *Megastigmus specularis* Walley. Interestingly, another wasp also emerged from these seed that we believe is a primary parasitoid of *M. specularis*. General descriptions of the life cycle and habits of seed chalcids in the Appalachian region were published in the early 1960s but no additional information has been published since. Identification of infested Fraser fir seed is particularly challenging because infestation occurs on immature cones and mature seed are small, light-weight, and frequently filled with resinous material so that their weight is similar to infested seeds. Thus, separation of infested seeds via wind columns or gravity tables is almost impossible. Our preliminary observations indicate that chalcid wasps favor certain Fraser fir families for oviposition but more data is required to confirm this hypothesis. Our main goal is to generate basic information about how the chalcid life cycle is correlated with Fraser fir seed physiology, assess clonal variation and learn more about possible natural chalcid parasites that in the long term will help us improve Christmas trees seed orchards and clone banks and to be prepared for changes in pest behaviors, outbreaks and future invasions.

Abstract: Oral Presentation

Somatic Embryogenesis of Fraser Fir

John Frampton, Lilian Matallana and Robert Thomas
NCSU Christmas Tree Genetics Program

What is somatic embryogenesis?

Inside every plant seed there is an embryo or undeveloped young plant. In our case, there is a tiny Fraser fir tree in each seed waiting to develop. Somatic embryogenesis (SE) is a laboratory process whereby we make exact copies of these embryos. The copies originate from somatic cells, i.e., cells forming the plant body rather than from ovules and pollen that in nature produce embryos through fertilization. If we successfully develop SE methods for Fraser fir, we could use these copies to plant whole fields of Christmas trees.

SE is a complex process divided into four major steps: 1) starting/establishing the embryogenic culture (initiation), 2) multiplication to make 1000's of copies of the original embryo (proliferation) 3) maturation of embryos into plantlets and 4) transfer of the plantlets from laboratory culture to soil. After the initiation step, we can use liquid nitrogen (cryopreservation) to store some of the culture for decades, and then revive it whenever needed.

> "If we successfully develop SE methods for Fraser fir, we could use these copies to plant whole fields of Christmas trees."

While it is theoretically possible to obtain somatic embryos from any source of plant tissue, in practice for conifers, the best initiation success occurs when immature zygotic (natural) embryos are used as the starting material. For Fraser fir, this involves collecting immature cones during late June and extracting embryos from the seed. Alternatively, embryos from full-term (mature) seed can be used (Figure 1) but initiation success is generally lower and may decrease further with storage time of the seed.

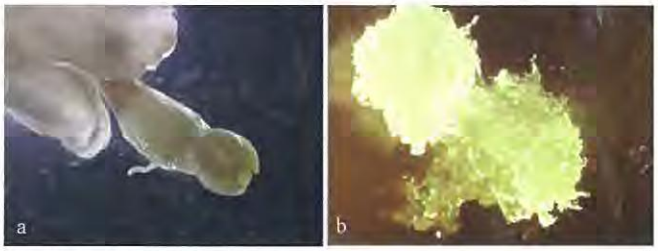

Figure 1. *A somatic embryo emerging from a mature Fraser fir embryo (a) and rapidly multiplying masses of Fraser fir somatic embryos (b). Reprinted with permission from* ***New Forests.***

Why do we want to develop SE for Fraser fir?

SE is basically a cloning technique that can theoretically produce hundreds of thousands of seedlings of the same genotype, that is, genetically identical seedlings. If SE could be further developed into a commercially viable propagation system for Fraser fir, uniform Christmas tree fields of superior clones selected for fast growth, excellent quality and good postharvest needle retention could be established. Uniformity would help cultural efficiencies in shearing, fertilization and pest control as well as yield a more uniform product for wholesale customers. 'Choose and cut' growers could plant a mixture of clones with known desirable traits to offer a variety of superior choices for their customers.

Another benefit of improving SE methods for Fraser fir would be to provide a pathway for genetic engineering in the future. SE would enable the development of genetic transformation of Fraser fir, that is, insertion or manipulation of DNA affecting desirable traits. Genetic transformation of forest tree species is usually performed by inserting genetic constructs into SE cultures, producing embryos, and then selecting and propagating the transformed embryos for further evaluation. Inserting genes related to *Phytophthora* resistance may be possible. Colleagues in the College of Environmental Science and Forestry at the State University of New York (ESF SUNY) have transformed American chestnuts with candidate resistant genes and the transformed seedlings will be challenged with *Phytophthora* at NCSU this summer to evaluate their resistance. If appropriate SE and genetic transformation techniques were available, a similar strategy could be explored for Fraser fir.

Current Status of SE for Fraser Fir

There are four papers in the scientific literature concerning Fraser fir SE. The most recent one (Pullman et al. 2016), reports significant improvements to the SE process for Fraser fir. It describes experiments conducted over a 10-year period in Dr. Gerald Pullman's laboratory at the Institute of Paper Science and Technology, Georgia Tech. Dr. Ulrika Egersdotter conducted some of the early research in this lab with funding from NCCTA. The seed for this research originated from Fraser fir seed orchards of the N.C. Division of Forest Resources (2003-2007) and the N.C. Premium Fraser Fir Seed Coop (2009-2012) so that the techniques reported are applicable to currently used families in the N.C. Christmas tree industry.

The initiation step of SE is critical. Once cultures are captured from individual seed, they can be multiplied and used for various experiments in order to improve the downstream steps of SE. The 2016 paper demonstrated the relationship between starting material and initiation success. When starting from mature seed, the initiation success averaged about 11% under the best conditions. However, when immature embryos were used as the starting material, initiation rates improved and typically averaged 20-30% or higher. Further, the developmental stage of the immature embryo was important with Stage 3 (Figure 2) yielding the highest initiation rate. So, there is a brief window during late June every year to collect starting material to have maximum success in initiating Fraser fir SE cultures.

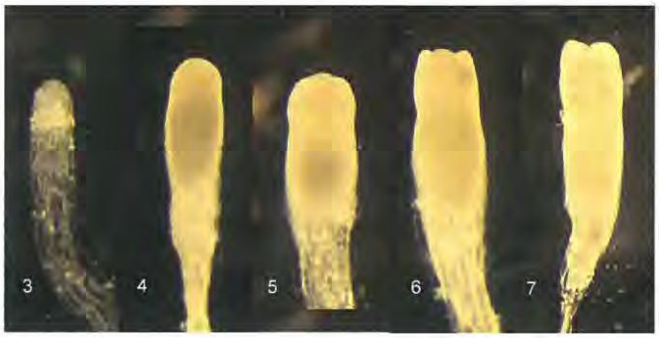

Figure 2. ***Stages of development of Fraser fir embryos from cones collected during late June and early July. The best initiation success of somatic embryogenic cultures occur with Stage 3 embryos but Stages 2-5 also give acceptable initiation success. Reprinted with permission from New Forests.***

In addition to the importance of the starting material, Dr. Pullman published many improvements to the components of the media used for the initiation of SE cultures and the downstream SE steps. He also demonstrated that SE cultures of Fraser fir could remain viable after cryostorage at -30° F. This would allow cultures to be easily preserved while clonal seedlings are grown and field-tested for Christmas tree characteristics and subsequently used to propagate the best clones. Dr. Pullman was also able to get somatic embryos to germinate and form a root (Figure 3).

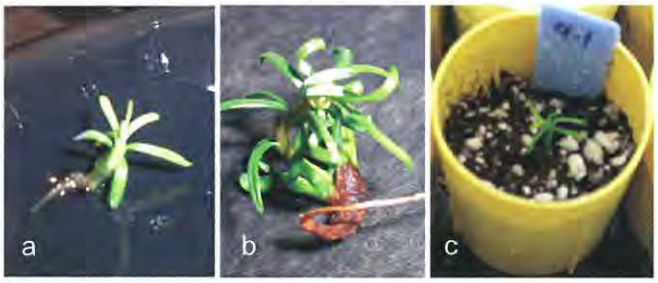

Figure 3. ***Somatic seedlings of Fraser fir with a root and shoot growth. Reprinted with permission from New Forests.***

New NCSU Project and Team Member

Recently, the NCSU Christmas Tree Genetics team was awarded a two-year grant through the NCDA&CS Specialty Crop Block Grant Program for a research project to continue improvement of Fraser fir SE techniques.

We are pleased to welcome Robert Thomas who joined us during April to work on this project. And, Robert is thrilled to be back at NCSU! He received a Master of Science degree from the NCSU Botany Department (now Plant & Microbial Biology) in 1993 and has worked for 18 years at companies including Syngenta (formerly Ciba Geigy) and ArborGen (formerly MeadWestvaco), where he improved loblolly pine, cottonwood, sweetgum, eucalyptus and even corn. We are grateful to have Robert's expertise to accelerate our progress on this project.

Our team plans to build on the recently published Fraser fir SE techniques. Specifically, the project objectives are to: 1) improve our knowledge of the laboratory steps of somatic embryogenesis for Fraser fir, 2) evaluate greenhouse practices for producing somatic seedlings of Fraser fir for field testing in the N.C. Mountains and 3) cyrostore Fraser fir clones with high capacity to produce somatic embryos for use in the development of genetic transformation technology.

> "We would like to improve initiation from mature seeds so that SE could be started anytime of the year."

We have already started efforts to initiate SE cultures from stored mature Fraser fir seed from the N.C. Premium Fraser Fir Seed Coop orchard. We would like to improve initiation from mature seeds so that SE could be started anytime of the year. But, we are also making plans for a large initiation effort when the optimum window for initiating from immature embryos occurs during June-July. Our team is excited about this research and will keep growers informed of our progress. Ultimately, we hope to enable the use of SE technology and genetic transformation in the commercial production of genetically improved planting stock for Fraser fir Christmas tree growers.

Literature on Somatic Embryogenesis of Fraser Fir

Rajbhandari, N. and A. Stomp. 1997. Embryogenic callus induction in Fraser fir. *HortScience* 32:737–738.

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Kim, Y.W., R. Newton, J. Frampton and K.-H. Han. 2009. Embryogenic tissue initiation and somatic embryogeneis in Fraser fir (*Abies fraseri* [Pursh] Poir.). *In Vitro Cellular & Developmental Biology – Plant*. 45:400-406.

Pullman, G.S., K. Olson, T. Fischer, U. Egertsdotter, J. Frampton, and K. Bucalo. 2016. Fraser fir somatic embryogenesis: high frequency initiation, maintenance, embryo development, germination and cryopreservation. *New Forests*. 47:453-480.

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WHY

Should I Become a Member of the North Carolina Christmas Tree Association?

To promote the traditions and memories of celebrating Christmas with a Real Tree.

To protect and grow your business. NCCTA works to stop legislation and regulations that are negative to you and others growing, wholesaling and retailing Real Christmas Trees and to support beneficial legislation and regulations.

To be part of a community of Christmas Tree professionals and share the knowledge and experiences of thousands who share your concerns, questions and needs.

FALL/WINTER 2018

Limbs & Needles

Serving the Christmas Tree Industry in the South

INSIDE:

When Should You Start Cutting Fraser Fir Christmas Trees?

Getting Familiar with Pesticide Families

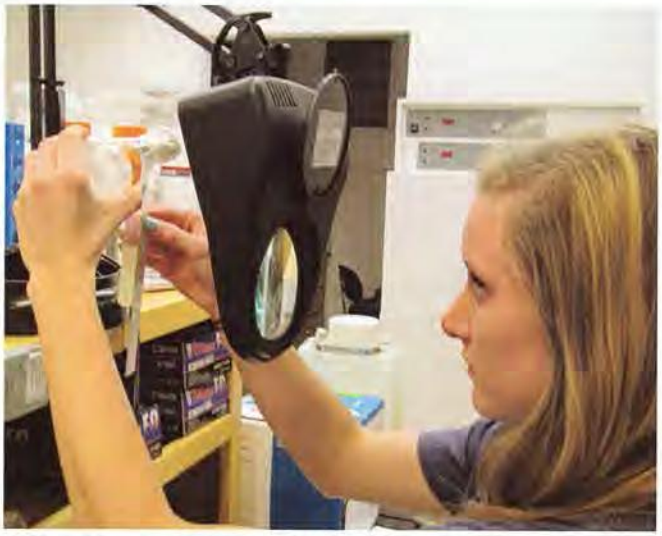
NCSU Undergraduate Students Help Advance Christmas Tree Research

CTPB Update: Millennial Families Target for CTPB Campaign

NCSU Undergraduate Students Help Advance Christmas Tree Research

John Frampton, Lilian Matallana and AnneMargaret Braham
NC State University

Over the years, the Christmas Tree Genetics Program at North Carolina State University has employed numerous undergraduate students to assist ongoing research projects. They work part-time while taking classes during the fall and spring semesters. Some students continue working full-time during the summer. Often students are hired through the Provost's Professional Experience Program (PEP) that provides meaningful research opportunities to students and helps campus research programs leverage funds by paying 75% of the students' wages. Recently, we have had an outstanding group of students assisting our research, so we would like to showcase their contributions.



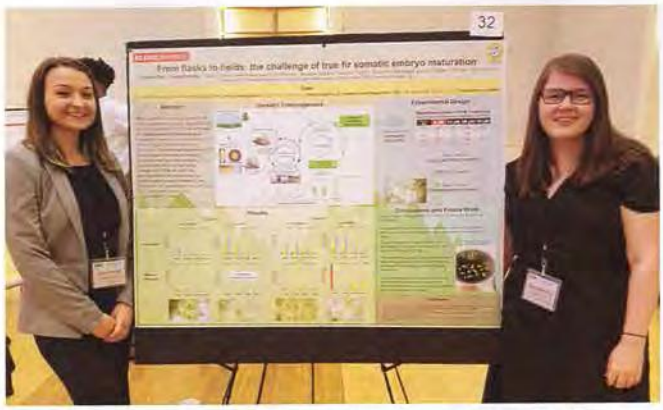
Audrey Bowen measures SCV (settled cell volume) to assess the growth rate of her liquid suspensions of fir somatic embryos.

Somatic Embryogenesis Project

Somatic embryogenesis (SE) is a laboratory cloning method and a necessary biotechnological tool to apply new gene editing techniques. Fraser fir SE research at NCSU began in 2016 supported by funds from the NCDA & CS Specialty Crop Block Grant Program. Starting in 2017, three students have assisted with this project: Audrey Bowen (Environment Technology & Management), Sophie McNatton (Genetics) and Danielle Pavic (Biological Sciences). Each student developed and carried out a research project to help further our SE efforts.

Audrey compared growing embryogenic Trojan fir cultures on gelled medium in petri dishes to suspension cultures in flasks of liquid medium. She found that liquid suspension cultures grew faster and produced more mature embryos than cultures on gel, which could greatly reduce the cost of cloning fir via SE. Audrey presented a poster entitled *Growth and Maturation Rates for Embryogenic Trojan fir Proliferated on Gelled Medium or as Liquid Suspension Cultures* at the NCSU Undergraduate Research Symposium during April 2018.

Sophie wrote a grant proposal called *Effect of Cytokinin-Auxin Interactions on Maturation of Fraser fir Somatic Embryos* and was awarded $500 from the Office of Undergraduate Research (OUR). In addition to this project, Sophie teamed with Danielle to investigate the effect of an auxin antagonist (PCIB) on both the growth and conversion to mature embryos of Fraser and Trojan fir SE cultures. They jointly presented a poster entitled *From Flasks to Fields: The Challenge of true Fir Somatic Embryo Maturation* at the Undergraduate Research Symposium. Theirs was judged to be among the outstanding presentations. Both students graduated spring semester but Dani-



The research poster and presentation of Danielle Pavic (left) and Sophie McNatton (right) was judged outstanding at the NCSU Undergraduate Research Symposium.

elle is continuing to work on this project while Sophie is pursuing graduate education at Duke University.

Chalcid Wasp Infestation Patterns in a Fraser fir Seed Orchard

During the summer of 2016, while dissecting hundreds of immature Fraser fir seeds for the SE project, tiny larvae were found inside some seeds and we observed differences in infestation rates from seeds originating from the different orchard clones. These preliminary results motivated us to identify these parasites and study the infestation and adult emergence patterns among different clones in a seed orchard in Ashe County managed by the N.C. Premium Fraser fir Seed Cooperative. The North Carolina Christmas Tree Association sponsored this research.

Kelly Goode, a senior majoring in Genetics and Plant Biology, helped to develop insect rearing methods and tracked the emergence of adult insects from seed lots that were exposed to different cold and darkness treatments. The adult insects were stored for further taxonomical classification and some specimens were provided to NCSU Insect Museum. The specimens were identified by their morphological characteristics with the help of two experts in North America, Drs. Matthew Bertone and Roger Burks, and Dr. Petr Janšt in the Czech Republic. We were able to identify two chalcid wasps, namely *Megastigmus specularis* and its parasitoid *Mesopolobus*. We observed that the longer we kept the seeds in the dark

Kelly Goode (left) dissects Fraser fir seeds under the stereo microscope while counting and collecting chalcid wasp larvae. Kelly (right) collects stored seeds from the N.C. Premium Fraser Fir Seed Coop in order to assess clonal variation in infested seeds.

and cold, the shorter the time *M. specularis* adults took to emerge. The most adults were detected when seeds were stored for approximately 3 to 4 months. In the case of *Mesopolobus*, there was no correlation between the number of days that seeds were stored and the number of days to emerge but the highest number of adults emerged after 4 to 5 months of storage.

Other interesting results from our study showed that there was a difference in clonal infestation rates among clones and that clonal infestation rankings correlated across two consecutive years. Finally, we were able to confirm that the machine the co-op uses to clean and separate seed (based on weight) is largely effective at separating infested from non-infested seeds.

Kelly presented partial results from this study during two important events: The N.C. Undergraduate Research and Creativity Symposium at Campbell University and the NC State Undergraduate Research Slam. Her talks were both entitled *How the Wasps stole Christmas Trees*.

Characterizing the Needle Abscission Zone

The commercial implications of post-harvest needle abscission on the Christmas tree industry are well known, as some consumers opt for artificial trees instead of real trees due to the "mess" from needle loss. Our group has been one of the pioneers in the study of needle abscission in conifers combining transcriptome (gene expression) and phenotypic (observed characteristics) data. Recently Kelly Goode was awarded $2,000 to develop a summer project entitled *Histological Characterization of Postharvest Needle Abscission in Fraser Fir*. Kelly works under the direct supervision of Dr. Lilian Matallana, and together they have standardized different protocols to microscopically visualize the abscission zone in Fraser fir. Our goal is to integrate previous transcriptome data analysis with the histology (microscopic structure of tissues) of abscission zones in order to identify structural changes associated with variation in gene expression. Kelly was selected to represent NC State during the National Undergraduate Symposium at the University of Central Oklahoma last April. From the ~4,000 national submissions to the event, 17 students represented NC State. Kelly was one of 14 NC State students who received full travel fund grants from the Office of Undergraduate Research. ***Kelly was the only NC State student invited to give an oral presentation at this national event.*** Her talk was entitled *Cell Separation: The Christmas Story You Haven't Heard*.

Eric Bolick inventories families of Fraser fir seedlings in a greenhouse at the NCSU Horticultural Research Laboratory (HFL). The inventory will be used to design genetic field trials to be established in the mountains of Western NC during spring of 2019.

Greenhouse and Field Projects

During the summer of 2018, Eric Bolick, a rising senior in Forest Management, assisted with greenhouse and field activities for the Christmas Tree Genetics Program. A native of Garner, NC, Eric is exploring genetics and tree improvement aspects of forestry with thoughts toward future study. Culture of plant material in greenhouses at the Horticultural Field Lab is an ongoing duty for Eric and includes watering, fertilizing, weeding, and occasional pest management. An initial major task this summer was transplanting grafted Virginia pine selections into larger pots, staking them as necessary, and trimming rootstock. Next spring, these grafts will be used to establish a new Virginia pine seed orchard. Eric traveled to the Mountain Research Station, in Waynesville, to help maintain the Fraser Fir Clone Bank there and braved poison ivy to trim rootstock, verify clonal ids, and install tags with new clone numbers. Currently, he is helping to take an inventory of Fraser fir seedlings that will be used to design genetic field trials scheduled for establishment in the mountains next spring. These seedlings will also need to be individually tagged, their heights assessed, and then arranged into planting design order prior to their move to the mountains. Eric is a fine example of the many student partners that have helped move Christmas tree research at NCSU forward. ▲

Christmas Tree Association
Become a Member!
Discover the advantages and benefits on page 11.

NC STATE UNIVERSITY

From flasks to

[1]Danielle Pavic, [2]Sophia McNatton, [3]Rober

Our goal is to establish efficient **protocols**

Abstract

NC STATE UNIVERSITY

Forestry and Environmental Resources

Growth and Maturation Rates for Embryogenic Trojan fir (*Abies equi-trojani*) Proliferated on Gelled Medium or as Liquid Suspension Cultures

Audrey L. Bowen, Robert Thomas, John Frampton

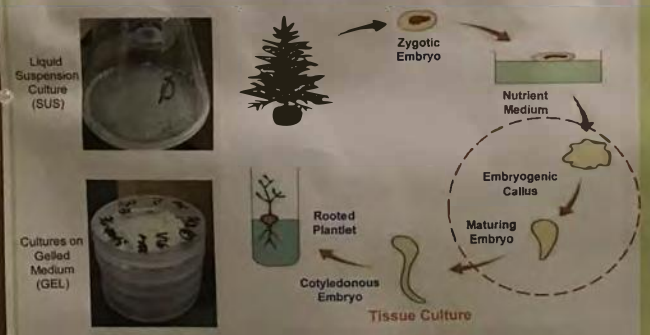
Abstract

This study is the first report of somatic embryogenesis in Trojan fir (*Abies equi-trojani*), a native of northwestern Turkey that has potential as a Christmas tree species in the U.S. Our findings encompass growth rates of immature embryo stock cultures as well as the subsequent production of cotyledonary stage plantlets. Three half-sibling genotypes of Trojan fir were proliferated on a nutrient medium that included 1% sucrose and 4.4uM of cytokinin 6 benzylaminopurin (BAP). The medium was either gelled with agar and poured into Petri dishes or used as a liquid in Erlenmeyer flasks on a gyratory shaker. For all three genotypes, the growth rate was 2 times greater in suspension culture conditions, concurring with scientific reports for model embryogenic conifer species. Next, we compared the two tissue sources for an important downstream process, the conversion of immature embryos into mature, cotyledonary stage plantlets. Embryo maturation was achieved by placing tissue from either source on gelled medium with 4% maltose and 37.8uM abscisic acid (ABA). Our findings will help to determine if rapidly growing suspension cultures can be an effective component of a Trojan fir somatic embryogenesis clonal propagation platform.

Background

- Trojan fir is a narrow conical evergreen native to Turkey and a candidate Christmas tree species in Europe and North America. Our group is currently field testing Trojan firs in Western NC
- This species has disease and drought tolerance allowing it to survive in challenging conditions where the NC native Fraser fir cannot
- Christmas trees are a $100 million annual business in North Carolina
- Somatic embryogenesis is a cloning technique used to produce millions of copies of elite pines & spruces for the timber industry; we'd like to develop a similar program for Christmas trees (Kurt et al, 2016)
- The most advanced & efficient programs utilize liquid suspension cultures to generate immature somatic embryos
- It is important to verify that the fast growing liquid cultures perform as well as the traditional gel grown tissue in downstream processes such as embryo maturation

Somatic Embryogenesis Steps



Research Objectives

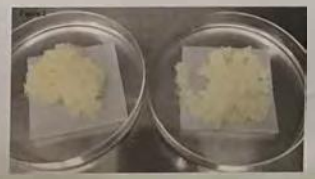
- Compare liquid culture to traditional gelled culture in terms of the proliferation rate for immature somatic embryos
- Verify that immature embryos derived from liquid culture are still functional in the downstream process of embryo maturation

JUSTIFICATION:

- The use of liquid culture systems may greatly reduce the cost of cloning fir via somatic embryogenesis.

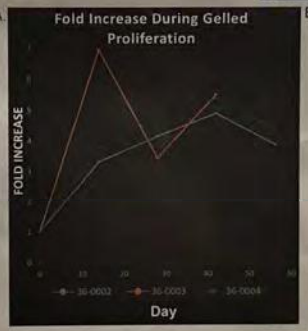
Methods

- Embryogenic cultures of Trojan fir were initiated and grown following the methods of Pullman et. al. (2016). Tissue was proliferated using medium 2226 in either a solidified or a liquid form (GEL & SUS, respectively). GEL cultures consisted of 20 ml medium in 100x15 polystyrene petri plates inoculated with four x 250 mg clumps of embryogenic tissue and sealed with Parafilm™. SUS were grown in 250 ml Nephalo flasks (an Erlenmeyer flask with side arm for estimating settled cell volume [SCV]) filled with 40 ml of medium, inoculated with 1000 mg of tissue, Parafilmed, then swirled at ~ 100 rpm on a gyratory shaker. Both environments were ~ 22C with 24 hours of darkness
- GEL growth rates were monitored by weighing the inoculum on day zero, weighing the callus clump after 2 weeks of growth, and dividing final mg weight by day 0 weight. SUS growth rates were estimated by SCV. SCV is measured by swirling a flask and pouring a homogeneous sample into the sidearm. After 15 minutes tissue fell to the bottom of the arm, leaving a layer of liquid above. The mm height to tissue was divided by the total height of the column, resulting in a % (e.g. 15 mm of tissue in a 100 mm column =15% SCV.)
- For maturation, tissues from GEL or SUS were placed on medium 1891 (Pulman et al. 2016). Good cotyledon stage embryos (Figure 1 a) were tallied at day 42 (SUS) to day 56 (GEL)

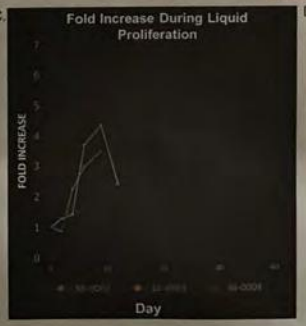


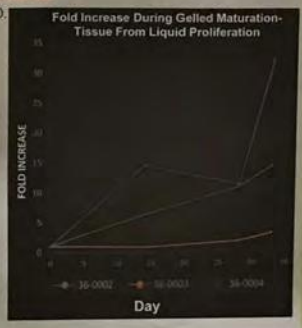
Data & Results

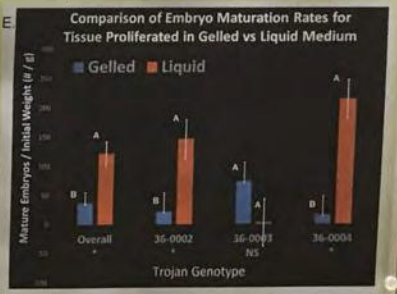
Gelled Data



Liquid Data







- For 2 of 3 genotypes tested, tissue grown in suspension produced more high quality embryos than comparable tissue grown on traditional gelled medium
- Means with different letters are statistically different at $p<0.05$

Conclusion

- Liquid culture results in, conservatively, at least 3x faster proliferation than traditional gelled cultures
- For 2 of 3 genotypes assayed, liquid grown somatic embryos performed as well or better than comparable embryos grown on gelled medium
- The results indicate that after screening for responsive Trojan fir genotypes, liquid culture processes can be used to greatly enhance the productivity of cloning via somatic embryogenesis

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Kurt Y., J. Frampton, F. Isik, C. Lindgren, and G. Chastagner. 2016. Variation in needle and cone characteristics and seed germination ability of Abies bornmuelleriana and Abies equi-trojani populations from Turkey. Turkish Journal of Agriculture and Forestry. 40:169-176.

PROJECT TITLE GAP Assistance to Growers

PROJECT SUMMARY

Food safety issues have caused many companies to demand increased vigilance of their growing and harvesting practices. Major retail stores such as Wal-Mart, Lowes, Food Lion and Harris-Teeter and North Carolina Public Schools all require their produce suppliers to be GAP certified and have at least a $2,000,000.00 insurance policy. Therefore, it is almost impossible for growers to move their produce through these market channels without GAP certification. The cost of certification is relatively high for small acre producers and it is crop specific. The cost for certification could be $600.00 -$1200.00, or more, per crop. This project alleviated a portion of this burden and helped growers gain a strategic foothold and open a new marketplace for them. This project is a continuation of previous GAP assistance programs.

PROJECT APPROACH

Throughout the project NCDA&CS promoted the Gap cost share program throughout the entire state. Use of social media and of press releases and public presentation concerning the Gap cost share were made. Furthermore, applications were downloaded from the website, completed and sent to this office to be processed with accompanying proof of performance. Once all documentation was made the funds were issued. The anticipated goal of 60 growers assisted per year was exceeded by 2 to a total of 122 growers over the 2 year period.

GOALS AND OUTCOMES ACHIEVED

The anticipated goal of 60 growers assisted per year was exceeded by 2 to a total of 122 growers over the 2 year period.

BENEFICIARIES

The beneficiaries were growers of fresh fruits and vegetables a total of 122 growers were assisted over a 2 year period.

LESSONS LEARNED

The only challenge for reimbursing growers for GAP certification is from the fact that only new growers or growers who have been in the program for no more than 1 year can apply. More growers have become GAP certified in the past; now most growers have been certified for more than three years.

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