

**SERA-14 Information Exchange Group Progress Report 2007
GRAPE RESEARCH AND EXTENSION ACTIVITIES
UNIVERSITY OF FLORIDA
INSTITUTE OF FOOD AND AGRICULTURAL SCIENCES (IFAS)**

MID-FLORIDA RESEARCH AND EDUCATION CENTER

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In 2006, we obtained USDA APHIS approval for field testing via the “notification system”. In January and April 2007, respectively, transgenic vines were established in the field at the University of the Virgin Islands and on site at the Mid-Florida Research & Education Center. In the Virgin Islands, the vines all contained a gene called VVTL-1 that was recovered originally from grape and then re-engineered for increased expression before insertion back into *Vitis vinifera*. In Florida, the same vines were planted. However, the predominance of vines in Florida contain proprietary antibacterial lytic peptide genes that will be evaluated for Pierce’s disease resistance.

While greenhouse testing showed exceptional resistance among the transgenic lines, we do not yet have data for the field, which is a more demanding environment. We expect to have results for fungal disease first, but PD evaluation will take several years.

Most of the transgenic plants are from the variety Thompson Seedless. However, transgenic Merlot, Seyval Blanc and Syrah (Shiraz) are either in the field or greenhouse testing phases. In addition, a number of other genes and genetic elements are under development for grape and should have application in other crops as well. We continue to publish and seek patent protection for new inventions. Patents are needed to establish value of the technology and might generate the amount of financial investment needed to develop and commercialize transgenic grapevines. Publications and patents are listed below.

Publications (2006/2007)

Journal articles:

Li, Z.T., S. Dhekney, M. Dutt, M. Van Aman, J. Tattersall, K.T. Kelley and D.J. Gray, Optimizing *Agrobacterium*-mediated transformation of grapevine, *In Vitro Cell. Dev. Biol. Plant.* 42, 220-227, 2006.

Dhekney, S.A., Z.T. Li., M. Van Aman, M. Dutt, J. Tattersall, and D.J. Gray, Genetic transformation of embryogenic cultures and recovery of transgenic plants in *Vitis vinifera*, *Vitis rotundifolia* and *Vitis* hybrids, Proc. 2005 Int. Symp.

Biotechnol. Temperate Fruit Crops & Tropical Species, ACTA Hort. 738, 743-748, 2007.

Dutt, M., Z.T. Li, K. Kelley, S.A. Dhekney, M. Van Aman, J. Tattersall, and D.J. Gray, Transgenic rootstock protein transmission in grapevines, Proc. 2005 Int. Symp. Biotechnol. Temperate Fruit Crops & Tropical Species, ACTA Hort. 738, 749-753, 2007.

Li, Z.T., S.A. Dhekney, M. Dutt, M. Van Aman, J. Tattersall, K. Kelley and D.J. Gray, Isolation and characterization of the 2S albumin gene and promoter from grapevine. Proc. 2005 Int. Symp. Biotechnol. Temperate Fruit Crops & Tropical Species, ACTA Hort. 738, 759-765, 2007.

Dutt, M., Z.T. Li, S.A. Dhekney and D.J. Gray, Transgenic plants from shoot apical meristems of *Vitis vinifera* Thompson Seedless via *Agrobacterium*-mediated transformation. Plant Cell Rep. (in press).

Abstracts:

Li, Z.T., S. Dhekney, M. Dutt, M. Van Aman, J. Tattersall, K.T. Kelley and D.J. Gray, Optimizing *Agrobacterium*-mediated transformation of grapevine, In Vitro Cell. Dev. Biol. Plant. 42, 220-227, 2006.

Dhekney, S.A., Z.T. Li., M. Van Aman, M. Dutt, J. Tattersall, K.T. Kelley, and D.J. Gray, Disease resistant transgenic grapevine constitutively expresses *Vitis vinifera* thaumatin-like protein. 2006 Soc. In Vitro Biol. Ann. Meeting, Abstract Supplement, 2006.

Gray, D. J., Z.T. Li, S. A. Dhekney, M. Dutt, M. Van Aman, J. Tattersall and K. T. Kelley, Screening disease resistant transgenic grapevines for field tests. 2006 Soc. In Vitro Biol. Ann. Meeting, In Vitro, 42, 20A, 2006.

Dutt, M., D. J. Gray, Z. T. Li, S. Dhekney and M. M. Van Aman. Micropropagation cultures for genetic transformation of grapevine. HortScience 41(4), 972A, 2006.

Dutt, M., Z. T. Li, S. Dhekney and D. J. Gray. Characterization of a composite promoter from genomic sequences of grapevine. HortScience 41(4), 1053A, 2006.

Gray, D. J., Z.T. Li, S. A. Dhekney, M. Dutt, M. Van Aman, J. Tattersall and K. T. Kelley, Screening disease resistant transgenic grapevines for field tests. 2006 Soc. In Vitro Biol. Ann. Meeting, In Vitro, 42, 20A, 2006.

Gray, D. J., Z.T. Li, S. A. Dhekney, M. Dutt, D. L. Hopkins, T. W. Zimmerman, Field testing of transgenic grapevine for bacterial and fungal disease resistance.

2007 Annual Meeting of the American Society for Horticultural Science, HortScience 42:858, 2007.

Dhekney, S.A., Z.T. Li., M. Dutt, T. W. Zimmerman, and D.J. Gray, Greenhouse Screening and Field Testing of Transgenic Grapevine for Fungal Resistance. 2007 Annual Meeting Society for In Vitro Biology, In Vitro, 43, S40, 2007.

Patents:

Gray, D. J. and R. Scorza, Disease resistance in *Vitis*, European Patent No. 1138767, 2006.

Gray, D. J., J. Subramanian, and R E. Litz, Regeneration system for grape and uses thereof. Australian Patent Ser. No. 2002301408, 2006.

Li, Z. and D. J. Gray, Bi-directional dual promoter complex with enhanced promoter activity for transgene expression in eukaryotes, US Patent 7,129,343, 2006.

Scorza, R. and D. J. Gray, Disease resistance in *Vitis*, US Patent No. 7,151,203 B2, 2006.

Scorza, R. and D. J. Gray, Disease resistance in *Vitis*, Argentine Patent No: AR007631B1, 2006.

Subramanian, J, and D. J. Gray, Pathogen resistant grape plants. US Patent No. 6,995,015, 2006.

Grape Pathology/Pierce's Disease Research
D. L. Hopkins

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

The overall goal of this project is to develop a biological control system for Pierce's disease (PD) of grapevine that would allow the production of *V. vinifera* in areas where PD and the sharpshooter vectors are endemic. A biological control strain of *X. fastidiosa*, EB92-1, has already successfully controlled Pierce's disease in *Vitis vinifera* Cabernet Sauvignon for 10 years in the MREC vineyard and is currently being evaluated in commercial vineyards in Florida. This biocontrol is currently being evaluated in tests in commercial vineyards in different geographical locations and in various genotypes of grapevine. These tests in Florida include *V. vinifera* cultivars that may be adapted to warm climates such as 'Barbera', 'Muscat', 'Ruby Cabernet', 'Chambourcin', 'Chardonnay' and 'Chenin Blanc' and American species or hybrid cultivars (Eastern grapes) such as 'Cynthiana' and 'Chambourcin.' At least two replicated tests each with Merlot and Chardonnay are planned for vineyards in California. In Georgia, biocontrol tests will be conducted in several commercial vineyards that have PD problems. Cultivars in Georgia will include 'Mourvedre', 'Cabernet Franc', and 'Vidal Blanc.'

Tests in South Carolina include ‘Seyval Blanc’, ‘Neptune’, ‘Jupiter’, ‘Mars’, and ‘Venus.’

The successful completion of the proposed research could lead to an effective control of Pierce’s disease and other diseases caused by *X. fastidiosa* that is environmentally friendly. Since the biocontrol strains would be used in areas in which *X. fastidiosa* occurs and causes disease, there should be no problem associated with the utilization of this biological control method. The strains utilized in this study are naturally occurring and are not genetically modified in any way. This project should yield results within the next 5 years and if the control is successful, there should be a biological control for Pierce’s disease available for commercial use in vineyards.

Publications

Refereed Journal Articles:

Reddy, J.D., S.L. Reddy, D.L. Hopkins, and D.W. Gabriel. 2007. ToIC is required for pathogenicity of *Xylella fastidiosa* in grapevine. *Mol. Plant-Microbe Interact.* 20: 403-410.

Hopkins, D. L. 2005. Biological control of Pierce’s disease in the vineyard with strains of *Xylella fastidiosa* benign to grapevine. *Plant Dis.* 89:1348-1352.

Abstracts:

Hopkins, D. L. 2007. Biological Control of Pierce’s Disease (*Xylella fastidiosa*) in Various Grape Genotypes. *Proc. Of 11th Int. Conf. Plant Path. Bacteria*, p 169.

GRAPE RESEARCH AT THE NORTH FLORIDA RESEARCH AND EDUCATION CENTER

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Grape research at the NFREC-Quincy has included variety improvement, culture and management and physiology. More recently, the plant-insect-disease interactions associated with Pierce’s disease has been a major focus. Pierce’s disease is caused by the bacterium, *Xylella fastidiosa* and is vectored by leafhoppers. Both *X. fastidiosa* and the leafhopper vectors feed exclusively on xylem fluid. Pierce’s disease precludes the culture of European grapes (*Vitis vinifera*) in the southeastern United States. There is no cure of Pierce’s disease and control of the vector is impracticable because of its ubiquitous nature. Since 1989 we have been investigating the pivotal role of xylem fluid chemistry on the

behavior and performance of this bacterium and the primary leafhopper vector, *Homalodisca vitripennis* (formerly known as *Homalodisca coagulata*). Xylem fluid is over 95% water and is the most dilute food source encountered by herbivores. The adaptations of the leafhopper to subsist on xylem fluid include extremely high feeding rates, ammonotelism, an unprecedented assimilation efficiency of organic compounds and the selection of host plants diurnally and seasonally with the highest nutrient content. Pathogenesis occurs as xylem vessels become plugged with bacteria and bacteria by products (biofilm). The bacterium shows a different pattern of planktonic growth, aggregation and biofilm formation depending on the chemistry of xylem fluid or growth media.

Publications:

Andersen, P.C., B.V. Brodbeck, R.F. Mizell III and S. Oden. 2005. The abundance and feeding of *Homalodisca coagulata* (Hemiptera: Auchenorrhyncha) on *Vitis* genotypes in north Florida. *Environ. Entomol.* 34(2):466-478.

Andersen, P.C. 2006. Performance of 20 muscadine grape cultivars in north Florida. *J. Amer. Pomol. Soc.* 60 (3):129-135.

Andersen, P.C., B.V. Brodbeck, S. Oden, A. Shriner and B. Leite. 2007. Influence of xylem fluid chemistry on planktonic growth, biofilm formation and aggregation of *Xylella fastidiosa*. *Current Microbiol.* (in press).

Alves, E., B. Leite, R.C. Marucci, S.F. Pascholati, J.S. Lopes and P.C. Andersen. 2007. Retention sites for *Xylella fastidiosa* in four sharpshooter vectors (Hemiptera:Cicadellidae) by scanning electron microscopy. *Current Microbiology* (submitted).

Brodbeck, B.V., P.C. Andersen, S. Oden and R.F. Mizell III. 2007. Preference-performance linkage of the xylem feeding leafhopper, *Homalodisca vitripennis* (Hemiptera: Cicadellidae). *Environ Entomol.* (submitted).

Mizell, R.F. III, C. Tipping, P.C. Andersen, B.V. Brodbeck and W. Hunter. 2007. A behavior model for the glassy-winged sharpshooter, *Homalodisca vitripennis*. *Environ Entomol.* (submitted).

Shriner, A.D. and P.C. Andersen. 2007. The effect of oxygen on the growth and biofilm formation of *Xylella fastidiosa*. *Appl. and Environ. Microbiol.* (in progress).

Shriner, A.D. and P.C. Andersen. 2007. Media component effects on growth and biofilm formation of *Xylella fastidiosa*. *Current Microbiol.* (in progress).

Leite, B., P.C. Andersen, M.L. Ishida, B.V. Brodbeck, L. Marques, M.E. Olson and M.R. Braga. 2007. Calcium bridging is critical for *Xylella fastidiosa* aggregation. *Physiol. And Molec. Plant Pathol.* (in progress).

Mizell, R.F. III and P.C. Andersen. 2007. Trap response of *Homalodisca vitrapennis*: color, size and sensitivity. Environ. Entomol. (in progress).

FOOD SCIENCE AND HUMAN NUTRITION DEPARTMENT, GAINESVILLE

Evaluation of Grape Varieties and Lines for Wine Potential in Florida – 2006 *Charlie Sims*

This project established a wine evaluation program designed to produce wine on a small scale from promising cultivars and genetic lines, evaluate this wine, and disseminate this information to growers and the wine industry. Wines were made from several promising cultivars/lines starting summer 2006 using a standardized procedure. Wines were subjected to evaluation by researchers and winemakers familiar with Florida grapes and wines. Findings from this research will be presented at the annual FGGA meeting and reports will be produced and distributed. This work is intended to be on-going (wines have already been produced from the 2007 harvest) and will help expand and improve the grape varieties available for wine production in Florida.

Wines were produced from 10 breeding lines and varieties during the 2006 season and evaluated. All 3 white bunch grape wines (Stover, Chardonnay, CB 81) had good quality and similar ratings. The red bunch grape wines from Chambourcin and Cynthiana also had good sensory ratings, although Cynthiana had a very high acidity. The breeding line W 1662 seemed to have less potential for a high quality wine. The two muscadine breeding lines that are being considered for release as new cultivars produced acceptable wines, although AA11-68 was not rated as high.

Results are needed for more than one growing season to better predict wine potential, and additional lines and varieties need to be evaluated. Also, all of these varieties and lines are still young vines and changes in quality may occur as the vines mature. Several wines are currently being produced from the same and additional varieties and lines from the 2007 season.

Enology, Grape Processing & Utilization – *Charlie Sims & Bob Bates*

The continuing goal is to optimize and popularize the beneficial properties of Florida grapes, grape products, and byproducts. There are now over 20 Florida wineries in operation with several more on the drawing board. An additional 100 acre wine grape vineyard and another machine harvester are now operational in state. We work quite closely with established and new 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 well over 1,000

commercial wines from U.S. and international wineries, as well as several hundred hobby wines from Florida residents.

These activities result in increased membership and participation in the FGGA, greater appreciation of local commercial wines, a growing demand for Florida grapes cultivars for planting and winemaking, and interest in grape activities and wines in other Southeast states.

Grape Phytochemical Research ***Sue Percival***

In collaboration with Mount Sinai Medical College, we are looking at how red wine (both Cabernet Sauvignon and Muscadine) and compounds derived from these wines play a role in preventing cognitive dysfunction associated with Alzheimer's disease (AD). Using Tg2576 mice, a transgenic mouse that shows AD-type amyloid beta-protein (A β) neuropathology (brain tangles), we tested whether moderate consumption of Cabernet Sauvignon modulates AD-type brain tangles and the deterioration in cognitive ability. We found that Cabernet Sauvignon significantly reduced AD-type deterioration in cognitive ability (spatial learning function) and brain tangles relative to control Tg2576 mice that were treated with either a comparable amount of ethanol or water alone. Chemical analysis showed the Cabernet Sauvignon used in this study was very low in resveratrol (0.2 mg/L), 10-fold lower than the minimal effective concentration shown to promote A β clearance in vitro. This study supports epidemiological evidence indicating that moderate wine consumption, within the range recommended by the FDA dietary guidelines of one drink per day for women and two for men, may help reduce the relative risk for AD clinical dementia.

Publication:

Wang, Ho L, Zhao Z, Seror I, Humala N, Dickstein DL, Thiyagarajan M, Percival SS, Talcott ST, and Pasinetti GM. Moderate consumption of Cabernet Sauvignon attenuates A β neuropathology in a mouse model of Alzheimer's disease. *FASEB J.* 2006 Nov;20(13):2313-20.

Between 1998 and 2004, FGGA in association with the VAC provided funds to initiate health benefits of grape and grape products to Susan S. Percival at the University of Florida. The data derived from some of these studies was used to develop a USDA grant which was funded from 2001-2004. The publications from this work caught the eye of Drs. Pasinetti and Ho at Mount Sinai Medical School of Medicine (MSSM) and we began a collaboration on the influence of grapes and grape products on the development of Alzheimer's Disease. Subsequently, Dr. Pasinetti developed a Center proposal to the National Institutes of Health which received a very high score and will likely be funded:

Center of Excellence for Research on Complementary and Alternative Medicine for Alzheimer's Disease with an Emphasis on Grape-derived Polyphenolic Compounds

Researchers across multiple academic disciplines at MSSM, Albert Einstein, Purdue University, UAB, in collaborations with scientists at Rutgers University, UCLA, University of Florida, and Texas A&M will establish a dynamic and interactive Research Center to critically examine the potential role of grape-derived polyphenolic compounds to prevent, attenuate and possibly to arrest Alzheimer's disease cognitive deterioration.

Grape Pigments and Phytochemical Research

Steve Talcott recently left the University of Florida for Texas A&M. It is hoped that he will continue his productive grape research, with value to the Southeast grape industry.

CITRUS RESEARCH AND EDUCATION CENTER, LAKE ALFRED

Russ Rouseff & Ozan Gurbuz (Visiting Scientist from Turkey)

This work is now completed with one publication:

Gurbuz, O.; Rouseff, J. M.; Rouseff, R. L., Comparison of aroma volatiles in commercial Merlot and Cabernet Sauvignon wines using gas chromatography - Olfactometry and gas chromatography - Mass spectrometry. J. Agric. Food Chem. 2006, 54, (11), 3990-3996.

EXTENSION ACTIVITIES

Mid-Florida Research and Education Center

Dennis Gray - Receives several calls per week from growers, hobbyists and potential new growers. Developed a website to provide help for growers (www.mrec.ifas.ufl.edu/grapes/grape-resources.asp). 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 – As Department Chair allocates resources and encourages grape programs.

Bob Bates - Retired with emeritus status 6/30/03. Still responds to grower and winemaker inquiries and judges state and regional wine competitions. Bates, John Mortensen, retired IFAS grape breeder, and Jaing Lu, Florida A&M are initiating a history of the Florida Grape Industry and the people behind these

developments. The narrative will include prominent individuals in other states who have contributed substantially to success of grapes in Florida. Both are on the FGGA Board of Directors.

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 six years; Plant Disease Clinic Monday & Friday 9-4; **Max Griggs** - Escambia County.