

SERA-IEG REPORT (2003-2004)
GRAPE RESEARCH AND EXTENSION ACTIVITIES
UNIVERSITY OF FLORIDA
INSTITUTE OF FOOD AND AGRICULTURAL SCIENCES

MID-FLORIDA RESEARCH AND EDUCATION CENTER, APOPKA, FL
GRAPE BIOTECHNOLOGY - Dennis Gray

Development of disease-resistant grape cultivars for Florida via genetic engineering biotechnology (FDACS/VAC-supported project).

In vitro selection of disease-resistant grape cultivars for Florida and their use for discovery of unique resistance genes (FDACS/VAC-supported project).

Establishment of a new research vineyard at the Center.

The following activities are ongoing:

1) Muscadine grape selections - replicated trial

A one-plus acre muscadine variety trial is well-established. The trial is composed of established varieties and seven selections from the MREC breeding program arranged in a complete replicated block design. First data were taken in 2003. Yields are increasing as vines mature. Data will be taken in 2004, but full production is not anticipated until 2005 - 06. To date, several selections appear to be promising, but it is still unclear as yet whether or not they will exceed existing commercial varieties in desirable traits.

2) Grape genetic engineering research

Methodology to accomplish genetic transformation in a number of vinifera varieties and rootstocks is now routine at MREC. We continue to develop new genetic constructs and test them in model varieties, notably 'Thompson Seedless'. The primary gene/trait of interest is Pierce's disease resistance. Inoculation of transgenic plants with *Xylella fastidiosa*, causative agent of PD in the greenhouse shows delayed symptom development among various lines. However, we believe that advanced genetic constructs will yield even better results. Currently, inoculated plants are being

evaluated under greenhouse conditions. Approximately 200 independent transgenic plant lines will be evaluated this summer and fall. If plants with sufficiently high levels of resistance are identified, we will begin planning for field tests.

3) A new website for grape growers

In response to significant demand, a new website was created at MREC to provide support to new grape growers in July 2004. The site address is

<http://www.mrec.ifas.ufl.edu/grapes/> Establishment of this site was overdue. The “official” University of Florida grape program was terminated in 1990, before use of internet-based information became commonplace. Therefore, much of the voluminous information created by the historic Leesburg viticulture research program was never published on the web. In a few instances, documents had to be re-created to make them available.

Patents

Gray, D. and R. Scorza, Disease resistance in *Vitis*. Mexican Patent No. 9810813. Sept. 18, 2003.

Gray, D. J. and Z. T. Li, Multi-functional genetic markers and uses thereof. Provisional US Patent Ser. No. 60/465,015, 2003.

Li, Z. T. and D. J. Gray, Nucleotide sequences of 2S albumen gene and its promoter and uses thereof. US Patent Application Ser. No. 10/382,066, 2003.

Refereed publications

Gray, D. J., Plant growth and development - artificial seeds. Encyclopedia of Applied Plant Science, Elsevier Science Ltd., Oxford, 2003, 1305-1310.

Gray, D. J., S. Jayasankar and Z. Li, Vitaceae (Grape *Vitis* spp.), In: R. E. Litz (Ed.), Biotechnology of Fruit and Nut Crops, CAB International Wallingford, U.K., 2004, (In Press).

Li, Z. and D. J. Gray, Effect of five antimicrobial peptides on the growth of *Agrobacterium tumefaciens*, *Escherichia coli* and *Xylella fastidiosa*, *Vitis* 42, 2003, 95-97.

Li, Z., S. Jayasankar and D. J. Gray, Bi-directional duplex promoters with duplicated enhancers significantly increase transgene expression in grape and tobacco. *Trans. Res.*, 13, 2004, 143-154.

Jayasankar, J. and D. J. Gray, In vitro selection for disease resistance in plants – an alternative to genetic engineering. *AgBiotechNet*, 5, 2003, 1- 5.

Abstracts from presentations at National/International scientific meetings

Gray, D. J., Z. J. Li, M. Van Aman, N. Barnett, A. Raveendran and K. Kelley, Applications of somatic embryogenesis and transformation in grapevine improvement, Invited presentation, 2003 Congress on In Vitro Biology, *In Vitro Cell. Dev. Biol.* 39, 2003, 7A.

Dutt, M., M. Van Aman and D. J. Gray, Liquid culture for rapid in vitro propagation of *Vitis*, 2004 Congress on In Vitro Biology, *In Vitro Cell. Dev. Biol.* 40, 2004, 56A.

GRAPE PATHOLOGY/PIERCE'S DISEASE RESEARCH - D. L. Hopkins

Pathogenic and Genetic Characterization of Pathotypes of *Xylella fastidiosa* from Various Hosts.

Biocontrol of Pierce's Disease of Grape with weakly virulent strains of *X.*

fastidiosa. In a 4-year test in the MREC, Leesburg vineyard, one weakly virulent *X. fastidiosa* strain provided significant control of Pierce's disease of grapevine in Cabernet Sauvignon on Freedom rootstock. Five of six treated vines survived five years and fruited. All six of the untreated vines died. Other weakly virulent strains of *X. fastidiosa* provided some biocontrol of Pierce's disease but were less effective, with 2 or 3 surviving vines.

In a new, replicated experiment in the MREC vineyard in Apopka, the most effective strain listed above and two new strains of *X. fastidiosa* that are weakly virulent to grapevine were included in a test to further develop cross protection for the biocontrol of Pierce's disease. These three strains are being evaluated in three different grape cultivars, Chambourcin, Cynthiana, and Cabernet Franc. There are plans to include other grape genotypes and bacterial strains in additional tests in the MREC vineyard and in commercial vineyards. The objective is to develop a biological control system that would allow the production of Pierce's disease susceptible grapevines in Florida.

The most effective strain of *X. fastidiosa* is being evaluated for its effect on movement and colonization of grapevine by virulent PD strains. Strains have been labeled with

fluorescent proteins to visualize the association of the biocontrol strain with the pathogenic strains in the grape plant. The objective of this study is to gain an understanding of the mechanism of the biological control. This work is part of the Ph. D. thesis problem of Robin Oliver.

Publications

Refereed Journal Articles

Hopkins, D. L. 2004. Biological Control of Pierce's disease in the vineyard with strains of *Xylella fastidiosa* benign to grapevine. Plant Disease (Submitted).

Wichman, R. L., C. M. Thompson, and D. L. Hopkins. 2004. Genetic and pathogenic diversity among strains of *Xylella fastidiosa*. Phytopathology (In Prep.)

Abstracts

Hopkins, D. L., R. L. Wichman, and C. M. Thompson. 2003. Molecular and pathogenic characterization of various pathotypes of *Xylella fastidiosa*. Proc. of 8th Int. Congress of Plant Pathology. 2:284.

CITRUS RESEARCH AND EDUCATION CENTER, LAKE ALFRED

Russ Rouseff & Ozan Gurbuz (Visiting Scientist from Turkey)

In cooperation with Steve Talcott, they are investigating the effects of various enzyme and yeast treatments on wine phenolics in muscadine wine and wine by-products. The influence of fermentation and storage conditions on muscadine phenolics and related antioxidant activity and stability are being evaluated.

FOOD SCIENCE AND HUMAN NUTRITION DEPARTMENT, GAINESVILLE

ENOLOGY, GRAPE PROCESSING & UTILIZATION - Bob Bates & Charlie Sims

The goal is to optimize and popularize the beneficial properties of Florida grapes, grape products, and byproducts. There are now over 12 Florida wineries in operation with several more on the drawing board. We work quite closely with commercial wineries and individuals committed to both commercial and hobby winemaking.

In cooperation with Florida Grape Growers Association (FGGA) and FGGA County Chapters, workshops and Hobby Wine Competitions are conducted annually. The FGGA also hosts a commercial wine competition at the Florida State Fair in Tampa every February. This event draws over 1,000 wines from U.S. and international wineries. These activities result in increased membership and participation in the

FGGA, greater appreciation of local commercial wines, and a growing demand for Florida grapes cultivars for planting and winemaking.

GRAPE PHYTOCHEMICAL RESEARCH - Sue Percival

In view of the well documented health value of grape phytochemicals, especially in muscadine cultivars, we are conducting a coordinated focus on the occurrence, distribution, and efficacy of these substances in grapes. Compounds of major interest are: ellagic acid and its precursors, quercetin, resveratrol, and anthocyanins. Studies involve the beneficial effects of these phytochemicals regarding human health, especially related to prevention of cancer and aging.

Dr. Susanne Mertens-Talcott has finished her research on “Anticarcinogenic effect of flavonoids from muscadine wine (*Vitis rotundifolia*) on signal transduction and cell cycle regulation in blood cell lines and non-tumourous cells” and graduated Summer 2004 with her doctorate. Robin Minor (PhD candidate) will initiate studies on wine phytochemicals and their anti-aging characteristics in human cells.

In 2004 and 2005, Dr. Percival traveled to NCI, NIH, Washington DC and Texas A&M, College Station Texas to present a seminar entitled: “Bioactive components of red wine that influence cancer-preventing immunity.”

Publications:

- 2004 Mertens-Talcott, S.U. and Percival, S.S. Ellagic Acid and Quercetin Interact Synergistically with Resveratrol in the Induction of Apoptosis, and Cause Transient Cell Cycle Arrest in Human Leukemia Cells. *accepted: Cancer Letters.*
- 2004 Mertens-Talcott, S.U. Polyphenolics From Red Wine In Cancer Prevention: Influence On Apoptosis In Human Leukemia Cells And Antioxidant Effects In Human Subjects *Ph.D Dissertation*
- 2003. Mertens-Talcott, S.U. Talcott, S.T., Percival, S.S. Low concentrations of quercetin and ellagic acid synergistically influence proliferation cytotoxicity and apoptosis in MOLT-4 human leukemic cells. *J.Nutr.* 133:2669-2674,
- 2002 S.S. Percival, S.T. Talcott Health Benefits of Red Wine: Dietary Importance - *Encyclopedia of Food Science & Nutrition*, 2002. 6209-6217. .
- 2002 Susan S. Percival, Charles A. Sims, Stephen T. Talcott, Immune Benefits of Consuming Red Muscadine Wine. *EDIS.*

2000 Wine Modifies the Effects of Alcohol on Immune Cells of Mice S.S. Percival and C.A. Sims. J. Nutr. 130:1091-4.

Publications In process:

Ellagic acid interacts in a potentiating manner with quercetin on p21^{WAF1/CIP1}, p53 and MAP-kinases without affecting intracellular ROS or quercetin stability. To be submitted to Journal of Nutrition.

Cell-protective and antioxidant effects of an acute consumption of red muscadine and cabernet sauvignon wine in human plasma and peripheral mononuclear cells. To be submitted to American Journal of Clinical Nutrition.

Abstract/Presentations at National Meetings:

Susanne Mertens-Talcott, Joonhee Lee, Stephen T. Talcott, Susan S. Percival. Alterations of vital cellular parameters and apoptosis in Caco-2 human colon carcinoma cells by polyphenols-rich fractions from muscadine grapes (*Vitis rotundifolia*) IFT Technical Abstract, 2004.

Susanne U. Mertens-Talcott, Joshua A. Bomser, Stephen T. Talcott, Susan S. Percival Quercetin and Ellagic Acid Act Synergistically in the Induction of Apoptosis and p21^{WAF1/CIP1}-involved Signal Transduction in Human MOLT-4 Leukemia Cells FASEB, 2004

Meri P. Nantz, Cheryl A. Rowe, Susanne Mertens-Talcott, Susan S. Percival, Damage to immune cells by ethanol is prevented in mice consuming red wine. FASEB, 2004

Grants Funded/Submitted Recently:

Health Benefits of Red Muscadine Wine – FI Department of Agriculture and Consumer Services in conjunction with the Viticulture Advisory Committee.

GRAPE PIGMENTS AND PHYTOCHEMICAL RESEARCH - Steve Talcott

Currently, three graduate students are conducting research full or part time on muscadine grapes, organized into the following projects:

Processing and modeling enzyme inhibition (PPO) for high hydrostatic pressure processing of muscadine grape juice.

Maturity and cultivar evaluation of ellagitannins and antioxidant polyphenolics in muscadine grapes and grape juices.

Anthocyanin copigmentation reactions for nutrient retention in processed muscadine grape juice.

Isolation and identification of antioxidant polyphenolics responsible for color degradation (enzymatic and non-enzymatic) in muscadine grape juice.

Accomplishments: Modifying functional properties of natural pigments (anthocyanins) are valuable to the food industries in the U.S. due to its potential impact in multiple areas. Specifically, preventing degradation of antioxidant phytochemicals, alleviation of visual color loss, reducing visible sediments, reduction of processing loss, and overall retention of nutritional and quality characteristics are paramount. The concept of anthocyanin copigmentation for incorporating antimicrobial, antioxidant, and enzyme inhibiting compounds into anthocyanins for novel uses within the food industry has thus far proved effective as a means of extending shelf life characteristics.

Research Papers Published or Submitted for Publication:

Red Clover Isoflavonoids Act As Copigments to Muscadine Grape Anthocyanins.
Stephen T. Talcott, Janelle E. McGuinness, and Carmen H. Brenes.

Phytochemical and Color Retention of Copigmented and Processed Muscadine Grape Juice. S. T. Talcott, Carmen H. Brenes, D. M. Pires, and David Del Pozo-Insfran.

Ellagic Acid and Flavonoid Antioxidant Content of Muscadine Wine and Juice
S.T. Talcott and J.H Lee

Ellagic Acid and Ellagitannins Affect on Sedimentation in Muscadine Juice and Wine
Joon-Hee Lee and Stephen T. Talcott

Fruit Maturity and Juice Extraction Influences Ellagic Acid Derivatives and Other Antioxidant Polyphenolics in Muscadine Grapes. Joon-Hee Lee and S. T. Talcott

Polyphenolic and Antioxidant Content of Muscadine Grapes Influenced by Cultivars and Ripening. JoonHee Lee and Stephen T. Talcott

EXTENSION ACTIVITIES

Mid-Florida Research and Education Center

Dennis Gray - Receives several calls per week from growers, hobbyists and potential new growers. Advises on varieties and directs clients to other sources of information. Serves on the Board of Directors of the Florida Grape Growers Association and as a committee member on the Florida Department of Agriculture and Consumer Services Viticultural Advisory Council.

Don Hopkins - Provides growers with disease diagnoses and recommends control practices.

Food Science and Human Nutrition Department

Charlie Sims - "Best Practices for Florida Wines" UF Extension Bulletin SP 316.

Bob Bates - Retired with emeritus status 6/30/03. Still responds to grower and winemaker inquiries and judges state and regional wine competitions. Co-author of "Best Practices for Florida Wines".

Statewide Extension Service

Extension agents assist growers and hobbyists on an ad hoc basis. The following agents present yearly programs to assist growers:

John Jackson & Charles Fedunak - Lake County. Grape Field Day each year in August for the past five years.

Max Griggs - Escambia County. Plant Disease Clinic Monday & Friday 9-4.