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**Cool Climate
Oenology &
Viticulture Institute**



Outline

- Grapevine virus diseases – importance
- Grapevine viruses – how to test and eliminate?
- Grapevine certification standards – structure
- Clean Plant Programs – how different and why?
- Canadian perspective – what we have learned?

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Major grapevine virus diseases

Grapevine Red Blotch Virus

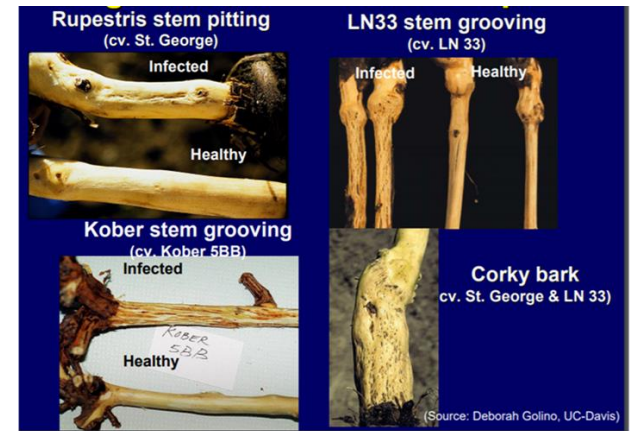


Grapevine leafroll-associated virus-3



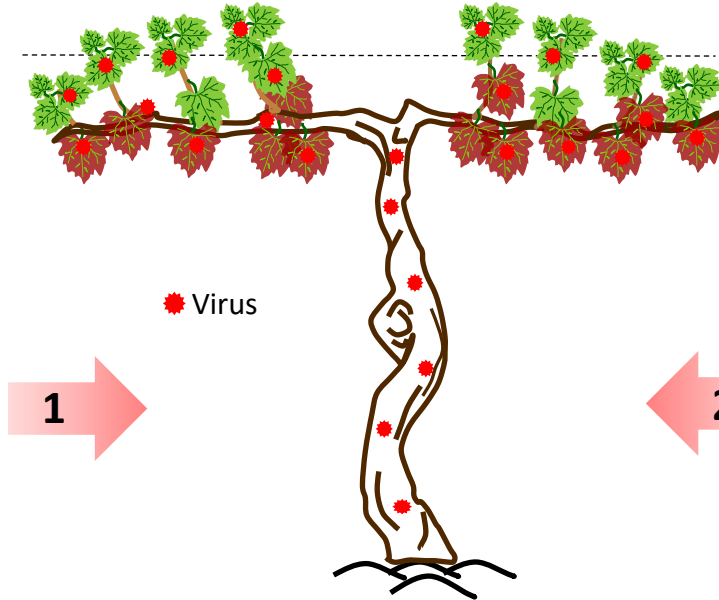
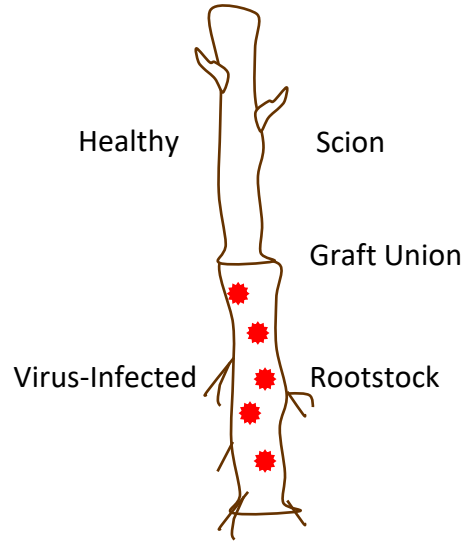
Grapevine fanleaf virus

Grapevine Pinot Gris Virus

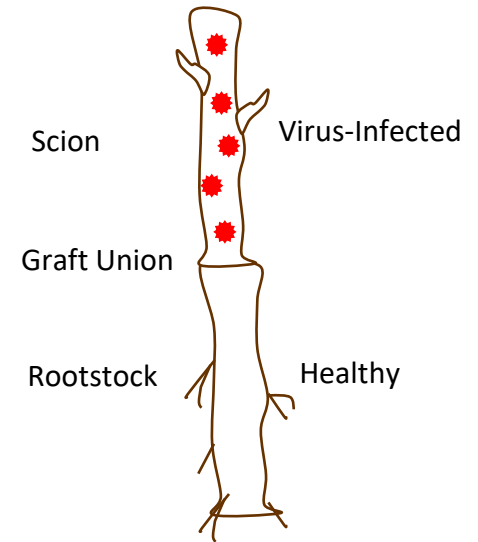


Primary Transmission

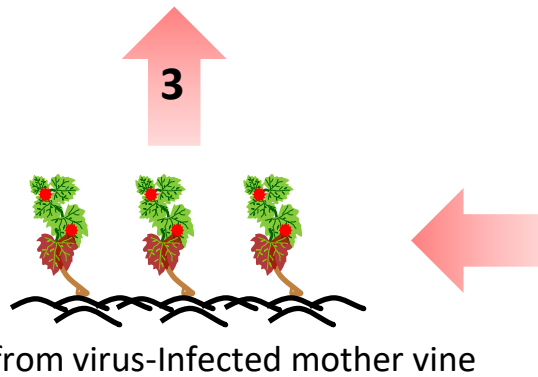
Virus-Infected Rootstock



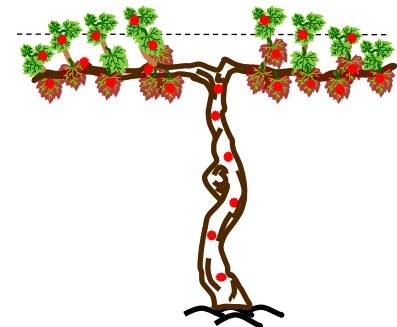
Virus-Infected Scion



Virus-Infected vine showing symptoms on mature basal leaves



Virus-Infected mother vine

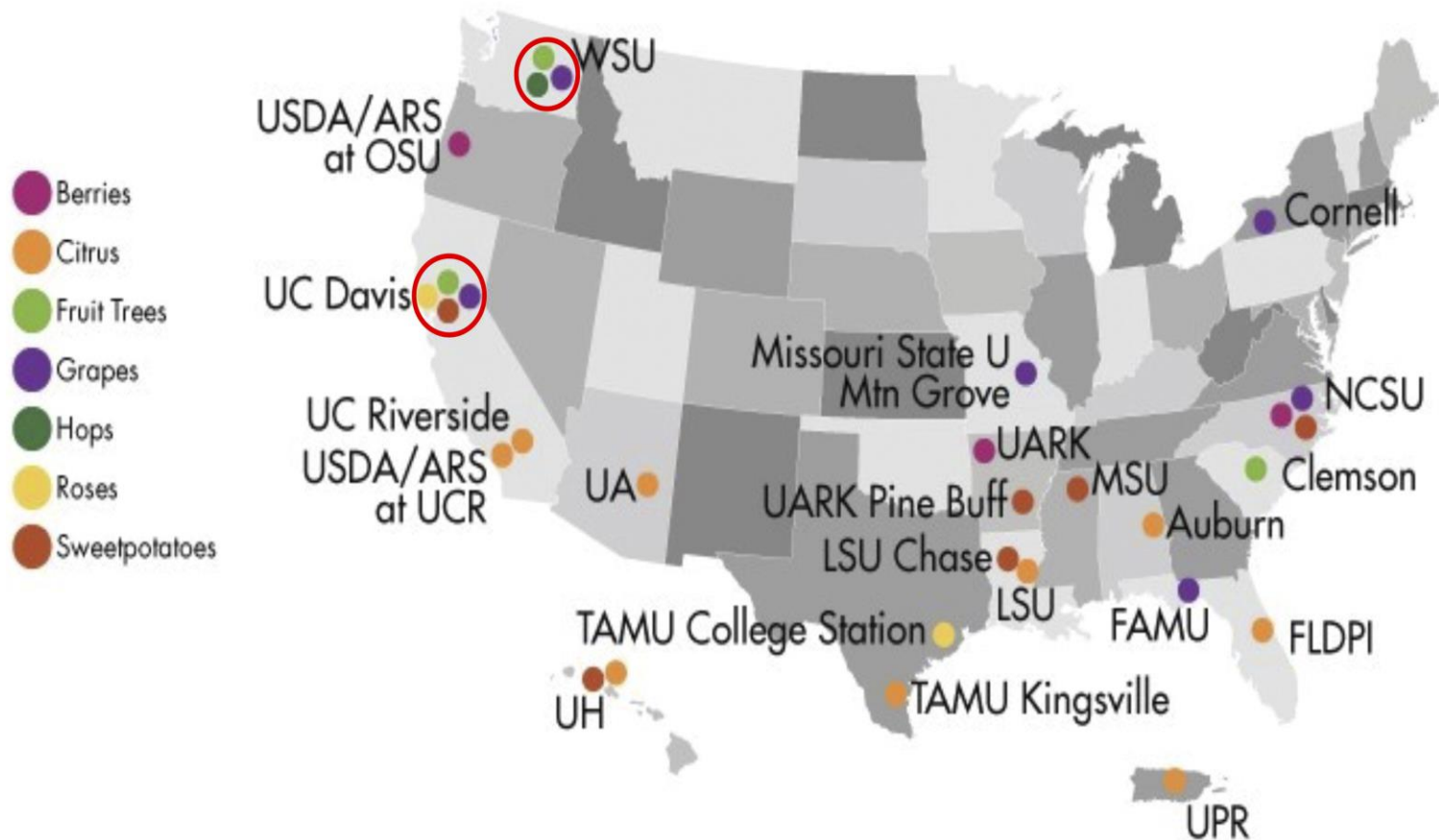


Centers of the National Clean Plant Network (NCPN) : USA

The U.S. Department of Agriculture (USDA) is investing over \$70 million in 374 projects through the Plant Protection Act's Section 7721 program.

- Agriculture plant pest detector dog teams: **\$6,265,992 allocated to California, Florida and nationally to support detector dog team training and maintenance for domestic pest detection;**
- Tribal organization's plant protection research, survey, outreach, and invasive pest mitigation efforts: \$1,545,290 in 6 states;
- National Honey Bee Survey: \$1,521,204 to support honey bee surveys in 41 states and territories.
- Box tree moth: \$890,137 to survey and protect American boxwoods from the invasive pest;
- **Stone fruit and orchard commodities: \$1,045,748 to support pest detection surveys** in 12 states, including Colorado, Massachusetts, New York, Texas, and Washington;
- Forest pests: \$1,240,130 for various detection tools, control methods development, and outreach to protect forests from harmful pests in 15 states, including Georgia, Illinois, Maine, Michigan, Ohio, Pennsylvania, Tennessee, and Virginia;
- Sudden oak death (*Phytophthora ramorum*) and related species: \$1,068,589 in 17 states, including Alabama, Florida, Louisiana, Michigan, Pennsylvania, and West Virginia, and nationally for survey, research, mitigation, and outreach;
- Northern giant hornet research and eradication efforts: \$1,097,052 in Washington;
- Invasive defoliating moths: \$1,456,893 to support surveys and enhance identification technologies in 16 states, including Alaska, California, Kentucky, Massachusetts, Nevada, and North Carolina
- **Certified, disease-free citrus planting materials: \$1,759,935** to protect American nurseries and growers from economic losses caused by citrus plant diseases.

Centers of the National Clean Plant Network (NCPN) : USA

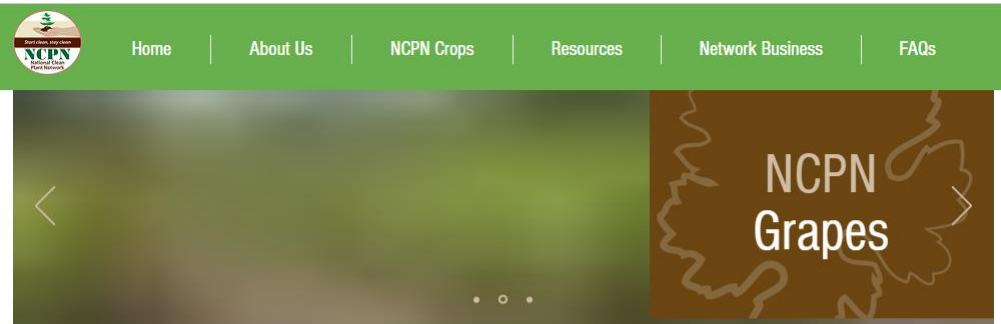


The 2014 Farm Bill made the NCPN a permanent program with dedicated funding.

Centers of the National Clean Plant Network (NCPN) : USA

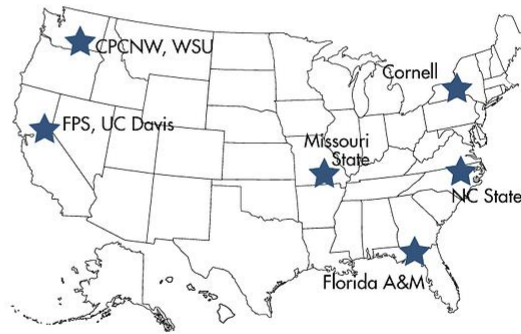
- **Fruit Trees:** Distributes disease-resistant plant material, providing over **500,000 cuttings** and **1.7 million** buds.
- **Grapes:** Maintain **over 1,000 grapevine selections** and distribute over **700,000 cuttings**, buds, and plants.
- **Berries:** Diagnose and clean new berry plant varieties, supplying disease-resistant mother plants to produce **~30 million** plants.
- **Citrus:** Over **1,000 citrus** selections and distributed over **60 million trees**.
- **Hops:** Maintains disease-resistant hops and distributes over **5,000** units for plant propagation.
- **Sweet Potato:** Maintain a collection of over **170 sweet-potato accession**
- **Roses:** Maintain and test over **600 rose selections**

Centers of the National Clean Plant Network (NCPN) : USA



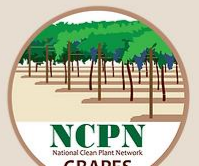
The National Clean Plant Network for Grapes (NCPN-Grapes) is a network of industry members, experts in plant pathology, regulators and clean plant centers that provides healthy grapevine material to the grape industry in the United States.

NCPN-Grapes clean plant centers produce and curate healthy vine stocks and distribute propagation materials for the production of healthy vines that are essential to the health and productivity of vineyards in the United States. The headquarters of NCPN-Grapes is at Foundation Plant Services at the University of California, Davis. FPS also manages the primary foundation vineyard for NCPN.



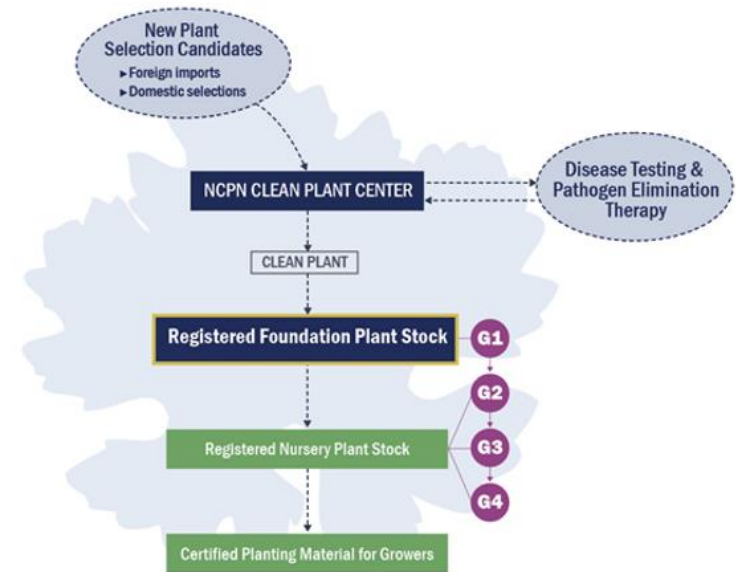
Our clean plant centers and programs:

- [Foundation Plant Services](#), University of California, Davis ([selections available](#)) ([order information](#))
- [Clean Plant Center Northwest](#), Washington State University ([selections available](#)) ([order information](#))
- [The Midwest Center of NCPN-Grapes](#), Missouri State University ([selections available](#))
- [The Eastern NCPN-Grapes Center](#), Cornell University
- [Micropropagation and Repository Unit](#), North Carolina State University



Six centers across the USA

Journey of Grapevine Selections through NCPN-Grapes



India Invests in Clean Plant Programs

‘Atmanirbhar Clean Plant Program’

\$196.0 million

The program will boost the availability of disease-free, quality planting material for high-value horticultural crops.

2/27/23, 10:02 AM

Govt plans 10 ‘Clean Plant Centres’ to boost fruit crop production | India News, The Indian Express

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Monday, Feb 27, 2023
EPAPER TODAY'S PAPER

 **The Indian EXPRESS**
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[News](#) / [India](#) / Govt plans 10 ‘Clean Plant Centres’ to boost fruit crop production

Govt plans 10 ‘Clean Plant Centres’ to boost fruit crop production

These centers will be set up under the ‘Atmanirbhar Clean Plant Program’, which was announced by Finance Minister Nirmala Sitharaman in the Union Budget 2023-24.

Written by [Harikishan Sharma](#) [Follow](#)
New Delhi | Updated: February 27, 2023 06:57 IST

 NewsGuard



Crops like apples, walnuts, almonds, grapes, mango, pomegranate, etc.,

New Zealand

Grafted Grapevine Standard

VERSION 4.0

Issued by New Zealand Winegrowers - 1 June 2021

Importance to GLRaV-3



Economic Benefits of Using Clean/Certified Planting Material

- **Leafroll disease:** \$61,750 - \$558,458 loss per acre (25 yrs) depending on location.
- **Red blotch disease:** \$5,434 - \$171,738 loss per acre (25 yrs) in California & Washington State.
- **Pierce's disease:** Costs California \$92 million annually.
- **Leafroll-3:** The screening program saves California \$90 million annually.

Source: <https://www.nationalcleanplantnetwork.org/clean-plant-process>

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- **Grapevine viruses – how to test and eliminate?**
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Testing in Certification

Grapevine Virus Diagnostics

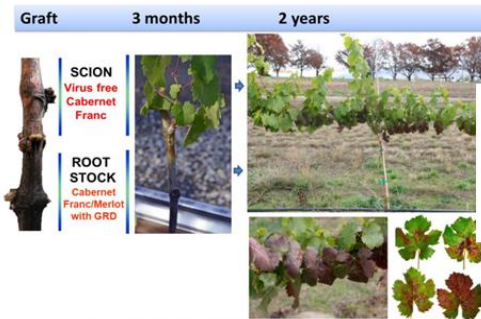
Symptom Based

Non-specific
Inaccurate



Biological Indexing

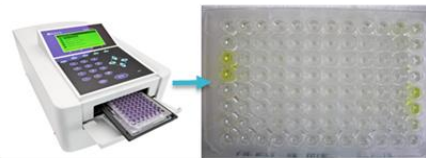
Symptom based
Labor intensive
Time consuming



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

Serological (ELISA)

Specific
No Abs for all viruses
Less sensitive than PCR



Molecular
(PCR, qPCR, ddPCR & NGS)

Highly specific
Post-PCR process
Multiplex
End-Point
Quantitative



High Throughput Sequencing (HTS) for virus detection



Quality Check

Total RNA → Ribo depletion
→ Ribo + poly A depletion

Total Nucleic acids

Small RNA

Viral NA enrichment → Oligo probes
→ dsRNA

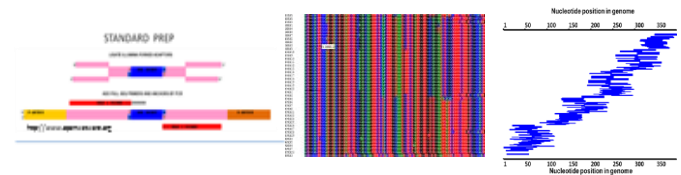
HTS sequence reads

Quality Check \ Adaptor sequence filter

De novo assembly of sequence reads

Blastn: host genome \ plant virus database

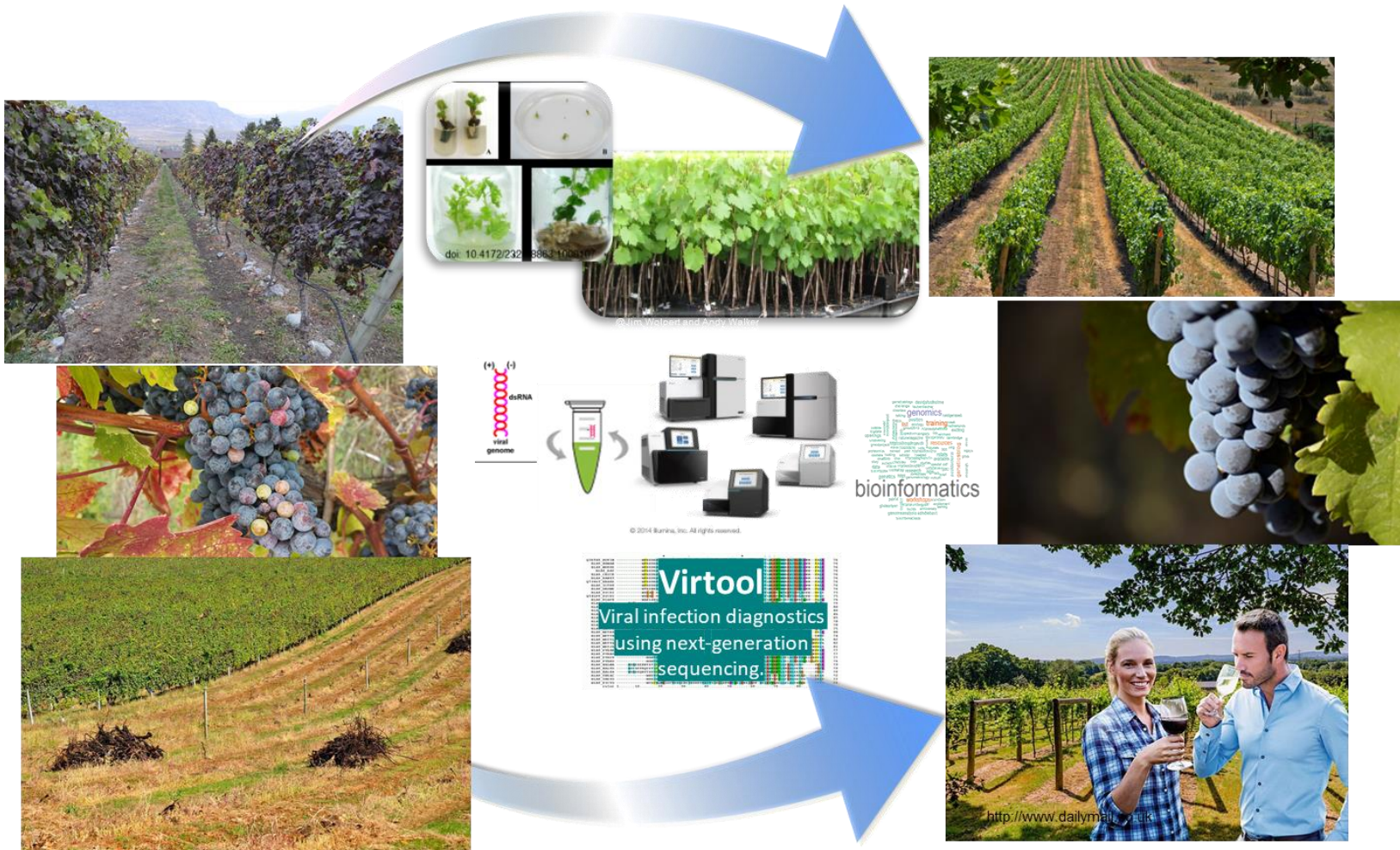
Virome discovery \ diagnosis



Virtool

Viral infection diagnostics using next-generation sequencing.

CLEAn plAnt extraction Sequencing SEquencing Diagnostics (CLEANSED) for Clean Grapevines in Canada



CLEAN plant extraction SEQUENCING Diagnostics (CLEANSED) for Clean Grapevines in Canada



Brock University



Canadian Food
Inspection Agency

Agence canadienne
d'inspection des aliments

Canada

Canadian Grapevine Certification Network

CGCN · RCCV

Réseau canadien de certification de la vigne



GenomeCanada



Genome
British Columbia

Leading > Investing > Connecting



Ontario Genomics



GenomeQuébec



compute canada



Conseil des vins
du Québec



University
of Victoria

illumina®



UNIVERSITÉ DE
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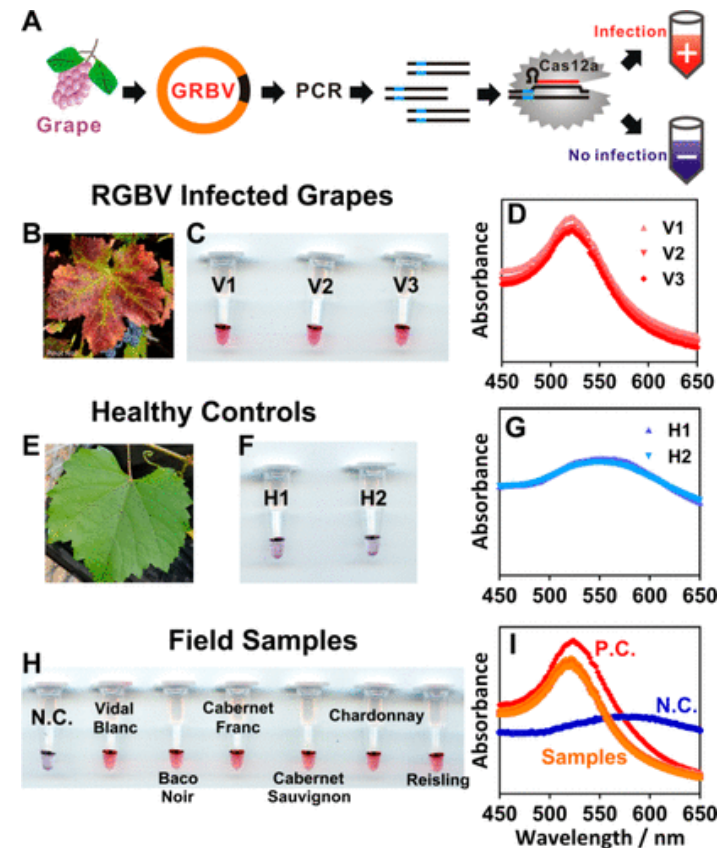
Other Detection Methods

Plasmonic CRISPR Cas12a assay for GRBV

(A) Workflow for visual detection of GRBV infections in red-fruited and white-fruited grapevine samples.

(B) Target recognition using CRISPR-Cas12a and the activation of incriminate ssDNase activity.

(C) Color development using plasmonic DNA functionalized AuNPs.



Yongya Li et al., Naked-Eye Detection of Grapevine Red-Blotch Viral Infection Using a Plasmonic CRISPR Cas12a Assay

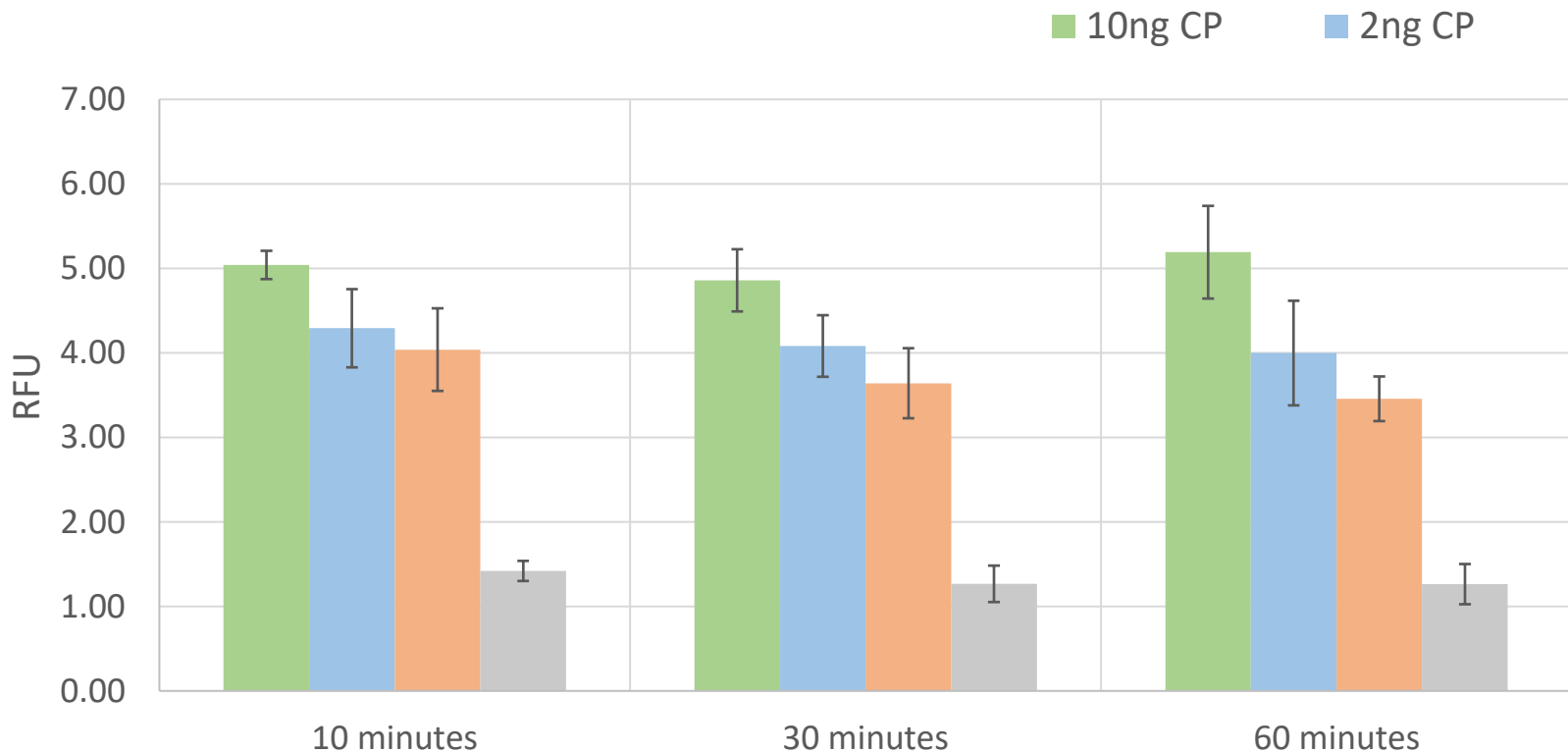
Analytical Chemistry. 2019. 91. 18. 11510-11513

Cool Climate Oenology and Viticulture Institute, Brock University

Department of Chemistry, Centre for Biotechnology, Brock University

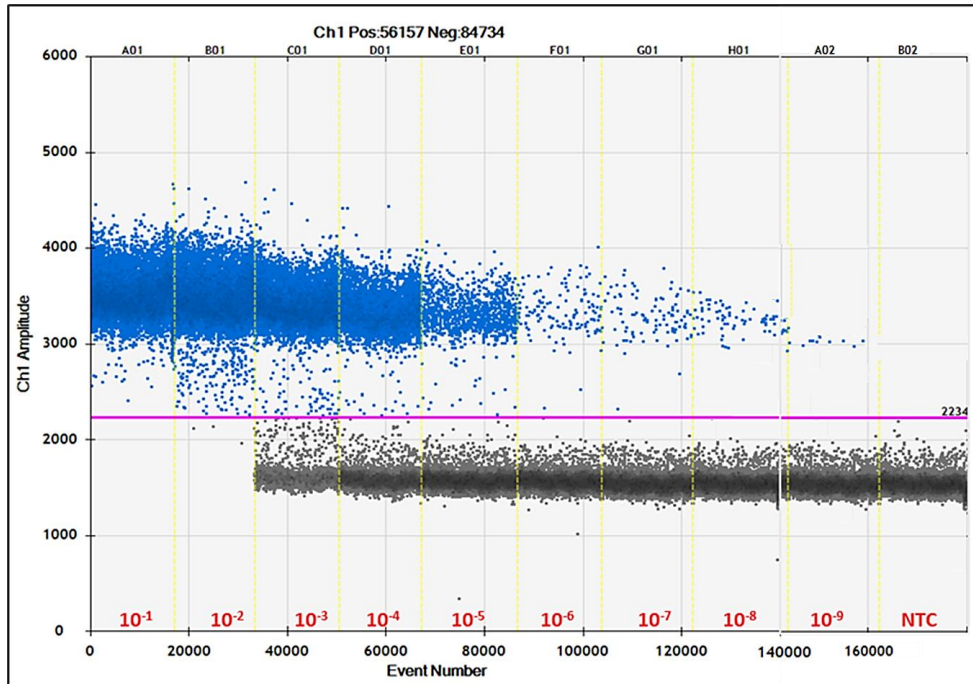
Other Detection Methods

Relative fluorescence level of CRISPR-Cas13 reaction with synthetic mRNA from the coat protein gene (CP) of GLRaV-3



CCOVI virus testing lab (Unpublished data)

Sensitivity of droplet-digital PCR assay for Grapevine Pinot gris virus



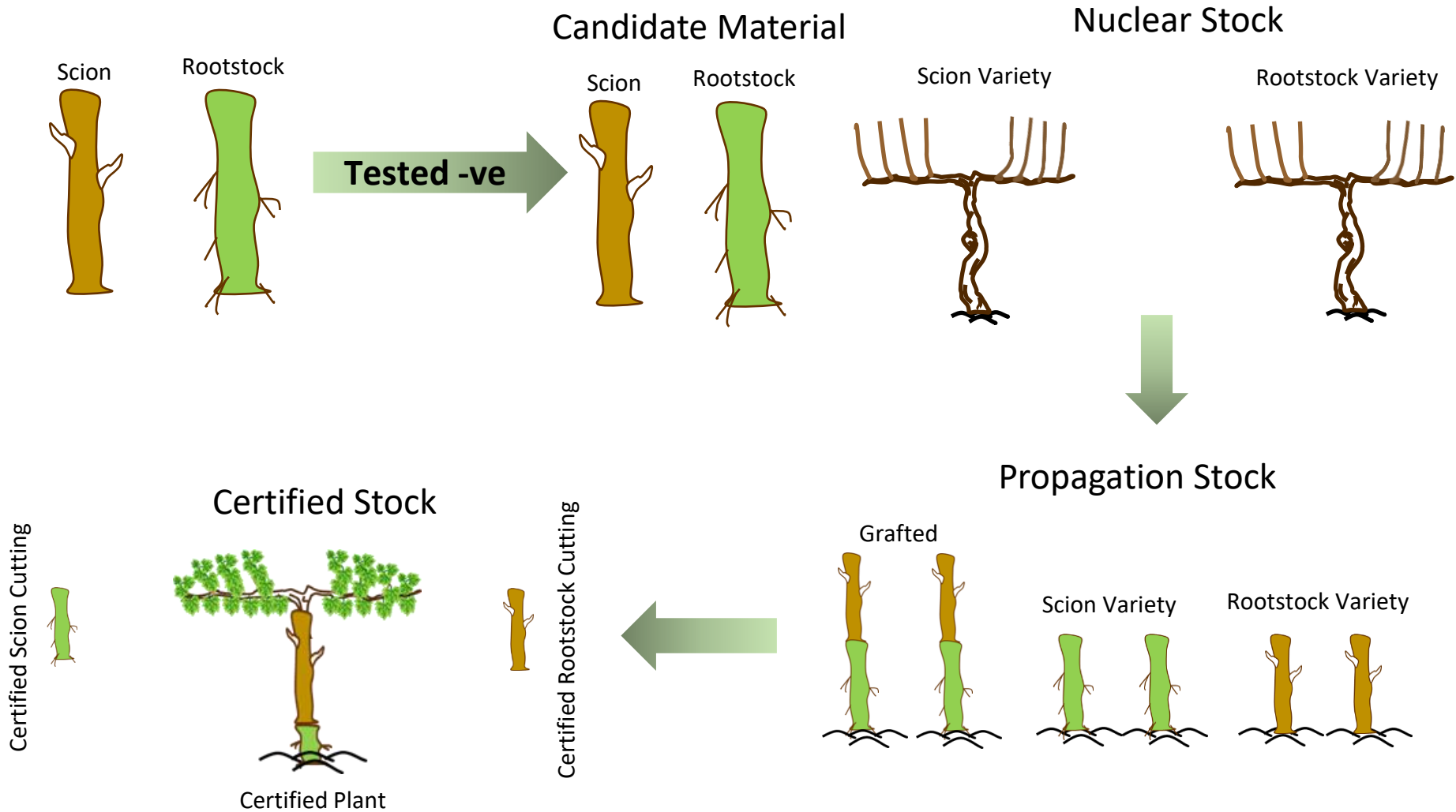
- 10-fold serial dilution of GPGV-MP plasmid DNA was tested
- 2uL template was used for each reaction
- All the negative droplets were excluded from the positive droplets by gating threshold values
- The lowest detection limit was recorded at 10⁻⁹ dilution (100 ag/uL)

Vemulapati et al. 2024 (Unpublished data)

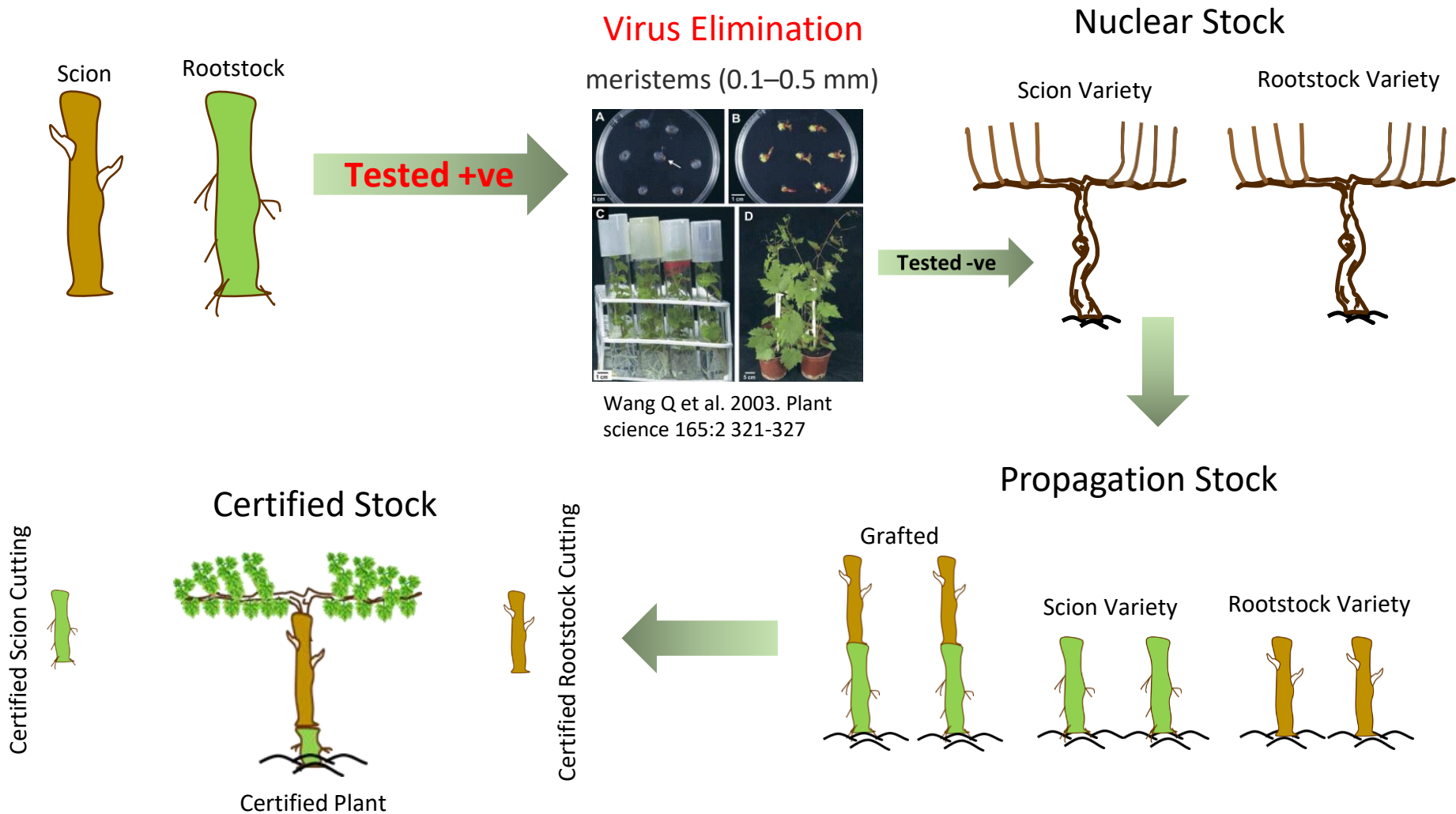
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Clean Plant Program - Certification Stages



Clean Plant Program - Certification Stages



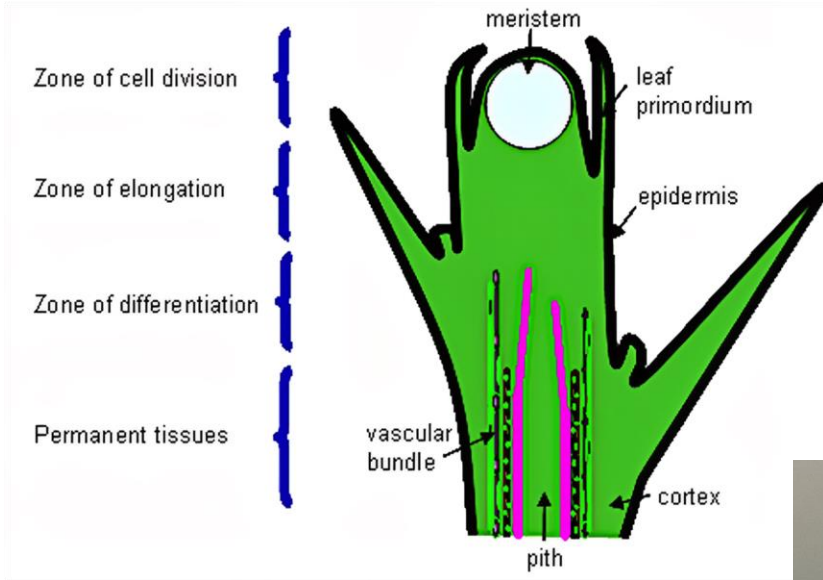
Wang Q et al. 2003. Plant science 165:2 321-327

Elimination of the Crown Gall Pathogen, *Agrobacterium vitis*, from Systemically Infected Grapevines by Tissue Culture

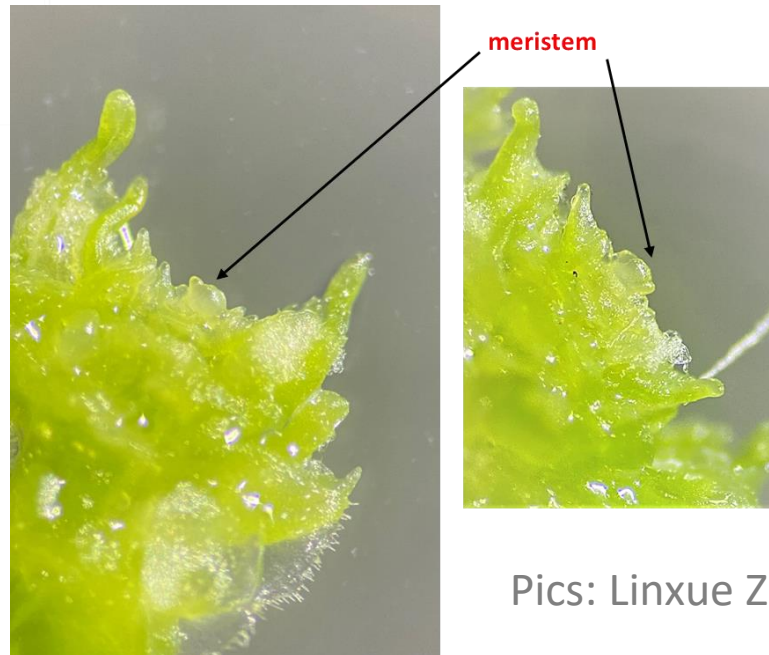
Luz Marcela Yepes, Tom Burr, Cherie Reid, Marc Fuchs
Am J Enol Vitic. July 2019 70: 243-248; DOI: 10.5344/ajev.2019.18083

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



Pics: Linxue Zhang

EPPO Guidelines on Sanitation

1. Heat Treatment

- Variable levels of efficacy
- Recommended for 2yr and older cuttings
- $38 \pm 1^{\circ}$ C and 16–18 h

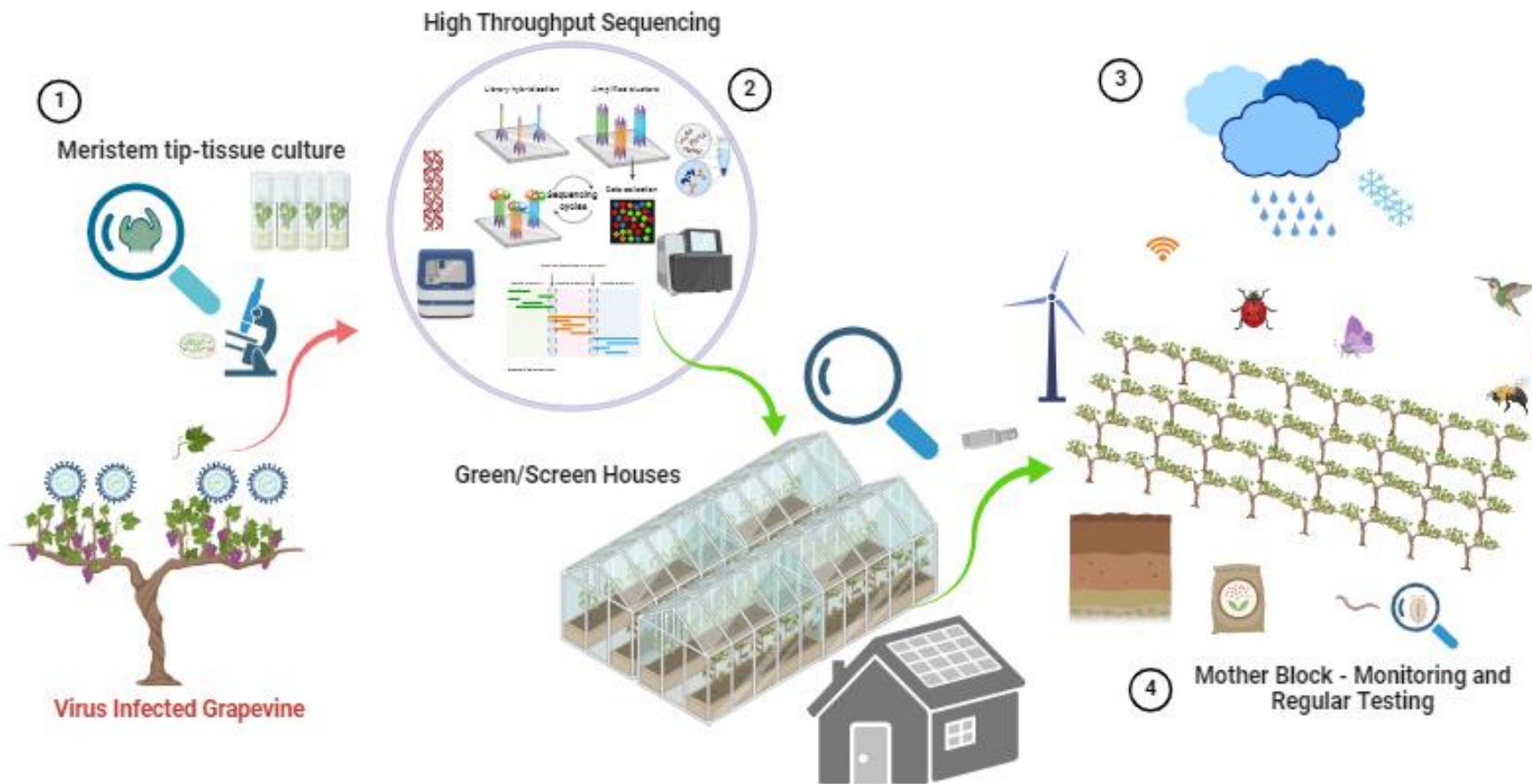
2. Meristem Shoot Tip Culture

- Shoot tips 0.4-0.6 mm-long with meristematic dome
- Grown in artificial media in controlled conditions
- Test the plantlets for viruses



Pics: Linxue Zhang

Clean Plant Program for Grapevines



What is a Certified Grapevine?

- **Clean Plant Center acts as a source:** They provide clean grapevine materials, not enough for entire vineyards, but to initiate propagation by nurseries.
- **Nurseries scale up production:** Nurseries receive the clean materials and multiply them to meet the needs of grape growers.
- **Certification ensures quality:** Nurseries must follow CGCN guidelines for pest management and virus testing to maintain certification.
- **Standards require frequent updates:** Check the CGCN website for the specific certification program and requirements.



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 clean plant programs?
5. How to ensure clean certified propagating material is available to nurseries/growers
6. Once the clean vines are planted, how to prevent the infection?

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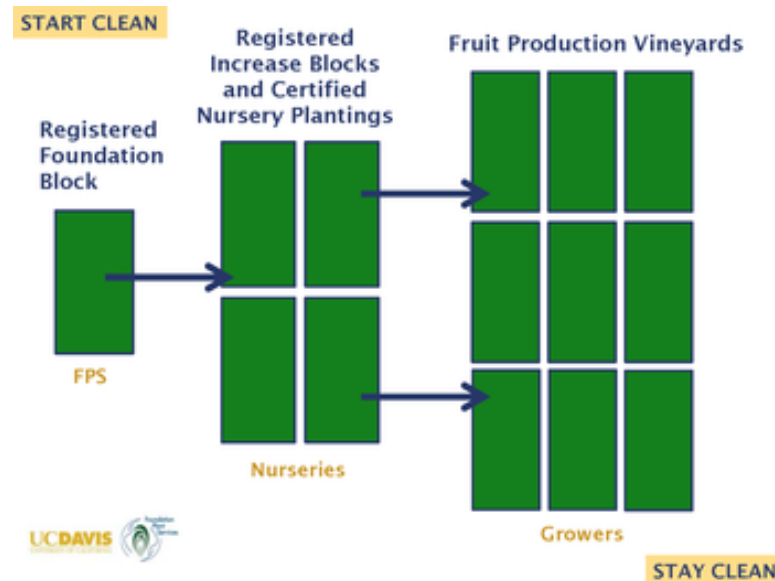
Quarantine Regulations: Canada

- Canada regulates the imports of grapevines from foreign countries through 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.

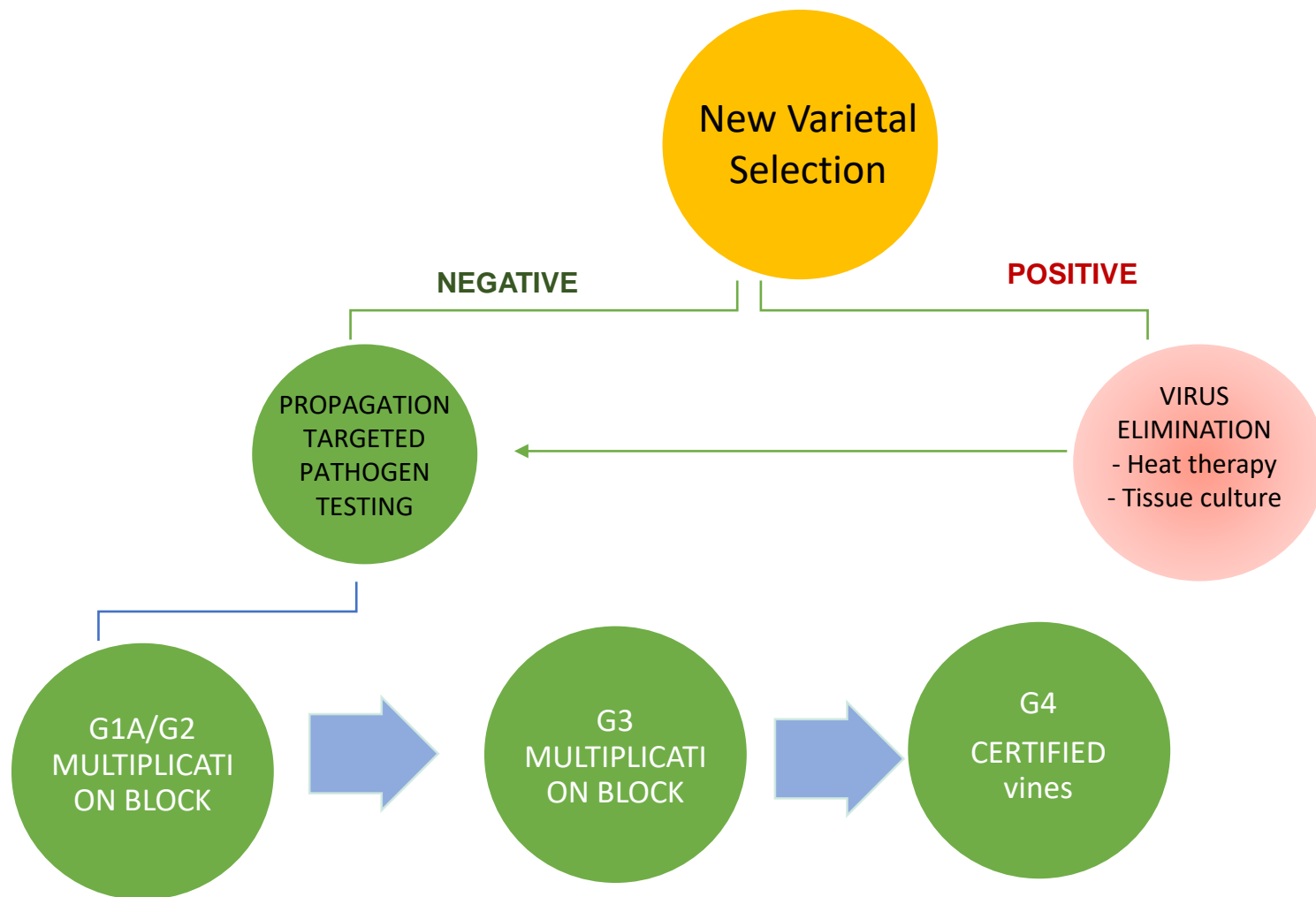
National Clean Plant Network (NCPN) – The USA

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



Long-Term Certification Standard



- ✓ Isolated, virus-tested block
- ✓ Inspected regularly for disease symptoms
- ✓ Retested on a 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	O	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	

Canadian Grapevine Certification Network (CGCN-RCCV)

- The nursery/custom propagator both need to have a signed contract with the CGCN-RCCV.
- Grapevine samples will be collected, and vines will be GPS-mapped for traceability.
- Leaf/Cane samples will be tested for viruses of concern at a CGCN-RCCV-approved lab.
- Sample collection and virus testing is at 50/50 cost-share between the grower/nursery and the CGCN-RCCV.
- The nursery remits a per vine levy (currently \$0.10) to CGCN-RCCV on each vine sold

Clean Plant Program - Structure

Science, Molecular diagnostics,
Taxonomy, and Epidemiology
Systematics studies



Research

Network, Educational Programs,
regional/crop-specific knowledge
transfer activities



Outreach

Imports/Exports: Surveys,
Developing Sanitary Protocols
Policy Changes



Quarantine

Acknowledgments



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Grape Growers
of ONTARIO



Ontario
Research Fund



Agriculture and
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Genome Canada



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



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Ministry of Agriculture,
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Agence canadienne
d'inspection des aliments

Canada



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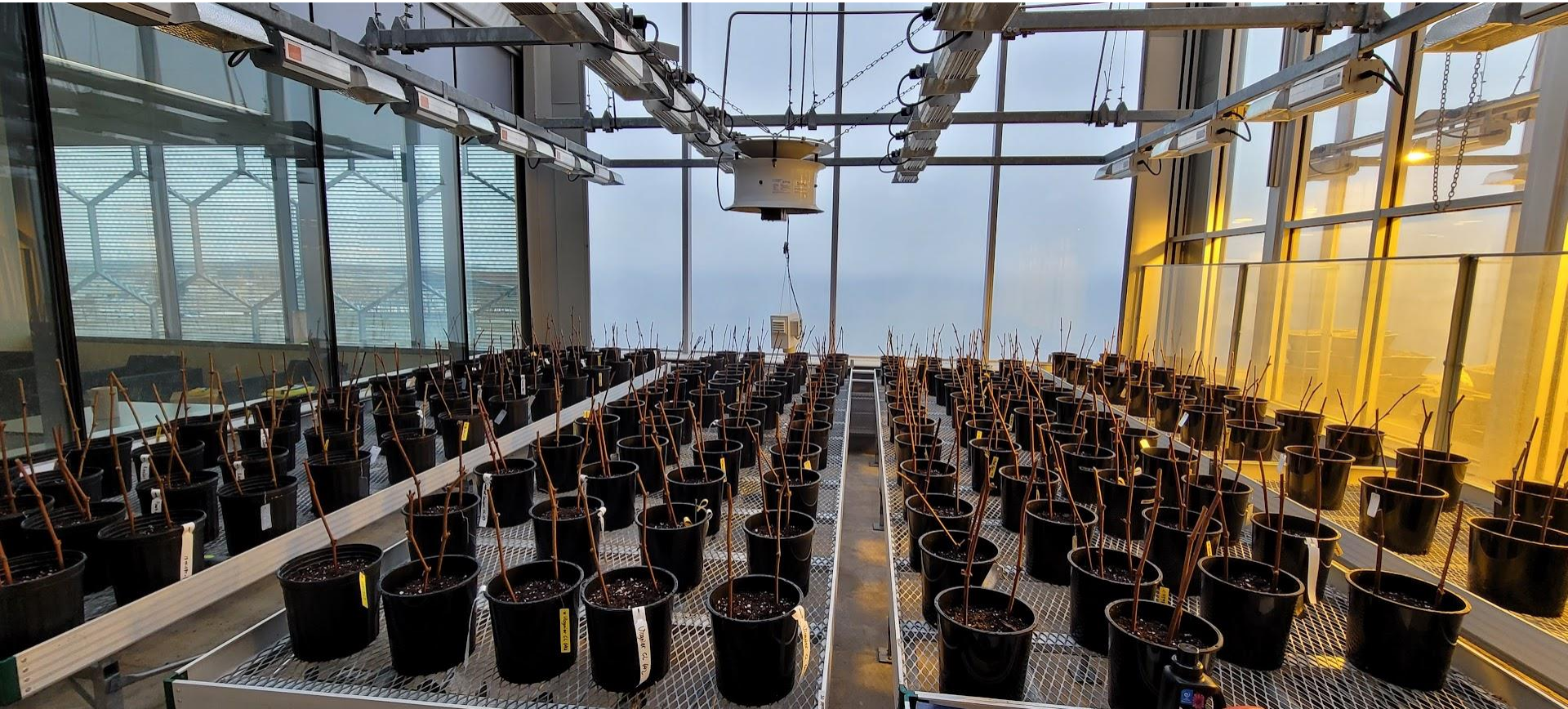
Virus Testing LAB



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



Grapevine Virus Culture Facility



We need “start clean stay clean” programs

