

RESPONSE OF *VITIS VINIFERA* TO *IN VITRO* VIRUS ERADICATION TECHNIQUES

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Introduction

- **Global Significance**

Vitis vinifera is the world's most significant fruit crop, supporting the global wine, medicinal, and commercial sectors. (Kang & Jeong, 2025)

- **Viral Threats**

High susceptibility to viral diseases compromises longevity, with grafting ensuring vertical transmission (Basso et al., 2017)

- **Sanitation Challenges**

Viruses are intracellular and resist surface disinfection, necessitating complex eradication protocols (SS., 2022)



Viruses

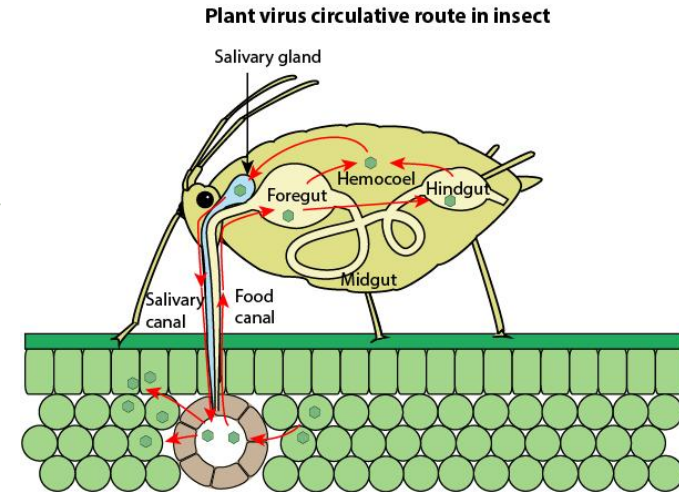
Viruses are small infectious agents, composed of one or more nucleic acid strands surrounded by a protective protein coat (Pearson, 2017).

Globally, **almost 100** different viruses are known to infect *Vitis vinifera* (Fuchs, 2023)

Easily transmissible by

- insect vectors such as aphids, mealybugs, spider mites, nematodes, treehopper protists and some species of fungi (Bahder, 2016, Tatineni & Hein, 2023);
- grafting, pruning operations and other mechanical damage;

They are widespread globally, except for vein-clearing and **red-blotch diseases (GRBD)**, which are known to be restricted within the United States (Martelli, 2017; Cieniewicz et al., 2020). However, recent studies have shown that **GRBD has spread already in European vineyards** (Reynard et al., 2021; Krenz et al., 2023)





Viruses

Generally, **visual symptoms** of viruses include mottled and deformed leaves, translucent veins, leaf-rolling symptoms, vine decline, and red blotch



Grapevine red blotch virus (GRBV)
Source: Reynard et al., 2022



Tomato Ringspot Virus (ToRSV)
Source: www.cgen-rccv.ca



Grapevine pinot gris virus (GPGV)
source: www.agriculture.vic.gov.au



Grapevine leafroll-associated viruses (GLRaV)
source: www.cgen-rccv.ca

Management

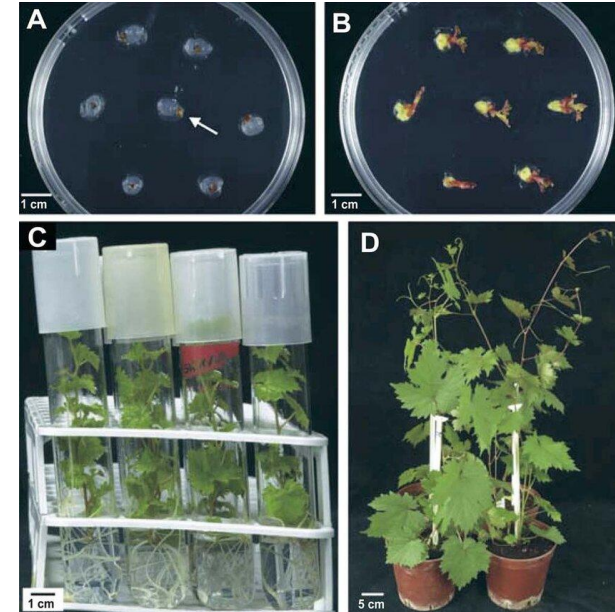
- Reducing the inoculum by removal all infected plants (Fuchs, 2020);
- Control of vector populations;
- Prevention of introducing the viruses into the vineyards – use of virus free planting material (Golino et al, 2017);

!!! Because viruses lack their own metabolic processes, there are no pesticides capable of combating them (Bhojwani and Dantu, 2013).

The advantages of establishing vineyards with planting material produced from virus-tested foundation stocks are substantial. These gains span economic, viticultural, winemaking (including fruit composition and wine chemistry), and environmental areas.

Generally, virus elimination is carried out *in vitro* by **isolating the meristem**. Worldwide, the production of planting material for vegetatively propagated species is based on *in vitro* multiplication.

The main methods used for plant virus eradication are **meristem culture**, **thermotherapy**, **chemotherapy**, and **cryotherapy**. Typically, these techniques are applied in combination to achieve higher efficiency in plant virus removal.



Shoots and plants obtained from cryopreserved meristems of *V. vinifera* 'Bruti'
Source: Wang et al., 2003

Materials and methods



Protocol identification

Examination of *in vitro* sanitation evolution, ranging from foundational Thermotherapy (1961) to modern dsRNA application, Chemotherapy and Cryotherapy.

Quantitative Assessment

Rigorous analysis of data focusing on the critical balance between viral eradication efficiency and the survival rates of explants.

Comparative Optimization

Evaluate current protocols to guide the integration of advanced, non-transgenic technologies into standard operations.



Results and discussions

Viral Pathogens: The Biological Trap

Systemic & Incurable: Unlike fungal or bacterial diseases, viral pathogens are systemic - they remain for life.

Vertical Transmission: Viruses are passed from mother to offspring with almost 100% efficiency.

The Necessity: The industry relies entirely on *ex situ* sanitation. Without it, viral load accumulates over generations.

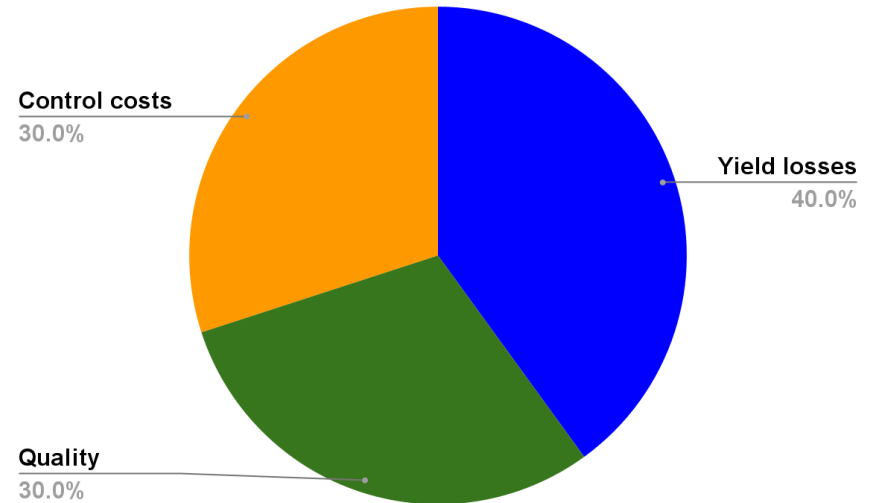


Results and discussions

Economic Impact

- **Yield loss:** Reduced tonnage and fruit set.
- **Quality penalties:** Lower sugar content, higher titratable acidity.
- **Control costs:** The “Hidden Cost” – vineyards are removed at year **15** instead of year **30**.

Data adapted from Atallah et al. (2012)



Thermotherapy: A Historical Evolution

Thermotherapy represents a heat treatment used in plant tissue culture to eliminate viruses and other pathogens from infected plant tissues.

Temperatures of 35-42°C → high enough to kill the virus, but low enough for the plant tissue to survive.



Source: bluestar-ee.com

Parameter	Gilford & Hewitt (1961)	Papakosta et al. (2025)
Objective	Single Virus (GFLV)	Multiple Co-infections
Temp/ Duration	37.8°C / 60-90 days	38°C / 40 days
Key Outcome	2% Regeneration rate	83% Sanitation rate
Limitation	Callus inactivity	Requires molecular tests

Thermotherapy

Miljanić et al. (2022) recommends min 6 weeks to max 3 months in growth chamber, at **36-38 °C**, with a 16- hour light photoperiod.

Then surface sterilization and **isolation of 0.1 – 0.2 mm meristematic tips** and **micrografted of sectioned hypocotyls**.



100 % elimination for 8 viruses: GRSPaV (grapevine rupestris stem pitting-associated virus), **GPGV** (grapevine Pinot gris virus) **GFLV** (grapevine fanleaf virus), **GLRaV-3** grapevine leafroll-associated virus 3, **GFkV** (grapevine fleck virus), **GRVfV** (grapevine rupestris vein feathering virus), **GSyV-1** (grapevine Syrah virus-1) and **RBDV** (raspberry bushy dwarf virus).

39.2 – 42.6% for 2 viroids: HSVd (hop stunt viroid and **GYSVd-1** (grapevine yellow speckle viroid 1)



Thermotherapy and meristem isolation in *Vitis vinifera*

Source: Miljanić et al., (2022)

Chemotherapy: Antiviral Agents

The Mechanism

Utilizes antiviral drugs (Ribavirin + Oseltamivir) in the growth medium.

The Trade-off: High doses kill the virus but are lethal to the plant – *Phytotoxicity*

Case study: Guță et al. (2014)

1. V1=40 mg/L ribavirin + 40 mg/L oseltamivir;
2. V2=20 mg/L ribavirin + 40 mg/L oseltamivir – optimal dosage for viral elimination and plant survival
3. V3=20 mg/L ribavirin + 80 mg/L oseltamivir;



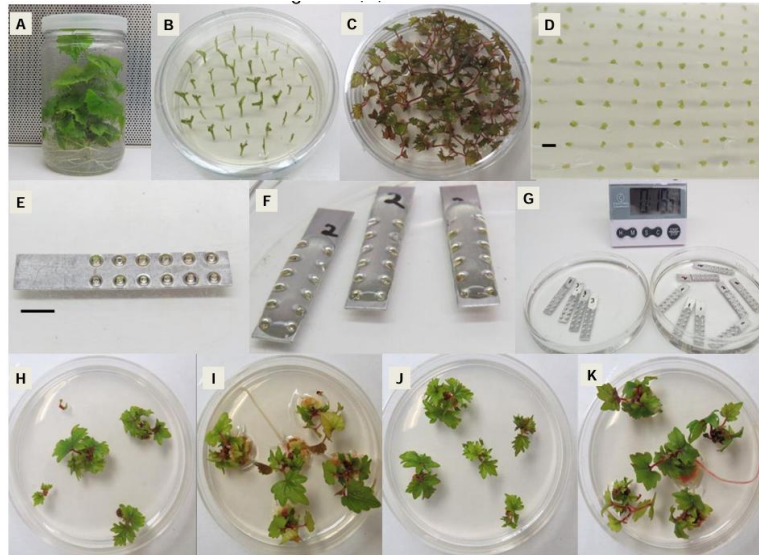
Chemotherapy: Antiviral Agents

Skiada et al. (2012) used antivirals such as **inosine 5'-monophosphate dehydro genase inhibitors tiazofurin (TR), ribavirin (RBV) and mycophenolic acid (MPA)** for elimination of GRSPaV (Grapevine rupestris stem pitting-associated virus)

Severe phytotoxic effects, evaluated 40 days into the culture period, were noted in explants exposed to antivirals, especially those treated with elevated TR doses.

- **highest GRSPaV elimination (80%)** in 'Agiorgitiko' was obtained with 10 μgml^{-1} TR, 30 μgml^{-1} RBV and 20 μgml^{-1} MPA
- lower elimination rates (37.5 – 53.5 %) were observed in the case of 'Malagouzia', with the highest ones were achieved after treatments with 15 μgml^{-1} TR and 80 μgml^{-1} MPA

Cryotherapy: Targeted Eradication



V cryo-plate procedure for *in vitro* grapevine
 Source: Bettoni, 2018

Mechanism of Action

Explants are exposed to **liquid nitrogen (-196°C)** to freeze their shoot tips

Infected cells: Highly vacuolated – they freeze, burst, and die
Meristem Cells: Densely cytoplasmic – they “vitrify” and survive

Efficacy vs. Tradition

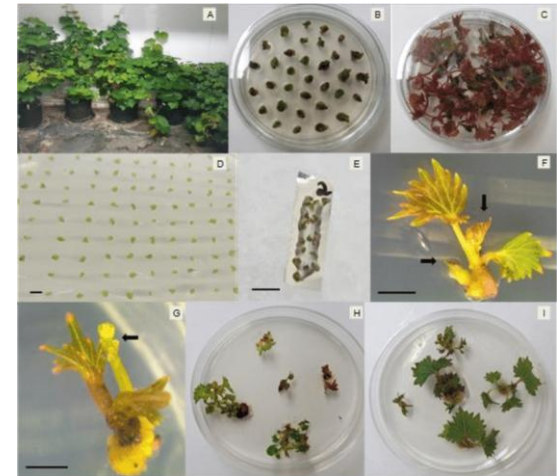
Wang et al. (2003) demonstrated a **97%** eradication rate of GVA using cryotherapy on 0.5–2.0 mm shoot tips.

Cryotherapy

Pathirana et al. (2015) used **droplet-vitrification technique** for elimination of **GLRaV-3** (Grapevine leafroll associated virus-3), **GLRaV-1** (Grapevine leafroll associated virus-1) and **GLRaV-2** (Grapevine leafroll associated virus-2) in ‘Chardonnay’, ‘Lakemont Seedless’, ‘Pinot gris’ and ‘Sauvignon blanc’

Bi et al. (2018) obtained high regeneration rates (43 – 59%) of **GLRaV-3-free** material using **droplet-vitrification**, by culturing 1.0 mm shoot tips on medium with **0.3 M sucrose**, **0.16 mM glutathione** and **0.14 mM ascorbic acid**, followed by treatment with **0.16 M glycerol** and **0.4 M sucrose** to freezing in liquid

GLRaV-3 was not present in **apical dome (AD)** and LPs 1–4, but it was detected in the basal shoot tip region, approximately 0.5 mm from the AD, as well as in LP 5 and more mature tissues



Cryotherapy by droplet vitrification procedure of *Vitis* shoot tips
Source: Bettoni, 2018

Future horizons: dsRNA & BioClay



Exogenous dsRNA sprayed topically



Triggers Plant RNAi



Virus RNA is destroyed

Overcoming Instability

Naked dsRNA degrades rapidly (5-7 days) when exposed to **environmental factors**.

The solution: BioClay (Layered Double Hydroxide nanosheets). This nanotechnology prevents wash-off, extending protection to several weeks.

(Minter et al., 2017)

Conclusions

Evaluation of Eradication Protocols:

Thermotherapy

Dominant standard due to accessibility.

Constraint: High mortality (fragile meristems).

Cryotherapy

High disinfection rates via cellular differentiation.

Constraint: Regeneration depends on sizing and requires special equipments

Chemotherapy

Drives prompt, effective antiviral response.

Constraint: Phytotoxicity & growth inhibition.

dsRNA

Novel, non-GMO, immune response trigger.

Constraint: Environmental sensitivity

1. Diagnostic Precision

Status confirmation requires **molecular tests** (PCR) rather than serological assays to avoid missing latent infections.

2. Adaptive Protocols

Parameters must be tailored to the pathogen's specific resistance (e.g., heat tolerance) while respecting grapevine physiology.

Virus control is not an expense, it is the biological insurance policy for your vineyard's future.

References

- Gifford, E. M., & Hewitt, W. B. (1961). The use of heat therapy and in vitro shoot tip culture to eliminate fanleaf virus from the grapevine. *American Journal of Enology and Viticulture*, 12(3), 129-130.
- Guță, I. C., Buciumeanu, E.-C., & Vișoiu, E. (2014). Elimination of Grapevine fleck virus by in vitro Chemotherapy. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 42(1), 115–118.
- Kang, C. M., & Jeong, R. D. (2025). Inhibition of hop stunt viroid by exogenous double-stranded RNA in micropropagated grapevine plantlets. *The Plant Pathology Journal*, 41(4), 507.
- Kang, C. M., Kim, M. J., Hong, J. S., & Jeong, R. D. (2025). Managing Plant Viruses in Tissue-Cultured Apple and Grapevine: Strategies for Detection and Eradication. *The Plant Pathology Journal*, 41(5), 545.
- Mitter, N., Worrall, E. A., Robinson, K. E., Li, P., Jain, R. G., Taochy, C., ... & Xu, Z. P. (2017). Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses. *Nature plants*, 3(2), 1-10.
- Papakosta, E., Cara, O., Merkuri, J., & Cara, M. (2025). Recovery from Viral Infections through in Vitro Techniques in the Local Grapevine Cultivar “Shesh I Zi”. *J Envi Sci Agri Res*, 3(4), 1-5.
- SS, O. (2022). Micropropagation of grapevine (*Vitis Vinifera* L.) Cvs. red globe and Superior. *Iraqi Journal of Agricultural Sciences*, 53(4), 833-849.
- Tatineni S, Hein GL. Plant Viruses of Agricultural Importance: Current and Future Perspectives of Virus Disease Management Strategies. *Phytopathology*. 2023 Feb;113(2):117-141.
- Fuchs, M. Grapevine viruses: a multitude of diverse species with simple but overall poorly adopted management solutions in the vineyard. *J Plant Pathol* 102, 643–653 (2020). Golino D, Fuchs M, Sim S, Farrar K, Martelli G.P. 2017a. Improvement of grapevineplanting stocksthroughsanitary selectionand pathogen elimination. In: Grapevine viruses: molecular biology, diagnostics and management. Meng B, Martelli GP, Golino DA and Fuchs M (eds), Springer Verlag, Cham, pp. 561-580
- Reynard, JS., Brodard, J., Dubuis, N. *et al.* Screening of grapevine red blotch virus in two European ampelographic collections. *J Plant Pathol* **104**, 9–15 (2022).
- M. Pearson, 2017 *Viral Diseases*, in Editor(s): Brian Thomas, Brian G Murray, Denis J Murphy: *Encyclopedia of Applied Plant Sciences* (Second Edition), Academic Press, 137-147 ISBN 9780123948083,
- Cieniewicz EJ, Qiu W, Saldarelli P, Fuchs M (2020) Seeing is believing: Lessons from emerging viruses in grapevine. *J Plant Pathol*. [https:// doi.org/10.1007/s42161-019-00484-3](https://doi.org/10.1007/s42161-019-00484-3)
- Martelli GP (2017) An overview on grapevine viruses, viroids and the diseases they cause. In: Meng B, Martelli GP, Golino DA, Fuchs M (eds) *Grapevine viruses: molecular biology, diagnostic and manage ment*. Springer Verlag, Cham, pp 31–46
- Krenz B, Fuchs M, Thompson JR. Grapevine red blotch disease: A comprehensive Q&A guide. *PLoS Pathog*. 2023 Oct 12;19(10):e1011671. doi: 10.1371/journal.ppat.1011671. PMID: 37824437; PMCID: PMC10569545.
- Bhojwani, S.S., Dantu, P.K. (2013). Production of Virus-Free Plants . In: *Plant Tissue Culture: An Introductory Text*. Springer, India. https://doi.org/10.1007/978-81-322-1026-9_16
- Pathirana, R., McLachlan, A., Hedderley, D., Carra, A., Carimi, F. and Panis, B. (2015). REMOVAL OF LEAFROLL VIRUSES FROM INFECTED GRAPEVINE PLANTS BY DROPLET VITRIFICATION. *Acta Horti*. 1083, 491-498
- Bi, Wen-Lu & Hao, Xin-Yi & Cui, Zhen-Hua & Pathirana, Ranjith & Volk, Gayle & Wang, Qiao-Chun. (2018). Shoot tip cryotherapy for efficient eradication of grapevine leafroll-associated virus-3 from diseased grapevine in vitro plants. *Annals of Applied Biology*. 173. 261-270. 10.1111/aab.12459.

Thank you for your attention!

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