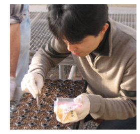
Spring 2014

VitisGen *Voice*

Mapping the way to the next generation of grapes

Third Annual VitisGen Meeting of Project Directors, Collaborators, and Industry Advisory Panel Members

The third annual VitisGen meeting of Project Directors, Collaborators, and Industry Advisory Panel Members was held February 20–21, 2014 at the New York State Agricultural Experiment Station in Geneva, New York. Over the span of the two-day meeting, a total of 41 VitisGen participants attended in person (including 15 Project Directors and 5 Industry Advisory Panel Members). The purpose of the meeting was to review the previous year's progress; to identify and prioritize action plans for the coming year; to begin discussions on the future of VitisGen; and to encourage continued industry collaborations and dialogue.

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Each VitisGen team (Breeding, Genotyping, Phenotyping, Trait Economics and Extension and Outreach) presented a report focused on their major accomplishments in years 2 and 3 and underscored upcoming plans. Discussion sessions were held after each team presentation to facilitate and encourage continued dialogue within the project and with the Industry Advisory Panel. In addition to team reports, guest speaker Dr. Ian Dry (CSIRO) gave a presentation on *"The use of molecular breeding techniques for germplasm improvement in the Australian Wine Industry."* The Industry Advisory Panel provided feedback and Project Directors, Collaborators, and the Advisory Panel discussed future plans and action items for the coming year. New this year, a poster session featured VitisGen related research, and presenters included project directors, postdoctoral associates, graduate students, and technicians from Cornell University, the USDA-ARS Grape Genetics Research Unit, and Missouri State University. ■

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Highlights from VitisGen Team Reports

Genotyping

The Genotyping team continued to make progress on the Genotyping by sequencing (GBS) pipeline. GBS was performed on individuals from 62 families and several diversity panels. SNP calling was completed using the new reference genome and 20k to 50k SNP markers were generated, per population. A tutorial was produced to demonstrate how this SNP data can be used to identify marker-trait analyses and the tutorial was distributed internally and used by project participants during the GBS data analysis workshop at the project meeting. The pipeline to produce 5,000 marker semi-automated genetic maps is being refined and will be applied to the core mapping populations.

Breeding

The Breeding team has maintained the core VitisGen populations. One core population was discarded during the year due to severe cold damage, but the population was replaced with two new populations. As a result, there are now 19 core populations. Almost 5,000 leaf samples were submitted to the genotyping center for SSR processing and resulting SSRs were used for marker-assisted selection at the USDA-ARS Parlier, the University of Minnesota, Florida A&M University, and Cornell University breeding programs. Phenotypic analysis of selected individuals confirmed the accuracy of the markers used. Finally, breeders arranged schedules and shipped back-up populations to E. & J. Gallo Winery and coordinated efforts to ship two more populations for back-up in 2014.

Phenotyping

The three phenotyping centers continue to receive plant materials from breeders and have completed trait evaluation on some of the core populations.

Powdery Mildew

The powdery mildew phenotyping center received leaf samples from six populations during the 2013 field season. With replicated datasets from multiple assays in hand, the team is currently evaluating the best populations and strategies for powdery mildew resistance phenotyping in 2014.

Low Temperature

The low temperature responses phenotyping center developed a method to transform chilling fulfillment data, which combines chilling units and rate of budbreak into one value. The center also completed chilling fulfillment and freezing tolerance phenotypes for three core populations over two years and returned the data to breeders.

Fruit Quality

The fruit quality center received fruit samples from eight populations, six were frozen for HPLC and spectrophotometry-based analyses for sugars, organic acids, yeast assimilable nitrogen, and low-molecular weight phenolics, and the remaining two sets were vinified to investigate tannin extractability. During the past year, the center also developed a new GC-TOF-MS method to phenotype off-odorants and a workflow for HPLC and spectrophotometry-based analyses.

Trait Economics

The trait economics team developed a survey for growers and producers to identify the growers' perspectives on top priority traits and their willingness to pay for specific traits, and also to estimate the cost savings from varietal innovations. Additionally, the trait economics team evaluated the returns to alternative varietal innovations for powdery mildew resistance. Outputs include:

- budgets for representative vineyards in California for wine, table grapes, and raisins
- industry-level analysis of powdery mildew management costs
- evaluation of environmental costs
- two working papers (see "Recent Publications", p. 6).

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VitisGen Focuses on Resistance to Most Important Disease in Grapes

Powdery mildew infections appear as gray or white "powder" on the surface of berries and leaves. Berry infections reduce the quality of the fruit at harvest.

Powdery mildew, *Erysiphe necator*, is the most common pathogen affecting *Vitis vinifera* grapes world-wide. This pathogen infects leaves and young berries, leaving the crop unusable for use as fresh fruit, raisins or for quality wine production.

Until 1845, powdery mildew was a minor pathogen, affecting only wild, North American varieties of grapes found east of the Rocky Mountains—but it was then discovered in Europe, in a glasshouse in Margate, England. Because it is easily spread by both wind and the distribution of plant material, by 1850 the pathogen had spread across Europe, with a 70% reduction in grapevine production seen in France and Germany alone.

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In the face of this massive loss of yield, growers turned to sulfur. Historically, sulfur had been used to treat plant fungal pathogens, and it was successfully deployed against grape powdery mildew. By 1858, sulfur dust was being used regularly to treat powdery mildew infections. This brought European grape and wine production levels back up to what they had been prior to the pathogen's discovery. By 1859, however, powdery mildew reached the California grape industry, and history repeated itself in North America.

Current data shows that growers can spend as much as 23% of their gross revenue just on powdery mildew control. In the U.S. alone, grape growers apply 18 million pounds of sulfur annually to manage powdery mildew. New fungicides have also been developed to manage powdery mildew in addition to sulfur. Because it can produce multiple generations of spores each year, powdery mildew is capable of developing resistance to many of these new fungicides if they are overused.

This problem is compounded by the fact that most of today's *Vitis vinifera* grape varieties are clonally propagated from a small pool of ancestors, limiting the available genetic diversity the plant has available to resist powdery mildew infections. The original

Continued on page 4.

"Highlights" (cont.)

Extension and Outreach

The extension and outreach team re-designed the project website, distributed its first project newsletter, and produced and published two short YouTube videos. The first video is "VitisGen—Breeding Crosses" featuring Cornell University's Bruce Reisch and his breeding program and the second is "VitisGen—Tracking Resistance", which highlights the powdery mildew phenotyping center. ■

VitisGen Vocabulary

Pathogen

Anything that can produce disease, such as virus, bacterium or fungus.

Phenotype

The detectable outward manifestation of a specific genotype. Phenotypes result from the expression of an organism's genes as well as the influence of environmental factors and the interactions between the two.

Powdery Mildew

A disease caused by many different species of fungi in the order Erysiphales. Infected plants display the distinctive symptoms of white or gray powdery spots on the leaves and stems, with the lower leaves most affected. Powdery mildew grows well in environments with high humidity and moderate temperatures and can result in significantly reduced crop yields. In an agricultural setting, the pathogen can be controlled using chemical methods, genetic resistance, and careful farming methods.

Resistance

The ability of an organism to defend itself against a disease.

"VitisGen Focuses" (cont.)

varieties were selected for attributes such as flavor or color, not for powdery mildew resistance, because the pathogen had not yet appeared in Europe. These grapes varieties are a long-established part of culture and tradition, and so there has been hesitation to accept new cultivars that are resistant to powdery mildew into the wine culture.

Because the ancestors of many of our modern grape cultivars were chosen long before powdery mildew appeared in Europe, they have had limited ability to develop resistance. Although significant genetic diversity exists in the broad *Vitis* family, consumers and growers have usually preferred certain varieties (e.g., Chardonnay, Riesling) and the result is limited genetic diversity in those genotypes.

VitisGen and Powdery Mildew Resistance

In VitisGen, grape breeders are using traditional breeding approaches to incorporate multiple powdery mildew resistance genes into new grape varieties. These varieties could potentially result in a dramatic reduction in fungicide applications, and thus reduce the overall costs and environmental impacts of grape production while still increasing yields and quality.

Consumers have significant name recognition and preferences for certain grape varieties (e.g., Concord, Chardonnay, etc.), however the current growing interest in locally produced, environmentally friendly agriculture may attract consumers to new varieties.

Identifying Powdery Mildew Resistance

Traditionally, the search for powdery mildew resistance has been a time and money consuming process. To make a cross between two different grape varieties, breeders collect pollen from one variety and transfer it to the flowers of a second variety. This alone can be a lengthy process: if the male flowers open later than the female ones, breeders have to collect the pollen, dry it, freeze it, and wait nearly a year to use it. Once the cross has been made, seeds are collected and

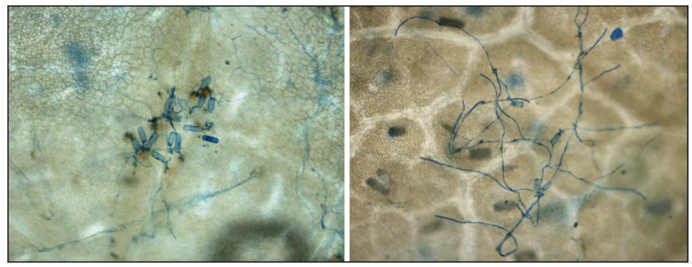
Powdery mildew infections on leaves can reduce photosynthesis, impacting the development of sugars and other compounds important for fruit quality and the health of the vine.

hundreds, or thousands, of offspring are grown. This population is tested for powdery mildew resistance, as well as favorable fruit quality characteristics, and candidates are selected for the next round of crossing (either back to one of the original parents or to another sibling with favorable qualities). This process continues over and over, year after year, until a plant is identified that excels in all the qualities that grape growers and consumers desire.

While this process has resulted in many grape varieties with favorable qualities, it can be difficult and time consuming to identify a characteristic, such as disease resistance, based solely on appearance. Sometimes a situation is obvious—for example, there is one healthy plant in a large group of sick ones. However, powdery mildew levels vary from year to year and from field to field, depending on temperature, humidity, and many other factors. A year with less powdery mildew pressure than usual may be a relief for a grape grower, but a frustration for a grape breeder—are plants with no powdery mildew in a specific year resistant or merely not exposed? Thus, grape populations are often grown for multiple years, and/or in multiple locations, before resistant selections can be made.

It can take over 30 years, in some cases, to develop a new grape variety, from the time the original cross is made to when the variety is publicly released. VitisGen is working to speed up and streamline this process by associating physical characteristics, such as disease resistance, fruit color or flavor, with specific molecular markers. These markers can be used by breeders early on in the evaluation of offspring from crosses to identify plants with advantageous characteristics, saving time, effort and money.

The phenotyping process actually begins with a few simple tools. Small leaf samples are harvested with a hole punch and then transferred to a controlled environment (otherwise known as a 9x13 glass baking dish), where they are each exposed to exactly the same amount of powdery mildew. Powdery mildew spores' are observed under a microscope to determine which individuals are resistant and which are susceptible. While this process is labor-intensive and time-consuming; it provides much more reliable observations than those from the field. (You can watch a video of this process on the Finger Lakes Grape Program's YouTube channel at http://www.youtube.com/watch?v=eFSqfL946j4).



Powdery mildew spores on leaf tissue from vines that are resistant (left) and susceptible (right) to infection. The presence of hyphae, the thin strands extending from the spores on the right, indicates that the fungus is infecting the tissue. Very few hyphae develop on the resistant tissue on the left

When resistant plants are identified, they can then be screened for potential molecular markers (specific genes or short sequences of DNA) associated with resistance. Once markers are identified, plant breeders can screen leaf samples from very young seedlings to determine if they should be used for further breeding crosses. No longer will they have to wait for years, painstakingly gathering powdery mildew observations in the field in unpredictably variable environmental conditions. No longer will they have to devote time, money, and vineyard space to growing thousands of susceptible offspring. VitisGen holds the promise to accelerate new variety development by decades, leaving everyone more time to enjoy the fruits of their labor. ■

Publications and Presentations

Recent Publications

Alston, Julian M., Fuller, Kate B., and Tumber, Kabir P. 2014. The Costs of Pierce's Disease in the California Winegrape Industry. *California Agriculture*. (In press.)

Alston, Julian M., Fuller, Kate B., Kaplan, Jonathan D., and Tumber, Kabir P. 2014. The Costs and Benefits of Pierce's Disease Research in the California Winegrape Industry. *Australian Journal of Agriculture and Resource Economics* 58, 1–21.

Fuller, Kate B., and Alston, Julian M. 2012. The Demand for California Winegrapes. *Journal of Wine Economics* 7(2), 192–212.

Fuller, Kate B., Alston, Julian M., and Sambucci, Olena S. 2013. "The Value of Powdery Mildew Resistance in Grapes: Evidence from California." Working Paper No. CWE-1401. Robert Mondavi Institute, Center for Wine Economics. Available from: http://vinecon.ucdavis.edu/publications/cwe1401.pdf

Sambucci *et al.* 2014. "The Costs of Powdery Mildew Management in California Grapes: an Estimate of Annual Pecuniary and non-Pecuniary Costs." Working Paper.

Presentations/Posters/Abstracts (2014)

Sacks G.L., Acree T.E., Kwasniewski M.T., Vanden Heuvel J.E., Wilcox W.F. 2013. The role of the vineyard in determining wine flavour chemistry. Proceedings of the 15th Australian Wine Industry Technical Conference, Sydney, Australia 2013.

Sacks, G.L. Studies of Wine Flavor Chemistry in Cool Climate US. 2013. *Australian Wine Research Institute Seminar Series*; Adelaide, Australia..

Sacks G.L., Acree T.E., Kwasniewski M.T., Vanden Heuvel J.E., Wilcox W.F. 2013. The effects of the vineyard on wine flavor precursor development. *15th Australian Wine Industry Technical Conference*, Sydney, Australia.

Sacks, G.L. Relating Grape Composition to Wine Composition. 2013. National Meeting and Exposition of the American Chemical Society (ACS). Indianapolis, IN, in *Abstracts of Papers of the American Chemical Society*: Vol. 246.

Sacks, G.L. Relating Grape Chemistry to Wine Chemistry. 2013. *American Chemical Society—AGFD Young Scientist Symposium*. Indianapolis, IN.

Ledbetter, C. Guest lecture and sampling of raisins bred strictly for fruit quality. 2013. Fresno Raisin Production Class, California State University, Fresno, CA.

Ledbetter, C. Current status of raisin breeding efforts at Parlier ARS. 2013. Board of Directors Meeting at Sun-Maid.

Fuller, Kate B. The Benefits of Certified Virus-Free Nursery Stock. 2013. Foundation Plant Services Annual Meeting, Davis, California.

Presentations/Posters/Abstracts (2014)

Ledbetter, C. Raisin breeding and evaluation efforts at ARS in Central California. 2014. Grape Day, Easton, CA.

Londo, JP and Hyma, KE. Evaluating wild grapevine germplasm with GBS: methods for conservation, preservation, genetics, and breeding. 2014. Allele Mining Workshop. PAG XXII, San Diego, CA.

Barba, P, Takacs, EM, Hyma, K, Sun, Q, Cadle-Davidson, LE, Reisch. Application of Genotyping-By-Sequencing in Crosses of Heterozygous Grapevines: Tools for Map Construction and Marker-Trait Association Testing. 2014. PAG XXII, San Diego, CA.

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