The Pipeline: From tissue culture to your vineyard.

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Sr. Extension Associate
Cornell University

Joshua Puckett
Production Manager
Foundation Plant Services, UC Davis
Grapevine Viruses

Nepovirus
Grapevine fanleaf virus (GFLV)
Arabis mosaic virus (ArMV)
Tomato ringspot virus (ToRSV)
Tomato blackring virus (TBRV)
Grapevine chrome mosaic virus (GCMV)
Strawberry latent ringspot virus (SLRV)
Raspberry ringspot virus (RpRSV)
Blueberry leaf mottle virus (BLMoV)
Grapevine Bulgarian latent virus (GBLV)
Artichoke Italian latent virus (AILV)
Grapevine Tunisian ringspot virus (GTRV)
Peach rosette mosaic virus (PRMV)

Closterovirus
Grapevine leafroll-associated virus 1 (GLRaV-1)
Grapevine leafroll-associated virus 2 (GLRaV-2)
Grapevine leafroll-associated virus 3 (GLRaV-3)
Grapevine leafroll-associated virus 4 (GLRaV-4)
Grapevine leafroll-associated virus 7 (GLRaV-7)

Vitivirus
Grapevine virus A (GVA)
Grapevine virus B (GVB)
Grapevine virus D (GVD)
Grapevine virus E (GVE)
Grapevine virus F (GVF)

Foveavirus
Grapevine rupestris stem pitting-associated virus (GRSPaV)

Marafivirus
Grapevine fleck virus (GFkV)
Grapevine rupestris feathering virus (GRVFV)
Grapevine asteroid mosaic virus (GAMV)
Grapevine redglobe virus (GRGV)
Grapevine Syrah virus-1 (GSyV-1)

Geminivirus
Grapevine red blotch-associated virus (GRBaV)

Other viruses
Grapevine vein clearing virus (GVCV)
Grapevine berry inner necrosis virus (GBINV)
Grapevine Algerian latent virus (GALV)
Grapevine line pattern virus (GLPV)

No Cure!
Within-Vineyard Spread
# Grapevine Viruses

## Nepovirus
- Grapevine fanleaf virus (GFLV)
- Arabis mosaic virus (ArMV)
- Tomato ringspot virus (ToRSV)
- Tobacco ringspot virus (ToRSV)
- Tomato black ring virus (TBRV)
- Grapevine chrome mosaic virus (GCMV)
- Strawberry latent ringspot virus (SLRV)
- Raspberry ringspot virus (RPRSV)
- Blueberry leaf mottle virus (BLMoV)
- Grapevine Bulgarian latent virus (GBLV)
- Artichoke Italian latent virus (AILV)
- Grapevine Tunisian ringspot virus (GTRV)
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## Closterovirus
- Grapevine leafroll-associated virus 1 (GLRaV-1)
- Grapevine leafroll-associated virus 2 (GLRaV-2)
- Grapevine leafroll-associated virus 3 (GLRaV-3)
- Grapevine leafroll-associated virus 4 (GLRaV-4)
- Grapevine leafroll-associated virus 7 (GLRaV-7)

## Vitivirus
- Grapevine virus A (GVA)
- Grapevine virus B (GVB)
- Grapevine virus D (GVD)
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- Grapevine Syrah virus-1 (GSyV-1)

## Geminivirus
- Grapevine red blotch-associated virus (GRBaV)

## Other viruses
- Grapevine vein clearing virus (GVCV)
- Grapevine berry internal necrosis virus (GBINV)
- Grapevine Algerian latent virus (GALV)
- Grapevine fine pattern virus (GLPV)
How did they get here?
• Self Propagation
• Collection from production blocks
• Nursery collection from customers
Virus and Vector Identification started in ‘70s

- Collection in Winter
- Trueness to type
- Leaf symptoms not visible in dormant grapes
Grapes in the East

- 1800s to 1960s
  *Native Labrusca-type grapes dominant*

- 1940s to 1970s
  *French Hybrids widely planted*

- 1970s to present
  *New hybrids (Cornell, MN, Ark, Private)*

- 1980s to present
  *Widespread V. vinifera plantings in the East*
## Wine Grape Varieties Grown in the Finger Lakes and ‘North Country’

### Native American
- Concord
- Niagara
- Catawba
- Delaware
- Elvira
- Diamond
- Isabella
- Fredonia
- Dutchess
- Ives

### Interspecific Hybrids (‘French Hybrids’)
- Aurore
- Baco noir
- Dechaunac
- Seyval blanc
- Marechal Foch
- Leon Millot
- Chancellor
- Rougeon
- Chambourcin
- Cascade
- Colobel
- Chelois
- Vignoles
- Vidal Blanc
- Vincent
- Ventura

### Interspecific Hybrids (Geneva)
- Cayuga White
- Traminette
- GR-7 (numbered)
- Melody
- Chardonel
- Noiret (2006)
- Corot Noir (2006)
- Valvin Muscat (2006)

### Vitis vinifera
- Chardonnay
- White Riesling
- Cabernet Franc
- Pinot Noir
- Cabernet Sauvignon
- Gewurztraminer
- Merlot
- Lemberger
- Viognier
- Sauvignon Blanc

### Cold-Hardy (MN)
- Frontenac
- Frontenac Gris
- Marquette
- La Crescent

### Cold Hardy (Private)
- Brianna
- Eidelweiss
- LaCrosse
- St Pepin

### Interspecific Hybrids (Geneva)
- Cayuga White
- Traminette
- GR-7 (numbered)
- Melody
- Chardonel
- Noiret (2006)
- Corot Noir (2006)
- Valvin Muscat (2006)
Tomato Ringspot Virus

- DeChaunac

**Acreage**

Markets: 600
Ringspot virus: -72%

**Tonnage**

Markets: 3500
Ringspot virus: -85%
Tomato Ringspot Virus

- DeChaunac

Impact in Finger Lakes:
~2000 acres out of production

-72%

-85%
Leafroll in the Finger Lakes

V. *vinifera* cv. Cabernet franc (top), Chardonnay (bottom); Lemberger (right)
Leafroll in Replants in 2005

Fig. 6. Decrease in sugar content (in Brix°) in berries from vines with leafroll disease symptoms relative to healthy vines in 14 different vineyard blocks (A-N).
Crown Gall Disease on Grape

Initiated at grafts in nursery and vineyard

At base and disbudded sites of rootstocks
What is the National Clean Plant Network (NCPN)?

- A national network of clean plant centers, scientists, regulators and educators
- Focused on providing healthy planting stock of vegetatively propagated specialty crops to nurseries and growers
Participating Crops:

1. Fruit Trees
2. Grapes
3. Berries
4. Hops
5. Citrus

Others interested in joining

www.nationalcleanplantnetwork.org
Farm Bill 2008

NCPN - Section 10202:

- pathogen diagnosis and elimination
- existing federal/state clean plant centers
- clean plant material for industry
- consult with state DOAs and universities
- funding - $5 million/year x 4 years ($20m total)
NCPN Grapes Clean Plant Centers

WSU Prosser
UC Davis
Missouri State
Cornell
Florida A&M
NCPN Grapes Clean Plant Centers

Infrastructure Pipeline: From 2008 to Present

WSU Prosser
Cornell
Florida A&M
The Pipeline: From Introduction to Certified Stock

Imports

Domestic varieties

Clean Plant Centers

Phytosanitary Status Unknown

Unknown
Certification

Diagnostics & Therapy

- Diagnostic tests for 30 pathogens (ELISA, DNA)
- Woody Indexing (2 yr)
- Virus elimination (meristem culture 2 yr)
- Retesting
Certification

Diagnostics & Therapy

• Budwood/cuttings distributed
• 3-4 yr to full production
• Mist-propagated plants (earlier)

FPS Mother blocks

• Mist-propagated vines in greenhouse (1 Yr)
• Grafted, planted into field
• 2-4 years to full production
  (50 3-bud cuttings own rooted)
• Testing by NYS Dept Ag/Markets

Audit & Quality Control
Certification

Imports

Clean Plant Centers

Diagnostics & Therapy

Clean Plant

FPS Mother blocks

Nursery Mother blocks

Nursery Production Blocks

Vineyards

150 vines
X 50 2-3 bud
= 7,500 vines

Domestic varieties

Audit & Quality Control
Certification

Imports

Clean Plant Centers

Diagnostics & Therapy

Import to Commercial Vineyard: 10-17 years

Domestic varieties

Clean Plant

Nursery Mother blocks

3-4 YR

2-6 YR

3-5 YR

2 YR

Nursery Production Blocks

Vineyards

Audit & Quality Control
Expected Benefits of Certification

• **Viticultural Benefits**
  • Improved cleanliness of the planting material
  • Improved fruit quality
  • Improved yield
  • Improved productive lifespan of vineyards

• **Economic Benefits**
  • Reduced losses due to poor vine establishment
  • Increased profitability
  • Limited penalty for poor fruit production
  • Enhanced competitive edge
  • Enhanced reputation
Foundation Plant Services

The Components of Delivering Clean Grape Material

Josh Puckett
Production Manager
FPS - UC Davis
Foundation Plant Services:

- Produces, tests, maintains and distributes elite disease-tested plant propagation material
- Provides plant importation and quarantine services, virus testing and elimination
- Coordinates release of UC patented horticultural varieties
- Links researchers, nurseries, and producers

College of Agricultural & Environmental Sciences
New Grape Selection
- Foreign imports
- Domestic selections
- New varieties

Disease Testing

Retesting
Tests positive

Disease Elimination Therapy
- Tissue culture
- Heat treatment

All tests negative

FOUNDATION

Provisional Foundation vines

Professional Identification

ID not correct
Remove

ID verified correct

Registered Foundation vines

To Nurseries and Growers
Biannual Visual Inspection

“Every grapevine in a foundation block shall be periodically retested at least once every five years, by FPS, for grapevine fanleaf virus, tomato ring spot virus, and grapevine leafroll associated viruses.”

Primary Increase Block

Secondary Increase Block

Certified Nursery Planting

“Primary and secondary increase blocks shall be tested by the Department for grapevine fanleaf virus, tomato ring spot virus, and leafroll associated viruses at least once every five years.”

Pathogen Detection

- Herbaceous host indexing
- Woody (field) index
- ELISA
- RT-PCR
- High throughput sequencing (HTS)
Woody index Procedure

- Chipbud 2 buds each into minimum of 6 indicator plants per variety
  1. St. George
  2. Cabernet franc
  3. LN-33
- Plant in field
- Observe leaf and trunk symptoms
Woody index Procedure

- Disbud, wax, root and pot up indicator plants
- Chipbud 2 buds/plant, 6 plants/variety
Woody index Procedure

1. Cut buds off of dormant canes of candidate variety.
2. Cut matching notch in indicator plant.
3. Place candidate bud into notch.
4. Wrap with budding rubber bands.

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1. Cutting the indicator plant.
2. Cutting the buds off the canes.
3. Placing the candidate bud into the notch.
4. Wrapping the bud with rubber bands.
Woody index Procedure

- Harden off
- Read budtake
- Rub off candidate buds
- Plant in field
Read Indicator Symptoms

Cabernet franc

LN33

St. George
Herbaceous index Procedure

- Inoculate:
  - *Chenopodium amaranticolor*
  - *Chenopodium quinoa*
  - *Nicotiana clevelandii*
  - Cucumber ‘National pickling’
Dust herbaceous host indicators with carborundum powder
Inoculate indicators with ground leaf tissue with nicotine buffer
Rinse leaves with water
Herbaceous index Procedure

- Read symptoms between 7 days and 21 days post inoculation
Laboratory Diagnostics

ELISA

PCR
Pathogen Elimination
Meristem shoot tip culture

6-12 months

Cut to < 0.5 mm

Treated
2011 – 156 selections
2012 – 124 selections
2013 – 110 selections
2014 – 135 selections
2015 – 128 selections
From Treatment to Foundation

Test to confirm successful treatment
Note: The green arrow represents a best case scenario in which a grape introduction tests negative for all viruses and establishes rapidly in the vineyard.

The purple arrows represent best case scenarios in which tissue culture treatment successfully eliminates virus and the vine establishes rapidly in the vineyard.
Distribution of Tested Grape Material

**CUTTING:** Cuttings are usually rooted directly in the field or greenhouse. The proximal cut is flat and adjacent to the bottom bud, while the distal cut is slanted at an internode (approximately 5 buds per stick). Length is 16'-18".

**BUDSTICK:** Budsticks are typically used for chip budding onto already established rootstock or for bench grafting. The proximal cut is flat and adjacent to the bottom bud, while the distal cut is slanted at an internode (approximately 5 buds per stick). Length is 16'-18".

**GRAFTSTICK:** Graftsticks are typically used for top working established vines by cleft grafting or side whip grafting. Both cuts are flat and at the internode (approximately 5 buds per stick). Length is 16'-18".

<table>
<thead>
<tr>
<th>Size</th>
<th>#1 Size (1/4&quot;-3/4&quot;)</th>
<th>#2 Size (Approx 1/4&quot;)</th>
<th>#3 Size (Less than 1/4&quot;)</th>
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<tbody>
<tr>
<td>5 to 24 units/variety</td>
<td>$5.00 each</td>
<td>$4.75 each</td>
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<td>25+ units/variety</td>
<td>$3.00 each</td>
<td>$2.75 each</td>
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**Rooted, Ungrafted Grape Mist Propagated Plants (MPPs)**

Prepared year-round by custom order only. Delivery to be arranged. Estimate of delivery time will be provided.

Green cuttings rooted under mist and established in 4" pots.

MPPs are prepared on a first-come, first-served basis. Time required to prepare MPPs depends on time of year requested, the availability of source vines/mother plants and the number of other orders currently being prepared.

$4.50 each
Flowchart of Mist Propagated Plants (MPPs) from Foundation Plant Services to Nurseries to Growers

YEAR 1

Mist Propagated Plants (MPPs) Available at FPS
Spring to Fall

YEAR 2

At nursery

Nursery Grows and Propagates MPP; Plants in Increase Block

Winter
Grow MPP; continuously take cuttings and root (10 to 30 possible)

Spring
Plant 10 to 20 vines in Increase Block (IB)

Summer
Grow IB vines

YEAR 3

At nursery

California Registered Green - growing Benchgrafts Available

Winter
Harvest cuttings from IB, (10 to 20 cuttings/vine), make benchgrafts for sale

Spring
Continue to Grow IB vines

Summer
Harvest cuttings (50 to 100 cuttings/vine), make benchgrafts

YEAR 4

At nursery

California Registered Green - growing and Dormant Benchgrafts Available

Winter

Spring

1 plant
10 - 30 plants
80 - 450 plants
400 - 2,500 plants
New Russell Ranch Foundation Vineyard

established 2010, Davis California

October, 2012

October, 2015
1) Microshoot tip tissue culture therapy

2) Negative test results - long list of pathogens - index, herbaceous, ELISA and PCR tests (35+)
Qualification of Russell Ranch Foundation

Microshoot tip tissue culture therapy required

<table>
<thead>
<tr>
<th>Group</th>
<th>Pathogen</th>
<th>Symbols</th>
<th>ELISA</th>
<th>qPCR</th>
<th>PCR</th>
<th>Herb. Index</th>
<th>Woody Index</th>
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<td>Tomato ringspot virus</td>
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Key:
- X Test performed at FPS.
- X gen. = ELISA using generic antibody which detects GLRaV-4, 5, 6, 9 and Car. in a single test.
- qPCR= quantitative PCR= real time RT-PCR with TaqMan probe; PCR= will include RT-PCR for RNA viruses.

Negative test results - long list of pathogens - index, herbaceous, ELISA and PCR tests (35+)

* Newly identified viruses continue to be added
Russell Ranch Foundation Vineyard

Planted

- **2011**: 575 Vines, 208 selections
- **2012**: 569 Vines, 236 selections
- **2013**: 569 Vines, 314 selections
- **2014**: 809 Vines, 312 selections
- **2015**: 693 Vines, 355 selections
Russell Ranch Foundation

- 2010 Protocol selection # indicates source selection#
  Ex. Chardonnay 04.1 indicates 2010 Protocol Qualification of TC treated Chardonnay 04
- Most scion selections represented with two field planted vines.
- Most rootstock selections represented with 2-12 field planted vines depending upon popularity.
- Availability depends upon vigor of cultivar and development of the vine.

2011 ~ 30#1, 30#2, 20 #1
2012 ~ 15#1, 10#2, 10#3
2013 ~ 5#2, 10#3
2014, 2015 ~ Brush
Thank you!