

# Maine Agricultural Center Integrated Research and Extension Grant Proposals: 2013–2014

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## **MAC132: Assessment of Compost Properties as Predictors of Compost N Supply to Tomatoes Grown in High Tunnels**

**Principal investigator: M. Susan Erich, Mark Hutchinson, Mark Hutton**

### **Background:**

Although there is considerable literature exploring parameters for indicating compost stability and maturity, significantly fewer studies have compared quality differences among finished composts. Composts are produced using different methods and different feedstocks. Carbon content is variable, with some composts containing a significant fraction of mineral material including inorganic nutrients taken up by plants, minerals consumed by animals as part of their diets (for composts containing animal manures), and soil particles incorporated during mixing (for composts produced in mounds or windrows on the ground). Both the organic material and the inorganic fraction may affect compost quality and its rate of decomposition after incorporation in soil.

Soil in high tunnels is quite different from field soil in Maine. Because high tunnels are irrigated systems, the soil in them has less run-off and more salt buildup than soil in open fields. Salts, including nitrates, build up because natural rainfall is unavailable and evaporation is increased due to higher temperatures in high tunnels. Also, the increased temperature and more consistent moisture in the high tunnels results in higher yields, which can create nutrient deficiencies in the soil. The increased temperature and moisture will likely cause higher compost carbon and nitrogen mineralization rates in the high tunnels, which means compost N availability may be higher in high tunnels as compared to field soil.

This study will compare four composts that are commercially produced in Maine. The composts have different feedstocks and are produced by a variety of methods. Composts will be assessed in the laboratory; and they will be applied to soil in caterpillar tunnels to look at the relationship between N mineralization rates under high tunnels and compost chemical and biological properties. Composts with C:N ratios of less than 20 are likely to supply plant-available nitrogen to the soils, whereas composts with C:N ratios of over 30 could immobilize the soil N. Literature suggests that when compost is added to field soil, less than 15% of the total N is mineralized during the first growing season (Amlinger et al, 2003), however studies on N availability from compost application in hoop houses are scarce. This study will look at N recovery by tomato plants and evaluate relationships between compost mineral content, lignin, fiber and both CO<sub>2</sub> and N release in laboratory incubations and N release in the field. Additionally, the nutrient levels in the plants will be looked at during early fruiting to determine whether any nutrient deficiencies occur.

### **Objectives:**

- Objective 1: Assess the properties of the organic and inorganic fractions of a range of finished composts.
- Objective 2: Assess compost properties as predictors of compost quality as estimated by short-term C and N mineralization rates.
- Objective 3: Assess the N availability from each of the composts to tomatoes grown in high tunnels at Highmoor Farm.

### **References:**

- Amlinger, F., B. Götz, P. Dreher, J. Gesztö & C. Weissteiner (2003). Nitrogen in biowaste and yard waste compost: dynamics of mobilisation and availability—a review. *Eur. J. Soil Biol.* 39: 107–116.

### **Description of research and/or extension education activities:**

We will use four composts produced in Maine. They are from Pineland Farms (beef manure, old hay, and wood shavings), Living Acres (dairy, poultry and turkey manures and wood shavings), Rainbow Valley (dairy manure, waste feed and refusals, fish and heifer bedding), and Kinney's (food waste, fish waste, heifer and horse bedding and wood shavings).

Objective 1: Composts will be dried at 60 °C and submitted to the Maine Agricultural and Forestry Experiment Station (MAFES) Analytical Laboratory for standard compost analysis. Neutral detergent fiber (NDF) will be estimated by using an automated Ankom 200 Fiber Analyzer (ANKOM Technology, Fairport, New York), as described by Hutchinson and Griffin (2008). Lignin will be estimated by digesting the NDF residue in 12 M H<sub>2</sub>SO<sub>4</sub>. Moist samples will be used to determine stability, as assessed by Dewar self-heating. Water extracts of the compost (10:1 water: compost, shaken, centrifuged, and filtered) will be analyzed for total C using a Shimadzu TOC-5000.

Objective 2: Jars (250 mL, fitted with septa-containing caps) containing nonamended, field-moist soil equivalent to 125 g of dry soil will be placed in an incubator 25°C for 20 days to pre-equilibrate. On the starting date, dried and ground composts will be mixed into the soil in each of the sample jars so that each sample receives 5g C kg<sup>-1</sup> soil of compost (Lannan et al., 2012). The unamended soil will also be mixed on the starting date. On each sampling date (approximately days 0, 3, 7, 10, 14, 21), three replicates for each treatment will be sampled using a gas-tight syringe. The level of CO<sub>2</sub> produced due to compost mineralization will be determined in each sample by injection into a Li-Cor LI-7000 CO<sub>2</sub> Analyzer. Inorganic N in extracts (1soil to 10 1N KCl) will be determined by an O.I. Apkem A/E analyzer for time 0 and at the end of the incubation.

Objective 3: Composts will be added to the soil of 4 caterpillar tunnels at the University of Maine's Highmoor Farm. Each caterpillar tunnel will be divided into six sections, approximately 5 ft by 15 ft. Four sections will be amended with a compost; one control section will have no compost or fertilizer amendment; and one control section will be amended with N-P-K but no compost. The compost will be applied at rates based on compost N content, so that an equal amount of total N will be added with each compost. The approximate rate of compost addition will be 30 yd<sup>3</sup> acre<sup>-1</sup>. Assuming an N content of 1% and a bulk density of 900 lb yd<sup>-3</sup> for compost, this addition rate is equivalent to 270 lb N acre<sup>-1</sup>. When actual values for N and bulk density are determined for the composts to be used, actual application rates will be adjusted up or down so that each compost is applied at a rate equivalent to 270 lb total N per acre. Supplemental fertilizer (P and K) may be used in equal amounts for each section containing compost; however N will not be supplemented in the sections with compost added or in the control section with zero inputs. Each caterpillar tunnel will serve as a replication. The 4 tunnels with 6 sections each will create 24 experimental units (treatments X replicates) in total.

Twelve samples of soil (3 from each caterpillar tunnel) will be collected before the compost is added. The samples will be submitted to the MAFES Analytical Laboratory for high tunnel soil analyses. At weeks 2, 4, 6 and 8 (after compost amendment) soil samples will be collected (one from each test area) and analyzed for nitrate and ammonium(1:10 soil:1N KCl extracts) and field moisture level. Once the growing season has ended, 24 samples of soil (one sample per test area) will be submitted for high tunnel soil analyses to determine post-season soil properties.

Tomatoes will be grown in the caterpillar tunnels, spaced 18 to 24 inches apart from each other, with 6 plants in each 15 foot long section. The middle 4 plants will be sampled. An indeterminant variety of tomatoes will be used. Integrated pest management methods will be used for disease and insect control. Plants will be compared for quality. The total weight of the fruit and number of disease occurrences will be documented. When the fruit is about golf ball size, 10-12 mature, fully-expanded leaves will be taken from below the last open flower cluster and submitted to the MAFES Analytical Laboratory for a total nutrient analysis. At the end of the season, a whole plant analysis will be completed for one tomato plant per test area to determine total plant N uptake.

## References:

- Hutchinson, M. & Griffin, T.S. (2008). Evaluation of Fiber Content Relative to Other Measures of Compost Stability. *Compost Sci. Util* 16:6-11.
- Lannan AP, Erich MS, Ohno T. (2012) Compost feedstock and maturity level affect soil response to amendment. *BiolFertil Soils* In Press.

## Expected outcomes and method for sharing outcomes:

We expect to find relationships between the compost chemical and biological properties determined in the laboratory and the N mineralization of the composts in the field, as well as tomato plant uptake of N. These predictive

relationships will be useful in developing recommendations for the use of compost to supply N to crops that are produced in high tunnels. Currently compost is often used in high tunnels by growers to build soil quality and supply plant nutrients, but there is little research –based information available to extension faculty to allow them to recommend optimal rates of compost for growers. Too little compost may not supply enough nitrogen for crops and too much may result in accumulation of nitrates and other nutrients in high tunnels.

This research project was developed by Ms. Kate Marshall, who is an M.S. degree candidate in the Department of Plant, Soil, and Environmental Sciences. Ms. Marshall is advised by Sue Erich, and both Mark Hutchinson and Mark Hutton are on her graduate committee. Ms. Marshall will be reporting results of this research at Highmoor Farm Field Days, Maine Vegetable and Fruit School, and American Society of Horticulture meetings.

## **Final Report**

### **Original project objectives that were met and significant findings:**

- Objective 1: Assess the properties of the organic and inorganic fractions of a range of finished composts

Four composts were collected from four different producers in the spring of 2013, and termed Beef, Food, Dairy, and Poultry, based on their major components. They ranged from 1.26 to 2.39% total nitrogen. Beef manure based compost had about 13%, and poultry manure based compost had about 28%, of its total nitrogen in the inorganic form, while the other two composts had <5% total nitrogen in the inorganic form.

- Objective 3: Assess the N availability from each of the composts to tomatoes grown in high tunnels at Highmoor Farm.

Total tomato yield for the Beef and Poultry treatments was not different than the inorganic fertilizer, while yield was lower for Food, Dairy, and unamended soil treatments.

### **Original project objectives that were not met:**

- Objective 2: Assess compost properties as predictors of compost quality as estimated by short-term C and N mineralization rates.

This objective is currently underway in a lab study. We emphasized completion of the field study and did not have time to begin the lab study last winter as planned.

### **Methods used to evaluate outcomes:**

We need to finish the 2014 field season before we are really in a position to assess the work and evaluate outcomes.

### **Integration of research and extension activities:**

The data generated from this project have been used in Extension programming (2014 Maine Vegetable School and 2013 Highmoor Farm Field Day). Additional presentations of the information are planned, e.g. 2015 Maine Ag Trade Show.

### **Educational material, publications, and programs:**

- 2014 Maine Vegetable School presentation: Compost for High Tunnel Tomatoes, *Kate Marshall*, March 10 (Portland) and March 11 (Bangor). Approximately 200 Maine, mixed vegetable and small fruit growers learned about the use of compost in high tunnels and that composts produced with different feed stocks have different properties.
- Research description in the field at Highmoor Farm Field Day and Summer Tour: *Kate Marshall*, Wednesday, July 31, 2013. Approximately 25 Maine vegetable growers were able to see first hand tomato plant health and growth of plants grown in plots amended with one of four composts.

**Research publications, abstracts, and presentations:**

- Planned poster presentation: *Kate Marshall, Susan Erich\*, Mark Hutton, Mark L. Hutchinson and Ellen Mallory*, Nitrogen Availability from Compost in High Tunnel Tomato Production, ASA, CSA, SSSA Annual meeting, Nov.2-5, 2014, Long Beach, CA.
- Poster presentation: *Kate Marshall, Susan Erich, Mark Hutton\*, Mark Hutchinson, and Ellen Mallory*, Compost for High Tunnel Tomato Production, American Society for Horticultural Science Annual meeting, July 2014, Rosen Plaza, Orlando FL, <https://ashs.confex.com/ashs/2014/webprogram/Paper19760.html>  
\*presenter

## **MAC133: Optimizing Irrigation Practices and Compost Application Rates within High Tunnels for Tomato Production**

**Principal investigator(s): Mark Hutton, Mark Hutchinson, David Handley**

### **Background:**

More than 60% of Maine mixed vegetable farms have at least one hoop house. Primarily, the structures are used for the production of early summer season tomatoes and/or fall salad greens. A recently published survey (Fitzgerald and Hutton, 2012) of new and experienced high tunnel growers in Maine identified irrigation technology and effective compost utilization within tunnels as growers' highest priority for more information. The design and installation of drip irrigation systems within hoop houses is relatively simple and straightforward. A review of current literature shows that drip irrigation in hoop houses is an expected practice. Yet, little research or educational material is available to help growers to maximize water use efficiency. The complicated aspect of irrigating inside a hoop house is determining when to irrigate, how long each irrigation event should last, and how much water to apply. Our survey supports the conclusions of Fereres et al. (2003); irrigation scheduling is usually based on grower experience and that most growers over-irrigate to ensure low soil moisture does not limit production.

The typical 14'x 96' hoop house tomato grower irrigates approximately three times a week with each irrigation event lasting between one and two hours. Assuming the grower is using high flow rate drip tape, between 180 to 360 gallons of water are applied with each irrigation event and up to 1080 gallons of water are applied inside the house per week regardless of soil condition or plant need. Soil type, soil organic matter, and soil structure control the available water holding capacity (AWC) of a particular soil. The maximum optimal duration of an irrigation event is well defined based on the AWC of a given soil. Irrigation events longer than the AWC maximum leach nutrients outside the effective root zone wasting nutrients, water and energy. Fluctuating soil moisture levels (dry to very wet) often result when irrigation is done based on a weekly schedule. Variable soil moisture negatively impacts tomato yield and quality by decreasing fruit set and increasing the amount of fruit culled because of cracking and blossom end rot. Soil moisture should be monitored on a daily basis either by feel (Maine Irrigation Guide, 2005), measurements made using *tensiometric* methods (tensiometers, resistance blocks or granular matrix sensors) (Muñoz-Carpena and Dukes, 2008) or *volumetric* method which estimate soil volumetric moisture by measuring the soil dielectric constant. (Mead et al., 1995).

Irrigation scheduling has been based on perceived water replacement using models based on evaporative transpiration losses combined with crop transpiration losses. More recent models are based on soil water tension measured by tensiometers and set points designed to maintain soil moisture in a defined range. The drawback with tensiometers is the lag time the tensiometer requires to respond to soil moisture changes. New technology based on volumetric soil moisture probes allow for real-time measurements which, when combined with quick response data interpretation capabilities create the possibility for precise effective irrigation scheduling.

Compost application has become a cornerstone of soil health and soil improvement practices on many farms in the Northeast, particularly in high tunnels. However, the lack of researched-based information on compost application rates in both high tunnels and open fields has led to excessive applications of compost and resultant accumulations of phosphorus and salts in the soil. There are no published studies examining the impact of compost application or application rates within high tunnels. Yet, there are published recommendation rates in excess of 5 cuft/100 sq.ft (78yds/acre) ([http://hoophouse.msu.edu/index.php?id=145&searched=compost+appplication&advsearch=oneword&highlight=ajaxSearch\\_highlight+ajaxSearch\\_highlight1+ajaxSearch\\_highlight2](http://hoophouse.msu.edu/index.php?id=145&searched=compost+appplication&advsearch=oneword&highlight=ajaxSearch_highlight+ajaxSearch_highlight1+ajaxSearch_highlight2)). Most commercial farms in Maine rarely exceed 12 yards/acre in open field situations. High compost rates will have broad ranging effects on soil health and quality and may possibly have significant impact on irrigation needs. Likewise, insufficient irrigation may lead to salt accumulation in the root zone of compost-amended soils.

The objectives of this project are:

1. Measure and document “typical” water use by Maine hoop house tomato growers, and a frequent, shorter duration irrigation protocol, calculate water use efficiency and compare the two irrigation regimes.
2. Determine if additions of organic matter increase water use efficiency
3. Develop baseline information in support of an NRCS grant application bringing state of the art irrigation technology to Maine vegetable growers
4. Demonstrate practical application of irrigation scheduling options via field days, presentations to growers, and web content.

### **Description of research and/or extension education activities:**

The research will be conducted in the 26x96” high tunnels located at Highmoor Farm. The experiment will be conducted in a split plot design using irrigation treatments as the main plot and compost rates as the sub-plot. There will be four replications of each irrigation practice. Five compost application rates 0, 10, 20, 55, 90 cu yd/acre will be randomly assigned within each irrigation main plot.

Two irrigation treatments will be employed. One designed to mimic “typical” grower practices: 2-3 irrigation events per week lasting between 1 and 2 hours. The second treatment will provide the equivalent of 1 acre inch of water to the crop split into 2 irrigation events per day. Tensiometers and volumetric soil moisture probes located in the 0, 10 and 90 yd compost plots will be used to monitor soil moisture through the course of the experiment. Water meters on the irrigation header pipes for each treatment will measure water consumption.

Highmoor farm compost will be used for the compost treatments. Prior to application the compost will be analyzed by the Maine Soils Lab. Fertility in the sub-plots will be adjusted based on soil tests to levels recommended for hoophouse tomatoes. The ratio of compost to fertilizer is expected to vary according to compost application rate. All treatment plots will receive the same fertigation applications of calcium nitrate based on standard hoop house tomato recommendations.

Weather data inside the hoop house will be collected through the course of the experiment using Hobo data loggers, including: soil temperature, air temperature at 1 and 2 meters, relative humidity, PAR. A 20 cm evaporative pan will be used to measure evaporation within the hoop house (Yuan et al. 2001).

‘Big Beef’ tomato transplants will be grown in the greenhouse and then transplanted into the hoop house during the first week of June. The plants will be planted at 15” within the row and six plants contained in each sub-plot. The plants will be trained and pruned to a single stem. Yield (number and weight of fruit graded as first, second and cull) and fruit quality (brix averaged by plot and harvest) will be measured from the center four plants of each plot.

### **References:**

- Fereres, E., D.A. Goldhammer and L.R. Parsons. 2003. Irrigation water management of horticultural crops. *HortScience* 38:1036-1042.
- Fitzgerald, C.B. and M.G. Hutton. 2012. Production practices and challenges with high tunnel systems in Maine. <http://www.nacaa.com/journal/index.php?jid=170>.
- Mead, R.M., J.E. Ayers and J. Liu. 1995. Evaluation of Sentek EnviroScan RT capacitance probe: Laboratory calibration and field analysis. A report submitted by the Water Management Research Laboratory ARS\_USDA, Fresno, CA.
- Muñoz-Carpena, R. and M.D. Dukes. 2005. Automatic irrigation based on soil moisture for vegetable crops. Fact Sheet AE 354 of the Dept. of Agr. And Bio. Engineering, Univ. of Florida. <http://edis.ifas.ufl.edu/>.
- Yuan, B.Z., Y. Kang, and S. Nishiyama. 2001. Drip irrigation scheduling for tomatoes in unheated greenhouses. *Irrig. Sci.* 20:149-154.

### **Expected outcomes and method for sharing outcomes:**

We expect to find differences in water use efficiency between ‘typical’ grower irrigation practices and frequent reduced volume irrigation. These differences may significantly impact tomato fruit yield, quality and costs of

production. This project forms the foundation for research optimizing hoop house irrigation practices and development of sensor based irrigation strategies as part of a NCRS CIG proposal

A range of compost application rates will help identify potentially excessive application amounts based on the economics of crop performance. The experiment will also investigate potential interactions with irrigation practices and compost application rates. .

The project will demonstrate to growers the importance of appropriate irrigation practices and appropriate compost application rates. Growers will be able to view the experiment at the Highmoor Summer Tour and by impromptu grower visits, presentations at grower meetings and web based reports.

## **Final Report**

### **Original project objectives that were met and significant findings:**

The objectives of this project are:

1. Measure and document “typical” water use by Maine hoop house tomato growers, and a frequent, shorter duration irrigation protocol, calculate water use efficiency and compare the two irrigation regimes.

Total water used was not different due to irrigation management strategy. Frequent and heavy rains leading to water infiltration into the house obscured differences due to irrigation management.

2. Determine if additions of organic matter increase water use efficiency

Five compost application rates 0, 10, 20, 55, 90 cu yd/acre produced slight increases OM however this did not result in and measurable difference in soil water holding capacity. Significant differences were observed due to compost application amounts. The 10 cu yd/ acre rate was significantly less than the fertilizer control or other compost application rates.

3. Demonstrate practical application of irrigation scheduling options via field days, presentations to growers, and web content

### **Original project objectives that were not met**

All of the original objectives were met.

### **Methods used to evaluate outcomes:**

These results indicate that there may be an upper limit to the economic and beneficial application of compost. It is not uncommon to see compost application rates within high tunnels to exceed 20 cu yd/acre. Our results indicate there is no yield benefit and that there is a rapid and dramatic increase in soluble salts, cation exchange capacity and phosphorous levels.

### **Integration of research and extension activities:**

The results of this project were used to develop educational programming on high tunnel vegetable production and presented to Maine small fruit and vegetable growers at the Highmoor Summer Tour (35 growers), Annual Meeting of the Maine Small Fruit and Vegetable Growers Association (125 attendees), High Tunnel Production School (35 attendees), and The Vegetable and Fruit School (200 attendees).

I have been asked to present the results of the 2013 and 2014 results of this research at the Mid-Atlantic Vegetable Growers Conference in Hershey, PA January 29, 2015

### **List of external funding both sought and received:**

Data from this experiment was used to support research proposals send to USDA AG Marketing and NorthEast SARE. We were asked to submit a full Research and Education proposal to NESARE however we did not receive funding.



### **Educational material, publications, and programs:**

There have been requests from Extension educators in five states for additional information regarding the results of this research project.

### **Research publications, abstracts, and presentations:**

- Poster presentation: *Mark Hutton\**, *Mark Hutchinson*, and *David Handley*, **Effect of Compost Application Rates on High Tunnel Tomato Yield**, American Society for Horticultural Science Annual meeting, July 2014, Rosen Plaza, Orlando FL, <https://ashs.confex.com/ashs/2014/webprogram/Paper19771.html>

## **MAC134: Improving Apple Rootstock Propagation Efficiency**

**Principal investigator:** Fang Geng, Renae Moran, Michael Day

### **Background:**

#### **Research Objectives:**

1. determine if apple shoots propagated by tissue culture can be invigorated by micrografting to seedling rootstock
2. compare the effectiveness of micrografting and chilling treatments on stimulating growth of microshoots by measuring anatomical differences in the development of shoot growth.

Woody plants that are difficult to propagate tremendously increase the nursery production cost. Apple is a great example. New cultivars are often in high demand, but only limited numbers are available to the industry. Conventional production of large numbers of stock plants take years to satisfy demand. Propagation of stock plants by tissue culture is a way to rapidly increase the number of available plants in a short period. However, in apple rootstock the slow growth of tissue culture microshoots greatly limits commercial production of highly desirable cultivars, such as Geneva cultivars (G.41, G.30, and G.935, and etc.), which are desired by their high disease-resistance, especially resistant to fire-blight, very good cold hardiness, and precocity.

Tissue culture is a method to rapidly develop new plants in an artificial medium under aseptic conditions from a small number of stock plants. However, high value dwarfing apple rootstocks exhibit stunting, or failure of the microshoots to elongate and proliferate, which slows down the propagation cycle. Reasons for this are unknown, but dormancy may be involved. Dormancy is a condition that prevents growth and protects plants from seasonal environments stress. In the case of apple, a one to two month period of cold temperatures is needed to overcome dormancy. In our preliminary research cold treatments have effectively stimulated apple microshoots to rapidly proliferate and elongate. Another possible reason is the growth stage of the tissue from which microshoots are propagated. In many woody plant species, tissue cultures derived from juvenile (pre-reproductive) donor plants are associated with ease of propagation. Apple cultivars, particularly dwarfing rootstocks, have undergone phase change from juvenile to reproductively mature states, but it may be possible to rejuvenate them by grafting to seedlings. In other species, plantlets derived from tissue culture have been successfully grafted onto juvenile rootstock, but this technique has not been tested as a way to improve apple propagation.

Propagation of plantlets by tissue culture can be inhibited by the requirement of shoots to experience a critical cold period to break an inherent state of dormancy. As the shoot grows new cells are produced by a small region of specialized stem cells, the shoot apical meristem, located at its tip. Shoot elongation is caused by the continuing division and elongation of daughter cells subtending the shoot apical meristem. During dormancy, cell division and elongation in the shoot tip are repressed, and plantlets derived from tissue cultures often need a dormancy-breaking cold period to stimulate shoot growth. This phase of our study will compare the relative efficiency of micrografting and cold-treatments in stimulating the growth of plantlets. An anatomical study will test the hypothesis that stunted microshoots are the results of reduced or absent activity in the elongation zone of shoot growth, and this condition is alleviated by either cold treatments or micrografting onto seedling rootstock.

### **Description of research and/or extension education activities::**

Single nodal stems of G.30 will be cultured on solidified MS media without any hormone. One set of cultures will be directly cultured at  $23 \pm 2^{\circ}\text{C}$  (unchilled) and another set will be exposed to  $4^{\circ}\text{C}$  for 6 weeks (chilling), and then will be transferred to culture room. The sprouts (chilled and unchilled) will be transferred to new solidified MS media with GA<sub>3</sub> (0.5mg/L), BA (1.0mg/L), and IBA(0.1mg/L) for proliferation. In this phase, eight microshoot tips (replicates) per set will be randomly collected after the first 7, 14 and 28 days of culturing. Chilled apple seeds will be germinated *in vitro* following seed coat removal and surface sterilization. Seeds will be cultured for about three weeks after which they will be used as rootstocks. Terminal and axillary buds of cultured G.30 apple shoots will be micrografted onto

seedling rootstock. After grafting, the survival rate and shoot length will be measured. Ungrafted microshoots will serve as the control for comparison with grafted shoots.

Chilled and unchilled shoot tips will be excised and immediately fixed in formaldehyde-acetic acid-alcohol and stored at 4 °C until examined. Fixed micro-shoot tips will be processed for viewing by light microscopy (LM), transmission electron microscopy (TEM; Philips EM-201), and scanning electron microscope (SEM; AMR 1820). For LM and TEM, microshoots will be dehydrated using acetone (50%, 70%, 95%, and 100%), followed by infiltration and molded using epoxy resin. Thick and thin sections will be cut using a Sorvall Porter-Blum MT2-B ultra-microtome. Thin sections on the cleaned copper grids will be stained with uranyl acetate and lead citrate. Thick sections on clean slides will be stained with 1% toluidine blue. Prepared thin and thick sections will be imaged respectively by TEM and LM. Tissue preparation for SEM is the same as for TEM except using ethanol (30%, 50%, 70%, 80%, 85%, 90%, and 100%) for dehydration. Then tissues will be cryofractured under liquid nitrogen and dried using Tousimis Samdri PVT-3 Critical-Point Dryer. The dried tissues will be mounted on aluminum stubs and sputter-coated with gold and palladium. The prepared specimen will be examined in a SEM. If micrografting proves successful, shoot tips of grafted and ungrafted plants will also be examined using SEM and TEM.

The anatomical characteristics of the shoot tips (elongation zone size, vascular tissue size, cell lignification, cell thickness, cell number, and cell size) in chilled and unchilled shoots (elongated and stunted microshoot tips) will be compared. All the images will be analyzed using UTHJSCSA, Image Tool for windows (version 3.0; University of Texas Health Science Center, San Antonio, TX, USA).

### **Expected outcomes and method for sharing outcomes:**

Results will be used to develop an improved method for difficult-to-propagate woody ornamental plants and fruit trees. New highly desired cultivars can be more rapidly mass propagated making them commercially available to apple growers and nurseries in Maine.

Results will be shared with 1) growers through an oral presentation at the Maine Ag. Trades Show; 2) propagators and tissue culture commercial labs; 3) other specialists and researchers at scientific meetings (NC-140 meeting and American Society for Horticultural Science Annual Conference), and through publishing articles in HortScience and Journal of Plant Biotechnology: Plant Cell, Tissue and Organ.

## **Final Report**

### **Original project objectives that were met and significant findings:**

1. To measure anatomical differences in the development of shoot growth to determine the cause of poor shoot growth during micropropagation.

The anatomy of normal and stunted *in vitro* (under sterile conditions) apple shoots were compared to identify traits associated with dormancy and stunting of shoot growth in the apical meristem. Stunted shoot apices had significantly larger cells and fewer cells than normal shoots, but the cell shape was similar. Cell walls in stunted shoots were thickened, and the plasmodesmata were constricted, in contrast to normal shoot cell walls that were thinner with unconstructed plasmodesmata. Primordial cells in stunted shoots appeared to have precipitates, unlike normal cells. Both traits are associated with the dormant state indicating that shoot growth may be limited by endodormancy.

### **Original project objectives that were not met:**

2. To determine if apple shoots propagated by tissue culture can be invigorated by micrografting to seedling rootstock

Due to the small size of the plants, a good graft union could not easily be achieved. Seeds were germinated *in vitro*. During the grafting procedure, they became dehydrated and subsequently died. The few grafts that survived failed to heal and also died.

**Methods used to evaluate outcomes:**

The anatomical characteristics of the shoot tips (elongation zone size, vascular tissue size, cell lignification, cell thickness, cell number, and cell size) in elongated and stunted microshoot were compared by light microscopy and transmission electron microscopy. Outcomes of this research will be shared with the intended audience by publishing in a peer-reviewed journal and by presenting results at a conference.

**Integration of research and extension activities:**

Preliminary results were shared with members of the NC140 (tree fruit researchers) at the annual meeting in Boise, Idaho, Nov. 2013 with members who collaborate with rootstock nurseries in the US and internationally.

**Educational material, publications, and programs:**

- One undergraduate student, Nicholas Rowley, Sustainable Agriculture student, participated in this research as part of his PSE senior capstone project. He produced a poster in class summarizing his results on micrografting.

**Research publications, abstracts, and presentations:**

- Geng, F., R. Moran and D. Zhang. 2014. In vitro apple shoot proliferation and elongation using growth regulators. American Society for Horticultural Science Annual Conference. Orlando, Florida, July 2014. <http://ashs.confex.com/ashs/2014/webprogram/Paper19844.html>
- Geng, F. 2014. Enhancing micropropagation efficiency of apple rootstocks by identifying limitations of microshoot elongation. Doctoral Dissertation. University of Maine.

## **MAC135: Exploring the Market Potential of Aronia Berries**

**Principal investigator(s):** Angela D. Myracle, Lois Stack, David Handley

### **Background:**

#### **Aronia background**

*Aronia melanocarpa*, commonly known as aronia berry or black chokeberry, is a native Maine shrub that offers untapped potential as a small fruit crop with health benefits as a food or nutraceutical. Aronia berries are a rich source of phytonutrients including anthocyanins, proanthocyanidins, flavonols and phenolic acids, in amounts that exceed those of blackberries, blueberries and cranberries [1-3]. The antioxidant content of aronia is among the highest of any fruit [4]. In addition, it is thought to possess anti-cancer compounds; a potential area of lucrative research [5]. Aronia fruit are high in sugar (12-20%); however, they have a high acidity and are much more palatable when utilized in value added products such as jams, jellies, wine and baked goods [4, 6]. Aronia offers many benefits as a functional food, and during a time when these markets are developing, it offers much potential to enhance Maine's economy [4], but are very astringent, and much more palatable when utilized in a value-added products such as jams, jellies, juice, wine and baked goods [4, 6]. Aronia has many potential benefits to offer as a functional food at a time when this market is expanding both locally and nationally. The development of aronia as a commercial crop in Maine offers much potential to enhance the state's economy [4]. . As a nutraceutical, aronia has many potential health benefits. The antioxidant content of aronia is higher than that of many fruits such as cranberry and blueberry [4]. In addition, it is thought to possess anti-cancer compounds that are an unexplored research aspect [5].

Goal: Establish plantings of Aronia in the University of Maine's Lyle E. Littlefield Ornamentals Trial Garden in Orono and Highmoor Farm in Monmouth to evaluate growth and production characteristics and determine which cultivars grow best in Maine, leading to recommendations for small berry growers that will best match the characteristics of Aronia cultivars to specific end uses (ornamental, juice and other food products, nutraceutical, dye source, etc.).

#### **Aronia uses**

Aronia berries are used to make many value-added products such as jams, jellies, juice, pies and wine. In addition, they can be used as a natural dye. Aronia has good attributes as landscape shrubs with ornamental and wildlife value. As an ornamental shrub with , it is easy to grow, can be utilized as a hedgerow shrub due to its dense habit, and has stunning red fall color and a dense growth habit to provide native wildlife habitat or make a good hedgerow. In addition to providing berries that can be used for human consumption and wildlife food [4]. The plants are pest resistant and can be grown organically, thus reducing the need for pesticides.

Aronia is self pollinating, although many native; eliminating the need to provide pollinators for a fruit crop, but the wildlife value of this plant in landscapes is high. Many pollinators, including Halictid bees and Syrphid flies, are known to visit the flowers. The dark, glossy attractive berries can be used for human consumption and wildlife food. Several species of birds eat the fruits in fall and winter [4]. The plants appear to have few, if any, pest problems and offer good potential for organic production reducing the need for pesticides and increasing its consumer appeal.

#### **Economic impact**

Aronia berries can be utilized to make a wide range of value-added products including jam, jellies, juice and wine. There is also interest in the fruit to make supplements for the nutraceutical industry. A mature plant can yield 15 -30 pounds of fruit [7]. One pound of fresh frozen berries presently retails for \$10.00 from internet suppliers. Other aronia products being marketed on internet sites include: dried fruit \$8.00/ 8oz, jelly \$6.00/ 8oz, juice concentrate \$30/ 16oz, wine \$14.00-21.00/ bottle. Berries can also be utilized to produce functional food, an area that and this market potential is relatively unexplored. Funds from this proposal would be utilized to make several test products with aronia to evaluate the bioactive stability of the berries in various foods. Products such as yogurt, cheese, breads, cakes, cookies and pies can be tested in the pilot plant to help develop recipes for utilizing the berries. This will provide pilot data for the submission of a grant to the Maine Technological Institute to develop food products based on aronia with the goal of enhancing regional, national and international demand for this locally grown fruit.

**Description of research and/or extension education activities:**

The test garden in the Littlefield Garden provides a location on the University of Maine, Orono campus where shrubs can be planted for observation and evaluation. It is also an excellent environment for students to learn more about the ornamental and cropping traits of this plant. The Highmoor farm facility in Monmouth is presently used for small fruit demonstration and research plantings and is accessible to many berry growers in southern Maine. Dr. Myracle's lab is equipped with instruments to evaluate the antioxidant capacity of Aronia. She has access to high pressure liquid chromatography (HPLC) equipment to analyze the phytochemical composition of the fruit. These data will contribute to publications that provide potential growers and processors information needed to successfully utilize and market this crop. In addition, Dr. Myracle has access to the Mathew Highland pilot plant in Hitchner Hall. This facility can be utilized to evaluate preservation methods for aronia berries to optimize stability of bioactive compounds, as well as small scale product development for food items containing aronia. In addition Highmoor Farm offers additional area to establish aronia plants for growth and gathering data to assemble fact sheets for extension activities and for filming video for education purposes. David Handley is the small fruit specialist and will help lead educational efforts in aronia berry production for this project. Lois Stack specializes in ornamental landscape and will offer expertise of how to utilize this bushshrub for home gardens, and landscape purposes with the added benefit of having local edible fruit.

Data to be collected from the planting include:

- Plant traits: growth rate, plant habit, floral display effectiveness and timing, pest pressure, hardiness, fruit set, fruit size, yield, fruit harvest dates;
- Ecological: Specific pollinators supported by flowers, bird species supported by fruits;
- Fruit: Bioactive composition, soluble solids, titratable acidity, dry matter, firmness.

**Expected outcomes and method for sharing outcomes:**

- Video: The Littlefield Test Garden and Highmoor Farm will provide an excellent environment to collect still photo and video on the planting and growth of aronia. The process of propagating and establishing aronia can be documented and made available on the University of Maine Cooperative Extension website, [www.umaine.edu/agriculture/home/aronia](http://www.umaine.edu/agriculture/home/aronia). In subsequent years, the aronia plants can be monitored for full maturity, flowering and berry production. In addition, Dr. Stack will produce a video on the importance of aronia as a plant for ornamental and wildlife landscapes.
- Fact sheets: A short series of fact sheets will educate end users about various aspects of aronia:
  - Aronia establishment and culture
  - Aronia health benefits and recipes
  - Aronia value added products and market potential

The development of these fact sheets will be an ongoing process beyond the time frame for this proposal, but they will provide opportunities for students to be involved with the project and will help develop a strong inter-disciplinary collaboration between Dr. Myracle's lab and the extension programs both in Orono and at Highmoor Farm. This will open future doors for submitting proposals that will further aronia research in Maine.

Establishing aronia in the Littlefield test garden will allow for many additional research projects to be developed beyond the scope of this proposal. The establishment of the plants and the collection and evaluation of fruit will generate extension and research publications, including peer-reviewed publications in the future. Possible topics include: phytochemical composition of aronia grown in Maine and bioavailability studies of aronia in rodent models and the impact on chronic disease and inflammation.

## **MAC137: Winter Moth Development on Blueberry and Apple**

**Principal investigator:** Eleanor Groden, Kaitlyn O'Donnell

### **Background:**

Outbreak populations of the winter moth, *Operophtera brumata*, which is an invasive pest in North America, were found for the first time in Maine in 2012 causing severe defoliation on a variety of deciduous trees and shrubs in Harpswell and Vinalhaven. This insect, which is native to Europe is known to feed on over 12 different genera of host plants (Simmons *et al.* 2012) and has been particularly pestiferous on commercial apple and highbush blueberry (*Vaccinium corymbosum*) in both introduced areas in North America and in its native range in the United Kingdom. The larvae cause severe defoliation in the spring and damage flower buds, which negatively affects fruit production (Holliday 1977; Horgan *et al.* 1999). After developing and feeding on leaf and flower buds for about six weeks, the larvae drop to the ground and pupate in the soil. They emerge as adults in the late fall and after mating, the females lay their eggs on the bark of the host trees. The timing of hatch is very important to winter moth survival. Larvae hatch in early spring at the time of their host plant bud burst. If hatch is not synchronized with the host plant's phenology, the larvae will disperse from the tree by spinning a silk thread and ballooning off of the branch in search of a new host plant. Larval survival is low when hatch is not synchronized and dispersal is necessary (Holliday 1977). It is not clear how winter moth egg hatch in Maine will be synchronized with bud development in its important agricultural hosts, blueberry and apple.

The winter moth was introduced into North America in Nova Scotia in the 1930's, where it has been successfully controlled by an introduced parasitic fly. In the 1970's it was then introduced into western areas of British Columbia, Washington and Oregon state. More recently, the winter moth has become a serious pest in southern New England. Severe defoliation in Massachusetts in the 1990's was originally blamed on other native larval defoliators such as the bruce spanworm. However, it is now thought that this defoliation was caused by the winter moth, whose presence in Massachusetts was confirmed in 2000 and has since been confirmed in Rhode Island, Connecticut, Long Island and southern Maine (Elkinton *et al.* 2010). After the defoliation in Harpswell and Vinalhaven, monitoring programs this past fall have now confirmed the presence of this insect in 33 towns from Kittery to Rockland and areas of Mount Desert Island (Donahue pers. comm. 2013). It is thought that it has the potential to spread further throughout the Maine coast. Because the outbreak in Maine is so recent, there is no information about the phenology of the winter moth in relation to its host plants, particularly the agriculturally important crops that it is known to feed on. There is also no information available about the potential for winter moth to become a serious pest of lowbush blueberry (*Vaccinium angustifolium*). It does feed on other closely related plants in the genus *Vaccinium*, including the European blueberry *Vaccinium myrtillus* (Nestby *et al.* 2011), high bush blueberry (*V. corymbosum*) and species of cranberry. Winter moth has been reported and studied as a serious pest of highbush blueberry in British Columbia, Washington, Oregon, and Nova Scotia and has more recently been described as a pest in highbush blueberry in Massachusetts and in cranberry bogs in southeastern Massachusetts (Sylvia 2010). Because this pest has such a broad range of hosts and is present in Maine where lowbush blueberry cultivation is an important industry, it is necessary that we study the winter moth as a potential pest of lowbush blueberries as well as highbush.

This project will investigate the potential for winter moth to become a serious pest of apples and blueberries in Maine. The timing of winter moth egg hatch and larval feeding relative to host plant development of both highbush and lowbush blueberries and apples will be monitored within the current infestation area in Harpswell, Maine. Evaluation of larval feeding, development, and survival on the different host plants will be determined.

### **Description of research and/or extension education activities:**

We have identified sites in the infestation area in Harpswell, ME, where winter moths were trapped and abundant egg masses have been laid on host plants with apple and low bush blueberries in the landscape. Host plants with egg masses will be located, tagged and monitored twice per week to determine the timing of hatch of the larvae. Surveys of flower and leaf development of apples and blueberries in the area will be conducted to assess the average and range of plant development when the new larvae are looking for food. Sleeve cages will be used to then track the



feeding, development and survival of the larvae from hatch to pupation on the different host plants. Hatched larvae on tagged plants will be transferred to sleeve cages set up on highbush and lowbush blueberry and apple plants. Because synchrony of budburst with larval hatch is important to larval survival we will be testing newly hatched larvae with different stages of leaf out in the apple and blueberry plants. Therefore, we will use both planted and naturally occurring plants plus potted blueberry clones and apple varieties that we bring to the study site to capture the variation in host plant phenology that growers would experience in their fields and orchards. Larval feeding damage, growth and survival will be monitored 3 times per week until pupation.

In addition to field studies, we will be conducting feeding experiments in the lab with reared larvae. Larvae will be placed in a petri dish with a newly leafed out host plant stem (oak, maple, lowbush blueberry, beach or cherry). Larvae will be allowed to feed on the host plants and their survival, growth and amount of foliage eaten will be measured. We have observed low survival rates in the lab during preliminary trials, and will be investigating the potential source of the mortality, in particular, any role of insect pathogens. Dead caterpillars will be surface sterilized and divided into two groups, one which will be set up in multi-well plates and incubated for evidence of infection by nematodes, and one for which individuals will be squashed and observed microscopically for signs of infection with microsporidia, NPV (viruses) or other pathogens. Similarly, caterpillars which die in the field experiments will be set up and observed for evidence of infection. Pathogens will be identified and considered for further investigation relative to their potential for biological control.

Harpswell residents have expressed interest in helping with the project and several individuals participated in pheromone trapping efforts in the area this past December and January. We will have a citizen science component of the project involving property owners in Harpswell to help with monitoring egg hatch and host plant development throughout the spring.

### **Expected outcomes and method for sharing outcomes:**

It is expected that winter moth larvae will develop more quickly, weigh more, and experience greater survival when egg hatch is synchronized with host plant budburst. This study will identify the time at which apple varieties and blueberry varieties/clones will be of greatest risk for damage in areas where this pest is established in Maine. It is also expected that low bush blueberry will be a favorable host for winter moth larvae and is therefore at risk if winter moth populations remain high and spread further throughout the state. We expect to recover either or both microsporidian and virus infection in winter moth larvae. The prevalence of these natural mortality agents may impact their potential for spread. Integrated pest management plans will then need to be developed for dealing with this pest. We will be sharing the outcomes of the project with the interested residents of Harpswell through informal talks, and with members of Maine's agricultural community at the Maine Agricultural Trade Show next winter. This proposed project will be part of a Masters thesis to be completed and published in the future.

## **Final Report**

### **Original project objectives that were met and significant findings:**

Objective: Investigate the potential for winter moth to become a serious pest of apples and blueberries in Maine.

1. The timing of winter moth egg hatch and larval feeding relative to host plant development of both highbush and lowbush blueberries and apples will be monitored within the current infestation area in Harpswell, Maine.

Natural populations of winter moth in Harpswell, ME were monitored regularly to determine the timing of egg hatch and larval development.

In 2013 eggs began hatching around April 18 and continued hatching until the final week in April. At the time of winter moth hatch, host plant buds were swollen and most were opening by the first week in May. In 2014 winter moth egg hatch did not begin until April 27 and continued to hatch until May 10. However, oak buds did not open



until the second week of May. In both years of study, birch, maple and cherry buds tended to be the first to fully open and were synchronized with winter moth hatch.

2. Evaluation of larval feeding, development, and survival on the different host plants will be determined.

Feeding, development and survival of winter moth on different host plants were evaluated in two experiments in 2013 and 2014. In the first experiment, newly hatched larvae were released onto the target plants in sleeve cages in May in order to monitor survival under field conditions on the different host plants. The cages were monitored for winter moth damage to leaves as well as winter moth survival. In the second experiment, winter moth larvae collected from the field were set up on different host plant buds and leaves in petri dishes in the laboratory in order to monitor their development, feeding and survival on target host plants.

While overall mortality in sleeve cages was high, survival was higher on apple and oak in sleeve cage experiments during both years of this study. Leaf material inside of sleeve cages was severely damaged if not completely defoliated in the case of oak and apple and in some lowbush and highbush blueberry plants as well. The winter moth consumed both leaf and flower buds of lowbush and highbush blueberry.

In the second experiment, winter moth survived and developed more successfully when fed on oak and apple leaves. Additionally, these lab feeding experiments showed that winter moth successfully feeds and develops on lowbush blueberry, although survival was not as high as on apple and oak.

3. The presence of pathogens that could contribute to mortality of winter moth caterpillars was assessed in order to identify potential future biological control agents.

Caterpillars collected from different host plants in the field were examined for the presence of pathogens.

Preliminary microscope work on deceased caterpillars during the summer of 2013 showed the presence of occlusion bodies that are likely the winter moth virus (NPV). Further investigation using molecular techniques to identify and determine the prevalence of this virus in the Harpswell population are underway. In collaboration with the University of Massachusetts at Amherst we have produced positive samples collected from lowbush blueberry, highbush blueberry and apple during the 2013 field season.

### **Original project objectives that were not met:**

Screening for pathogens in winter moth caterpillars collected over both years of study has not yet been completed but is ongoing. We will continue to screen individual caterpillars for pathogens throughout the rest of the fall and compare the incidence of pathogens between caterpillars collected off of different host plants.

### **Methods used to evaluate outcomes:**

1. In the first year of this study sleeve cages were set up on seven different host plants in Harpswell Maine, an area with high winter moth defoliation. These host plants included red oak, apple, white birch, red maple, highbush blueberry, lowbush blueberry, and pin cherry. Four sleeve cages per host plant were set up on three different dates in year one. In year two, five sleeve cages per host plant were set up on two different dates. The purpose of the staggered set date was to explore the importance of the timing of winter moth hatch for survival. We placed 20 newly hatched larvae in each sleeve cage in year one and 15 in each sleeve cage in year two. If the leaf material inside of the sleeve cage was depleted, the cage was moved to a new branch. When it was approaching time for pupation peat moss was placed in the bottom of each sleeve cage as a pupation medium. At the end of winter moth development, the sleeve cages were collected and the peat moss was sifted to find winter moth cocoons. The number of surviving winter moth cocoons was analyzed by each different host plant using a one-way ANOVA. A two way ANOVA was performed to determine whether set date and plant type had an effect on winter moth survival.
2. Each week during the winter moth feeding period, 50 caterpillars were collected from each of the seven host plants listed above in Harpswell Maine. These caterpillars were set up in petri dishes with moistened filter paper and leaves from the host plant they were collected from. In year one we placed ten caterpillars per

dish and in year two we placed five. Leaves and filter paper were changed out every few days and we recorded the number of live caterpillars, removed all dead caterpillars and recorded how many were pupating. At the end of the experiment the surviving pupae were weighed and set up in cups with peat moss. A survival analysis in JMP was performed for each separate collection date. A two-way ANOVA was also performed in JMP to determine whether the pupal weights were different between collection dates as well as between host plant types.

3. Dead caterpillars removed from lab feeding experiments in year one were squashed and inspected for NPV occlusion bodies using a compound microscope. Each week during both year one and year two, 50 caterpillars were collected per host plant, frozen live and stored at -80°. Caterpillars from the last collection period of year one (June 6, 2013) were used for DNA extraction and PCR following the protocols published by Burand *et al.* 2011.

### **Integration of research and extension activities:**

Extension activities included a presentation on research methods, results and important winter moth facts as well as the potential methods of control for this insect given at the Maine Agricultural Trade show in January of 2014. We will present further findings from the second year of study at the next Ag Trade show in January of 2015.

### **List of external funding both sought and received:**

1. University of Maine Graduate Student Government grants Fall of 2013 - \$637.50, Spring 2014 - \$567.33
2. University of Maine IPM grant Spring 2013 - \$2,980.00
3. MAFES funding for sample analysis of station projects – Fee for service

### **Educational material, publications, and programs:**

We will be publishing a cooperative extension fact sheet on winter moth in lowbush blueberry with Dr. Frank Drummond.

### **Research publications, abstracts, and presentations:**

#### **Presentations:**

- O'Donnell, K. and E. Groden. 2014. *Winter moth development and survival on seven different host plants*. University of Maine Graduate Student Expo, Orono, ME, April 3, 2014.
- O'Donnell, K. and E. Groden. 2014. *The relationship between the winter moth (*Operophtera brumata*) and its host plants*. Northeast Forest Pest Council Conference, Quebec City, Quebec, Canada, March 18 2014.
- O'Donnell, K. and E. Groden. 2014. *Outbreak of winter moth in Harpswell Maine*. Agricultural Tradeshow, Augusta, Maine, January 9, 2014.