



# **Potential Implications and Management of Grapevine Viruses in Mexico: A Review**

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**Abstract:** Worldwide, virus infections in grapevines are of concern due to the potential for economic loss. Although the grape industry in Mexico is relatively small and focused mainly on the local market, production dates back to the time of the Spanish colonization. This manuscript discusses the findings on grapevine viruses in Mexico. Nine viruses have been identified in the last fifty years, including grapevine red blotch virus (GRBV), grapevine leafroll-associated virus 3 (GLRaV-3), grapevine fanleaf virus (GFLV), and grapevine virus A (GVA). Important information is provided about these viruses and viral pathogens that have not yet been reported in Mexico, but represent an ongoing threat to plant health and grapevine production in other viticultural regions of the world. Strategies for virus control in vineyards are described. The information discussed here should be shared with growers and stakeholders to prevent future negative impacts on the Mexican grapevine industry and to save ancient grapevine accessions.

Keywords: grapevine; grape production; Mexico; virus; conservation; epidemiology; disease control

# 1. Introduction

Mexico has over 37,000 hectares of vineyards, the majority dedicated to the cultivation of table and wine grapes, with an estimated production of 400,000 tons per year (Consejo Mexicano Vinicola; https://www.uvayvino.org.mx; accessed: 30 November 2022). The grapevine industry generates jobs for 500,000 day-laborers, which makes it the fourth source of employment in the agricultural sector after the fruit tree, berry, and vegetable industries (Instituto Nacional de Estadística y Geografía; https://www.inegi.org.mx; accessed: 30 November 2022). Currently, grapes (*Vitis* spp.) are cultivated in fourteen Mexican states, with Baja California, Sonora, and Zacatecas being the major states of production (Figure 1). The increasing popularity of wine in Mexico has led to the establishment of many new vine-yards. Red cultivars grown in Mexico include Cabernet Sauvignon, Carignan, Pinot Noir, Pinot Gris, Petite Syrah, Malbec, Merlot, Ruby Cabernet, Tempranillo, and Nebbiolo. White cultivars include Chardonnay, Chenin, Sauvignon Blanc, St. Emilion, Macabeu, Muscat Blanc, Malaga, French Colombard, Ugni Blanc, and Traminer (International Organisation of Vine and Wine; https://www.oiv.int/index.php/what-we-do/statistics; accessed: 30 November 2022).



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Figure 1. Main grapevine producing states in Mexico indicated in red color.

Although the industry of grape production is relatively new in Mexico, interestingly, some of the oldest vineyards in the American continent are situated in the country. According to official information from the Mexican government, the history of viticulture in Mexico started with the first vines that were brought by the Spanish conquerors (https://www.gob.mx/inaes/articulos/historia-de-la-viticultura; accessed: 30 November 2022). The Spanish conquerors grafted *V. vinifera* from Spain onto wild grapevines (*V. labrusca, V. rupestris* and *V. berlandieri*) that they discovered in what is now Mexico [1]. Vineyards began to spread from Mexico City, towards the northern regions: Querétaro, Guanajuato and San Luis Potosí. In 1900, a pest (grape phylloxera) destroyed many vineyards in the country [2]. As a result, the preservation of old vineyards became a priority in order to preserve these sources of genetic diversity [3,4].

In 2020, Fuchs updated a list of grapevine viruses from Martelli, resulting in 86 different viral agents [5,6]. Additionally, at least other 10 viruses have been discovered in this plant during the last years [7–10], making grapevine the woody crop with the highest number of viral agents known in a single crop. Some of these pathogens can be spread in the field by natural vectors (insects, mites, and nematodes) and all can be transmitted by vegetative propagation and grafting. Yeh et al. [11] and Fuchs et al. [12] reviewed the economic impact of some of these viruses on grapevine. For example, the annual impact of grapevine leafroll-associated virus 3 (GLRaV-3) is estimated at \$90 million US dollars in California [13], and losses to grapevine red blotch virus (GRBV) were estimated at \$2200 US dollars per hectare in Washington [14], both located in the United States.

A relatively new technology used to detect plant pathogens is high throughput sequencing (HTS). HTS generates a large amount of sequence information from the plant, including its genome and any pathogenic and non-pathogenic agents present [15]. New viruses have been discovered that cause disease and economic loss, as well as new viruses that have an unknown impact on the grapevine production. The use of HTS could lead to a better understanding of the potential cause of disease in vineyards. However, further work is required in order to demonstrate a causal relationship between detected pathogens and observed symptoms.

García-Reséndiz and Carrillo-Tripp [16] enlisted nine grapevine viruses associated with viral diseases in Mexico: grapevine fanleaf virus (GFLV), grapevine leafroll-associated viruses 1, 2 and 3 (GLRaV-1, GLRaV-2, GLRaV-3), grapevine viruses A and B (GVA, GVB), grapevine rupestris stem pitting-associated virus (GRSPaV), grapevine fleck virus (GFkV), and GRBV. However, as discussed by these authors, most of the reports lack detail and

follow-up or they were published in local media of difficult access. Since their publication describes the chronology of studies of grapevine viruses in Mexico, this aspect will not be discussed here. Instead, this manuscript describes the possible future of grapevine viruses in Mexico, including some of the major viral pathogens, viral complexes, and diseases in this crop. The potential for damage, management strategies, and control measures are discussed.

#### 2. Viruses Identified in Mexico

# 2.1. Grapevine Red Blotch Virus

GRBV belongs to the genus *Grablovirus* in the family *Geminiviridae*, it has a singlestranded circular DNA genome (3.2 kb long) encapsidated in a geminate virus particle. The main symptoms caused by GRBV appear as red patches (blotches) at the interveinal area or occasionally on the edges, sometimes red veins, as well as chlorotic and irregular-shaped areas in the case of white-berried cultivars. Fruit development and sugar content may be affected, and yield may be reduced. The need to rogue and replace infected vines can be a significant cost to vineyards, making it crucial to identify and control the disease at an early stage [17]. The virus has been detected in Baja California, the most important wine producing region in Mexico [18,19]. Infected plants showed typical red blotch symptoms.

GRBV is a systemic, phloem-limited pathogen. It is graft-transmissible, resulting in spread by vegetative propagation [20]. This virus is also transmitted by an insect vector [21,22]; GRBV transmission by the three-cornered alfalfa hopper (*Spissistilus festinus*) has been demonstrated in the greenhouse [23]. The possibility of other vectors is still being pursued. The grape leafhopper (*Erythroneura ziczac*) was reported to transmit GRBV. However, as its feeding is restricted to mesophyll cells, it is not believed that it can transmit a phloem limited virus [21,22]. The virus has been detected in the gut of different taxa of Hemiptera: Aphididae, Cixiidae, Delphacidae, Membracidae, and Phylloxeridae and more than 17 species of Cicadellidae [22,24]. Consequently, they are considered potential vectors of GRBV, however no GRBV transmission has been demonstrated [25].

#### 2.2. Grapevine Leafroll-Associated Virus 3

GLRaV-3 is the type member of the genus *Ampelovirus* in the family *Closteroviridae*. Long, filamentous virions 1400–2200 nm in length and 10–12 nm in diameter contain monopartite linear RNA genomes of approximately 18.6 kb [26]. GLRaV-3 is considered the most economically important virus of grapevine and the main causal agent of the disease known as grapevine leafroll. Red cultivars exhibit a reddish-purple pigmentation, while white grapevine cultivars exhibit either a light-yellow coloration or a chlorotic molting; until the leaves eventually roll downwards. Both typically exhibit symptoms post-veraison. Finally, depending on the impact on fruit yield and quality and concentration of sugars in the berries, growers may lose profit over long production periods, until the plants are replaced [27]. Monroy-Corral [28] reported GLRaV-3 in commercial vineyards in Baja California with a incidence of 24%.

GLRaV-3, a phloem-limited virus, can be transmitted by grafting [29,30]. Additionally, mealybugs, soft scale and scale insects are known vectors [31,32]. Tsai and collaborators [33] demonstrated the efficiency of transmission by the vine mealybug (*Planococcus ficus* Signoret) able to transmit GLRaV-3 up to 4 days after acquisition; this being characteristic of semi-persistent viruses [34].

#### 2.3. Grapevine Fanleaf Virus

It is the oldest known virus (genus *Nepovirus*, family *Secoviridae*) infecting grapevine, with polyhedral particles about 30 nm in diameter. The bipartite genome consists of two positive-sense, single-stranded RNA molecules of 7326–7342 (RNA-1) and 3730–3817 (RNA-2) nucleotides, each encapsulated in different particles and both required for infectivity [35]. Plants infected with GFLV often show growth abnormalities, such as vine weakening and shortening or deformation of internodes, in addition to leaf yellowing or deforma-

tion (typical fanleaf symptom) and leaf vein clearing. This virus threatens production profits by reducing plant longevity, graft compatibility, and the grapevine's ability to propagate [36,37]. In 1968, plants with symptoms of fanleaf were observed in the northern part of Mexico, and later the presence of GFLV was confirmed in Aguascalientes [38]. A later study determined a 6.7–37.5% incidence of this virus among different commercial vineyards in Aguascalientes [39].

GFLV is transmitted persistently by the dagger nematode (*Xiphinema index*, family Longidoridae), which feeds on the grapevine roots [40,41]. The nematode can acquire virus particles from infected plants and releasing them when its stylet is inserted into the parenchyma tissue of growing root tips. GFLV can be retained by the vector for extended periods in the absence of host plants [42,43]. The virus can be transmitted by vegetative propagation and mechanical inoculation [44]. The presence of GFLV in the embryo is rare, but it occurs in the pollen of grapevine and herbaceous hosts and in endosperm of grapevine seeds. Finally, this pathogen can also be transmitted through seeds of *Chenopodium amaranticolor*, *C. quinoa*, and soybean [44,45].

#### 2.4. Grapevine Virus A

GVA is a phloem-limited virus. It is the type member of the genus *Vitivirus*, family *Betaflexiviridae*, with flexuous, filamentous particles, and a single-stranded positive-sense RNA genome (7.5 kb long). In the case of grapevine cultivars infected with GVA, a reduction in shoot growth and sugar accumulation in berries, as well as vine senescence has been observed [46]. GVA is associated with the rugose wood disease. Virus infection can lead to yield reductions, and less profits due to poor fruit quality and replacement costs. Some strains of GVA are considered causal agents of Shiraz disease, reducing the lifespan of infected vineyards to a maximum of six years, as production no longer sustainable [47]. In 2019, GVA was identified along with different GLRaV species in Mexican vineyards [28].

GVA is transmitted by several species of mealybugs and soft scale insects in a semipersistent manner [31,48,49]. There are no reports of seed transmission for this pathogen [50–52].

## 3. Viruses Not Identified in Mexico: Potential Threats

## 3.1. Grapevine Pinot Gris Virus

GPGV (genus *Trichovirus*, family *Betaflexiviridae*) with virions of non-enveloped, flexuous, filamentous morphology and a linear single-stranded RNA genome of 7.3 kb in length. Recent studies involving phylogenetic analyses support the possible existence of symptomatic and asymptomatic strains [53]. However, another study suggests that regardless of the GPGV isolates, infected grapevines display symptoms, but later these plants develop asymptomatic leaves [54]. Currently, in Europe, GPGV is considered a major pathogen of grapevine [55].

Although grapevines infected with GPGV are often asymptomatic, they can sometimes show leaf distortion, chlorotic mottling, stunting, and smaller internodes that usually develop early in the growing season, resembling symptoms caused by mite damage [56]. Yield and quality losses can occur, affecting commercial production [57]. Recently, it was demonstrated that GPGV is present in seedlings developed from seeds of infected grapevine plants [58].

GPGV is transmitted semi-persistently by the grape erineum mite (*Colomerus vitis*, Acari: Eriophyidae) [59]. This mite penetrates the cells inserting only its mouthpart into the outermost of epidermal cells [60]. Many plants are reported hosts of GPGV, including *Silene latifolia* subsp. *Alba*, *Chenopodium album* and plants belonging to the genera *Ailanthus*, *Asclepias*, *Crataegus*, *Fraxinus*, *Rosa*, *Rubus*, and *Sambucus* [61,62]. GPGV is transmitted by grafting, while mechanical transmission in herbaceous plants has not been observed [59].

## 3.2. Arabis Mosaic Virus

ArMV (genus *Nepovirus*, family *Secoviridae*) is a grapevine pathogen of European origin [63], with a large natural host list, including annual and perennial species. ArMV

has a bipartite genome consisting of two positive-sense, single-stranded RNA molecules of approximately 7300 nucleotides (RNA-1) and 3800 nucleotides (RNA-2), each encapsulated in separate polyhedral virus particles of 30 nm diameter. ArMV has been reported to cause stunting, deformation, leaf mottling, and flecking, which may initially be perceived as mild leaf yellowing [64,65]. It is a major cause of losses in the wine and table grape industry worldwide and poses a challenge to sustainable and profitable vineyards [35].

ArMV is spread by the nematode *X. diversicaudatum*, in a persistent manner [66]. It is transmitted more efficiently by adults compared to larvae, but is not retained through moulting and is not passed from female to progeny [67]. The virus is mechanically transmissible to *C. amaranticolor*. However, vegetative propagation is the principal means of dissemination [64]. Seed transmission of ArMV is not confirmed in grapevine [68,69].

## 4. Viral Complexes: More Viruses More Problems

Grapevine leafroll disease is one of the most important grapevine viral diseases, having negative impact on wine, table grape, and rootstock cultivars. Typical symptoms associated with this disease are interveinal reddening and downward rolling of leaf margins in red cultivars, and interveinal chlorosis and downward rolling of leaf margins in white cultivars [70]. Currently, six different virus species in three genera are associated with leafroll disease (Table 1). Given the possibility of multiple viruses, it is difficult to correlate the presence of symptoms with a specific virus, and the possibility of coinfection by GLRaV species exists.

Table 1. Virus species associated with grapevine leafroll disease.

Genus	Species Name	Virus Acronym
Ampelovirus	Grapevine leafroll-associated virus 1	GLRaV-1
	Grapevine leafroll-associated virus 3	GLRaV-3
	Grapevine leafroll-associated virus 4	GLRaV-4
	Grapevine leafroll-associated virus 13	GLRaV-13
Closterovirus	Grapevine leafroll-associated virus 2	GLRaV-2
Velarivirus	Grapevine leafroll-associated virus 7	GLRaV-7

According to the International Committee on Taxonomy of Viruses (2021 Release), nine different viruses are formally classified as vitiviruses (genus *Vitivirus*, family *Betaflexiviridae*) and are known to infect grapevine: grapevine viruses A, B, D, E, F, G, H, I and J (GVA-J). Some of these viruses are associated with the etiology of rugose wood disease in grapevine. A disease complex with worldwide distribution and linked to different syndromes affecting the bark, cambium tissue, and woody cylinder of grapevines [46]. Symptom expression depends on the virus–host combination and environmental conditions. For example, GVA has been associated with Kober 5BB stem grooving on Kober 5BB (*V. berlandieri* × *V. riparia*) [71,72], GVB has been identified as the putative causal agent of corky bark in LN 33 (Couderc 1613 × *V. berlandieri*) [73], and GVD has been implicated in growth reduction in cv. Freedom (1613-59 × Dog Ridge 5) [74].

More recently, five new viruses have been discovered in grapevine and proposed as members of the genus *Vitivirus*. These new viruses were tentatively named grapevine viruses K, L, M, N and O (GVL-O) [8,75–77]. Unlike other previously known vitiviruses, particularly GVA, B, and D, the biological significance of the novel viruses is still largely unknown, including effects on grapevine performance and transmission mechanisms.

In 2018, a field survey was launched to determine the incidence of grapevine-infecting vitiviruses in California, USA [78]. Results of this work revealed that GVG, H, I, J, and L were present in California, predominantly occurring as mixed infections with GVA. Furthermore, grapevines carrying up to six different vitiviruses were identified, and the agronomic significance of such coinfection is still a pending question.

Vitiviruses are often detected in coinfection with members of the family *Closteroviridae* (GLRaV-1, -2, and -3), resulting in synergistic interactions that can lead to lethal effects

in several scion and rootstock combinations [79]. Although vitiviruses by themselves do not cause symptoms on common grapevine scion and rootstock combinations, a mixed infection of a vitivirus with a *Closteroviridae*-virus may create a serious problem in the field, especially if one of the susceptible rootstocks has been used for propagation. In the case of Mexico, there are reports of such combination, however, the lethal effect has not been investigated [28]. For example, Velásquez et al. [80] describe the presence of GVB and GLRaV-2 in different cultivar/rootstock combinations with a rugose wood symptomatology. Finally, vitiviruses are transmitted by members of several insect genera of mealybugs and scale insects (*Pseudococcus, Planococcus, Heliococcus, Neopulvinaria, Parthenolecanium, Cavariella,* and *Ovatus*) in a semi-persistent manner [48,52]. The vectors of vitiviruses are all shared with GLRaV-1 and -3, which are commonly detected in coinfections with vitiviruses as discussed before. Thus, insect species mediate the short distance spread of these grapevine viruses, while long-distance dissemination occurs primarily through contaminated propagation material [81].

#### 5. Management and Control of Grapevine Viruses

The majority of grapevine virus spread occurs by planting or grafting virus infected materials. Planting healthy stock generated by certification programs is the most effective and least expensive means of controlling grapevine viruses [82–85]. For viruses that can be transmitted by vectors, primary introduction of the virus into a vineyard and secondary spread within the vineyard after introduction must be prevented. When a vine is infected, there is no chemical treatment or agricultural action that can cure the vine; it must be removed (rogued) from the field. Specific management strategies for vector-spread viruses of economic importance are as follows. The mealybug and soft scale insects that transmit the ampeloviruses GLRaV 1, 3, and 4, which cause grapevine leafroll disease, can be spread downwind or be carried by workers' clothing, field equipment, or birds [86]. Vector control can be more effective if the vector species in the field are known. Pheromone traps can be used to quickly detect and identify vectors [87]. Mealybug and scale insects can differ in their preferred location on the vine at different times of the year, the number of generations they have per season, their fecundity, and the ability to transmit the viruses [88–91]. The use of control methods, including contact and systemic insecticides, parasitizing insects, pheromones for mating disruption, and planting vector-tolerant rootstock, can be tailored to specific vectors [92–95].

It is critical to detect and remove infected vines. Infected red grape varieties may be visually detected in the late summer and fall when leaves turn red and leaf edges roll downward. Leafroll disease in white grape varieties is more subtle and can be detected by enzyme-linked immunosorbent assay (ELISA), reverse transcription PCR (RT-PCR), and loop-mediated isothermal amplification (LAMP) [96], or by grafting red grape variety scions onto the white grape vines to serve as field indicators of leafroll presence in the fall [94]. Once infected vines are discovered, they should be removed immediately to prevent them from serving as a source of inoculum for the rest of the vineyard. Prior to roguing, an infected vine should be treated with a systemic insecticide and the soil around the vine should be drenched with pesticide. The vine should also be treated with an herbicide to kill the roots of the infected plant. This will ensure that viruliferous vectors are destroyed and the infected roots do not remain in the soil to serve as inoculum for new generations of vector. After removal of the vines, the soil should be left fallow for a season and any volunteer sprouts removed. If symptomatic vines comprise 20-25% of the vineyard, it may be more economical to replace the entire block than to rogue individual vines [82,84,87].

GVA and GVB are causal agents of rugose wood and, like grapevine leafroll ampeloviruses, are spread by mealybugs and soft scale insects in a nonspecific manner [46]. Symptoms of GVA and GVB may not be observed on the stems of live grapevines, but the viruses can be detected by serological or molecular methods. Roguing infected vines and controlling vector species by methods outlined for the management of leafroll disease will be similarly effective for managing the spread of vitiviruses.

GRBV, like leafroll viruses, causes red symptomatic leaves in infected red grape varieties in late summer and fall. Confirmatory testing by molecular-based assays is recommended because there are many other causes of leaf reddening, such as petiole girdling by insects, Pierce's disease, crown gall, mite damage, poor root health, trunk injury, and nutrient deficiencies, such as magnesium or potassium deficiency. Roguing infected vines is an important way to reduce the presence of inoculum in the field. The only confirmed vector of GRBV is the three-cornered alfalfa hopper (*S. festinus*). Pesticides have not been effective in controlling the spread of the virus. However, there is a report that discing leguminous groundcover before eggs and flightless nymphs can develop wings may help to limit their populations [25,97]. A study that examined the economic costs of GRBV in different cultivars estimated that it is more cost-effective to replace the block once the virus has infected 30% of the plants than to replace individual vines [14].

Fifteen viruses in grapevine cause fanleaf degeneration and eight are spread by nematodes. The most economically important is GFLV, which is spread exclusively by the dagger nematode (*X. index*). This nematode is found in temperate grape-growing regions across the globe, withstands adverse conditions, retains virus for long periods, and lives up to 3.6 m deep in the soil. Proper nematode identification and the ability to detect viruliferous nematodes are key components of disease management. Nematodes can be spread through contaminated equipment, infested plants, soil transfer, workers' boots, and via water, such as streams, floodwaters, and water seepage. To manage fanleaf degeneration, infected vines must first be destroyed with systemic herbicide, vine and roots removed, soil disinfected, and a fallow period should follow. Replanting with vines grafted onto *X. index*-tolerant rootstock may be helpful for a time [98]. Efforts to cross protect grape by infecting with a mild strain of GFLV were not successful, due to decreased yields caused by the weaker strain [99]. Perhaps, in time, a newly discovered recessive gene for GFLV resistance can be used in rootstock breeding [28].

Although grapevine disease control strategies are well known, implementation can easily fail [98,100]. Critical to success is the development and maintenance of rigorous quarantine programs and modern certification programs with effective virus-testing methods to provide clean stocks. Also critical is the dissemination of knowledge and coordination of efforts to prevent grape growing regions from becoming infested with mealybugs, scale insects, or dagger nematodes. Chemical control measures are increasingly regulated, natural virus resistance in grapevines is rare, and there is a lack of public support for genetically modified grapevines (which could be engineered to be resistant to viruses), making the ability to control virus spread in the presence of abundant vectors doubtful. To protect Mexican viticulture, the focus must be on preventing the introduction and movement of virus-infected planting material within Mexico. To be successful, strict quarantine and virus-detection programs must be established.

#### 6. Conclusions and Perspectives

Few grapevine viruses have been reported in Mexico in recent decades. Although unofficial reports of virus symptoms in Mexican vineyards are common (Figure 2), very little virus diagnostic work using molecular (PCR) or serological assays (ELISA) has been undertaken. Field surveys have been conducted in Aguascalientes and Baja California only. The lack of information on the current phytosanitary status of vineyards opens the possibility of unintentional dissemination of pathogens via propagation material. In addition, virus-infected vines (symptomatic or asymptomatic) are potential inoculum sources for the infection of nearby vineyards through vector spread. More frequent virus screening by PCR or ELISA assays could prevent virus spread if this information was used to rogue infected vines. Finally, a virome analysis using HTS has not been performed in Mexico, which could identify all the viral agents affecting the national grapevine production, including viral complexes.



**Figure 2.** Virus-like symptoms observed in commercial vineyards located in Mexico. Symptoms include red and chlorotic spots on leaves, leaf deformation, vein necrosis and reduction in shoot growth.

Although some of the viruses described here are vectored by insects or nematodes, the main route of virus transmission is via the clonal propagation of infected plant material. To prevent the spread of viruses, it is critical to use certified (virus-tested) material. Unfortunately, the vast majority of this high-quality plant material is produced outside of Mexico, e.g., France and Spain, which drives up production costs. While we cannot ignore the risk of infection by GPGV and ArMV, common pathogens in Europe. The old vineyards located in Mexico represent a challenge for conservation, as virus infection negatively affects the tolerance of plants to biotic and abiotic factors. New efforts should focus on the creation of a national clonal germplasm repository. Mexico's unique old grape cultivars, as well as its wild grapevines could pass through a virus elimination program to establish the virus-free germplasm repository. Aiming to replicate, a successful case of pathogen-tested foundation

plants program is the National Clean Plant Network (NCPN) established in the United States.

Viticulture in Mexico is a growing agribusiness. There is a high potential to increase production due to the wide range of weather conditions and soil types favorable to production. In addition to Baja California and the famous Valle de Guadalupe, there are 14 grape producing states, where more than 40 different varieties are grown. Of the 37,000 hectares planted with grapes, currently, 12.5% of production is destined for winemaking, and most of the Mexican wine is consumed in the local market. The value of Mexican wines is growing at 13% annually and there is an incentive to increase export volumes The government and growers have agreed, through meetings that seek to promote this sector, to create a larger cultivated area, improve infrastructure, industrial equipment and mechanization, and improve production in nurseries.

In summary, the main objective of this review was to highlight the need for further research on grapevine viruses in Mexico in order to develop effective virus control strategies to prevent economic losses due to these viruses. History and other regions of the world have shown the impact of proper or improper management of viruses in viticulture.

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