# EPPO Datasheet: *Grablovirus vitis*

Last updated: 2021-11-08

## IDENTITY

**Preferred name:** *Grablovirus vitis*
**Taxonomic position:** Viruses and viroids: Monodnaviria: Shotokuvirae: Cressdnaviricota: Repensiviricetes: Geplafuvirales: Geminiviridae
**Other scientific names:** *GRBV, Grapevine cabernet franc-associated virus, Grapevine red blotch virus, Grapevine red blotch-associated virus, Grapevine redleaf- associated virus*
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**EPPO Categorization:** A1 list, Alert list (formerly)
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**EU Categorization:** Quarantine pest ((EU) 2019/2072 Annex II A)
**EPPO Code:** GRBAV0



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### Notes on taxonomy and nomenclature

Although red blotch disease is considered as an emerging disease only described in 2008 on Cabernet Sauvignon in an experimental vineyard at UC Davis (California) (Calvi, 2011), it appears that grapevine red blotch virus (GRBV) has been present in grapevine for a very long time, particularly in Californian vineyards. Supporting this notion, GRBV has been detected in herbarium grapevine samples collected in California in the 1940s (Al Rwahnih *et al.*, 2015a), demonstrating its presence in North American vineyards for more than 80 years. In 2011-2012, in Oregon and Washington States, the red blotch or red leaf disease was tentatively linked to a gemini-like virus called grapevine redleaf-associated virus (Poojari *et al.*, 2013). The first formal description of GRBV, under the name grapevine red blotch-associated virus dates back to 2011 (Calvi, 2011) and was achieved by high throughput sequencing (HTS) in grapevines exhibiting leaf symptoms of red blotches and red veins, with a foliar reddening initially suspected to be induced by leafroll-associated viruses (Al Rwahnih *et al.*, 2013). HTS data revealed the presence of a new member of the *Geminiviridae* family, which became the type-member of the new *Grablovirus* genus (Varsani *et al.*, 2017). The virus initially named *Grapevine red blotch-associated virus* (Al Rwahnih *et al.*, 2013) is nearly identical to another *gemini-like* virus found in declining Cabernet Franc in New York, reported as Grapevine cabernet franc-associated virus (Poojari *et al.*, 2013). The GRBV species name was finally changed to *Grapevine red blotch virus* after the unambiguous demonstration that it is the causative agent of the grapevine red blotch disease (GRBD) (Yepes *et al.*, 2018). GRBV shares close genomic and phylogenetic relationships with *Prunus geminivirus A* (PrGVA), a novel *Grablovirus* infecting *Prunus* spp. (Al Rwahnih *et al.*, 2018). Moreover, a distinct *Grablovirus* identified in wild *Vitis* species in California has been tentatively named *Wild Vitis virus 1* (WVV1) (Perry *et al.*, 2018). WVV1 genome has approximately 60% nucleotide similarity with that of GRBV but this second Grablovirus has not yet been detected in *Vitis vinifera*. Two phylogenetic clades (1 and 2) have been identified so far in GRBV, with clade 1 showing the largest diversity and clade 2 seeming to predominate in Californian vineyards (Al Rwahnih *et al.*, 2015b).

## HOSTS

The cultivated grape *Vitis vinifera* and hybrids of *V. vinifera* or of other *Vitis* species (e.g. those used for rootstocks) as well as free-living North American *Vitis* are the only known natural hosts of GRBV (Perry *et al.*, 2016; Bahder *et al.*, 2016a; Cieniewicz *et al.*, 2017). Due to the significant presence of the red blotch disease in North American vineyards, extensive searches for GRBV natural hosts have been performed there (Krenz *et al.*, 2014). Free-living grapevines found to be infected include hybrids of *V. californica* × *V. vinifera*, or hybrids between *V. californica* and grapevine hybrid rootstocks such as Rugerri 140 and *Vitis rupestris* St George (Perry *et al.*, 2016; Cieniewicz *et al.*, 2017). Many cultivars of *Vitis vinifera* have been found to be hosts for GRBV (Krenz *et al.*, 2014; Adiputra *et al.*, 2018) so it should be assumed that most if not all grapevine varieties could be hosts. Blackberry (*Rubus armeniacus*)

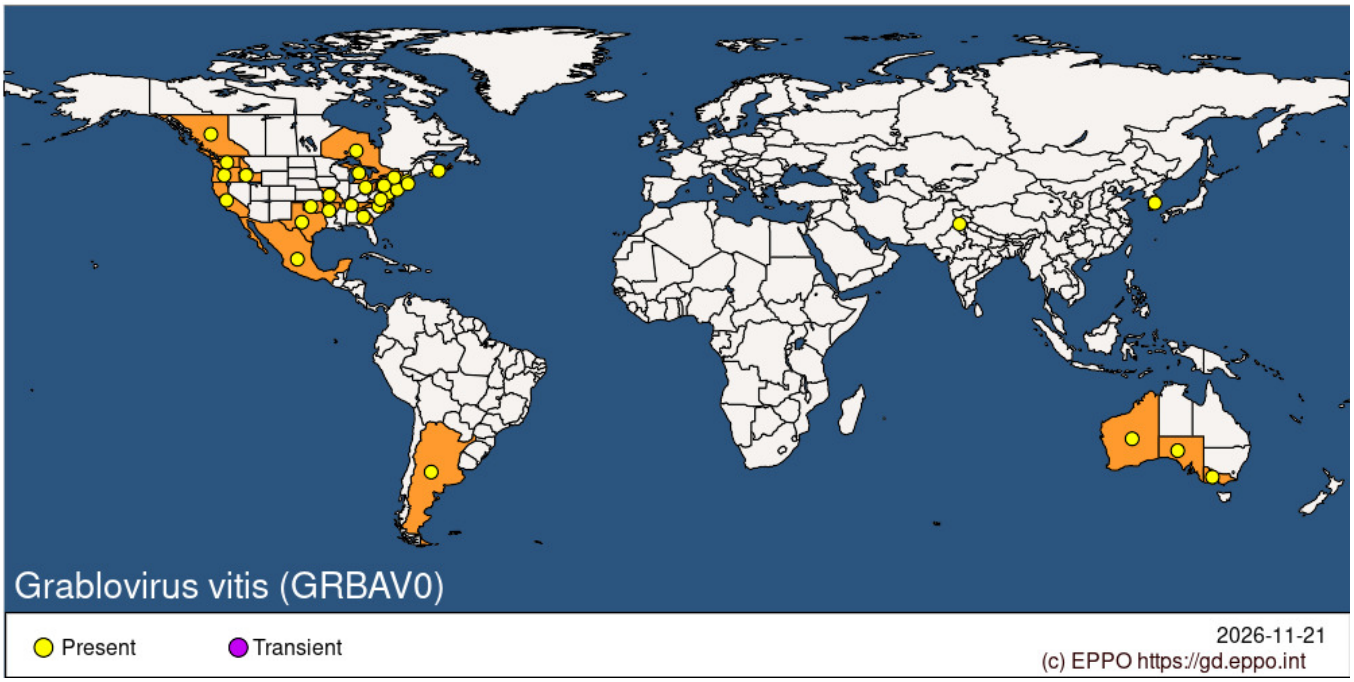
has been suggested as an alternative host of GRBV in Northern California, but tests indicated that the virus did not replicate in this host (Bahder *et al.*, 2016a) and this hypothesis has not been confirmed. No herbaceous natural or experimental hosts are known for GRBV (Cieniewicz *et al.*, 2019)

**Host list:** *Vitis californica*, *Vitis hybrids*, *Vitis riparia*, *Vitis sp.*, *Vitis vinifera*

## GEOGRAPHICAL DISTRIBUTION

GRBV is widespread in the major grape growing areas of the USA (Al Rwahnih *et al.*, 2013; Krenz *et al.*, 2014) and in some USA germplasm repositories. It is also present in the main grape growing regions of Canada: British Columbia and Ontario (Poojari *et al.*, 2017; Xiao *et al.*, 2018) and in Mexico (Gasperin-Bulbarela *et al.*, 2019). It has also been reported from South America (Luna *et al.*, 2019) and from Asia (Lim *et al.*, 2016; Marwal *et al.*, 2018). A report from Switzerland is related to grapevine material from North America introduced in a germplasm collection. These accessions were removed from the collection and the virus absence was confirmed in this country based on a survey of more than 3,000 grapevines from commercial vineyards (Reynard *et al.*, 2018).

In the absence of surveys, GRBV might be present at low frequency in other regions of the world as its symptoms can easily be confused with those of grapevine leafroll disease, of phytoplasma infections or with symptomatology due to abiotic stress.




**Asia:** India (Punjab), Korea, Republic of
**North America:** Canada (British Columbia, Nova Scotia, Ontario), Mexico, United States of America (Arizona, Arkansas, California, Connecticut, Georgia, Idaho, Maryland, Massachusetts, Michigan, Missouri, New Jersey, New York, North Carolina, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, Tennessee, Texas, Virginia, Washington)
**South America:** Argentina
**Oceania:** Australia (South Australia, Victoria, Western Australia)

## BIOLOGY

As with most other plant viruses, GRBV is graft-transmissible; several epidemiological studies suggest that its wide detection in most of the grape-growing regions of the USA might be correlated with dissemination through GRBV-infected grapevine propagation materials (Sudarshana *et al.*, 2015). In infected grapevines, GRBV gives systemic infections and spreads widely within the infected plants.

As with many other members of the *Geminiviridae* family, GRBV is assumed to be phloem-limited (Sudarshana *et al.,* 2015), suggesting that phloem-feeding vectors might be involved in its horizontal spread. Indeed, spread from red blotch-infected vineyards to neighbouring healthy plots, as well as clustering of infected vines strongly suggested that an aerial vector was involved in GRBV transmission. In a greenhouse study the three-cornered alfalfa hopper (3CAH), *Spissistilus festinus* (Say) (Hemiptera: Membracidae) has been experimentally shown to transmit GRBV (Bahder *et al.,* 2016b). Recent results suggest that this transmission is in a circulative, non-propagative mode (Flasco *et al.,* 2021). In California, the highest population density and ratio of viruliferous *S. festinus* were identified in June and July (Cieniewicz *et al.,* 2018a); a recent epidemiological study revealed an annual spread of 1 to 2% of GRBV in a Californian vineyard (Cieniewicz *et al.,* 2018b). Although *S. festinus* is polyphagous and can feed on vine, it has not been reported to reproduce on grapevine (Preto *et al.,* 2018). Nevertheless, this treehopper can feed and reproduce on a variety of plant species found in and around vineyards, including some cover crops and weeds [ *Vicia benghalensis* (purple vetch), *Trifolium alexandrinum* (berseem clover), *Lolium multiflorum* (annual ryegrass), *Fagopyrum esculentum* (buckwheat), *Triticum x Secale* (triticale), *Vulpia myuros* (Zorro fescue)]. The legume species (purple vetch and berseem clover) exhibited higher nymph emergence (Kron and Sisterson, 2020). In addition to *S. festinus*, GRBV has been detected by multiplex PCR in other phloem-sucking insects: *Colladonus reductus* (Cicadellidae), *Osbornellus borealis* (Cicadellidae) and *Melanoliarus* sp. (Cixiidae) but there is to date no proof that these other species might be able to transmit GRBV (Cieniewicz *et al.,* 2018b). A recent unpublished report suggests that the ragweed treehopper (*Entylia carinata*) and the two-marked treehopper (*Enchenopa binotata*) might be able to transmit GRBV (Smith *et al*., 2020).

In addition, vineyard observations validated the hypothesis of an association between GRBV spread dynamics and abundance of *S. festinus* populations (Cieniewicz *et al.,* 2019), demonstrating the epidemiological relevance of this Membracidae.

## DETECTION AND IDENTIFICATION

### Symptoms

The symptoms of GRBD are dependent on grapevine phenology. Overall vine vigour is not reduced by GRBV infection in the short term (Levin and Achala, 2020) but results from Reynard *et al.* (2018) showed clear detrimental effects of GRBV on grapevine physiology (vine vigour, leaf chlorophyll content, and gas exchange) and fruit quality. The virus changes vine carbon metabolism from the leaf (assimilation) to the whole vine level (partitioning), in turn having negative impacts on secondary metabolism in the short term and, potentially, vine productivity in the long term (Levin and Achala, 2020).

Symptoms occur on leaves and berries. They appear on the older leaves of the canopy between spring and summer, and migrate to the upper part of the plants at the end of summer or, beginning of autumn. For red-berried varieties, red blotches are visible on leaves early in the growing season, and can then coalesce during summer with variations in the red colour. On white-berried varieties, leaf symptoms consist of chlorotic spots, that can eventually evolve into necrotic lesions (Sudarshana *et al.,* 2015; Cieniewicz *et al.,* 2017). Accumulation of anthocyanins in leaves precedes other signs of senescence. On berries, besides impact on yield, a delay and heterogeneity in ripening are seen and fruit juice quality and anthocyanin accumulation are negatively affected through a hypothesized disruption in hormone signalling (Blanco-Ulate *et al.,* 2017). Anthocyanin concentration was generally reduced in GRBV positive vines, by between 18% and 30% with yearly variations in Pinot Noir (Levin and Achala, 2020). Total soluble solids are also reduced at harvest, and berries of red varieties exhibited reduced polyphenolic content (Girardello *et al.,* 2019; Martinez-Luscher *et al.,* 2019).

The similarities in symptoms on leaves and fruits between GRBD and grapevine leafroll disease are the most probable reason that red blotch disease took such a long time to be recognized. Grapevine leafroll-associated virus-3, which is regarded as the most important causal agent of grapevine leafroll disease, another phloem-limited virus, is thought to act via similar mechanisms which could explain the similarities in symptomology (Martinez-Luscher *et al.,* 2019). Symptom confusion on red-berried cultivars could also be made with those resulting from Pierce’s disease, and with arthropod damage as well as abiotic stresses such as nutrient deficiencies (magnesium and chloride) (Cieniewicz *et al.,* 2017).

### Morphology

GRBV virions visualization through electron microscopy has never been clearly documented, whether from crude sap homogenates or from attempts at particle purification. Nevertheless, being classified as a gemini-like virus, GRBV virions would be expected to be geminate, consisting of twined icosahedra of approximately 22 x 38 nm with a sedimentation coefficient of about 70S. GRBV has a monopartite genome consisting of a single-stranded circular DNA molecule of approximately 3.2 kilobases, encoding 6 partly overlapping open reading frames (ORFs). The gene expression strategy is similar to that seen in other of the *Geminiviridae* family members (Cieniewicz *et al.,* 2017; Vargas-Asencio *et al.,* 2019). The genome encodes 3 ORFs in the virion sense; V1 (CP of 25.3 kDa), V2 and V3 (hypothesized to be movement proteins). There are also 3 ORFs in the complementary sense, C1, C2 and C3. As *Geminiviridae* do not encode a functional DNA polymerase, replication is performed by recruitment of host factors enabling the viral DNA synthesis. The splicing of the complementary sense transcript produces a replication-associated protein (Rep), that is essential for virus ssDNA synthesis.

### Detection and inspection methods

A procedure for inspection of places of production of *Vitis* plants for planting is provided in Standard PM 3/85 (EPPO, 2018). Given the possible confusion with leafroll disease foliar symptoms, in particular in red-berried varieties, symptoms are not sufficiently reliable to establish the presence of GRBV in a vineyard. Moreover, it has not yet been possible to isolate viral particles from GRBV-infected plants, thereby hampering the development of serological reagents and assays that would facilitate GRBV detection using serological procedures such as ELISA. Nucleic acids-based methods are therefore the only available options for GRBV detection. PCR and real-time PCR multiplex tests have been developed with primers covering the genetic diversity of GRBV (Krenz *et al.,* 2014) and used in mother plants blocks, nurseries and vineyards. Spatiotemporal fluctuations in GRBV detection leading to erratic detection results during some periods of the year have been reported using PCR or real-time PCR. A recent well-documented methodological study (Setiono *et al.,* 2018), using an optimized real-time PCR, has proposed a useful guide for the reliable and sensitive detection of GRBV in field-grown grapevines, highlighting the variability of virus distribution in the vine and identifying the best sampling period. The study found that petioles of older leaves are the richest source of GRBV, while emerging leaves exhibit the lowest viral DNA content, similar to that which has been extensively reported for grapevine leafroll-associated viruses using ELISA assays. In the USA, the best period of sampling was shown to be between July and October, the virus reaching a ‘saturation’ titre at the end of the growing season (Setonio *et al*., 2018).

Mass spectrometry has also been applied to quantify GRBV in infected plants and identify potential biomarkers of viral infection, by detecting two GRBV proteins (coat protein and V2 protein) in tissue lysates (Buchs *et al.,* 2018). Finally, as an alternative to more classical methods, a novel plasmonic CRISPR Cas12a assay has also been recently proposed with successful detection of GRBV from grapevine samples from commercial vineyards, generating a colorimetric signal that can be exploited under field conditions without the need for bench instruments for readout (Li *et al.,* 2019).

## PATHWAYS FOR MOVEMENT

Given the efficient transmission of GRBV through vegetative propagation practices, the main pathway for long-distance movement is the circulation and trade of infected grapevine propagation materials. A minor entry pathway could be through the movement of viruliferous *Spissistilus festinus* vectors, on their own or hitchhiking on plants other than *Vitis*. In countries where it is present, the vector represents the main means of local spread of the virus.

## PEST SIGNIFICANCE

### Economic impact

GRBV infection has been shown to affect many aspects of vine physiology from leaf metabolism (Wallis and Sudarshana, 2016) to fruit development and ripening (Blanco-Ulate *et al.,* 2017). Economic losses can be severe and

are a consequence of a reduction in fruit and wine quality as well as reductions in vegetative growth and canopy development. The economic impact has been estimated in the grape-growing regions in the United States, on Cabernet-Sauvignon in California and on Merlot in Washington State and New York, reaching between 2 200 - 68 500 USD per hectare over a 25-year production period (Ricketts *et al.*, 2017). This is similar to the losses that have been estimated for the leafroll disease. However, while GRBV infection may not cause long-term productivity decline if vines are infected once mature, it remains unclear to what degree management practices post-infection and/or vine age at time of infection may influence the outcome (Levin and Achala, 2020).

**Control**

There is no curative treatment available to control the red blotch disease in vineyards, so all control strategies are based on prophylaxis or on control of insect vectors. In this context, a key control element is the use of GRBV-free planting materials (Cieniewicz *et al.*, 2017). Thus, implementation of GRBV testing worldwide in certification and quarantine programs to prevent the spread of this virus are well established or should be considered.

In US vineyards, Ricketts *et al.* (2017) suggested that roguing symptomatic vines and replanting with clean vines derived from GRBV-free stocks would minimize losses if GRBD incidence is low to moderate (below 30%), while a full vineyard replacement should be pursued if disease incidence is higher. Another economic study aiming at mitigating red blotch disease showed that in a 10 year-old Pinot Noir vineyard there were no significant effects of water deficits on foliar symptoms onset, but there was a more rapid progression of symptoms in a year with more severe water deficits (Levin and Achala, 2020). Although keeping GRBV positive vines well-watered may mitigate some of the negative effects of GRBD, this study also suggested that watering management may not improve overall fruit quality in GRBV infected vines (Levin and Achala, 2020).

The role of infected free-living vines as alternate hosts and GRBV reservoirs requires further investigation (Cieniewicz *et al.*, 2018b). Indeed, the directionality of GRBV spread between cultivated grapevines and wild *Vitis* remains an open question and a better understanding of the epidemiology of GRBV will be essential for the development of more efficient control strategies. In any case, the possibility of the existence of a reservoir in wild grapevines should not be dismissed in any eradication efforts (Cieniewicz *et al.*, 2018a; Cieniewicz *et al.*, 2018b).

Systematic control of the insect vector, *Spissistilus festinus*, using insecticides is currently not recommended (Cieniewicz *et al.*, 2019), but may complement other management strategies once the phenology of this insect and its efficiency as a vector are better understood (Cieniewicz *et al.*, 2017).

**Phytosanitary risk**

The phytosanitary risk is essentially linked to infected grapevine propagation material and seen as significant for the whole EPPO *Vitis* production area given the clear pathogenicity and potential for negative impact of GRBV. In the EPPO region, the risk is mitigated by the absence of the *Spissistilus festinus* vector but the possibility exists that some European species such as *Stictocephala bisonia* (Membracidae) might be able to transmit GRBV. EFSA (2019) considered that, except for those affecting the host, no eco-climatic constraints exist for GRBV. Therefore, it is expected that GRBV is able to establish wherever *Vitis* are cultivated, causing symptoms and having impacts on *Vitis* fruit yield and/or quality.

## PHYTOSANITARY MEASURES

As drafted in an EPPO PRA Report (EPPO, in preparation) based on the EFSA pest categorization (EFSA, 2019), appropriate phytosanitary measures to import *Vitis* plants for planting (other than seeds) into the EPPO region could require that these plants are produced in a pest free area or in a pest free place/site of production for GRBV established according to EPPO Standard PM 5/8 *Guidelines on the phytosanitary measure 'Plants grown under physical isolation'* (EPPO, 2016). The physical isolation should allow to prevent both the virus and the vector from entering the place/site of production. A system approach combining the absence of GRBV symptoms during the growing period, testing of the consignment for GRBV and treatment of the consignment for the vector can also be envisaged. A number of EPPO countries already ban the import of *Vitis* plants for planting (other than seeds) (e.g. EU countries: Annex VI, points 10 of Regulation 2019/2072 (EU, 2019)). Host plants for planting could also be

imported using post-entry quarantine (in the framework of a bilateral agreement).

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**Datasheet history**

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