Advances in Grapevine Certification Standards

Cool Climate Oenology & Viticulture Institute

Brock University

Outline

- Grapevine viruses – importance & detection
- Grapevine certification standards – structure
- Clean Plant Programs – how different and why?
- Canadian prospective – what we have learned?

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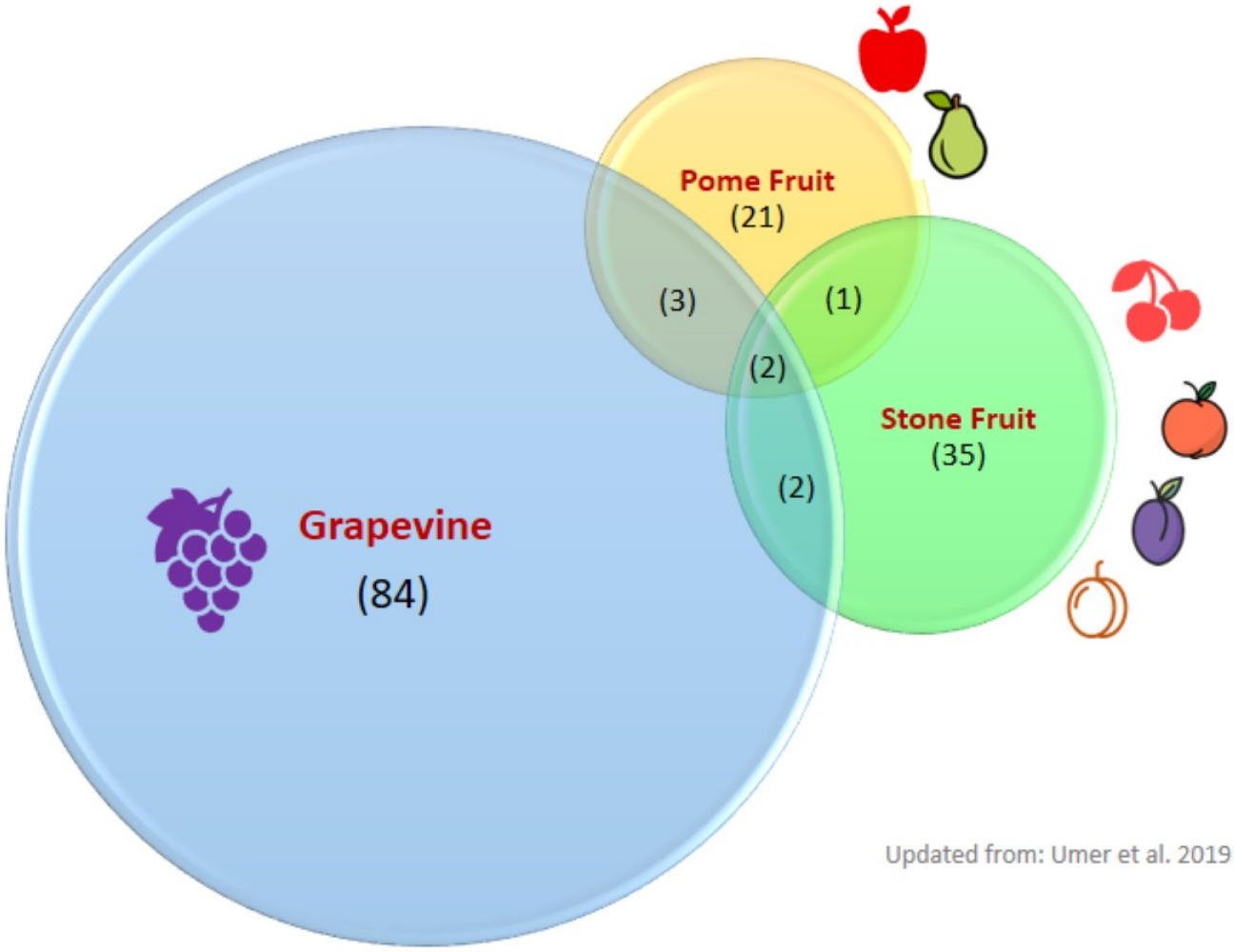
Virus cross-infections among fruit trees



Updated from: Umer et al. 2019

Grapevine Viruses

More than 80+ viruses

- Viruses cause serious production problems affecting rooting ability, graft take, vine vigour and fruit quality.

Major viral diseases

Grapevine leafroll disease (GLRD)

Grapevine leafroll-associated virus-1,-3,-4 and -7: *Ampelovirus*

Grapevine leafroll-associatedvirus-2: *Closterovirus*

Grapevine red blotch-associated virus (GRBaV)

Rugose wood (RW) complex

Grapevine rupestris stem pitting-associated virus (GRSPaV),
Grapevine virus A (GVA), *Grapevine virus B* (GVB),
Grapevine virus D (GVD).

Grapevine fanleaf degeneration complex

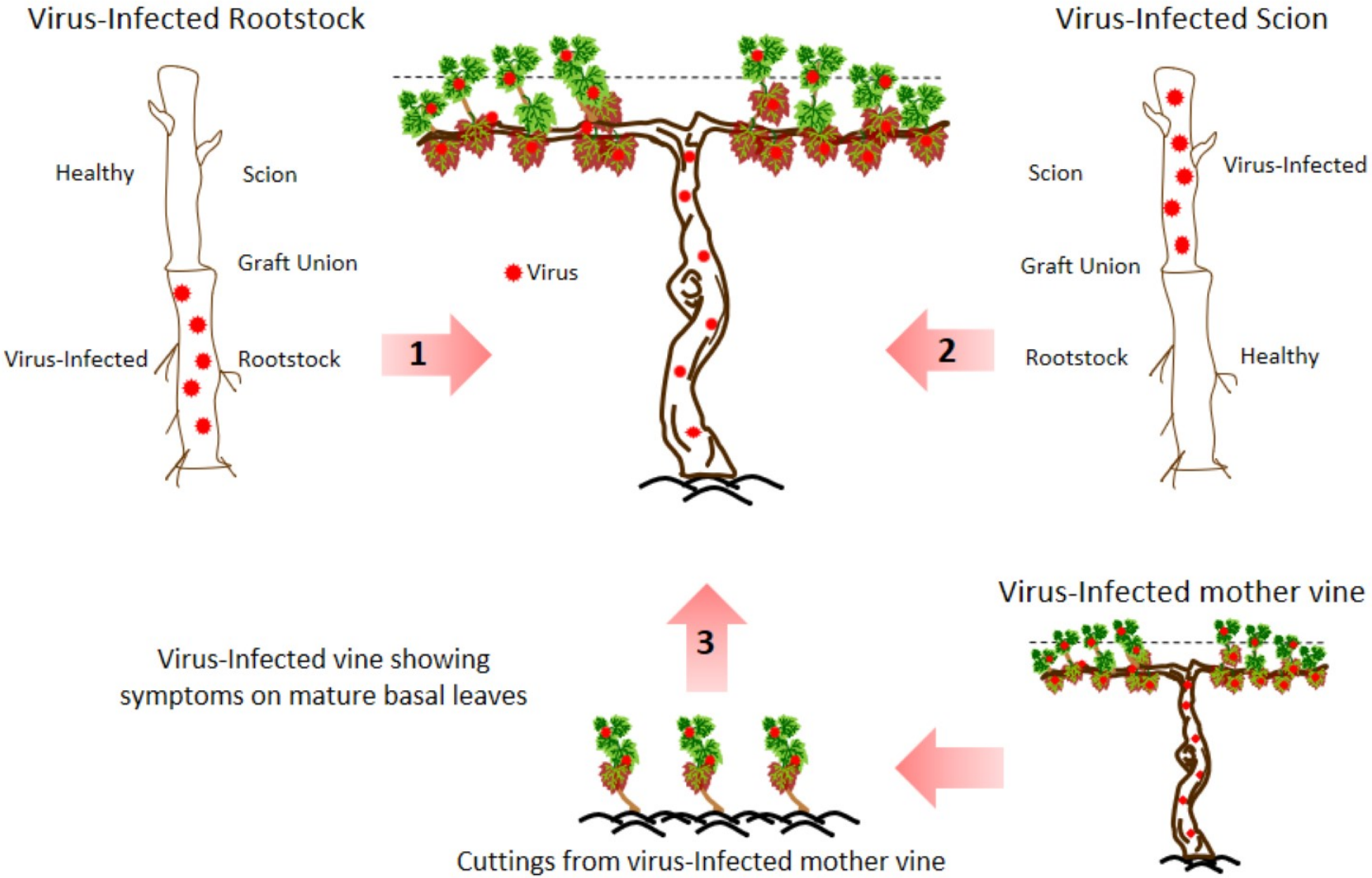
Grapevine fanleaf virus (GFLV)

Other viruses:

Grapevine fleck virus (GFkV)

Grapevine Pinot Gris Virus (GPGV)

Why?


Virus-Infected Rootstock
Healthy
Scion
Graft Union
Virus-Infected
Rootstock
Virus
1
2
Virus-Infected Scion
Scion
Virus-Infected
Graft Union
Rootstock
Healthy
3
Virus-Infected mother vine
Virus-Infected vine showing
symptoms on mature basal leaves
Cuttings from virus-Infected mother vine

Importance of Testing in Certification

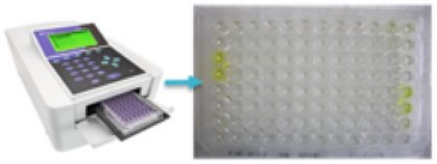
- Not all viruses show symptoms
- Source? Where did the virus come from?
- Plant Protection and Quarantine
- Disease Management
- The sustainability of grapevine production system depends heavily on the health status of the propagating planting material being free of grapevine pathogens.

Importance of 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



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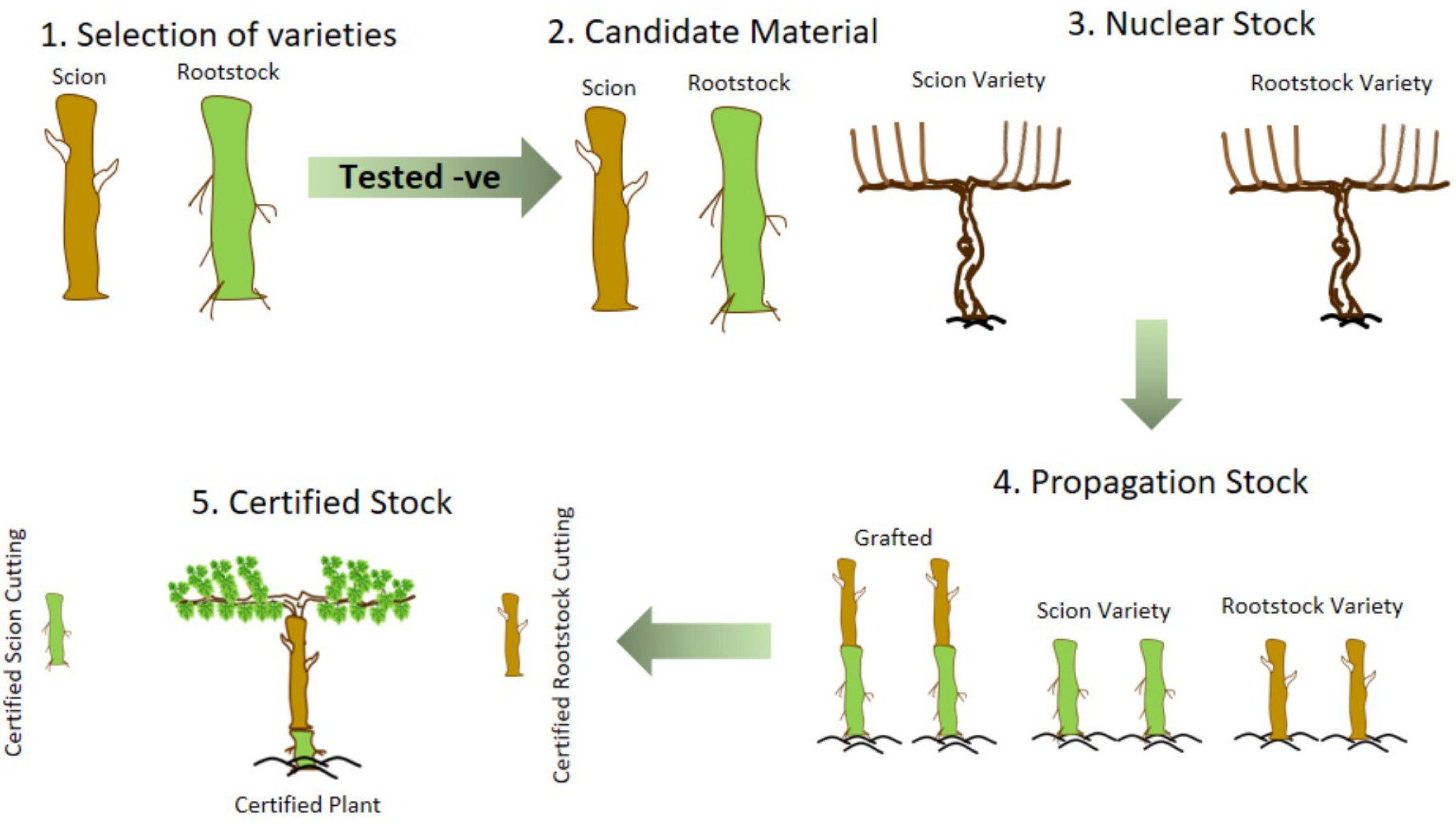
Certification Stucture

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

Certification Stages



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: 25 Jan 2021

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

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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. Once the clean vines are planted, how to prevent the infection?

Clean Plant Program

G1

- Mother vine selections screened for known pathogens (testing, biological indexing/HTS)
- 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

National Clean Plant Network (NCPN) – The USA

National Clean Plant Network for Grapes (NCPNG)

USDA's Animal and Plant Health Inspection Service (APHIS)

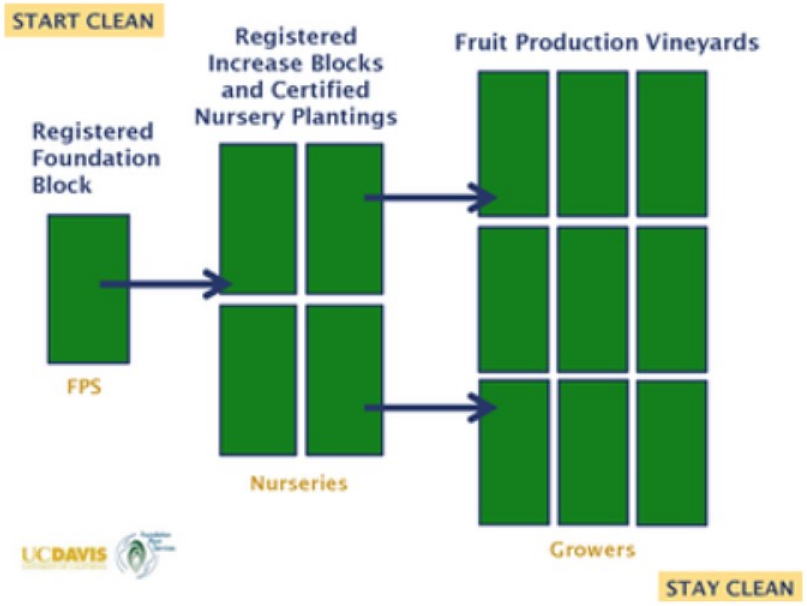
- California Department of Food and Agriculture (CDFA)
- Grapevine Registration and Certification (R&C) Program
- Established in 1956

http://ncpngrapes.org/Grape_Centers/

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

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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, and Washington.

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

List of CFIA Regulated Grapevine Infecting Viruses in Canada

Virus Name	Family	Genus	Directive*
Grapevine asteroid mosaic virus	*Tymoviridae*	*Marafivirus*	D-97-06, D-94-34
Grapevine Bulgarian latent virus (GBLV)	*Secoviridae*	*Nepovirus*	D-97-06, D-94-34
Grapevine chasselas latent agent	-	-	D-97-06, D-94-34
Grapevine (Hungarian) chrome mosaic virus (GCMV)	*Secoviridae*	*Nepovirus*	D-97-06, D-94-34
Grapevine corky bark virus	-	-	D-97-06, D-94-34
Grapevine enation agent	-	-	D-97-06, D-94-34
Grapevine vein mosaic or necrosis virus	-	-	D-97-06, D-94-34
Cherry leaf roll nepovirus (CLRV)	*Secoviridae*	*Nepovirus*	
Peach rosette mosaic nepovirus (PRMV)	*Secoviridae*	*Nepovirus*	D-97-06, D-94-34
Raspberry ringspot nepovirus (RRSV)	*Secoviridae*	*Nepovirus*	D-94-34
Strawberry latent ringspot nepovirus (SLRV)	*Secoviridae*	NA	D-94-34
Tomato black ring nepovirus (TBRV)	*Secoviridae*	*Nepovirus*	D-98-01, D-97-06, D-96-05, D-94-34
Tobacco ringspot nepovirus (TRSV)	*Secoviridae*	*Nepovirus*	D-97-06, D-94-34
Tomato ringspot nepovirus	*Secoviridae*	*Nepovirus*	D-97-06, D-94-34

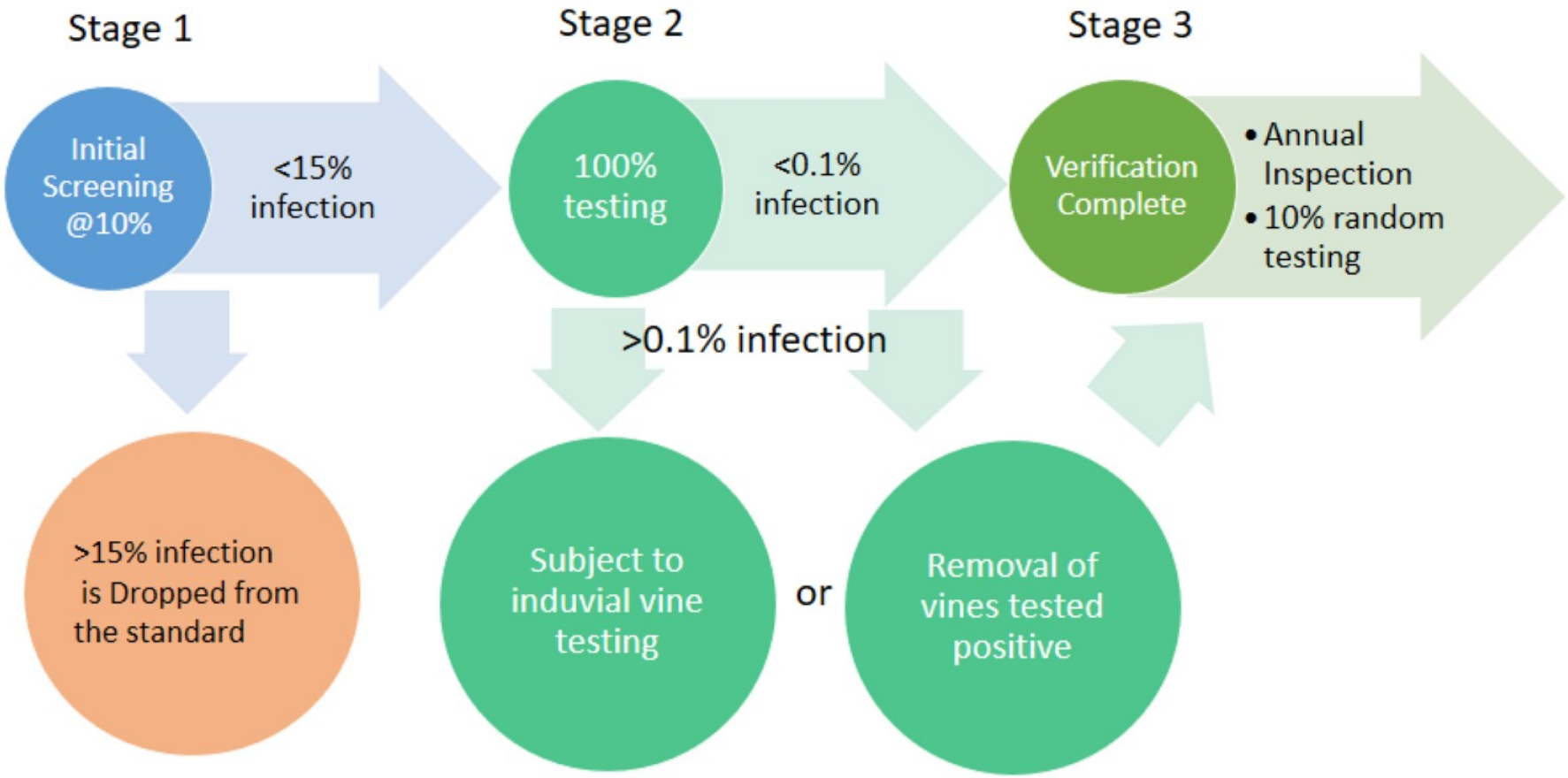
(http://inspection.gc.ca/plants/plant-pests-invasive-species/pests/regulated-pests/eng/1363317115207/1363317187811)

Canadian Grapevine Certification Network

CGCN-RCCV

Réseau Canadien de Certification de la Vigne

Interim Verification Standard



Canadian Grapevine Certification Network

CGCN-RCCV

Réseau Canadien de Certification de la Vigne

Interim Verification Standard

How it works

- The nursery and grape grower or winery both need to have a signed contract with the CGCN-RCCV.
- Grapevine field samples will be collected GPS mapped for traceability (to be able to eliminate positives)
- Samples will be tested for four viruses 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 to the grape grower/ nursery

Four Viruses

Grapevine leafroll-associated virus-1 & 3
Grapevine red blotch virus
Grapevine pinot gris virus

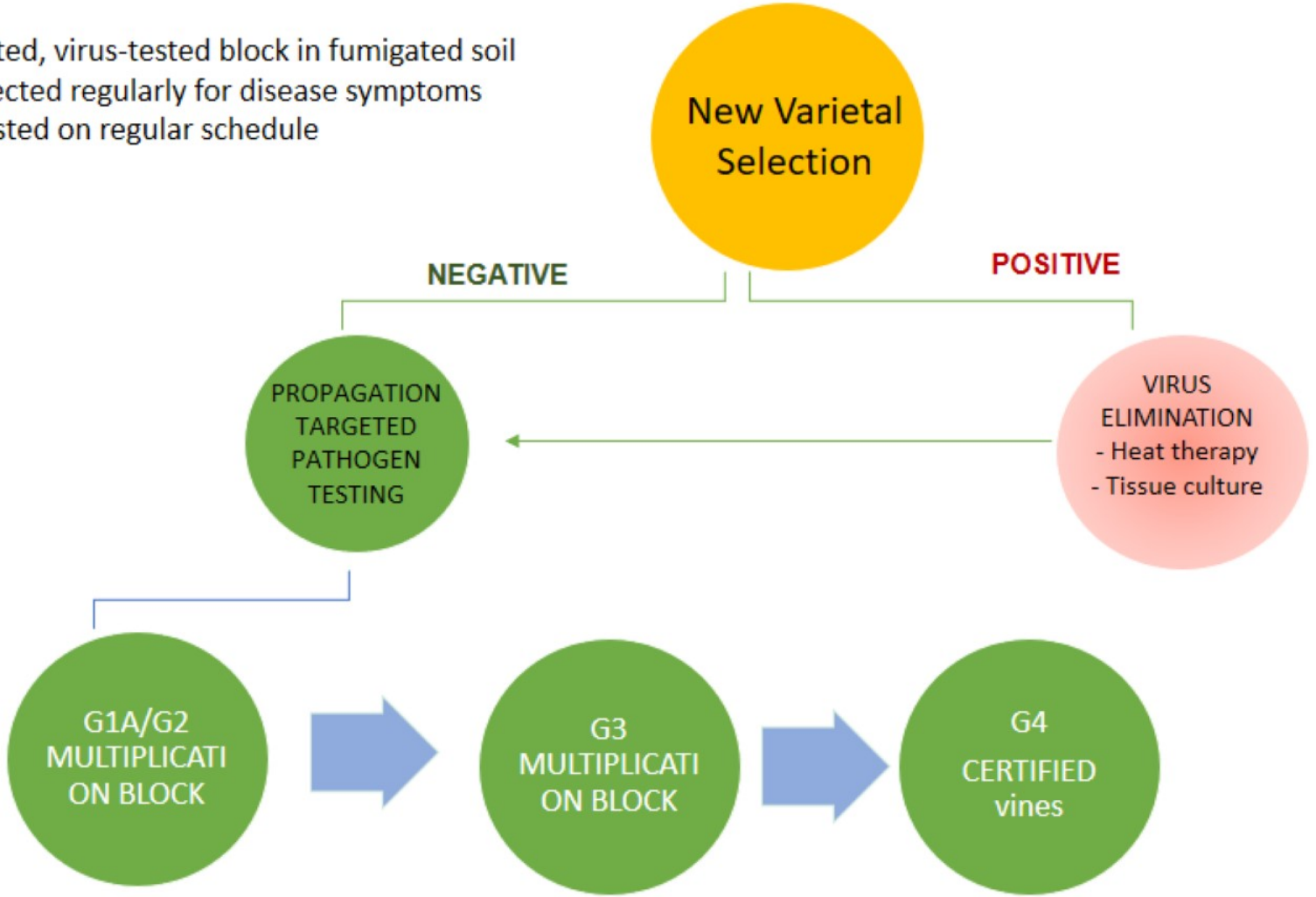
Canadian Grapevine Certification Network

CGCN-RCCV

Réseau Canadien de Certification de la Vigne

Long-Term Certification Standard

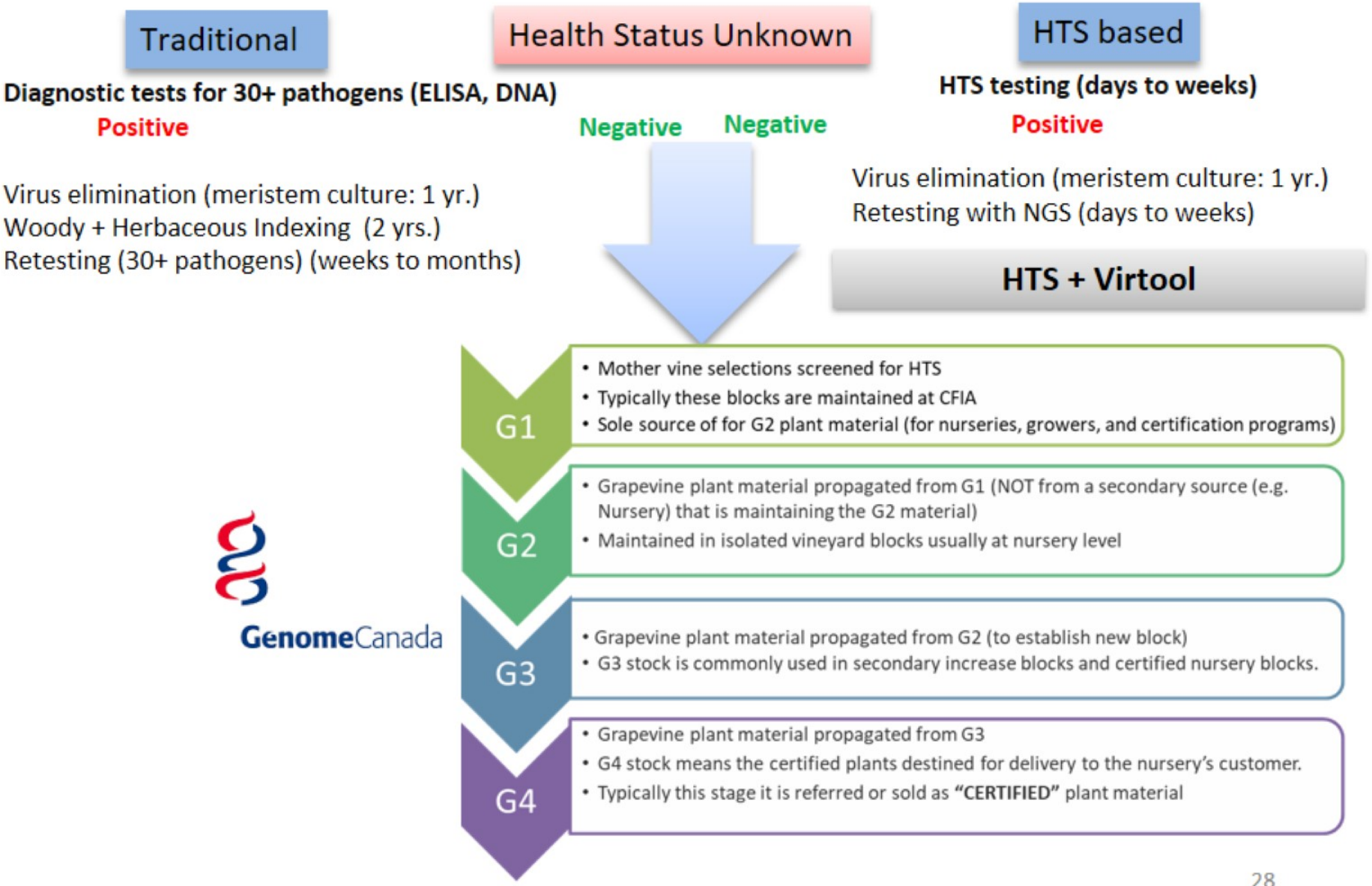
- ✓ Isolated, virus-tested block in fumigated soil
- ✓ Inspected regularly for disease symptoms
- ✓ Retested on regular schedule



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	

High Throughput Sequencing: Work in Progress



GenomeCanada

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

  in NZ: Grafted grapevine standard: v3.1: only GLRaV-3
- 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/G1 vineyards blocks or create new foundation/G1 vineyards
- Monitor and test the nuclear stock and increase blocks (G2/G3/G4) at regular intervals
- Revise standards of certification programs: Surveys; epidemiological research; advances in detection methods
- Multidisciplinary efforts are needed to remain strategically vigilant and always look forward

Courtesy: Dr. Marc Fuchs

Acknowledgments

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