Importance of Clean Plant Program for Grapevines

Cool Climate Oenology & Viticulture Institute

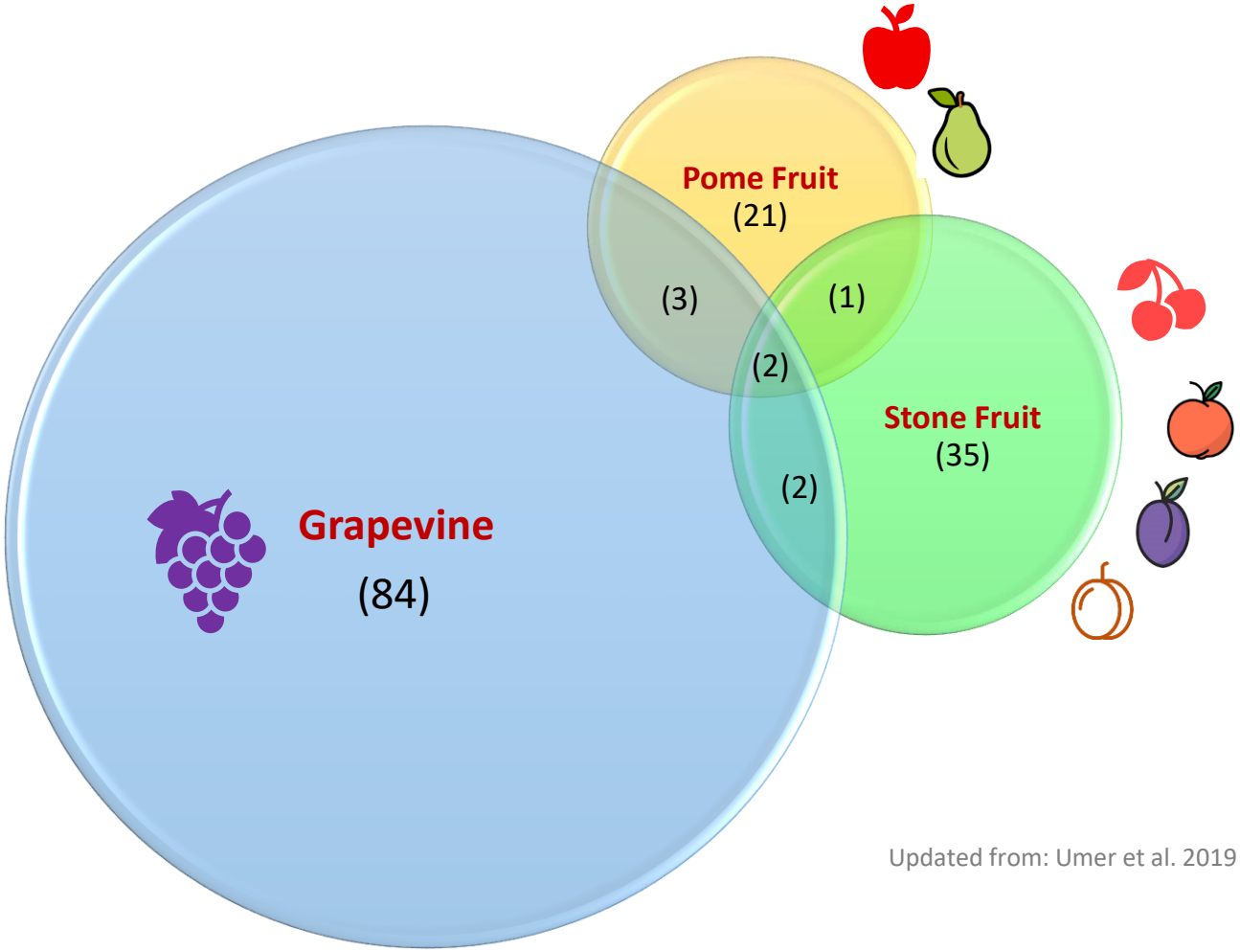
Brock University

Outline

- Graft transmissible agents– importance
- Grapevine certification standards – structure
- Clean Plant Programs – how different and why?
- Canadian prospective – what we have learned?

  – success Stories

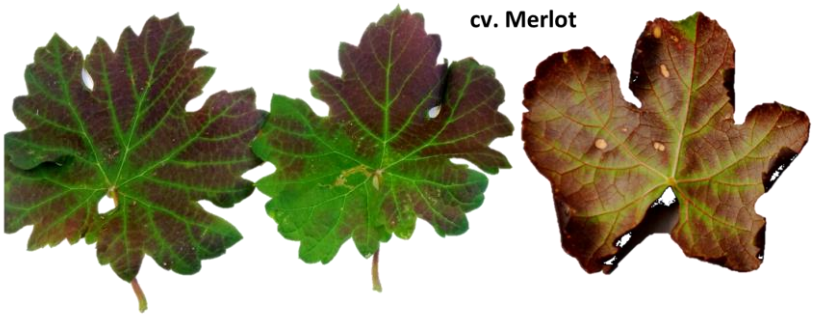
Virus cross-infections among fruit trees



Updated from: Umer et al. 2019

Major grapevine virus diseases

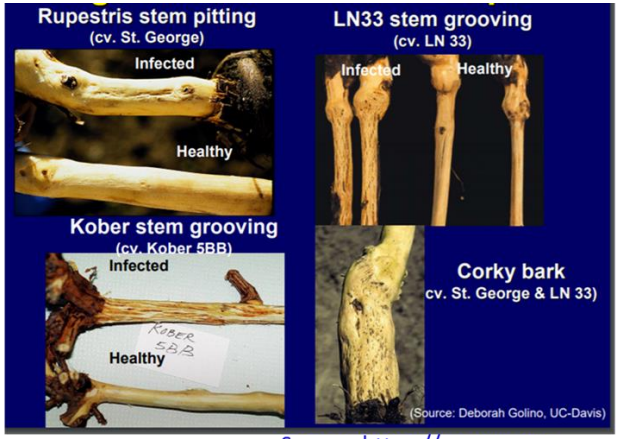
Grapevine leafroll-associated viruses



Grapevine red blotch virus

Grapevine fanleaf virus

Grapevine Pinot Gris Virus



Source: https://wawgg.org

Importance of Clean Plant Programs

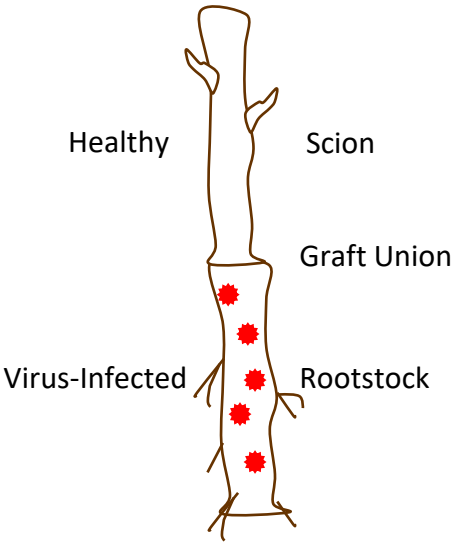
- Leafroll disease cause losses up to **$91,662** per acre over the lifespan of vineyard
- The annual impact of GLRaV-3 in California is estimated at **$90 million**
- Red blotch disease the losses estimated to be **$1,110/acre/year** in Napa County, California
- The amount of loss depends - disease prevalence, onset of the disease, yield reduction, price penalty on fruit quality, and grape growing region.

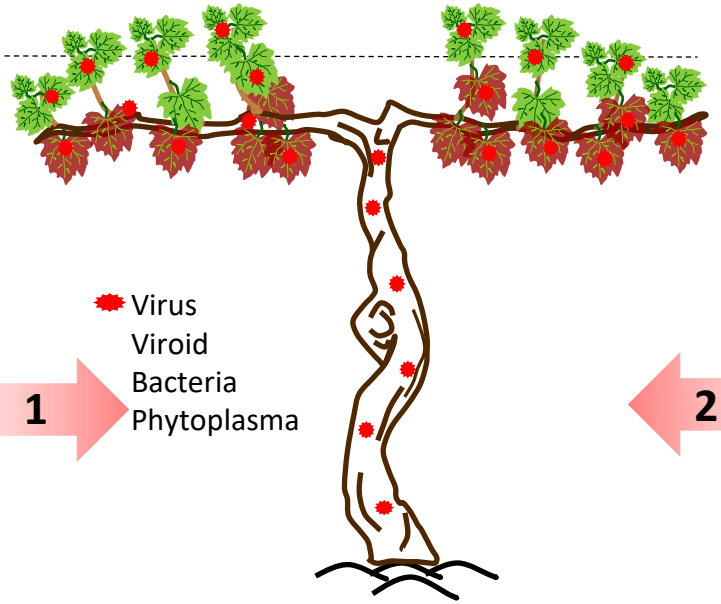
- Pierce's disease - $92 million in California
- Little cherry disease – reduced 12% production in 2020 in the PNW

(Cheon et al. 2020; Atallah et al. 2012; Ricketts et al. 2015 and 2017)

Why?

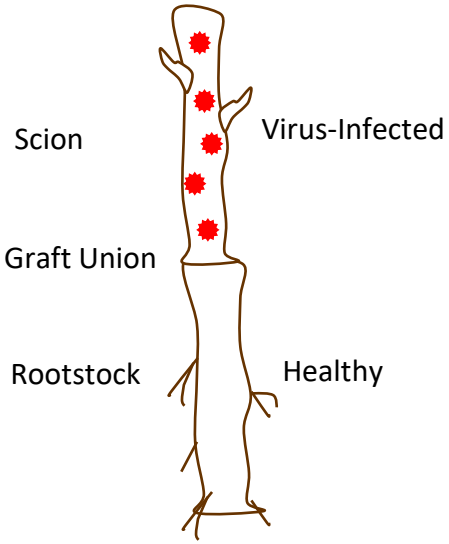
Virus-Infected Rootstock





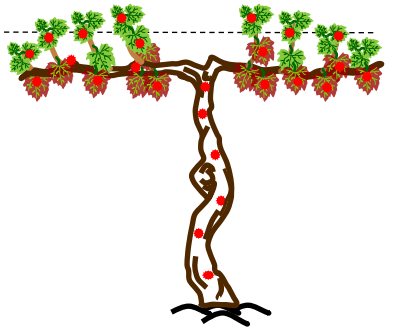
1

Virus-Infected Scion



2

Virus-Infected mother vine



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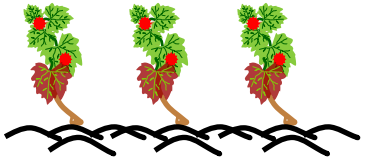


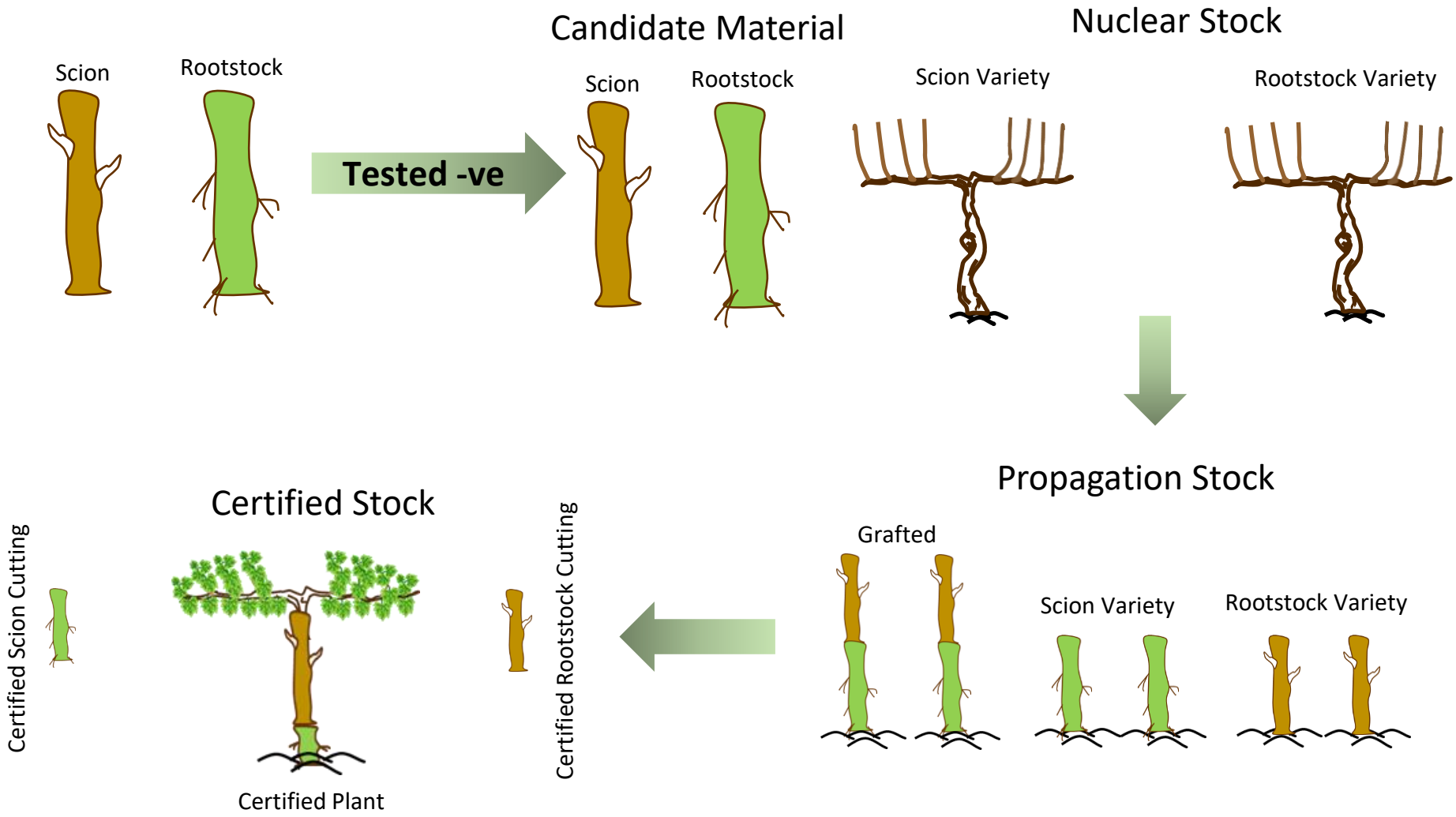
Cuttings from virus-Infected mother vine

Virus-Infected vine showing symptoms on mature basal leaves

Certification Stages: Clean Plant Program

- Selection of varieties and rootstocks
  - No symptoms
  - Serological and molecular tests
  - If tested positive: Elimination process
- Production of nuclear stock
  - Propagation by cuttings in isolation
  - Propagation by meristem-tip tissue culture/heat treatment
- Maintenance of nuclear stock
  - Best Management Practices
  - Regular re-testing
- Propagation block
  - Propagation from the nuclear stock
- Certified plants
  - Grafted or own-rooted
  - Standards of propagation blocks

Certification Stages



Certification Stages

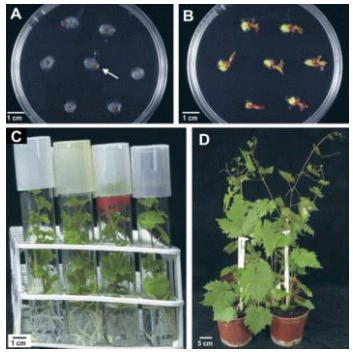
Scion

Rootstock

Tested +ve

Virus Elimination

meristems (0.1–0.5 mm)



Wang Q et al. 2003. Plant science 165:2 321-327

Nuclear Stock

Scion Variety

Rootstock Variety

Propagation Stock

Grafted

Scion Variety

Rootstock Variety

Certified Rootstock Cutting

Certified Stock

Certified Plant

Certified Scion Cutting

Elimination of the Crown Gall Pathogen, *Agrobacterium vitis*, from Systemically Infected Grapevines by Tissue Culture
Luz Marcela Yepes, Tom Burr, Cherie Reid, Marc Fuchs
Am J Enol Vitic. July 2019 70: 243-248; **DOI:** 10.5344/ajev.2019.18083

Clean Plant Program: Simplified

G1

- Mother vine selections screened for known pathogens (testing, biological indexing/NGS)
- Typically these blocks (Foundation or Nuclear) are maintained at the Clean Plant Centers
- Maintained in isolated vineyard block that is **regularly tested and monitored**
- Sole source of for G2 plant material (for nurseries, growers, and certification programs)

G2

- Grapevine plant material propagated from G1 (NOT from a secondary source (e.g. Nursery) that is maintaining the G2 material)
- Maintained in isolated vineyard blocks usually at nursery level
- Purpose is to increase the production of virus-tested vines to **support the supply chain** (G3 & G4)
- Need to follow guidelines developed by NCP to be maintained as **"registered"** propagation blocks

G3

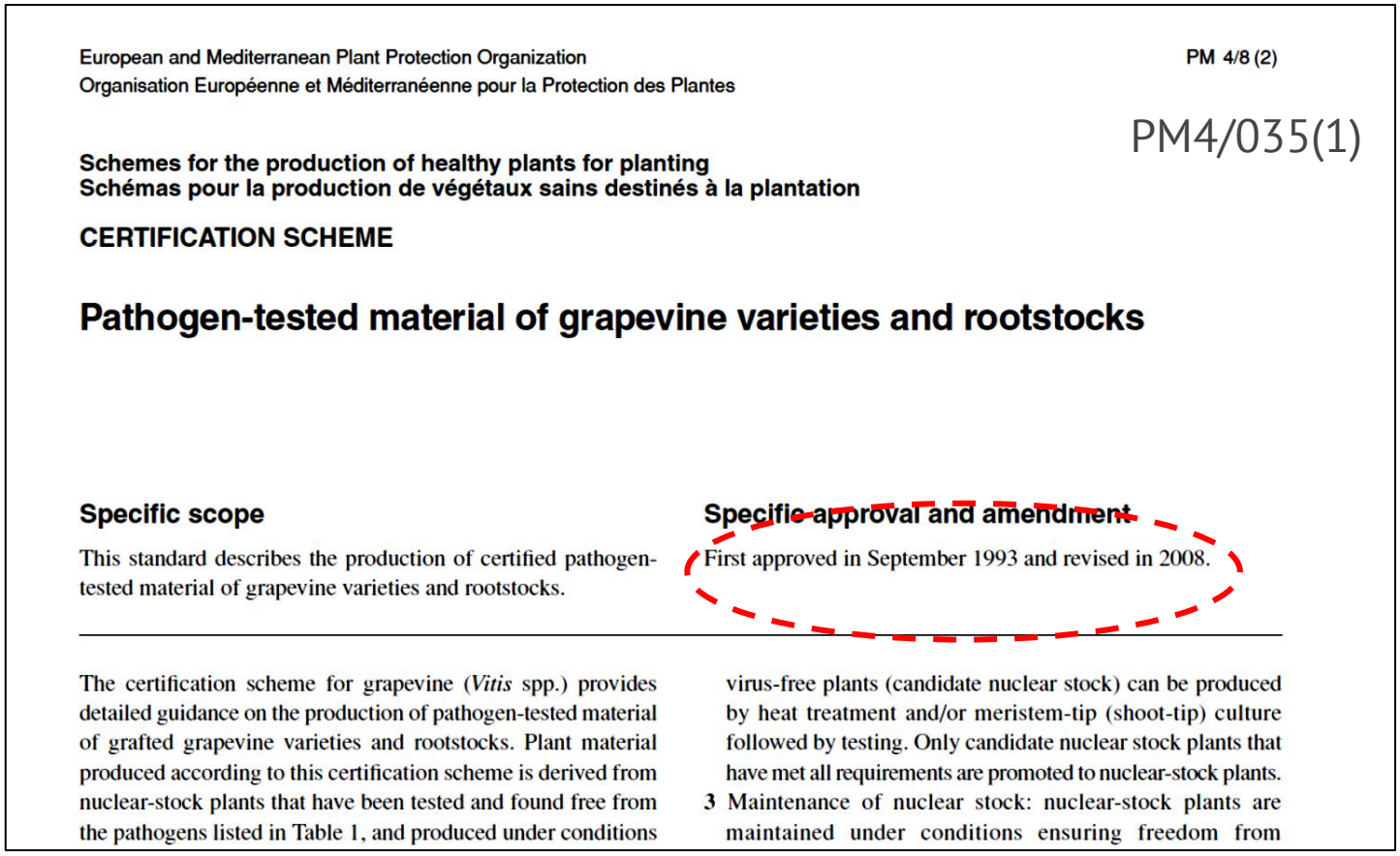
- Grapevine plant material propagated from G2 (to establish new block)
- G3 stock is commonly used in secondary increase blocks and certified nursery blocks.

G4

- Grapevine plant material propagated from G3
- G4 stock means the certified plants destined for delivery to the nursery's customer.
- Typically this stage it is referred or sold as **"CERTIFIED"** plant material

The 'Generation (G) level' concept is used to define plant material categories in clean stock certification programs. Signifies the degree to which plant stock is related to the original virus-tested plant material. Source: nationalclenplantnetwork.org

EPPO Grapevine Certification Standards



European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

PM 4/8 (2)

PM4/035(1)

Schemes for the production of healthy plants for planting
Schémas pour la production de végétaux sains destinés à la plantation

CERTIFICATION SCHEME

Pathogen-tested material of grapevine varieties and rootstocks

Specific scope

This standard describes the production of certified pathogen-tested material of grapevine varieties and rootstocks.

Specific approval and amendment

First approved in September 1993 and revised in 2008.

The certification scheme for grapevine (*Vitis* spp.) provides detailed guidance on the production of pathogen-tested material of grafted grapevine varieties and rootstocks. Plant material produced according to this certification scheme is derived from nuclear-stock plants that have been tested and found free from the pathogens listed in Table 1, and produced under conditions

virus-free plants (candidate nuclear stock) can be produced by heat treatment and/or meristem-tip (shoot-tip) culture followed by testing. Only candidate nuclear stock plants that have met all requirements are promoted to nuclear-stock plants.

3 Maintenance of nuclear stock: nuclear-stock plants are maintained under conditions ensuring freedom from

Source: https://gd.eppo.int/standards/PM4/ Downloaded on: 02 March 2023

EPPO Guidelines on Testing

1. Biological Indexing
   - Testing on indicator plants
   - Mandatory step – leafroll and rugose wood complex
   - Minimum of 3 replicates and 6-8 grafts for each

2. ELISA testing
   - Grapevine fanleaf and other nepoviruses; leafroll; vitiviruses; fleck
   - As a complement to but not as a substitute to other diagnostic methods
   - Antibodies are not available for all virus species

3. Molecular Testing
   - Higher sensitivity than ELISA and biological indexing
   - Highly specific

OEPP/EPPO Bulletin 38, 422–429

EPPO Guidelines on Testing

1. Grapevine degeneration complex
   - Arabis mosaic virus
   - Grapevine chrome mosaic virus
   - Grapevine fanleaf virus
   - Raspberry ringspot virus
   - Strawberry latent ringspot virus
   - Tomato black ring virus
2. Grapevine leafroll complex
   - Grapevine leafroll-associated virus 1
   - Grapevine leafroll-associated virus 2
   - Grapevine leafroll-associated virus 3
   - Grapevine leafroll-associated virus 4
   - Grapevine leafroll-associated virus 7
3. Grapevine rugose wood complex
   - Grapevine virus A
   - Grapevine virus B
   - Grapevine rupestris stem pitting associated virus
4. Grapevine fleck disease
   - Grapevine fleck virus
5. Grapevine phytoplasmas
   - Grapevine flavescence dorée
   - Grapevine bois noir and other yellows

OEPP/EPPO Bulletin 38, 422–429

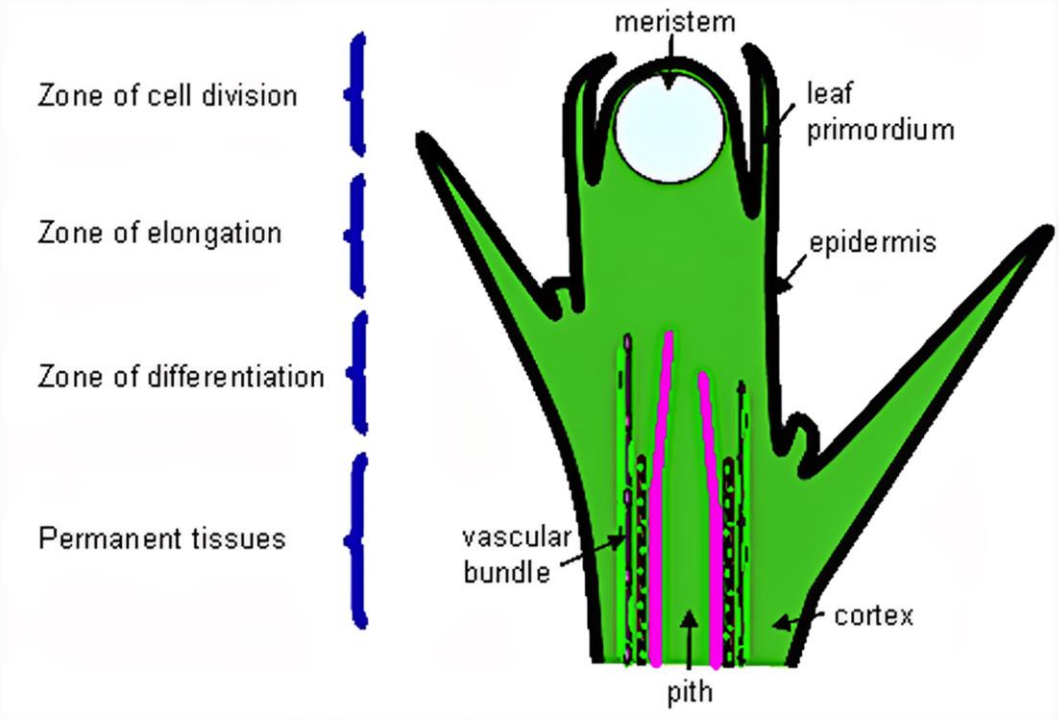
EPPO Guidelines on Sanitation

1. Heat Treatment
   - Variable levels of efficacy
   - Recommended for 2yr and older cuttings
   - 38 ± 1° C and 16–18 h

2. Meristem Shoot Tip Culture
   - Shoot tips 0.2-0.5 mm-long with meristematic dome
   - Grown in artificial media in controlled conditions
   - Test the plantlets for viruses

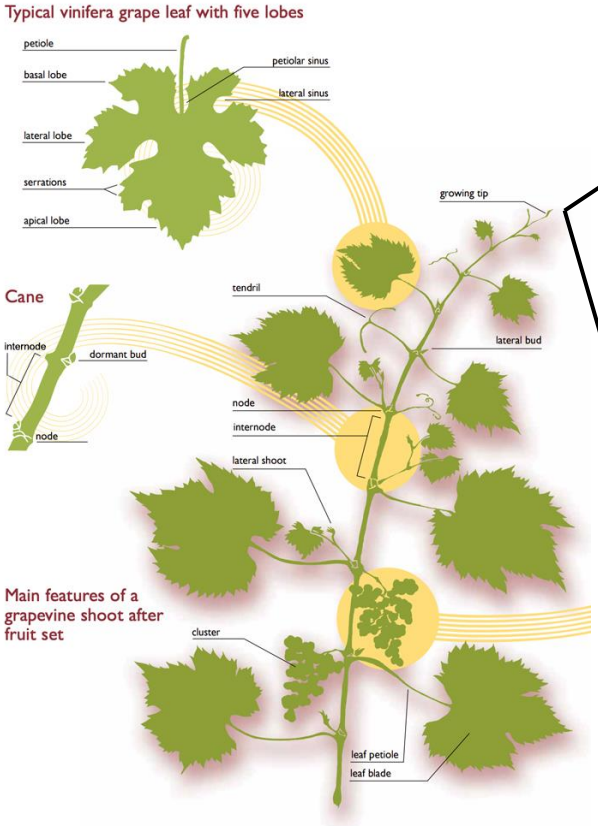
Meristem-tip tissue culture for Grapevines

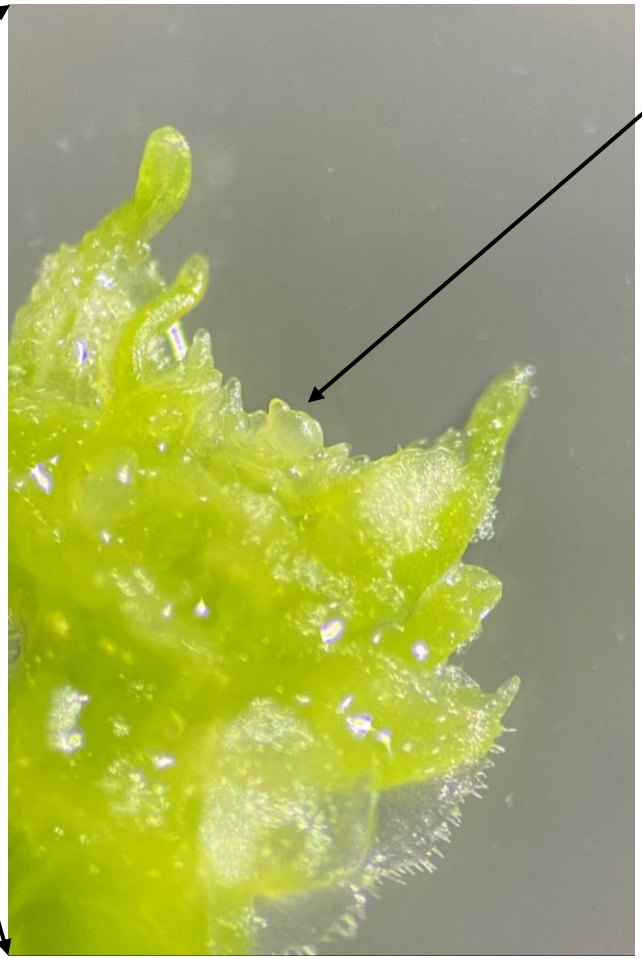
Why could meristem tissue culture eliminate viruses in plants?



- **Two ways** of virus movement in plants:
  Through the vascular bundle. Meristem tip has no structural vascular bundle;
  Through plasmodesmata. Virus movement speed is very slow in plasmodesmata, it can not catch up with the speed of cell division in meristem tip.
- Thus, the distribution of viruses in plants is uneven. Apical meristem tissues in infected plants usually contain no or very low concentrations of viruses, whereas tissues far down from the meristem tip may contain viruses.
- Meristem tissue culture is a promising technology for generating virus-free vines

Meristem-tip tissue culture for Grapevines

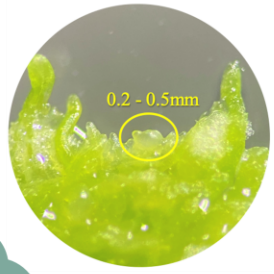




meristem

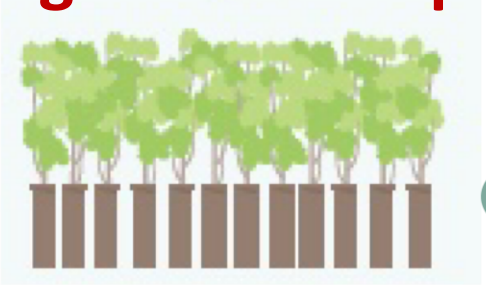
Credit: Linxue Zhang, CCOVI

Clean Plant Program for Grapevines



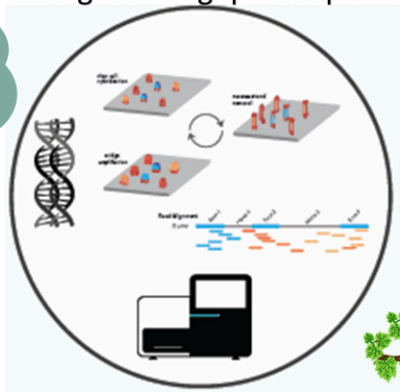
0.2-0.5 mm size meristem tips are cut under 100X microscope

Meristem tissue culture



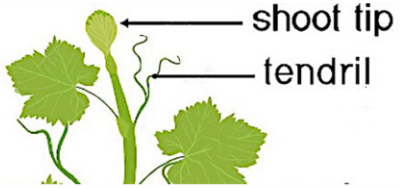
If HTS results show negative, vine introduced into the repository

High Throughput Sequencing



Propagate in the lab under controlled conditions

Propagate in greenhouse



Virus infected grapevine

Clean Plant Program: Virus Elimination

Virus free, healthy, true-to-type grapevine

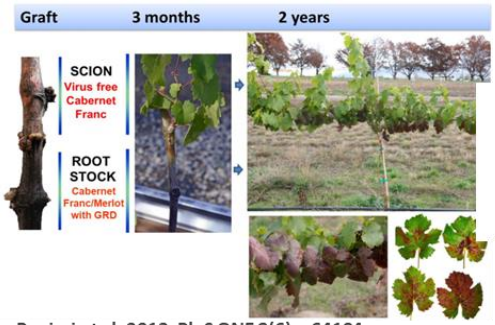
Canadian Grapevine Certification Network
CGCN · RCCV
Réseau canadien de certification de la vigne

Ontario GRAPE & WINE RESEARCH Incorporated

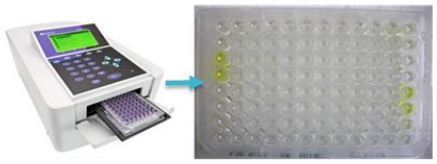
Testing in Certification

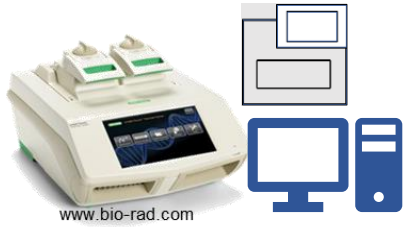
Grapevine Virus Diagnostics

Symptom Based	Biological Indexing	Serological (ELISA)	Molecular (PCR, qPCR, ddPCR & NGS)
Non-specific Inaccurate	Symptom based Labor intensive Time consuming	Specific No Abs for all viruses Less sensitive than PCR	Highly specific Post-PCR process Multiplex End-Point Quantitative

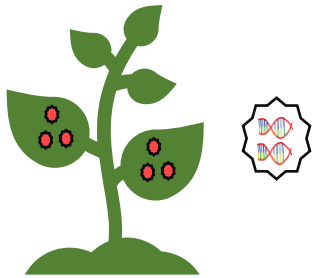


Poojari et al. 2013. PloS ONE 8(6): e64194





High Throughput Sequencing (HTS) for virus detection



Virus suspected plant sample

Quality Check

Total RNA → Ribo depletion
Total RNA → Ribo + poly A depletion

Total Nucleic acids

Small RNA

Viral NA enrichment → Oligo probes
Viral NA enrichment → dsRNA

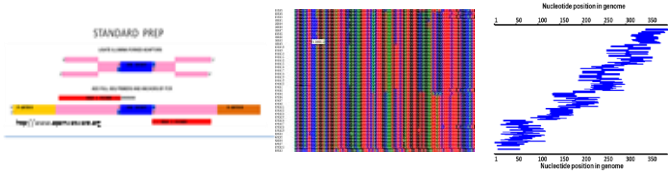
NGS sequence reads

Quality Check \ Adaptor sequence filter

De novo assembly of sequence reads

Blastn: host genome \ plant virus database

Virome discovery \ diagnosis



Virtool
Viral infection diagnostics using next-generation

High Throughput Sequencing: Clean Plant Program

Traditional

Diagnostic tests for 30+ pathogens (ELISA, DNA)

Positive

Virus elimination (meristem culture: 1 yr.)
Woody + Herbaceous Indexing (2 yrs.)
Retesting (30+ pathogens) (weeks to months)

Health Status Unknown

Negative **Negative**

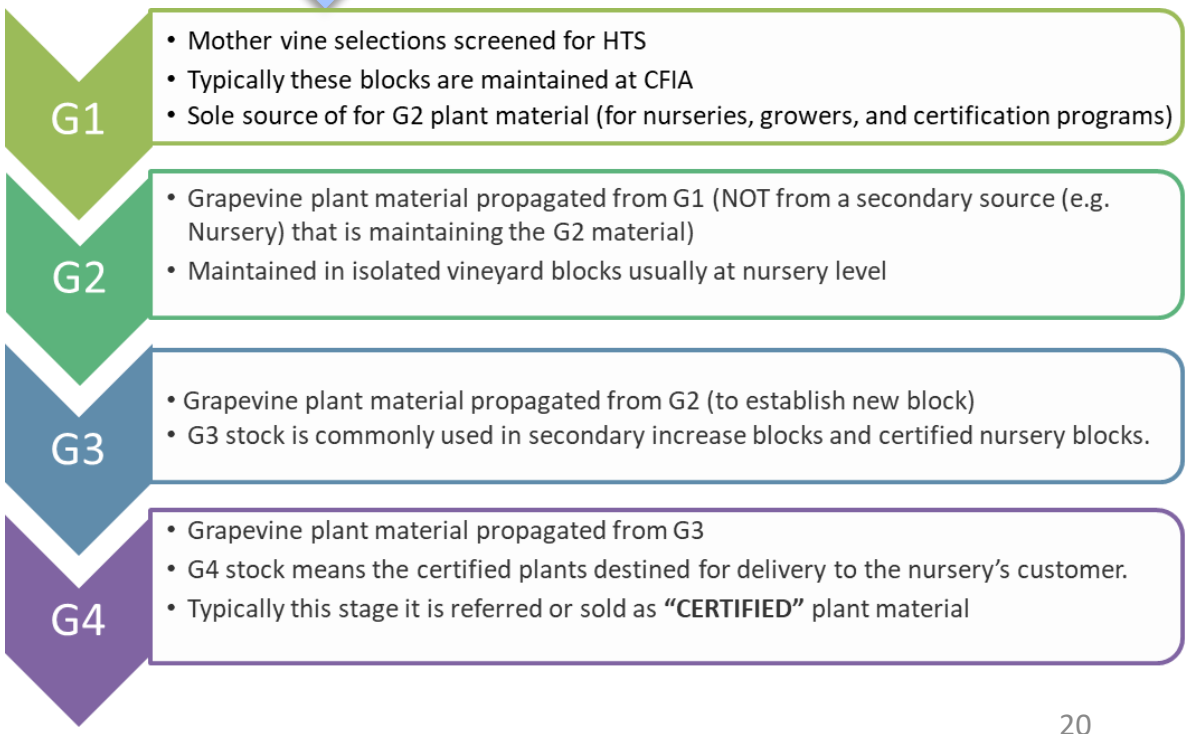
HTS based

HTS testing (days to weeks)

Positive

Virus elimination (meristem culture: 1 yr.)
Retesting with NGS (days to weeks)

HTS + Virtool

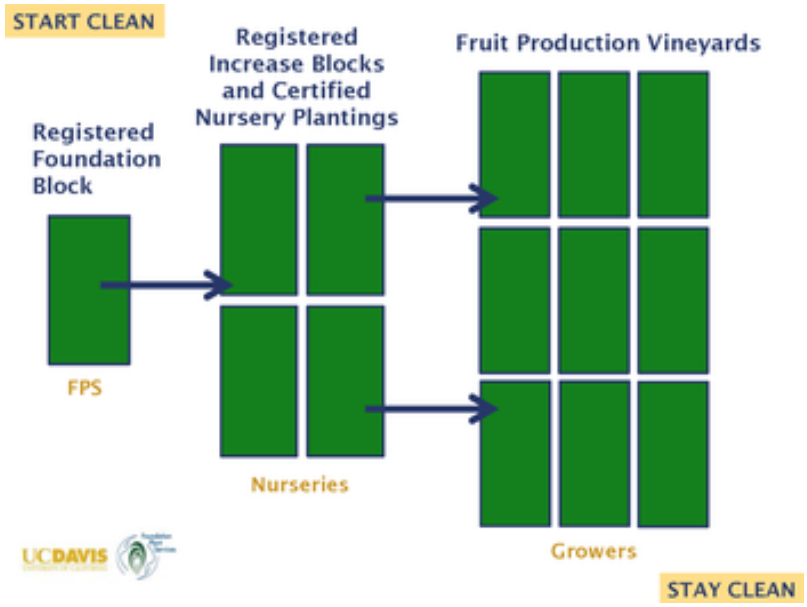


https://www.genomecanada.ca/en/clean-plant-extraction-sequencing-diagnostics-cleansed-clean-grapevines-canada

National Clean Plant Network (NCPN) – The USA

Grapevine Disease Testing PROTOCOL 2010

- PCR, qPCR, ELISA, herbaceous and woody indexing.
- 30+ viruses; Phytoplasma and Pierce's Disease
- Fast tracking the process with high throughput sequencing (HTS)
- Tested –ve with HTS: Provisional quarantine release



https://fps.ucdavis.edu/fgr2010.cfm

Quarantine Regulations: Canada

- Canada regulates the imports of grapevines from foreign countries though Canadian Food Inspection Agency (CFIA) Directive D-94-34.

- Canada allows the import of grapevine material certified under the US state certification programs: California, Oregon, New York, and Washington State.

- Other than the USA, France and Germany are the only countries with CFIA-approved nurseries certified to export grapevine material to Canada.

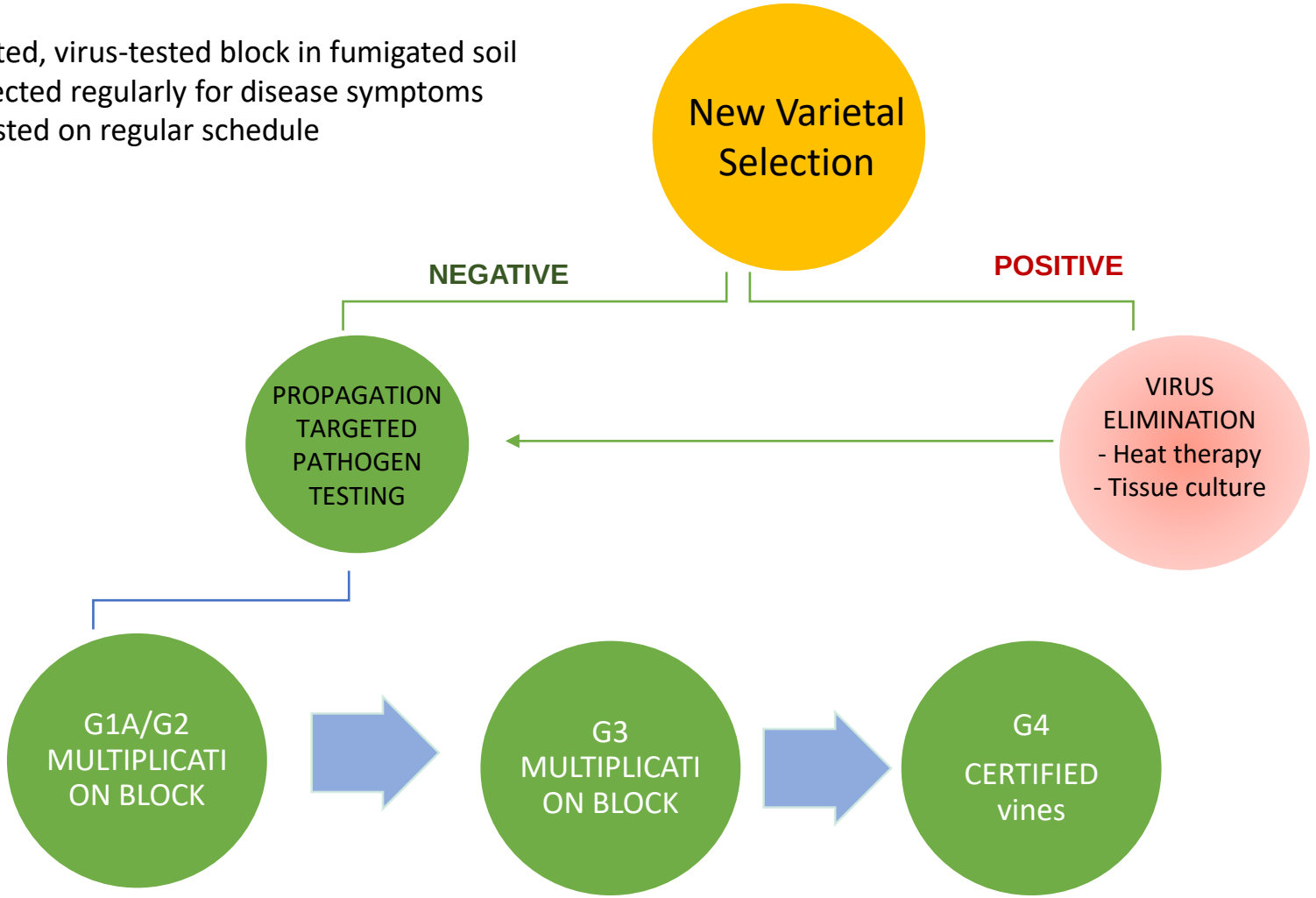
Canadian Grapevine Certification Network

CGCN-RCCV

Réseau Canadien de Certification de la Vigne

Long-Term Certification Standard

- ✓ Isolated, virus-tested block in fumigated soil
- ✓ Inspected regularly for disease symptoms
- ✓ Retested on regular schedule



CGCN-RCCV Long-Term Certification Standard

Viruses Tested	G1	G2	G3	G4
Arabis Mosaic Virus	X	O	O	
Grapevine Fanleaf virus	X	X	X	
Grapevine Leafroll Associated Virus 1	X	X	X	
Grapevine Leafroll Associated Virus 3	X	X	X	
Grapevine Leafroll Associated Virus 4 strains	X	O	O	
Grapevine Leafroll Associated Virus 7	X	O	O	
Strawberry latent ringspot virus	X	O	O	
Raspberry ringspot virus	X	O	O	
Tomato Ringspot virus	X	O	O	
Grapevine Fleck Virus	X	O	O	
Grapevine Leafroll Associated Virus 2	X	O	O	
Grapevine Leafroll Associated Virus 2 Red Globe Strain	X	O	O	
Grapevine Virus A (associated with grapevine Kober stem grooving disease)	X	O	O	
Grapevine virus B (associated with grapevine corky bark disease)	X	O	O	
Grapevine Virus D (associated with rugose wood disease)	X	O	O	
*Grapevine Virus E	X	O	O	
*Grapevine Virus F	X	O	O	
Grapevine red blotch virus	X	X	X	
Grapevine Pinot gris virus	X	X	X	
Grapevine asteroid mosaic-associated virus	X	O	O	
Grapevine rupestris stem pitting associated virus and its strains	X	O	O	
Raspberry ringspot virus	X	O	O	
Tomato black ring virus	X	O	O	
Phytoplasmas: Flavescence dorée, Bois noir, Australian grapevine yellows, Palatinate Yellow, Aster Yellows, X Disease	X	O	O	
*Crown Gall	X	O	O	

CLEan plAnt extractioN Sequencing SEquencing Diagnostics (CLEANSED) for Clean Grapevines in Canada

CLEan plAnt extractioN SEquencing Diagnostics (CLEANSED) for Clean Grapevines in Canada

Certification: work in progress

- USA: State certification programs (voluntary), harmonization efforts
- Europe: Clonal and sanitary certification (mandatory), country directives are stricter than EU directives
- Australia and New Zealand: Standards include GRSPaV and GVB
- South Africa: Viral diseases rather than viruses, bacteria and oomycetes

Courtesy: Dr. Marc Fuchs

Certification: Challenges and opportunities

- Revisit the health status of vines in foundation vineyards or create new foundation vineyards
- Revise standards of certification programs
- Multidisciplinary efforts are needed to remain strategically vigilant and always look forward

Courtesy: Dr. Marc Fuchs

Clean Plant Program

1. Which viruses are important?
2. Any virus infections can be tolerated?
3. Any virus (es) can be eradicated in a given geographic location?
4. How to adopt the latest diagnostic methods in clan plant programs?
5. How to ensure clean certified propagating material available to nurseries/growers?
6. Once the clean vines are planted, how to prevent the infection?

Success Stories: Grapevines

- At Foundation Plant Services (FPS) , UC Davis, a microshoot tip tissue culture selection was created in 2008; Qualified for the Russell Ranch Foundation in 2012 under the name Zinfandel 42.1.
- Zinfandel 42.1 was returned to Croatia as part of an exchange program to return the virus-tested material to its source country.
- The Croatian wine industry was revitalized as a result of the discovery of the origin of Zinfandel, with many new Croatian cultivars identified and developed into wines.

NCPN – grapevines

- Fuller et al. 2019: at FPS clean plant program for grapevines substantially exceeds the grower cost benefit ratio of 117 over 10 years

- Cheon et al. 2020: The program has an annual benefit of $70 million for the rest of the grape growing regions in California

Powdery Mildew-resistant "*Renstack*" Vines Released to the Public Domain

Tim Martinson, Bruce Reisch, Matthew Clark, Craig Ledbetter, Surya Sapkota, and Lance Cadle-Davidson

Timothy Martinson is a retired senior extension associate from Cornell AgriTech in Geneva, NY. **Bruce Reisch**, is a professor of horticulture, plant breeding and genetics at Cornell AgriTech. **Matthew Clark** is a professor of horticulture at the University of Minnesota in St Paul, MN. **Craig Ledbetter** is a research geneticist for the USDA-ARS Crop, Disease, and Genetics Research Unit of the San Joaquin Valley Agricultural Sciences Center in Parlier, CA. **Surya Sapkota** is a post-doctoral research associate with the USDA-ARS Grape Genetics Research Unit at Cornell AgriTech. **Lance Cadle-Davidson** is a research plant pathologist for the USDA-ARS Grape Genetics Research Unit at Cornell AgriTech.

PUBLIC AND PRIVATE GRAPE BREEDERS will soon have access to new powdery mildew-resistant grapevines produced by the USDA-funded *Vitis*Gen2 project. Eight "*Renstack*" vines that incorporate four to six different powdery mildew resistance loci have been released to Foundation Plant Services at the University of California, Davis (FIGURE 1, FIGURE 2).

These eight vines are the tangible product of a decade of progress in incorporating advanced DNA sequencing to identify DNA markers associated with powdery mildew resistance and use them for marker-assisted selection. Breeders have used resistant sources for variety improvement for more than a century but have not had an efficient way to screen new seedlings to identify the resistant ones without introducing the pathogen.

"Before marker-assisted selection, we could only observe whether or not powdery mildew was infecting our grapevine selections," said Bruce Reisch, Cornell University grape breeder and leader of the *Vitis*Gen2 project. "Reliable DNA markers allow us to know which resistance genes are present in each seedling we test."

Using marker-assisted selection, breeders are now able to "stack" several different loci and combine them into vines that have multiple sources of powdery mildew resistance. This strategy, it is thought, should make the resistance more durable (as each gene has a unique mechanism) and less likely to be affected by the chance occurrence of novel powdery mildew strains that can overcome the resistance.

BRUCE REISCH

FIGURE 1: Post-Doctoral research associate Surya Sapkota with a vine used to make cuttings at Cornell AgriTech in Geneva, NY before shipping to the Foundation Plant Services at the University of California, Davis.

Resistance Markers

To date, 15 powdery mildew resistance loci have been identified, originating from several different *Vitis* species native to the Caucasus regions of Europe, North America, and Asia (FIGURE 3).[1] Studies from 24 F1 mapping populations (siblings of crosses between susceptible and resistant vines) have localized these distinct loci on eight of the 19 grapevine chromosomes (chromosomes 2, 9, 12, 13, 14, 15, 18, and 19).

A marker for the first resistance locus, called Run1, was identified in 2000.[2] "Run" refers to Resistance to *Uncinula necator*. Following the revision of the scientific name to *Erysiphe necator*, subsequent resistance loci were identified as "Ren" followed by a number. As DNA sequencing techniques and mapping populations have progressed over the last 15 years, the rate of discovery of additional loci has accelerated, with the most recent locus described in September 2021 called Ren11.[3]

*Vitis*Gen2 Collaboration to Produce *Renstack* Vines

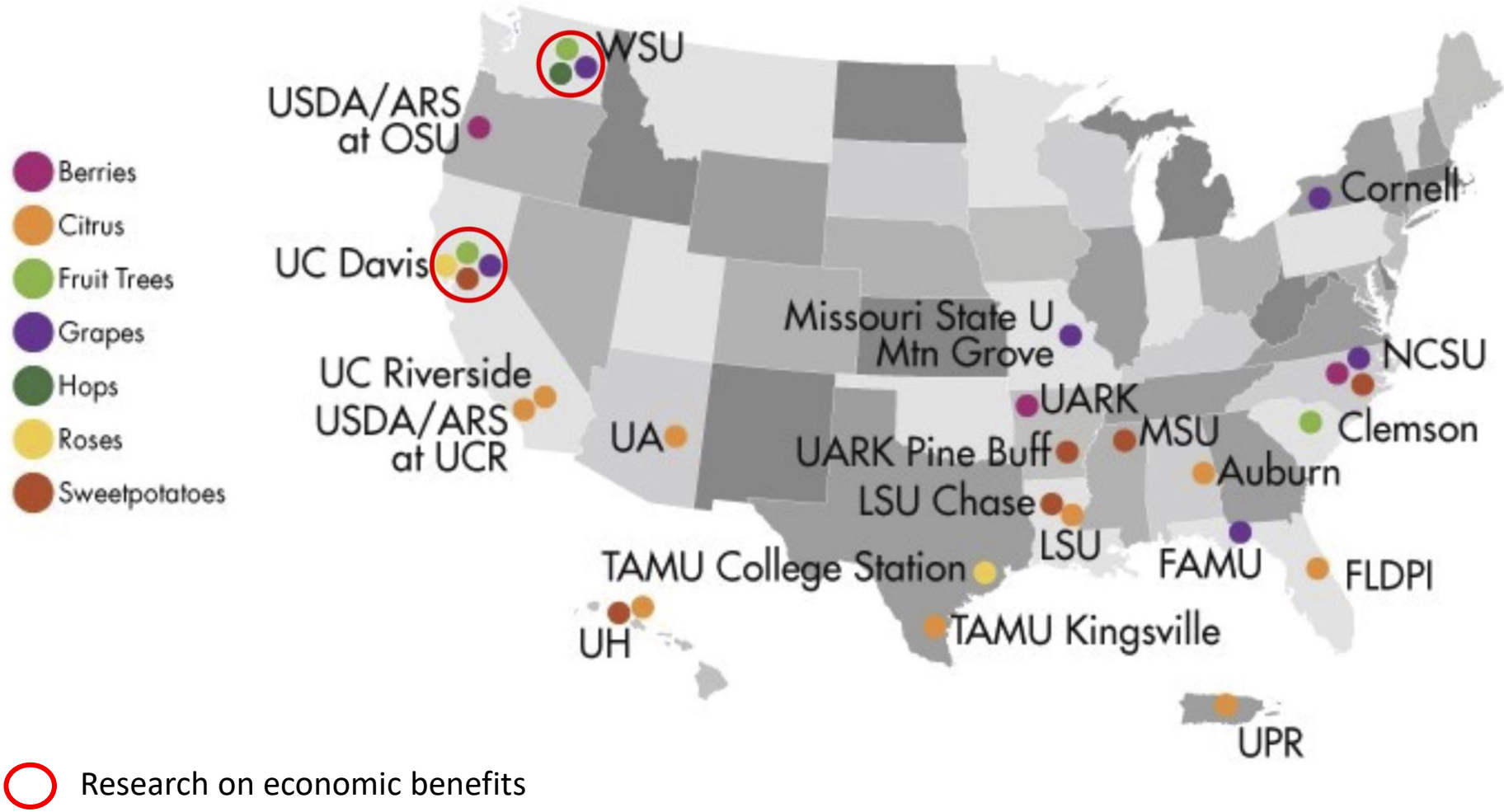
The *Vitis*Gen2 project brought together grape breeding programs at four institutions to make the crosses that produced the *Renstack* vines: Bruce Reisch's program at Cornell University, Craig Ledbetter's table grape breeding program at USDA-ARS Parlier, Andy Walker's program at University of California, Davis, and Matthew Clark's program at the University of Minnesota (FIGURE 4).[4,5]

TIM MARTINSON

FIGURE 2: Vines regenerated from Cornell cuttings at Foundation Plant Service's greenhouse, on the campus of UC Davis in Davis, Calif.

Success Stories: Grapevines

Success Stories: Grapevines and Beyond



M Fuchs et al. 2021. Plant Disease. https://doi.org/10.1094/PDIS-05-20-1061-FE

Success Stories: Grapevines and Beyond

- Clean seed program for sweetpotato started in California in the 1960s.
- New seed stock was needed to prevent “Russet Crack” and variety decline.
- Clean material and higher yielding, disease-resistant varieties have resulted in significant yield increases.
- In 1967, average yields in California were 5 tons/acre.
- In 2001, average yield had more than doubled to 12 tons/acre.
- Sweetpotato clean seed programs are established in California, Louisiana, North Carolina, and Mississippi.
- National Clean Plant Program (USA) – Education and outreach program

https://www.nationalcleanplantnetwork.org/success-stories-1

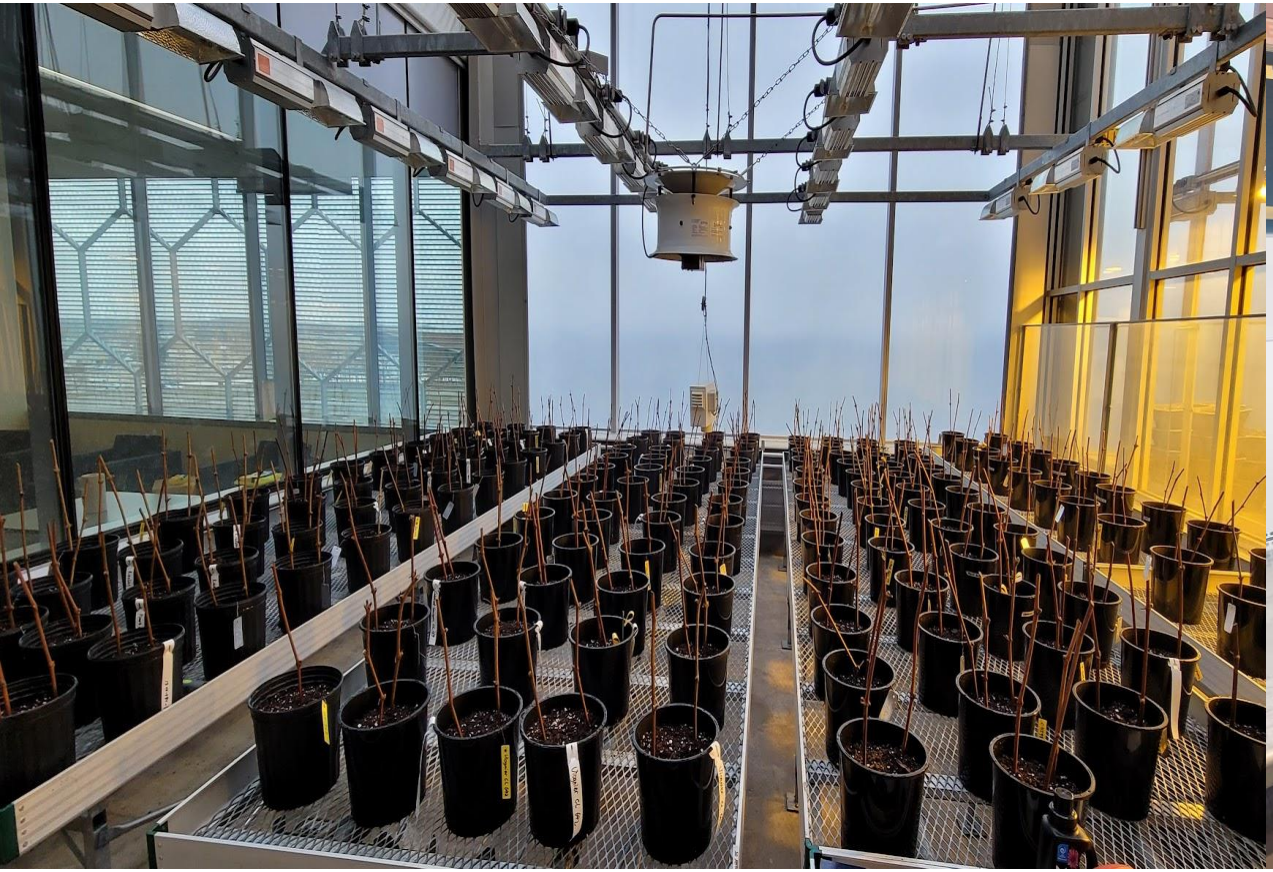
We need “start clean stay clean” programs

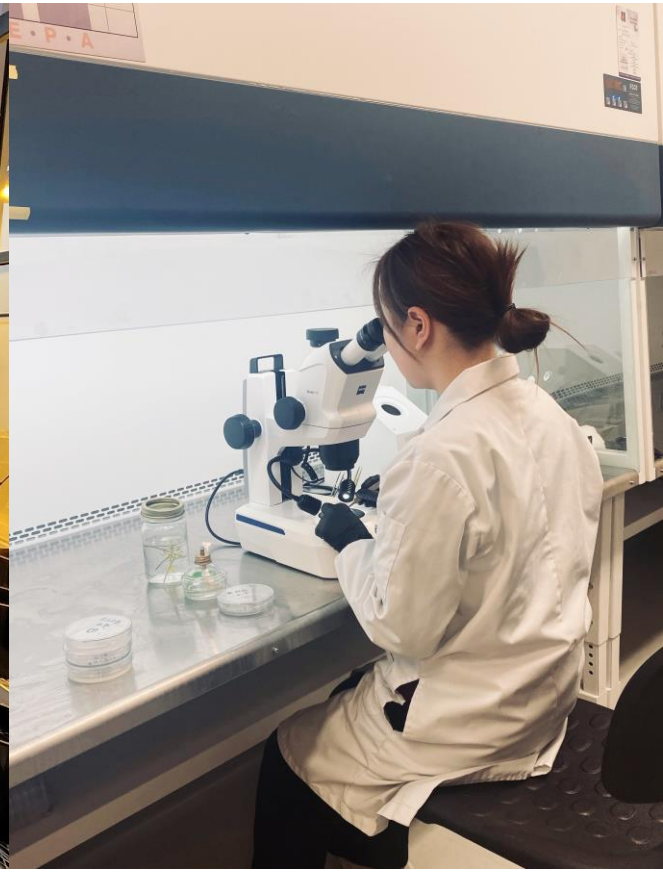
Virus Diagnostic LAB

Cool Climate Oenology & Viticulture Institute
Brock University

National Grapevine Germplasm Facility

"Development of micro-shoot tip tissue culture-based protocols for maintaining virus-free grapevine germplasm for elite and local varieties"





National Grapevine Germplasm Facility

Ontario GRAPE & WINE RESEARCH Incorporated

Brock University

Canadian Grapevine Certification Network
CGCN · RCCV
Réseau canadien de certification de la vigne

Grapevine Virus Culture Facility

Cool Climate Oenology & Viticulture Institute
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Acknowledgments

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Ontario Ministry of Agriculture, Food and Rural Affairs

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