FINAL PROJECT REPORT

Project Title: Consulting for the Pacific Northwest sweet cherry breeding program

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Cooperators: Nnadozie Oraguzie and other members of the cherry team (Matt Whiting, Cameron Peace, Amit Dhingra and Fred Bliss)

Other funding sources

Agency Name: USDA-CSREES NRI Plant Genome
Amount awarded: $400K, Aug 2009 – Aug2011
Notes: "The development of COS markers for comparative mapping in the Rosaceae and their application for understanding variation in fruit size". PI: Iezzoni. Develops and validates fruit size genetic markers for sweet cherry and new state-of-the-art marker development for cherry. Leveraged with WTFRC/OSCC funding.

Agency Name: USDA-CSREES Specialty Crops Research Initiative
Amount awarded: $7.2 mil plus equal matching, Sep 2009 – Aug 2013
Notes: "RosBREED: Enabling marker-assisted breeding in Rosaceae. Broad umbrella project on genetic marker development and application. Leveraged with WTFRC/OSCC funding.

Total Project Funding: $13,000

Budget History:

Item	Year 1: 2009	Year 2:	Year 3:
Salaries			
Benefits			
Wages			
Benefits			
Equipment			
Supplies			
Travel & expenses	$ 3,000		
Consulting fee	$ 10,000[a]		
Miscellaneous			
Total	**13,000**		

[a]These activities, which began in 2004, have historically been funded as a consulting arrangement. This was done so that Michigan State University would not be a shared "inventor" of the forthcoming sweet cherry cultivars. I then waived my personal "inventor" rights to any cultivars in exchange for a consulting fee that I donate to MSU to help support the MSU tart cherry breeding program.

OBJECTIVES:

1. Assisted in generating breeding populations. This includes developing the crossing plan, sourcing germplasm, and making crosses along with the breeding team.
2. Provided horticultural guidance. This is provided by site visits, phone consultations, and sharing results from my cherry research at MSU.
3. Provided genetic expertise. My cherry genetics team is currently developing the genetic infra-structure for the PNW sweet cherry breeding program in collaboration with C. Peace to include the generation of molecular markers and genotyping of many of the parents used in the program. This work is funded by USDA grants.

SIGNIFICANT FINDINGS/ACCOMPLISHMENTS:

- Reviewed and contributed to the crossing plan.
- Traveled to Prosser at the beginning of the bloom season to provide organizational and technical assistance to help the crossing team.
- Traveled to Prosser during the growing season to see the seedlings and review horticultural practices.
- Developed a photo-illustrated document outlining seedling growth benchmarks that can be used to implement a seedling growth tracking system so that potential problems can be identified and corrected in a timely manner.
- Provided specific information on the genetic control of fruit size and cherry skin and flesh color to C. Peace for validation in the breeding populations
- Provided C. Peace and N. Oraguzie with a database of genetic and phenotypic data that will be the cornerstone used to determine the genetic control of important phenotypic traits in the cherry breeding program.

RESULTS and DISCUSSION:

Assist in generating breeding populations & provide horticultural guidance.

In April I visited Prosser and assisted N. Oraguzie organize for spring crossing. This included review of the Best Management Practices, the crossing scheme, pollen viability testing, seeds in stratification and seedlings in the field. Numerous specific recommendations were made. In addition, I spent several hours with the entire pollinating crew at the Roza Farm where I demonstrated the best management practices for crossing activities and discussed the rationale for the various strategies.

In July, I visited Prosser to tour the seedlings in the field and the greenhouse. The seedlings in the field were growing nicely; however, survival of newly germinating seedlings continues to be problematic. To help address this problem, I developed a photo-illustrated document outlining seedling growth benchmarks that can be used to implement a seedling growth tracking systems (Fig. 1). I have suggested to N. Oraguzie that his team record seedling growth according to these benchmarks and make the data available to me on a weekly basis. This would make it possible for me to diagnose and help solve problems in a timely manner.

Provide genetic expertise

My cherry genetics team is currently developing the genetic infra-structure for the PNW sweet cherry breeding program in collaboration with C. Peace to include the generation of molecular markers and genotyping of many of the parents used in the program. This work is funded by USDA grants. Specific deliverables in 2009 include:

- A database (called FlexQTL™) containing genetic and phenotypic characterizations for the majority of the parental germplasm used in the breeding program, plus populations from MSU (NY x EF), WSU (PMR x Rainier) and France (Regina x Lapins)(Fig. 2).
- Knowledge of genomic regions controlling fruit size and skin and flesh color in cherry (Fig. 3)(Zhang et al., 2009; Sooriyapathirana et al, 2009). This information was shared with C. Peace for validation in the PNW sweet cherry breeding program.
- Identification of DNA marker polymorphisms in six parental selections used in the breeding program. This information is being used to design a high-throughput genotyping platform for sweet cherry with state-of-the art markers by spring 2010.

Collectively, these efforts provide the building blocks that will allow the cherry team to implement marker assisted breeding to increase the efficiency and success of the breeding program.

EXECUTIVE SUMMARY:

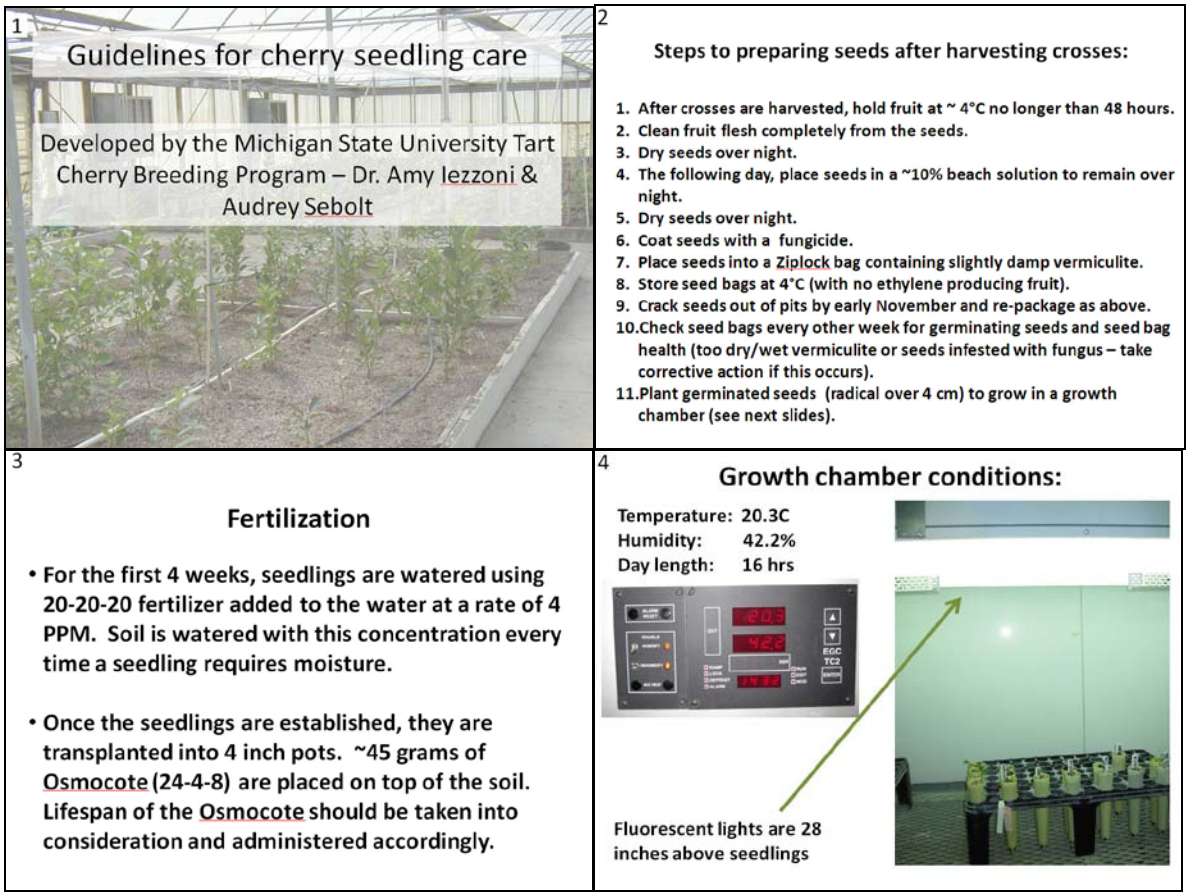
The building blocks of a successful breeding program include the use of diverse germplasm, generation of large numbers of progeny populations for evaluation, appropriate horticultural management of the breeding materials, the ability to identify and commercialize superior cultivar candidates, and judicious use of genetics knowledge. The goal of my consultancy with the PNW sweet cherry breeding program is to assist in our ability to excel at all of these objectives so that we can deliver superior sweet cherry cultivars to the Oregon and Washington industries as quickly as possible. In 2009, I provided knowledge and recommendations regarding breeding and horticultural practices, and advances made this year in cherry genetics. In addition, a photo-illustrated document outlining seedling growth benchmarks was developed to address problems with seedling survival. This document can be used to implement a seedling growth tracking system so that potential problems can be identified and corrected in a timely manner. In addition a core genetic database was developed and used to elucidate the genetic control of fruit size and color. This knowledge was provided to C. Peace for further validation within the sweet cherry breeding populations. Continued collaboration, whereby I contribute my time and knowledge of cherry germplasm, breeding and genetics, will help us achieve our collective vision of a cost-effective aggressive and successful sweet cherry breeding program.

LITERATURE CITED:

Sooriyapathirana SS, Khan A, Sebolt AM, Wang D, Bushakra JM, Lin-Wang K, Allan AC, Gardiner SE, Chagne D, Iezzoni AF. 200x. QTL analysis and candidate gene mapping for skin and flesh color in sweet cherry fruit (*Prunus avium* L.). Tree Genet Genomes (in review).

Zhang G, Sebolt AM, Sooriyapathirana SS, Wang D, Bink MCAM, Olmstead JW, Iezzoni AF. 2009. Fruit size QTL analysis in an F_1 population derived from a cross between a domesticated sweet cherry cultivar and a wild forest sweet cherry. Tree Genet. Genomes (in press).

Fig. 1. Guidelines for cherry seedling care.



5

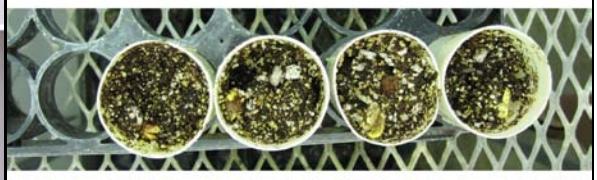
Seedling health in seed germination bags:

This an example of a healthy seedling and one that is ideal in length to plant. Radical is between 4-7 cm in length and appears to be healthy.

This is an example of a seedling that is beginning to show signs of poor health, as the end of the radical is brown. Reasons for poor health include: too much/little moisture in the seedling bags or not enough vermiculite in the seedling bags. Once the problem is identified, action should immediately be taken to ensure that seedlings are healthy. In this case, there was not enough vermiculite in the bag. Action: vermiculite was added as well as water.

6

Day 1: After seedlings are planted, they are watered until fully saturated with fertilizer/water. After watering, seedlings are examined and care is taken to be sure cotyledons are above the soil line.



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Day 2: Soil is still damp at the top and bottom of the cone-tainer. Seedlings were not watered.

8

Day 3: Soil is just beginning to dry out. Added ~ 50 ml of fertilizer/water.

9

Day 6: Soil is still moist on the surface and at the bottom of the cone. ~ 14 ml of fertilizer/water was added.

10

Day 10: Soil is still moist on the surface and at the bottom of the cone. No water was added.

11

Day 15: Soil is still moist on the surface and at the bottom of the cone. No water was added.

12

Day 21: Seedlings were watered (if dry) with fertilizer/water until soil was fully saturated (water dripping out of the bottom of the cone-tainer). The second seedling from the left required less frequent watering as it is much slower in growth. Continued care for the taller seedlings will involve watering every other day until soil is fully saturated. For the second seedling from the left, only as needed (much less frequently and less volume of water). Approximately on Day 28, seedlings will be transferred to 4 inch pots.

NOTE: These pictures and this timeline are for tart cherry. Sweet cherry seedlings will grow with a lot more vigor and apical dominance than tart cherry. So achieving these size measurements by Day 28 should not be a problem.

13

Suggested seedling watering schedule

Day 1 Watered soil until fully saturated	**Day 2 No water added**	**Day 3 ~ 50 ml of water**	**Day 4 No water added**	**Day 5 No water added**	**Day 6 ~ 14 ml of water**	**Day 7 No water added**
Day 8 No water added	Day 9 No water added	Day 10 No water added	Day 11 Watered soil until fully saturated*	Day 12 No water added	Day 13 No water added	Day 14 No water added
Day 15 No water added	Day 16 Watered soil until fully saturated*	Day 17 No water added	Day 18 ~ 50 ml of water	Day 19 No water added	Day 20 No water added	Day 21 Watered soil until fully saturated*

*Seedlings that were less vigorous were watered less frequently.

14

Seedling growth progression over the course of three weeks

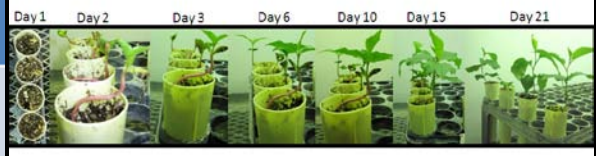

Fig. 2.B. FlexQTL genome coverage where the NYxEF linkage map is used as the backbone. Black boxes represent the locations of the genetic markers on the 8 *Prunus* linkage groups.

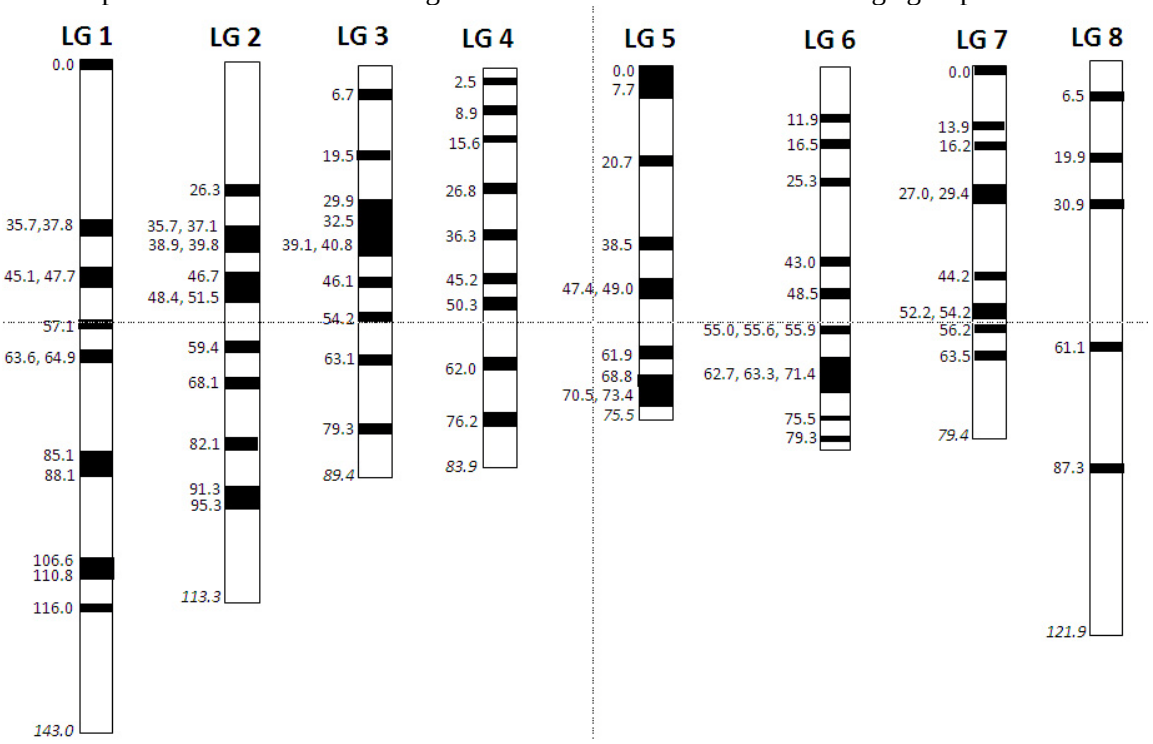

Fig. 3.A. Range of skin & flesh colors used in the genetic analysis. Skin Color Indexes are a modified version of the MSU Sweet Cherry Maturity Index. The flesh color index is the Washington State University Sweet Cherry Flesh Color Index. Skin color 1 and skin color 2 refer to bluch color and ground color, respectively.

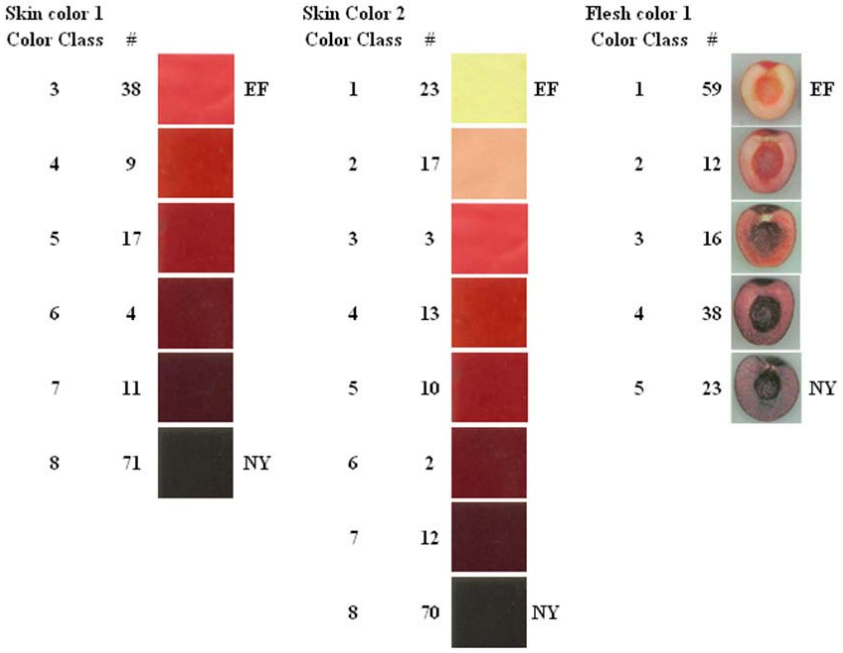

Fig. 3.B. Progeny distribution for skin color. Color scale (3-8) is for skin color 1 as defined in Fig. 3.A.

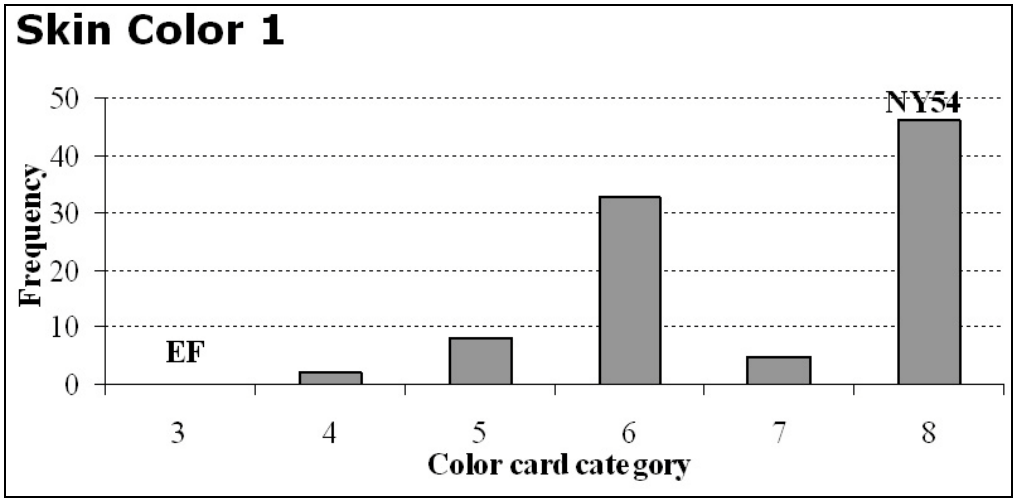

Fig. 3.C. Genetic markers on cherry linkage group 3, simplified by letters a, b, c, d, illustrate the contribution of this genomic region to the genetic control of flesh color. Only those progeny individuals that have the genetic markers termed "a", have dark skin and red flesh.