March 2019

National Clean Plant Network

NCPN Network News

Grapes Feature Edition

The beginning of NCPN and NCPN-Grapes

Members of the grape industry, along with the fruit and nut tree industry, were instrumental in founding the National Clean Plant Network in 2008. Representatives from nurseries, growers, wineries, land-grant universities, state regulatory agencies and USDA had been meeting since 2006 to organize and procure federal aid to maintain and improve existing clean plant programs for specialty crops. This was in response to eroded federal funding to state agricultural experiment stations, which in turn reduced funding to clean plant centers. At the time, it was the position of these institutions that practical service work did not serve their teaching and research mission. In addition, as faculty who worked on clean plant programs retired and were replaced by scientists with other interests, there was a risk of losing the body of knowledge and experience in managing clean plant programs.

The importance of clean plant programs may not always be apparent, especially if there is no major disease outbreak. This is a continuous challenge - clean plant programs tend not to be glamorous and can easily be taken for granted due to the simple fact that using clean planting material is a preventative measure to manage viral diseases, much like good sanitation or vaccination.

In grapes, the importance of clean plants became glaringly apparent in the 1950s when fanleaf degeneration was a serious concern. It was found that it could be controlled by using clean propagation material, which led to the formation of Foundation Plant Services (FPS) at the University of California, Davis in 1958.

FPS is currently the headquarters for NCPN-Grapes and manages a 30-acre foundation vineyard at Russell Ranch.

As is true for all the NCPN crop networks, NCPN-Grapes works to produce and maintain a clean, virus-tested source of foundation stocks, and distributes propagation material derived from clean stocks. For grapes, this means dormant woody cuttings, about 14 inches long with about 5 buds, as well as mist-propagated green-rooted cuttings.

Dormant woody grape cuttings are soaked to rehydrate them.

Small mist-propagated plants are generated more quickly than dormant woody cuttings because they are propagated from small diameter brush that is normally pruned off before vines are established in the foundation vineyard.

Grape viruses

Over 80 graft-transmissible agents (viruses, virus-like agents and phytoplasmas) are known to occur in grapes. Three of the more significant viruses in the United States, listed here, can greatly reduce yield and negatively affect fruit and wine quality, by reducing sugar content, affecting fruit color and increasing fruit juice acidity. The extent of the effects is dependent on many factors, including cultivar, rootstock, environment, and cultural practices. More information can be found on the NCPN-Grapes website http://ncpngrapes.org/.

Grapevine leafroll associated viruses (GLRaVs) are members of the family Closteroviridae; most of them are vectored by mealybugs and soft scales.

Grapevine fanleaf virus (GFLV) is a member of the family Secoviridae and is vectored by the dagger nematode Xiphinema index.

Grapevine red blotch virus (GRBV) is a member of the family Geminiviridae and transmitted by the three-cornered alfalfa hopper, Spissistilus festinus (Hemiptera: Membracidae) in experimental conditions.

Economic Benefits of Starting with Clean Planting Stock

The economic benefits of planting a vineyard with clean grape material has been studied and found to be enormous.

- In Napa County, California the economic costs of grapevine red blotch disease were estimated to be as high as $1,100/acre/year in 2015 dollars (or about $68,548 per hectare over a 25-year lifespan of a vineyard) in a scenario of high initial infection and high quality penalty (Ricketts et al., 2017). Roguing symptomatic vines and replanting with clean vines, derived from virus-tested stocks, should minimize losses if red blotch disease incidence is low to moderate (below 30%). A full vineyard replacement should be pursued if disease incidence is higher, generally above 30%. http://www.ajevonline.org/content/68/1/127

- Estimated costs of leafroll disease with no disease control ranged from $29,902 to $226,405 per hectare over a 25-year lifespan of a vineyard in California. If disease prevalence is between 5 and 10%, then roguing symptomatic vines, replanting with certified vines, and controlling for mealybugs can minimize losses to leafroll (Ricketts et al. 2015) http://www.ajevonline.org/content/66/2/138.

Grape Industry by the Numbers

Bearing acreage planted in the US: **1,000,700** (NASS 2017)

Grape fresh market value: **$1,576,519,000** (NASS 2017)

Grape processing (juice, wine, raisin) value: **$4,883,746,000** (NASS 2017)

Approximate number of clusters in a bottle of wine: **10**

Approximate number of clusters on a vine: **30**

Average yield per acre: **5-6 tons** (range 2 to 10 tons)

Most popular wine cultivars: Chardonnay, Cabernet Sauvignon, Pinot grigio/gris, Pinot noir, Sauvignon blanc, Merlot, Cabernet franc

Wine Export value: **$1.53 billion**

Top 5 wine export markets: European Union countries, Canada, Hong Kong, Japan, and China

Clean Plant Success Story: Blanc du Bois

Blanc du Bois is a hybrid bunch grape developed by crossing *Vitis vinifera* and native Florida grapes. It was bred in 1968 by University of Florida breeder John Mortensen specifically to be tolerant of Pierce's disease. This potentially devastating disease, caused by the bacterium *Xylella fastidiosa*, is especially challenging in the southeast. There is no treatment for Pierce's disease and the primary means of control is planting resistant or tolerant cultivars.

Blanc du Bois was bred to be tolerant to Pierce's disease, a devastating bacterial disease, especially in Southeastern US.

In Texas, Blanc du Bois showed great winemaking potential and researchers understood the value of starting with clean planting stock. Dr. Mark Black at the Texas AgriLife Extension Service submitted an 'Austin County, Texas' clone of Blanc du Bois (obtained from Cat Spring, Texas) to Foundation Plant Services in 2010. The original material tested positive for grapevine leafroll associated virus-3, and was successfully treated to eliminate virus in 2010 and planted in Russell Ranch Foundation Vineyard in 2013.

Clean Blanc du Bois planting stock has been distributed nationwide since 2014. Acreage of Blanc du Bois is on the rise and Texas leads in production. In 2017, Blanc du Bois ranked fifth in acreage of grape cultivars in Texas with 210 bearing acres. Now, new plantings of Blanc du Bois can all be sourced from certified material, leading to more productive and healthy vineyards.

The first commercial planting of certified Blanc du Bois, "Whitley Vineyards" in Montgomery, TX.

To learn more about Blanc du Bois, read Fritz Westover's article in *Wines and Vines* at: https://www.winesandvines.com/features/article/96941/Blanc-Du-Bois-Takes-Root.

The Pipeline: Progression of grape material from importation to vineyards

By law, all imported grapevines must be quarantined by a USDA-approved facility to prevent the introduction of foreign pests and diseases into the U.S. Scientists at Cornell University and the University of California at Davis hold permits to import grapevines. Foundation Plant Services (FPS) at UC Davis is the largest of these facilities and processes 50 to 100 foreign and domestic grapevine selections a year. The description below represents an ideal scenario of a foreign grape introduction being processed through the FPS pipeline from importation to State-certified material. However, each case is unique and there are many technical challenges that may delay the process.

Imports come to FPS through cultivar exchanges, breeders or an individual. We use the term *selection* to refer to grapevines in our collections rather than *cultivar* or *clone* because it signifies that the grape is from a single vine source. Since cuttings are harvested when the vine is dormant, most selections arrive at FPS between January and March.

The USDA National Plant Germplasm Quarantine Center was designed with measures such as small, compartmentalized greenhouse sections to isolate plants in quarantine. It is located remote from cropland in Beltsville, MD.

Prior to receiving the material at FPS, imported cuttings are visually inspected for pests and obvious pathogens at the USDA National Plant Germplasm Quarantine Center in Beltsville, MD. If they pass inspection, the canes are forwarded to FPS where they are inspected again by a county agricultural inspector. Canes are dipped in a fungicide, a pesticide and finally hot water (hot water dipping eliminates the phytoplasma Flavescence doree).

Next, the single-source vine canes are divided into two bundles; one is used for the woody index and one is used for propagation. For the woody index, dormant buds from the test canes (also known as the *candidate vine*) are chipbud grafted onto virus-sensitive indicator plants. The indicators will show disease symptoms if a pathogen was transmitted from the buds to the indicator.

A dormant bud from the candidate plant is chipbud grafted into an indicator plant. The indicator will show symptoms when it grows out over the next 18 months if a virus is present in the bud.

The indicator plants are planted in a field and observed several times over two growing seasons to see if they develop disease symptoms. Here the trunk of the cultivar LN-33 is being inspected for stem pitting symptoms in a destructive test that requires bark to be peeled away to see the phloem tissue underneath.

The Pipeline, continued

The bundle of canes for propagation is used to make plants for lab testing and tissue culture treatment. By December, these plants are often large enough to begin virus elimination therapy. Virus elimination is done using tissue culture techniques. More information about tissue culture, including a short video showing the meristem excision process, is available at http://fps.ucdavis.edu/fgrintroservices.cfm. In an effort to potentially shorten the release time, most selections are treated preemptively for virus elimination even before the results of virus testing are known. Also, to qualify for planting in the Russell Ranch foundation vineyard, selections must go through tissue culture for virus elimination even if virus tests are negative. This is to eliminate viruses that may not be known yet and for which there is no test.

Tissue culture for virus elimination requires an average of 7 months to grow from a piece of tissue <0.5 mm to a small plantlet about 6 cm.

Spring of the year after importation is the optimal time for another test: the herbaceous host index. Leaves and stems of the candidate vine are ground and inoculated to a panel of herbaceous indicator plants in the greenhouse. The indicator plants will show symptoms within a few weeks if the leaves of the candidate vine contained mechanically transmissible viruses, especially nepoviruses.

The herbaceous host index detects viruses that are mechanically transmissible. Four herbaceous host species are inoculated.

Meanwhile, a panel of qPCR tests is performed on the candidate vine. The panel includes tests for over 35 viruses, phytoplasmas and *Xylella fastidiosa* and is continually evaluated and improved to include the latest developments in grapevine virology. The panel of tests can be found at http://fps.ucdavis.edu/fgr2010.cfm. Members of the NCPN-G are world renown grapevine virologists and are the people responsible for discovering, characterizing, and testing technology for pathogen detection.

Lab testing is extensive. Over 35 pathogens are tested using state of the art technology.

Ground up leaf material from the candidate plant is rubbed onto Chenopodium amaranticolor. Symptoms will appear on the C. amaranticolor in 3 weeks if a mechanically-transmissible virus is present in the leaf material.

After two years of growth in the field, the results of the woody index are complete and test results from all the different tests are compiled and compared. If all tests are negative, FPS submits a letter to USDA requesting release from quarantine. By the time a selection is released and is planted in the foundation vineyard at Russell Ranch, at least three years have passed since importation. Now, the vines still need at least two years to grow onto the trellis system and become established before dormant canes are available.

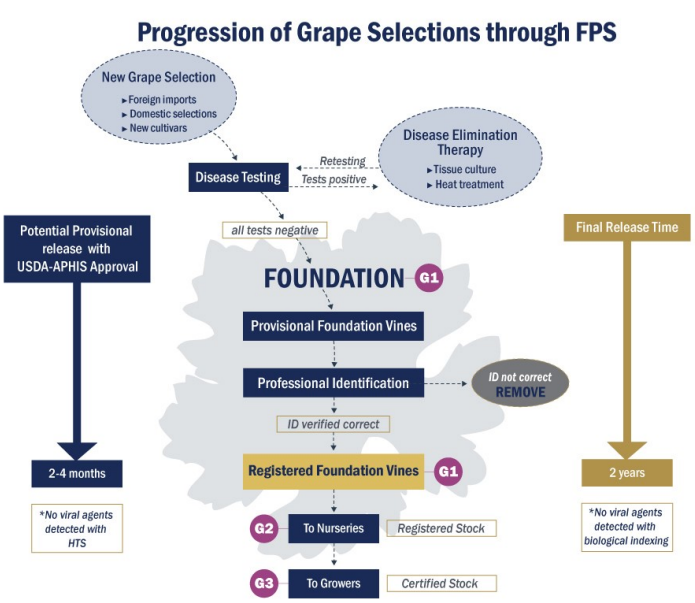
The Pipeline, continued

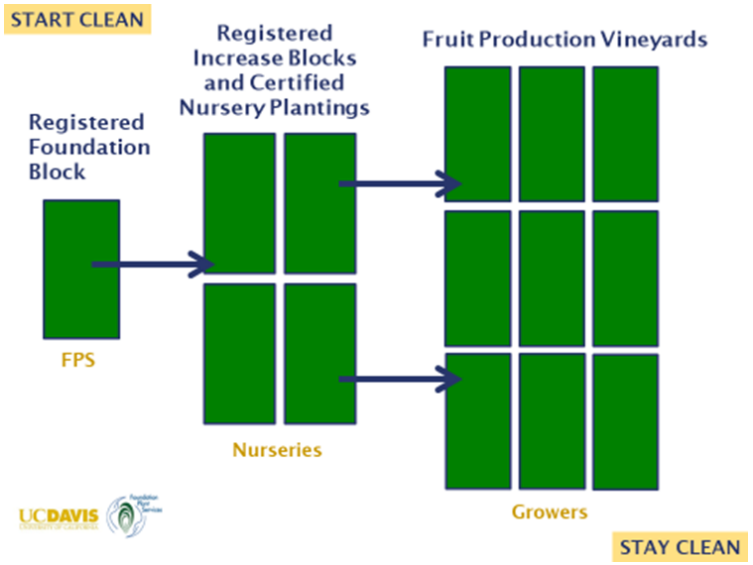
There are several methods employed to speed up the process. First, as mentioned earlier, therapeutics may be started before test results are complete. Secondly, FPS propagates green cuttings from plants before they are planted in Russell Ranch. This can reduce the time from import to availability to two years. Finally, high throughput sequencing (HTS) is used to test the plants. HTS is a powerful sequencing technology that can reduce the time required for testing to 2 to 3 months. More information is available on the HTS Factsheet on the NCPN website.

If a plant tests negative by HTS it can be released under *provisional quarantine* release status. Under provisional quarantine release status, nurseries may begin to propagate vines before the woody index is complete.

Approximately half of the selections introduced through FPS are found to be virus-infected. The virus-elimination treatment requires about one year and then testing is repeated to determine if the treatment was successful. Overall treatment success is over 87% but some virus infections are more difficult to eliminate than others. Treatment and subsequent testing may add three more years to the process.

The progression of domestic material through the pipeline is similar, with the exception of the dip treatments and USDA inspections.





Most foundation grapevine material is sold to nurseries to establish *registered increase blocks*. Increase blocks require another 3 to 4 years to mature. Nurseries harvest cuttings from their increase blocks to make dormant benchgrafts—plants that are grafted, rooted and dormant. It requires two years to produce a dormant benchgraft and they are grown in a block referred to as a *certified nursery planting*. From here, dormant benchgrafts are dug and sold to growers for the establishment of fruit production vineyards. Nursery blocks and plantings are tested and inspected by the CDFA on a scheduled and as-needed basis.

It's a long process. The time from when a selection is imported to when it is available to growers as clean, certified material can range from 10 to 20 years. Even so, demand for clean material remains high. Experience and economic studies have shown that planting with clean material greatly enhances the health of the vineyard and the fruit quality and quantity. The NCPN-Grapes centers continue to provide clean, virus-tested for the benefit of grape growers throughout the United States and enable the safe importation of varieties desired in the domestic marketplace.

A presentation entitled 'The Pipeline: From tissue culture to your vineyard' with more details and photos of each step was presented as part of a clean plant webinar series hosted by Cornell University. The presentation can be found on page 7 of the newsletter *Appellation Cornell* at http://ncpngrapes.org/Outreach_Materials/.

What does *certified* mean?

Certified means that the plant material has a been subjected to a comprehensive process established, authorized and performed by a State or other governmental entity to minimize the occurrence of regulated pests and diseases in planting stock. State-level certification focuses on testing for graft transmissible or systemic pathogens such as viruses.

Five states have grapevine certification programs: California, Missouri, New York, Oregon and Washington.

Common features of the programs:

- Participation is voluntary
- Registered blocks (foundations) are visually inspected and/or tested for significant grape pathogens as defined by the state
- Mechanism for tracking vines forwards and backwards
- Isolation requirements from commercial plantings
- Identity (true-to-type) requirements.

The California Grapevine Registration & Certification Program handles the highest volume of certified grapevine material. Best estimates, based on nursery self-reporting, are that approximately 50 million CDFA certified plants are sold annually. Regulations are described at:

Grapevine Registration and Certification Program from the California Dept. of Food and Agriculture
https://www.cdfa.ca.gov/plant/pe/nsc/nursery/grapevine.html

In New York, a certification program was reinstated in 2017. Nursery stock is tested for grapevine fanleaf virus, tomato ringspot virus, tobacco ringspot virus, grapevine leafroll-associated viruses and grapevine red blotch virus. A presentation describing the program can be found at:

Clean Plants for the Future of the Eastern Wine and Grape Industry from Cornell University
https://grapesandwine.cals.cornell.edu/sites/grapesandwine.cals.cornell.edu/files/shared/March%2031%20Clean%20Plants%20Slides.pdf

In Missouri, a grape nursery stock virus disease certification program was started by the Missouri Department of Agriculture in 1989. The regulations for this program can be found at the following link:

Missouri Plant Law Rules from the Missouri Dept. of Agriculture
https://www.sos.mo.gov/cmsimages/adrules/csr/current/2csr/2c70-10.pdf

In Washington and Oregon, the *Grape Planting Stock Certification Programs* are being revised to coordinate with similar programs in other western states, through a stakeholder working group made up of state regulators, university researchers and the wine and grape industry. The process began in June 2016 and will result in harmonized rules (starting in 2019), making interstate shipping of grapevines easier while protecting the state from harmful pests and diseases of grapevines. More information is available at:

Grape Planting Stock Certification from Washington Dept. of Agriculture
https://agr.wa.gov/plantsinsects/plantcertification/certprograms/grapeplantingstockcertification.aspx

Acquiring Healthy Grape Plants from the Oregon Dept. of Agriculture
https://www.oregon.gov/ODA/shared/Documents/Publications/NurseryChristmasTree/AcquiringHealthyGrapePlants.pdf

The Story of Zinfandel

The source of a selection is often a fascinating story. An example of such a story is the discovery of the origin of the grape cultivar Zinfandel in Croatia, which is told in a book by Nancy Sweet available on the FPS website.

http://fps.ucdavis.edu/grapebook/winebook.cfm?chap=Zinfandel

Following several plant exploration trips around Croatia by scientists from the University of Zagreb, a potential candidate for a "Zinfandel" clone in Croatia was identified in 2002; only a few vines of that candidate clone remained in Croatia at that time. Samples from the candidate selection, known in Croatia as Crljenak kaštelanski, were evaluated using DNA technology in Dr. Carole Meredith's lab at UC Davis. The DNA profile of the Crljenak vines matched that of California's Zinfandel and Italy's Primitivo. Dr. Meredith arranged for dormant material to be shipped to FPS in 2002 for inclusion in the public foundation grapevine collection at UCD.

The original material was tested from 2003-2005 and found to have virus. A microshoot tip tissue culture selection was created in 2008 and qualified for the Russell Ranch Foundation in 2012 under the name Zinfandel 42.1. It is believed that the Croatian "Zinfandel clones" offer genetic diversity for the variety in California, having developed for such an extended time in another location. Zinfandel 42.1 was also returned to Croatia as a part of an exchange program to return the virus-tested material to its source country. The Croatian wine industry was revitalized by the attention received in the story of the discovery of the origin of Zinfandel and many new Croatian cultivars have been identified and developed into wines as a result.

NCPN-Grapes Tier 2

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A Look Ahead: *What's Happening in the Network*

- **March 10-15, 2019** Joint IOCV-IRCHLB Conference, Riverside, CA
- **March 20-21, 2019** NCPN Communications Initiative, Sacramento, CA
- **April 11-12, 2019** NCPN-Roses Tier 2 meeting at TAMU, College Station, TX
- **April 15-16, 2019** NCPN Berries Tier 2 meeting at Cornell, Ithaca, NY
- **April 17-18, 2019** NCPN Economics Initiative at Cornell, Ithaca, NY
- **April 22-23, 2019** Southern Plant Board, Nashville, TN
- **June 5-6, 2019** NCPN Communications Initiative, Sacramento, CA

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