**SERA IEG-14 REPORT (2002-2003)**
**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)**

Since *Xylella fastidiosa*, a bacterium and the causal agent of PD, exists only in xylem vessels, it is important for antimicrobial gene expression to occur in the xylem sap. During 2001, transgenic Thompson Seedless grapevines were produced that contained the green fluorescent protein gene (GFP) and an antimicrobial lytic peptide gene. The plants fluoresced bright green, allowing the site of gene expression to be pinpointed. Plants with high expression in xylem tissues (i.e., green fluorescent veins) were selected. Xylem sap from these plants then was collected and tested for the presence of lytic peptide via ELISA. Plants with GFP expression in veins were shown also to have lytic peptide in xylem sap. These plants were inoculated with *Xylella*. While there appeared to be a range in symptom expression among various transgenic plants, all eventually had some sign of PD. This was possibly attributed to the fact that lytic peptide concentration in the xylem sap was below the concentration needed for antimicrobial activity to occur. To explore this possibility, new genetic constructs are being utilized, which should enable at least a 10-fold increase in lytic peptide concentration. In 2001, a US patent was issued to UF and USDA for the use of lytic peptide genes to control PD. A version of this patent that covers all microbial pathogens subsequently has issued in Chile and South Africa. The patent issued for use against fungal diseases in Australia as well. In September 2002, the foundational patent issued that covers the enabling culture system. This patent had previously issued in South Africa.

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

Selection of fungal toxin tolerant cell lines led to the regeneration of plants that expressed high constitutive levels of various pathogenesis-related (PR) proteins. These plants resist anthracnose disease, as well as Botrytis, in in vitro assays. In the greenhouse, plants are resistant to anthracnose but not downey and powdery mildews. Greenhouse inoculation with *Xylella* in 2001 showed that plants of one selected line resisted PD also (although this response did not subsequently hold-up during 2002). This suggests that in vitro selection using one fungal toxin activates a broad-spectrum disease resistance response that functions against some, but not all, pathogens. Greenhouse and field tests to confirm these observations are ongoing. To date, field studies do not show dramatic differences between selected and control vines, with the exception of ‘Stover’. ‘Stover’ was developed by UF and is relatively disease resistant. Selected plants exhibited less angular leaf spot when compared to non selected control plants.

**Establishment of a new research vineyard at the Mid-Florida Research & Education Center, Apopka, FL**

A four-acre vineyard was constructed during December 2001 to February 2002. Approximately 75% of the acreage has been planted. The vineyard supports the following activities: 1) A muscadine table grape variety trial to evaluate selections from the MREC breeding program, 2) Tests of plants from in vitro selection, 3) Tests of PD cross protection via use of non-pathogenic *Xylella* strains inoculated into *V. vinifera* cultivars, 4) A germplasm repository of historic vines and cultivars released over the years by the UF/IFAS breeding program.

**Awards**

In July 2002, the "Grapevine Biotechnology Research Team", comprising researchers at the MREC (Dennis Gray, Zhijian Li, Jay Subramanian and Donald Hopkins) and the USDA (Ralph Scorza, Appalachian Fruit Research Station) received the 2002 USDA Secretary's Honor Award. This is the highest award given annually by the USDA. It was given in recognition of research on genetic engineering of grape to combat Pierce's Disease.

**Patents**

Gray, D. J. and R. Scorza, Disease resistance in *Vitis*, Australian Patent No. 723058, 2001.
Gray, D. J. and R. Scorza, Disease resistance in *Vitis*, Chilean Patent , 2002.
Gray, D. J., J. Subramanian, and R E. Litz, Regeneration system for grape and uses thereof. South African Patent No. 20000591, 2001.
Gray, D. J., J. Subramanian, and R E. Litz, Regeneration system for grape and uses thereof. US Patent No. 6,455,312, 2002.
Scorza, R. and D. J. Gray, Disease resistance in *Vitis*, US Patent No. 6,232,528 B1, 2001.
*Also filed in relevant foreign countries.*

**Publications**

Book Chapter

Gray, D. J., S. Jayasankar, Z. Li, J. Cordts, R. Scorza and C. Srinivasan, Transgenic grapevines, *In:* G. G. Khachatourians, A. McHughen, R. Scorza, W. K. Nip and Y. H. Hui (Eds.), Transgenic Plants, Chapter 27, Marcel Dekker, 2002, pp. 397-405.

Refereed Journal Articles

Li, Z., S. Jayasankar and D. J. Gray, Expression of a bifunctional green fluorescent protein (GFP) fusion marker under the control of three constitutive promoters and enhanced derivatives in transgenic grape (*Vitis vinifera*). Plant Sci. 160, 2001, 877-887.
Li, Z., S. Jayasankar and D. J. Gray, An improved enzyme-linked immunoabsorbent assay protocol for the detection of small lytic peptides in transgenic grapevines (*Vitis vinifera*), Plant Molec. Biol. Reptr. 19, 2001, 341-351. (Note that the cover photograph for volume 19 is taken from our work).
Jayasankar S., D. J. Gray and M. VanAman, Direct seeding of grapevine somatic embryos and regeneration of plants, In Vitro Dev. Biol. Plant 37, 2001, 476-479.

Jayasankar S., B. Bondada and D. J. Gray, A unique morphotype of grape somatic embryos exhibits accelerated plant development. Plant Cell Rep. 20, 2002, 907-911.

Proceedings of Symposia
Gray, D. J., Z. Li and J. Subramanian, Tissue-specific expression of lytic peptides in transgenic grapevines via use of a GFP/NPTII fusion marker, Invited speaker, 2nd International Symposium on Biotechnology of Tropical and Subtropical Species, ACTA Hort., Academia Sinica, Taipei, Taiwan, Nov. 5 - 8, 2001. (In press)

Abstracts
Jayasankar S., B. Bondada and D. J. Gray, Histology and scanning electron microscopy of somatic embryo development n grapevine. 2001 Congress on In Vitro Biology, In Vitro Cell. Dev. Biol. 37, 2001, 42A.
Li, Z. J., S. Jayasankar and D. J. Gray, Expression of a GFP fusion marker under control of three constitutive promoters and enhanced derivatives in transgenic grape. 2001 Congress on In Vitro Biology, In Vitro Cell. Dev. Biol. 37, 2001, 22A.
Li, Z. J., S. Jayasankar and D. J. Gray, Use of a GFP fusion marker to select grapevine clones with tissue-specific transgene expression. 2001 Congress on In Vitro Biology, In Vitro Cell. Dev. Biol. 37, 2001, 43A.
Jayasankar, S., Z. J. Li, Hopkins, D. L. and D. J. Gray, Broad spectrum disease resistance in grapevine by in vitro selection. 10th Congress of the International Association for Plant Tissue Culture, Orlando, FL June 23-28. Final Program and Abstracts P-1251, 2002, 97-A.
Z. J. Li, Jayasankar, S. and D. J. Gray, Use of marker genes to target disease resistance gene expression in grape. 10th Congress of the International Association for Plant Tissue Culture, Orlando, FL June 23-28. Final Program and Abstracts P-1101, 2002, 58-A.

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

**Pathogenic and Genetic Characterization of Pathotypes of *Xylella fastidiosa* from Various Hosts**
Genomic DNA fingerprinting after digestion with rare-cutting restriction endonucleases, RAPD analysis, REP PCR and ERIC PCR are being used to define genetic relationships among pathotypes. Various hosts (16)-live oak, laural oak, sycamore, elm, red maple, sweet gum, oleander, grapevine, elderberry, etc.--were inoculated with different strains of *X. fastidiosa,* monitored for symptoms, and tested by culturing and PCR for the presence of the bacterium to determine pathogenic relationships among the strains. Florida strains of *X. fastidiosa* obtained from elderberry, grapevine, lupine, oak, oleander, and sycamore, as well as ATCC strains from mulberry, almond, plum, elm, periwinkle, ragweed, red oak, and grapevine were included in the project. Approximately 37 strains have been fingerprinted and were in the 3-year host range test. The genetic characterization is being compared with host specificity in an attempt to identify and characterize pathotypes of X. fastidiosa.

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

In greenhouse tests, weakly virulent strains of *X. fastidiosa* provided cross protection against Pierce's disease in V. vinifera 'Carignane'. 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.

Several strains of X. fastidiosa that are weakly virulent to grapevine will be utilized in new vineyard tests to further develop cross protection for the biocontrol of Pierce's disease. These weakly virulent strains were shown to multiply and systemically colonize grapevine; avirulent strains that were localized in the plant and did not systemically colonize grapevine did not provide cross protection. Strains that are highly virulent on their host of origin but also colonize grapevine, producing very mild or no symptoms, will also be evaluated. Strains of *X. fastidiosa* that provide cross protection against PD will be evaluated for their effect on movement and colonization by PD strains of X. fastidiosa in grapevine. This work is the M.S. thesis problem of Robin Oliver.

**Publications**

Book Chapters

Hopkins, D. L. 2001. Pierce's Disease. *The Encyclopedia of Plant Pathology*. Eds: Maloy, O. C., Murray, T. D. John Wiley & Sons, Inc., pp 771-772.

Hopkins, D. L. 2001. Xylem-Limited Bacteria (RLOs). *The Encyclopedia of Plant Pathology*. Eds: Maloy, O. C., Murray, T. D. John Wiley & Sons, Inc., pp 1212-1214.

Refereed Journal Articles

Wichman, R.L. and D.L. Hopkins. 2002. Differentiation of pathogenic groups of *Xylella fastidiosa* strains with whole-cell protein profiles. Plant Dis. 86:875-879.

Hopkins, D.L. and A. H. Purcell. 2002. *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. Plant Dis. 86:1056-1066 (Feature article).

Non-refereed Jornal Articles

Hopkins, D. L. 2001. Glassy winged sharpshooter transmitted maladies. Proceedings for the 17th Conference on Insect & Disease Management on Ornamentals, Society of American Florists, pp 35-40.

Abstracts

Wichman, R. L., C. M. Thompson, and D. L. Hopkins. 2002. Pathogenic and genetic relationships among strains of *Xylella fastidiosa* isolated from various hosts. Phytopathology 92S:86.

## FOOD SCIENCE AND HUMAN NUTRITION DEPARTMENT, GAINESVILLE

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

In view of the considerable interest among growers and wineries regarding the nutraceutical value of Florida grapes and cooperative research in both vineyards and wineries is in progress. The goal is to optimize and popularize the beneficial properties of Florida grapes, grape products, and byproducts.

The number of Florida wineries continues to grow and there are now over 10 in operation and several more on the drawing board. We work quite closely with commercial wineries and individuals committed to both commercial and hobby winemaking. A publication, "Best Practices for Florida Wine Production" has been produced and is available to wine interests in-state. It is a general introduction emphasizing the special challenges of viticulture/enology in Florida and includes insights gained by both researchers and industry professionals over many decades.

Hobby winemaking continues to attract individuals to Florida grapes. In cooperation with Florida Grape Growers Association (FGGA) and FGGA County Chapters, workshops and Hobby Wine Competitions are conducted annually. 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, Steve, Sue, colleagues, and students 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 isolation, identification, quantification of these phytochemicals as well as the influence of cultivar, cultivation conditions, maturity, processing (fresh fruit, juice, and wine), and storage upon the nutraceutical properties. We're looking into the mechanics and kinetics of action, including in vitro and in vivo interactions.

Susanne Mertens-Talcott (PhD Candidate) is continuing 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."

**Publications**
Low concentrations of quercetin and ellagic acid synergistically influence proliferation cytotoxicity and apoptosis in MOLT-4 human leukemic cells. J.Nutr. 133:2669-2674, 2003.
Health Benefits of Red Wine: Dietary Importance - Encyclopedia of Food Science & Nutrition, 2002. 6209-6217. S.S. Percival, S.T. Talcott.
Immune Benefits of Consuming Red Muscadine Wine. Susan S. Percival, Charles A. Sims, Stephen T. Talcott, EDIS, 2002.
Wine Modifies the Effects of Alcohol on Immune Cells of Mice S.S. Percival and C.A. Sims. J. Nutr. 130:1091-4, 2000

**Manuscripts in progress**
Quercetin and Reservatrol Interactions

**Grants Funded/Submitted Recently**:
1. Health Benefits of Red Muscadine Wine – USDA, NRI funded in 2002.

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.

**Student Work**
Ms. Janelle McGuinness is now employed at Schriber Foods (Monet, MO) after conducting her research project entitled: “Isoflavonoid Copigmentation Reactions and the Functional Properties of Muscadine Wine”.
Copigments, such as isoflavonoids found in red clover, were found to enhance the color properties of anthocyanins in muscadine wine. Copigmentation reactions between red clover isoflavonoids, predominantly formononetin, prunetin and biochanin A, and the anthocyanins present in muscadine wine were evaluated by measuring absorbance and wavelength shifts using a UV-vis spectrophotometer. The copigmentation effect was evaluated in several treatments including muscadine wine with red clover powder, red clover extract and steeped versions of the red clover powder and red clover extract. The different treatments were subsequently analyzed for their effects on color properties of anthocyanins evaluated using established spectrophotometric assays. Analysis by HPLC confirmed the identity and concentration of anthocyanins used for copigmentation reactions. The interaction between muscadine (Vitis rotundifolia) wine anthocyanins and red clover (Trifolium sp.) isoflavones (formononetin, prunetin and biochanin A) pre- and post-fermentation were investigated for nine weeks in a storage temperature study. Significant increases in color stability were observed in the wines that had been copigmented with red clover powder extract. This work has created an alternative for the increase of color stability in muscadine wine during storage.

**Summary of 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
Isoflavonoids from red clover (Trifolium pratense) were found to enhance overall color and thermal stability of anthocyanin 3,5-diglucosides present in muscadine grape juice through intermolecular copigmentation reactions. Predominant isoflavonoids present in red clover (RC) were formononetin, biochanin A, and prunetin and based on their high concentrations in solution likely acted as cofactors to anthocyanin copigmentation reactions influencing grape juice color. Hyperchromic shifts in anthocyanin color at 515 nm following addition of RC extracts were measured spectrophotometrically and individual isoflavonoids were quantified by HPLC. The solubility of RC isoflavonoids in water was determined to be relatively low but followed a quadratic solubility curve as ethanol was increased in solution, extracting up to 57% of the total in 20% ethanol at 60°C. Other polyphenolics and extraneous water-soluble compounds were easily removed from RC by steeping in hot water without appreciably affecting isoflavonoid concentrations or the magnitude of copigmentation. Color enhancement by ethanolic extracts of RC were found to increase up to an anthocyanin:cofactor ratio of 8, however extreme cofactor concentrations are

considered impractical for use in food-based applications. Little information is available on copigmentation reactions occurring in food systems and isoflavonoids proved to be a novel and effective anthocyanin cofactor enhancing color and stability of muscadine grape anthocyanins.

**Phytochemical and Color Retention of Copigmented and Processed Muscadine Grape Juice**

Stephen T. Talcott, Carmen H. Brenes, Danielle M. Pires, and David Del Pozo-Insfran

Muscadine (Vitis rotundifolia) grape juice was assessed for color and phytochemical stability as influenced by copigmentation with rosemary extract (RE), fortification with ascorbic acid (AA), and processing by heat or high hydrostatic pressure (HHP). Color and phytochemical stability are relatively low in juice, therefore the role of anthocyanin cofactors in the presence and absence of AA were assessed as a means to improve overall quality characteristics. Addition of RE from 0.1-0.4% v/v readily formed copigment complexes with anthocyanins and gave concentration-dependent hyperchromic shifts from 10-27% that correspondingly increased antioxidant activity (AOX). Physicochemical attributes following processing demonstrated that AA fortification was generally detrimental to quality, especially in the presence of RE, and resulted in overall anthocyanin, AA, and AOX losses. Although both thermal pasteurization and HHP were detrimental to juice quality, high pressure resulted in the greatest loss of these attributes. Since conditions of juice extraction were not severe enough to inactive residual enzymes, their presence likely influenced phytochemical and quality retention. The presence of quinones in solution may have been responsible for co-oxidation of anthocyanins and AA that were formed via enzymes such as polyphenol oxidase. Without added AA, total anthocyanin decreases were equal between all treatments but in the presence of AA anthocyanin processing conditions influenced degradation reactions. AA fortification did not affect AOX values during thermal pasteurization but HHP lead to a 25% overall decrease, implicating dynamic changes in antioxidant compounds under high pressure in the presence of anthocyanins and AA or due to the influence of oxidase enzymes. Although physicochemical attributes were enhanced by copigmentation, methods to inactivate residual enzymes and prevent degradation of anthocyanins in the presence of anthocyanins prior to processing should be addressed.

**Ellagic Acid and Flavonoid Antioxidant Content of Muscadine Wine and Juice**

S.T. Talcott and J.H Lee

Antioxidant properties of flavonoids and ellagic acid were characterized in eight wines and juices produced by various processing methodologies from red and white muscadine grape cultivars (Vitis rotundifolia). Juices and wines were produced by hot and cold-pressed techniques and additional wine produced following on-hull fermentation for 3, 5, and 7 days. Chromatographic conditions were developed to simultaneously separate anthocyanins, ellagic acid, and flavonols and correlated to a measurement of overall antioxidant capacity (AOX), and their changes monitored after storage for 60 days at 20 and 37 °C. Regression coefficients between concentrations of individual polyphenolics and AOX ranged from 0.55 for ellagic acid to 0.90 for kaempferol. Both red and white wines had higher AOX values after storage than juices made from an identical grape press, despite lower concentrations of individual polyphenolic compounds. Red wines fermented on-hull had higher initial concentrations of antioxidant polyphenolics as compared to a corresponding hot-pressed juice, but changes in AOX during storage were more affected by time than by storage temperature despite lower concentrations of flavonoids and ellagic

acid present at 37 °C compared to 20 °C. Oxidative or polymerization reactions significantly decreased levels of monomeric anthocyanins during storage with greatest losses observed for delphinidin and petunidin 3,5-diglucosides. Processing methods for muscadine wine and juice production were important factors influencing concentrations of antioxidant flavonoids and ellagic acid, while the role of fermentation and time had the greatest influence on retention of AOX properties during storage.

**Ellagic Acid and Ellagitannins Affect on Sedimentation in Muscadine Juice and Wine**
Joon-Hee Lee and Stephen T. Talcott
A mechanism for the formation of water-insoluble sediments in wines and juices made from red and white muscadine grapes (Vitis rotundifolia) was investigated as a function of processing methodology and storage. Sediments are considered quality defects in muscadine grape products, and may influence consumer acceptability and expansion of retail markets. Processing regimes included both hot (70 °C) and cold (25 °C) press techniques for wine or juice production and fermentations in contact with grape skins for 3, 5, and 7-days. Relationships between free ellagic acid (FE), total ellagitannins (ET), and total ellagic acid (TE) concentrations were evaluated initially in each product and in sediments that formed during storage for 50 and 120 days at 20 °C. Processing techniques influenced initial concentrations of these compounds and the extent of sediment formation. Following storage, juices generally had higher concentrations of FE in sediments compared to wines, but sedimentation was independent of initial FE or TE concentrations. Decreases in ET were observed for hot-pressed juice and skin-fermented wines after storage indicating their hydrolysis during storage and possible contribution to FE in sediments. However, quantitative analysis of the collected sediments revealed that no more than 12% FE by weight was actually present in the sediments, the remainder consisting of either unidentified compounds or conjugated forms of ellagic acid. This work elucidated a potential mechanism for the presence of FE in muscadine wine and juice sediments through ellagitannin hydrolysis and suggests that sedimentation from mechanisms other than ellagic acid precipitation may also contribute to wine and juice quality.

**Fruit Maturity and Juice Extraction Influences Ellagic Acid Derivatives and Other Antioxidant Polyphenolics in Muscadine Grapes**
Joon-Hee Lee and Stephen T. Talcott
Polyphenolic compounds including ellagic acid, ellagic acid derivatives, and anthocyanins were characterized in eight muscadine grape (*Vitis rotundifolia*) cultivars and evaluated for antioxidant capacity as influenced by two ripening stages and their location within the fruit (skin, pulp and juice). Methanolic extracts were used for quantification of each polyphenolic and antioxidant capacity, while ellagic acid and its glycosides were partitioned into ethyl acetate from hot-pressed juice and from extracts of skin and pulp. Novel chromatographic conditions were developed to characterize and quantify free ellagic acid, two ellagic acid glycosides, total ellagic acid, and anthocyanidins before and after acid hydrolysis. All polyphenolics generally increased as fruit ripened and the highest concentrations were located in the skins. Free ellagic, ellagic acid glycosides, and total ellagic acid ranged from 8-162, 7-115, and 587-1900 mg/kg respectively in the skin of ripe grapes, which was 82-87% of the total present in whole grapes. Hot-pressed juices contained considerably lower polyphenolic concentrations than were present in whole grapes with actual recovery varying widely among cultivars. Five anthocyanidins were present in each cultivar in variable concentrations (delphinidin > petunidin > malvidin+peonidin > cyanidin) and those with *o*-diphenolic substitutions, generally responsible for poor color stability, accounted for

78-96% of the total in fresh grapes and from 67-100% in juice. Antioxidant capacity was appreciably influenced by cultivar, maturity, and location in the fruit with good correlations to soluble phenolics found in both methanolic and ethyl acetate extracts (r=0.83 and 0.92, respectively). Ethyl acetate extracts, containing primarily flavonol glycosides and phenolic acids, contributed 12-29, 22-83, and 6-15% to the total antioxidant capacity present in the skin, pulp, and juice, respectively and indicated the high antioxidant content of anthocyanins. The diversity and antioxidant capacity of muscadine grape polyphenolics can provide useful information for the development of muscadine grape juice blends for desired quality and polyphenolic content.

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

Muscadine grapes are an important fruit crop in the southern United States with a unique phytochemical composition due to their ellagic acid, ellagic acid glycosides and ellagitannin composition. These compounds have been related to potential health-promoting benefits and exhibit good antioxidant properties. Limited data exists on these components of muscadine grapes and their resultant antioxidant capacity (AOX). These attributes were examined in 8 grape cultivars as influenced by two ripening stage and their location within the fruit (skin, pulp and juice). Ellagic acid and its glycosides were partitioned into ethyl acetate from hot-pressed juice and from extracts of skin and pulp. Solvent was evaporated and re-dissolved in citric acid buffer. Novel chromatographic conditions were developed to characterize and quantify free ellagic acid, two ellagic acid glycosides in ethyl acetate extracts and total ellagic acid and anthocyanidins in methanolic extracts (following acid hydrolysis) by HPLC. AOX was determined on these same isolates using the oxygen radical absorbance capacity assay (ORAC). Generally, evaluated phytochemicals increased as fruit ripened, with highest concentrations located in the skins. Free ellagic acid ranged from 8-162 and <0.5 –24.5 mg/kg while ellagic acid glycosides ranged from 6.8-115 and <0.5-12.8 mg/kg in the skin and pulp of mature grapes, respectively. Total ellagic acid varied from 587-1,900 mg/kg in skin and <0.5-455 mg/kg in pulp of mature grapes. Hot-pressed juice contained considerably less of each compound and yielded on average 38, 65, 56 and 46% of free ellagic acid, two ellagic acid glycosides and total ellagic acid, respectively, in juice of ripe fruits. Five anthocyanidins were separated and quantified in various ranges of each fraction from ripe fruits. AOX varied with cultivar and maturity and showed good correlations to the amounts of total soluble phenolics in both methanolic and ethyl acetate extracts (r=0.83 and 0.92, respectively). Results show the diversity of phytochemical composition in muscadine grape varieties and related changes associated with maturity and location within the fruit and data can be used to develop novel blending scheme to produce desired quality products.

## EXTENSION ACTIVITIES

### Mid-Florida Research and Education Center

Dennis Gray

Receives approximately two 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.

**Department of Food Science and Human Nutrition**

Charlie Sims
Produced “Best Practices for Florida Wines” UF Extension Bulletin SP 316.

Bob Bates
Retire with emeritus status 6/30/03. Responds to grower and winemakers. Co-author on “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
Plant Disease Clinic Monday & Friday 9-4

Max Griggs - Escambia County