Grapevine Viruses in Mexico

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About grapevine viruses in Mexico, nine viruses have been identified, 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.

Keywords: Grapevine Viruses; Mexico; virus

1. Grapevine Red Blotch Virus

GRBV belongs to the genus *Grablovirus* in the family *Geminiviridae*, it has a single-stranded 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 [1]. The virus has been detected in Baja California, the most important wine producing region in Mexico [2][3]. Infected plants showed typical red blotch symptoms.

GRBV is a systemic, phloem-limited pathogen. It is graft-transmissible, resulting in spread by vegetative propagation [4]. This virus is also transmitted by an insect vector [5][6]; GRBV transmission by the three-cornered alfalfa hopper (*Spissistilus festinus*) has been demonstrated in the greenhouse [7]. 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 [5][6]. 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 [6][8]. Consequently, they are considered potential vectors of GRBV, however no GRBV transmission has been demonstrated [9].

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 ^[10]. 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 ^[11]. Monroy-Corral ^[12] 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 $\frac{[13][14]}{}$. Additionally, mealybugs, soft scale and scale insects are known vectors $\frac{[15][16]}{}$. Tsai and collaborators $\frac{[17]}{}$ 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 $\frac{[18]}{}$.

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 [19].

Plants infected with GFLV often show growth abnormalities, such as vine weakening and shortening or deformation of internodes, in addition to leaf yellowing or deformation (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 [20][21]. 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 [22]. A later study determined a 6.7–37.5% incidence of this virus among different commercial vineyards in Aguascalientes [23].

GFLV is transmitted persistently by the dagger nematode (*Xiphinema index*, family Longidoridae), which feeds on the grapevine roots $^{[24][25]}$. 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 $^{[26][27]}$. The virus can be transmitted by vegetative propagation and mechanical inoculation $^{[28]}$. 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 $^{[28][29]}$.

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 [30]. 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 [31]. In 2019, GVA was identified along with different GLRaV species in Mexican vineyards [12].

GVA is transmitted by several species of mealybugs and soft scale insects in a semi-persistent manner $\frac{[15][32][33]}{[33]}$. There are no reports of seed transmission for this pathogen $\frac{[34][35][36]}{[36]}$.

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 [32] [38][39][40]. 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 [41]. 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 [42]. 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 [43][44][45][46]. 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 [47][48][49][50].

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) $^{[51]}$, or by grafting red grape variety scions onto the white grape vines to serve as field indicators of leafroll presence in the fall $^{[49]}$. 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 $^{[32]}$

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 [30]. 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 [9][52]. 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 [53].

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 [54]. 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 [55]. Perhaps, in time, a newly discovered recessive gene for GFLV resistance can be used in rootstock breeding [12].

Although grapevine disease control strategies are well known, implementation can easily fail [54][56]. 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.

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