Natural Transmission of Grapevine Vein Clearing Virus

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In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Plant Science

By

Matthew Manu

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NATURAL TRANSMISSION OF GRAPEVINE VEIN CLEARING VIRUS

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Matthew Manu

ABSTRACT

More than 60 million tons of grapes are produced annually in the world, making them one of the most widely grown fruit crops. Despite grapes’ economic and health benefits, biotic stressors, such as viruses, cause significant loss to the grape and wine industry. One such virus is grapevine vein clearing virus (GVCV) which seriously threatens grape cultivation in the Midwest region of the United States. This virus has caused the removal of seven commercial vineyards since its discovery in 2004. About 34% of Ampelopsis cordata wild vines are infected with GVCV and serve as a primary inoculum for the spread of the virus by grapevine aphids to commercial vineyards. About 40% of grapevine aphids carry GVCV and it takes only a few seconds for grapevine aphids to transmit this virus. However, the presence of GVCV in its vector does not mean automatic transmission to a new host. In a greenhouse study, in which grapevine aphids were fed only on GVCV-infected vines, their transmission efficiency was 28%, but the natural transmission efficiency of GVCV by grapevine aphids is unknown. In this study, I asked two questions: 1) What is the natural transmission efficiency of GVCV by grapevine aphids? 2) Can GVCV be transmitted vertically via seeds? To answer these questions, grapevine aphids were collected from their hosts at native sites and placed onto the leaves of Chardonel grapevines in the greenhouse. One year later, I collected leaf tissue from these Chardonel grapevines and tested them for GVCV by polymerase chain reaction (PCR). Only 3% of the Chardonel grapevines tested GVCV positive, which means grapevine aphids have low natural transmission efficiency of GVCV. I detected GVCV in the seeds of infected grapevines and A. cordata but did not find it in the seedling vines. This indicates that GVCV is seed-borne but not seed-transmitted. Knowledge of the natural transmission of GVCV by grapevine aphids and by seeds helps design strategies to prevent the spread of GVCV to vineyards.

KEYWORDS: plant viruses, badnavirus, seed transmission, grapevine, grapevine vein clearing virus, grapevine aphids, Ampelopsis cordata
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In the interest of academic freedom and the principle of free speech, approval of this thesis indicates the format is acceptable and meets the academic criteria for the discipline as determined by the faculty that constitute the thesis committee. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.
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INTRODUCTION

Grapes are one of the most cultivated fruit crops in the world and more than 60 million tons of grapes are produced worldwide with USA, China, Italy, and France being the major producers (Matthäus 2008). As the most important fruit crop in the United States, grapes were expected to generate about $276 billion for the economy in 2022 (Dunham and associates 2022). Grapes can either be eaten fresh or used to produce grape juice, grape seed extract, grape seed oil, jelly, vinegar, raisins, wine, and jam. Grapes can be classified as seedless, wine, table, and raisin grapes (Girard and Mazza 1998). About 80% of the grapes produced are used in wine making, 13% are sold as table grapes and the rest as resins, juice, and other products (Girard and Mazza 1998). The major species of grapes are *Vitis labrusca* and *Vitis rotundifolia* from North America, *Vitis vinifera* from Europe, and French hybrids (Girard and Mazza 1998). Grapes are abundant in polyphenols, which may have anti-oxidative, anti-diabetic, anti-cholesterol, and anti-platelet properties (Ma and Zhang 2017).

Despite the economic and health benefits of grapes, the grape and wine industry is under constant threat of diseases. Viruses cause the highest number of emerging diseases in plants (Fargette et al. 2006). Grapevine is a host to about eighty-six viruses (Howard et al. 2021; Martelli 2018). The presence of numerous viruses in this widely cultivated fruit crop is probably explained by (i) a very long history of domestication and coexistence, (ii) a dearth of sources of resistance in *Vitis* spp., and (iii) a massive trade of germplasm on a global scale (Cieniewicz et al. 2020). Examples of these emerging viruses in grape cultivation are grapevine red blotch virus, grapevine pinot gris virus, and grapevine vein clearing virus (Cieniewicz et al. 2020).
Grape cultivation in the Midwest region of the United States faces a major threat from grapevine vein clearing virus (GVCV) (Qiu et al. 2007; Zhang et al. 2011). This virus was discovered in 2004 after a grape grower in Augusta, Missouri noticed unusual translucent vein clearing symptoms and mosaic patterns on the leaves of “Chardonnay” grape cultivar in his commercial vineyard (Qiu et al. 2007). “Can these symptoms be as a result of nutrient deficiency or a disease?” he wondered. Plant pathologists and virologists were invited to look at the symptoms. “These symptoms may be associated with a virus,” says one of the plant virologists. “But which virus is specifically associated with these symptoms?” another virologist asked. To determine which specific virus is associated with these symptoms, leaf tissues of affected grapevines were collected and tested for the presence of known RNA viruses in grapevines. Though some of the known viruses were detected, none could be associated with the symptoms. To determine if the virus was novel, deep sequencing of three cDNA libraries was carried out and results revealed that the small viral RNAs shared many similarities with viruses in the genus Badnavirus in the Caulimoviridae family (Zhang et al. 2011). Also, results from polymerase chain reaction and gel visualization showed that the virus was a DNA virus, making it the first double-stranded circular DNA virus to be discovered in grapevines (Zhang et al. 2011). The virus's entire genome is 7,753 base pairs (bp) and encodes three open reading frames (ORF) (Guo et al. 2014; Zhang et al. 2011). Badnavirus ORF I and ORF II-encoded proteins have been discovered to be connected to virions (Jacquot et al. 1996). It is hypothesized that the self-encoded proteinase activity will convert the polyprotein encoded by ORF III into coat protein, movement protein, and reverse transcription (RT)/RNaseH (Hull et al. 2005). The name “grapevine vein clearing virus” was given to this novel virus because of its ability to clear the veins of its host (Fig. 1) (Zhang et al. 2011). Other symptoms associated with the virus include
short internode, reduced vigor, and reduction in the quality of the berries, leading to huge economic losses to the grape and wine industry (Qiu and Schoelz 2017). In the states of Missouri, Arkansas, Illinois, Indiana and Tennessee, GVCV was found in key *V. vinifera* cultivars such as Cabernet Sauvignon, Cabernet franc, Riesling, and interspecific hybrids Chardonel, Vignoles, Vidal Blanc, and Valvin Muscat (Beach et al. 2017; Hu et al. 2021). Since its discovery, this virus has caused the removal of seven commercial vineyards (Qiu and Schoelz 2017; Zhang et al. 2011).

![Fig. 1. A grape leaf showing vein clearing and chlorotic symptoms of grapevine vein clearing virus in a vineyard at the Missouri State Experiment Station in Mountain Grove, Missouri. Photo Credit: Dr. Wenping Qiu.](image)

"Fig. 1. A grape leaf showing vein clearing and chlorotic symptoms of grapevine vein clearing virus in a vineyard at the Missouri State Experiment Station in Mountain Grove, Missouri. Photo Credit: Dr. Wenping Qiu."
Aphis illinoisensis (grapevine aphid) is the transmission vector of GVCV (Petersen et al. 2019). Native to the North American continent, grapevine aphids infest wild A. cordata plants (Petersen et al. 2019). Aphids' widespread dispersal and long-distance migration provide countless opportunities for viruses to infect domesticated crops (Fereres et al. 2017). About 40% of single grapevine aphids carry GVCV and it takes a few seconds for grapevine aphids to transmit a virus to their host (Uhls et al. 2021). However, the presence of GVCV in its vector does not mean an automatic transmission to a new host. In a greenhouse experiment, grapevine aphids after feeding on GVCV-infected leaves had a transmission efficiency of 28% (Qiu, unpublished data). A key question this present research addresses is the natural transmission efficiency of grapevine aphids in transmitting GVCV to its host.

Native plants, including native wild vines, host viruses and serve as reservoirs and primary inoculum where they contribute significantly to the evolution and spread of plant viruses (Cooper and Jones 2006; Pagán 2022; Roossinck 2015). For instance, in free-living V. californica and its hybrids in California, grapevine leafroll associated virus-2 (GLRaV-2) and GLRaV-3, as well as grapevine virus A and grapevine virus B, were discovered (Klaassen et al. 2011). Also, in riparian areas that are far from and right next to commercial vineyards, grapevine red blotch virus (GRBV) was found in V. californica and hybrids (Bahder et al. 2016; Perry et al. 2016). Recently, GVCV was found in 34% of Ampelopsis cordata (Petersen et al. 2019), 10% of wild grapevines (Beach et al. 2017), and 8% of cultivated grapevines sampled (Schoelz et al. 2021) in Missouri and Arkansas.

Ampelopsis cordata, known as heartleaf peppervine, a member of the Vitaceae family coexists in habitat with native grapevines (Vitis spp.). A. cordata is native to 20 states, ranging from Nebraska to Texas. It is most common in Missouri, Arkansas, and Louisiana, and is
becoming less common on the East Coast (Petersen et al. 2019). These wild vines encroach on commercial vineyard borders, riverbanks, fence rows, and tree lines. The presence of GVCV in *A. cordata* and native *Vitis* may be a major source of inoculum for the spread of GVCV by grapevine aphids to commercial vineyards.

It is unknown whether the spread of GVCV is solely due to grapevine aphids or if grape seeds may also contribute to the spread. More than 25% of plant viruses can be vertically transmitted from parents to progeny through seeds (Cobos et al. 2019; Sastry 2013; Simmons and Munkvold 2014). Vectors and agents of dispersal such as birds, rodents, and human activities help distribute these seeds containing viruses to near and far areas sufficient to create an epidemic (Pagán 2022).

A virus enters the seed either by invading gametes early before fertilization or directly invading the embryo after fertilization through maternal tissues like the suspensor (Hull 2014). Given that grapevines are vegetatively propagated, the epidemiological significance of seed transmission would be negligible. However, because wild vines serve as virus reservoirs and as food for the vectors, seed transmission is important in grape cultivation especially among wild vines (Digiaro et al. 2017; Murant and Taylor 1965). For viruses that are retained by seed but not seed-transmitted and do not infect progeny plants, there are no epidemiological consequences. However, if seed transmission occurs, it has epidemiological implications, first by allowing for long-distance viral transmission via commercial seed trading, and second by creating primary infection sites for secondary propagation in viral epidemics (Campbell and Madden 1990; Maule and Wang 1996).

Badnaviruses, including Sweet potato pakakuy virus (SPPV), Sugarcane bacilliform virus (SCBV), Piper yellow mottle virus (PYMoV), Taro bacilliform virus (TaBV), Kalanchoe top
spotting virus (KTSV), Commelina yellow mottle virus (ComYMoV), Banana streak virus (BSV), and Cacao swollen shoot virus (CSSV), have been found to be transmitted through seeds at various rates (Balan et al. 2022; Daniels et al. 1995; Deeshma and Bhat 2014; Hareesh and Bhat 2010; Hearon and Locker 1984; Medberry et al. 1990; Macanawai et al. 2005; Quainoo et al. 2008; Zhao et al. 2020). Studies on seed transmission in CSSV revealed that the virus was present in the testa, cotyledon, and embryo. The CSSV infection was confirmed in every seedling that came from infected seeds (Quainoo et al. 2008). Both self-pollinated and cross-pollinated taro plants were found to transmit TaBV through seed (Macanawai et al. 2005). It is unknown if GVCV, a member of the genus badnavirus, can also be vertically transmitted by seeds.

I sought to determine whether GVCV is seed transmitted and the efficiency at which grapevine aphids naturally transmit the virus. I hypothesized that if grapevine aphids are efficient in transmitting GVCV, then the number of GVCV-infected Chardonel grapevines should be high after being fed on by the grapevine aphids. Also, if GVCV is a seed transmitted virus, then it will be detected in the seeds, cotyledons, and true leaves of seedlings. Information on the rate of natural transmission of GVCV by grapevine aphids and whether the virus is seed transmitted or not will not only add to the body of knowledge, but will most importantly, help in controlling the spread of this virus to reduce the economic loss to the grape and wine industry.

To achieve these objectives, grapevine aphids were collected from their hosts at native sites and placed onto the leaves of Chardonel grapevines in the greenhouse. I collected leaf tissue from these Chardonel grapevines one year later and tested them for GVCV. I also collected seeds from known and unknown GVCV infected vines as well as the cotyledons and true leaves of
their seedlings. The presence of GVCV in the samples was detected by polymerase chain reaction (PCR) assay.
LITERATURE REVIEW

Plant Virus Disease Pandemics and Epidemics

Worldwide, viruses account for around 47% of the pathogens responsible for epidemics of new and reemerging plant diseases (Anderson et al. 2004). Plant viruses were discovered in the 19th century when plant pathologists Dimitri Ivanovsky and Martinus Beijerinck in an experiment that transferred filtered extract from *Nicotiana tabacum* containing tobacco mosaic virus to healthy host and observed these host show symptoms of the tobacco mosaic disease (Bradamante et al. 2021). Pandemics of virus disease and significant epidemics threaten cultivated plants used for human and animal consumption, production of fiber, ornamental plants, and therapeutic products. When many plants contract a systemic virus infection that could lead to severe diseases, the loss of crop production or reduction of crop quality could be catastrophic. Major epidemics or virus disease pandemics that affect main crops necessary for food security can drastically reduce food supplies, leading to famine.

The global plant viral disease epidemics, which is fast getting worse, has been influenced by several causes. First, the brisk growth of multinational corporations' commerce in plants and plant products internationally is bringing harmful viral diseases to regions of the world where they were previously absent. There are three basic causes for this.

i) Trade globalization has created new opportunities for the large-scale transfer of agricultural products from one continent or far-off nation to another through agreements on free trade or tariff reductions. (ii) Developing nations have been able to increase their trade in foreign crop products thanks to reduced subsidies for developed country production. (iii) This trade has been made possible by the development of faster air and sea transportation as well as the
relaxation of plant quarantine laws to comply with updated World Trade Organization requirements (Jones 2009). Second, because crop plants are being transported from domestication hubs to distant nations or continents where they are grown in monocultures, harmful new virus diseases are spreading at an alarming pace (Jones 2009). Thirdly, due to climate instability brought on by global warming, plant virus disease epidemics and pandemics are becoming harder to control. This is due to the projected effects of climate change on virus hosts, virus vectors, and virus itself. It seems inevitable that global warming will both hasten the appearance of novel plant virus diseases and worsen the harm caused by viral epidemics in known pathosystems (Jones 2009).

It is getting more crucial to address issues regarding virus mobility across managed and natural ecosystems as well as the potential dangers caused by introduced and native viruses.

**Horizontal Transmission of Plant Viruses**

Undoubtedly one of the most crucial processes in plant virus ecology and epidemiology is transmission to new hosts since viruses are obligate, intracellular parasites whose long-term survival depends on such processes (Cooper and Jones 2006; Jones 2009; Jones and Naidu 2019; Thresh 1980). Transmission to new plants is known as horizontal transmission. Invading roots by zoosporic fungus-like organisms (protists) or ectoparasitic nematodes, vector transmission by winged insects or wind-borne mites, or contact transmission where mechanical damage occurs are common examples of horizontal transmission (Hull 2014; Jones 2018).

Most viruses are transmitted by insects, and aphids are responsible for 50% of these transmissions (Nault 1997; Ng and Perry 2004). Aphids are perfectly crafted for their vector roles. Without causing permanent harm, virions can be delivered into plant cells with piercing-
sucking mouthparts. Parthenogenetic reproduction allows aphid populations to grow at extremely rapid rates, which accelerates the spread of viruses over short and long distances. Aphids are also found worldwide, and over 200 diverse kinds of aphid vectors have been found (Nault 1997; Ng and Perry 2004).

Plant viruses are classified as nonpersistent, semipersistent and persistent depending on their transmission modes (Gray and Banerjee 1999). If a virus was not kept by the vector for over a few hours, it was termed nonpersistent. Viruses that were semipersistent were kept around for days or weeks. Both nonpersistent and semipersistent virus groups do not require a latent period and do not multiply in the vector. The nonpersistent and semipersistent viruses were discovered to exclusively associate with the epicuticle that lines their vectors' stylets (mouthparts) or foreguts, respectively. These viruses are sometimes called stylet-borne or foregut-borne viruses. Each time an animal molts, its cuticle (which includes the lining of its mouth and foregut) is shed, along with any viruses it may have picked up along the way. These viruses have all been categorized as noncirculative. The viruses are not ingested by the vector in the sense that they do not penetrate any vector cell membranes or enter the hemocoel of the vector (as for some arthropods or nematodes). Noncirculative viruses are carried externally on the vector surface (like some fungi do) or on the cuticle lining of the vector's mouthparts or foregut (Gray and Banerjee 1999).

On the contrary, once acquired, persistent viruses remained inside the vector for the rest of its existence. Internalization of the ingested virus is necessary for the successful transmission of persistent viruses. Viruses present in the spaces between organs of the vector (hemocoel) and connected with the salivary system have high chances of infecting host. These viruses are now known as circulative viruses. Circulative viruses are those that can only spread if they cross cell
membranes and are carried internally within the body cavity of the vector or fungus cells. Circulative viruses can be further classified as propagative viruses and nonpropagative viruses. Propagative viruses replicate both in their plant hosts and in their arthropod vectors. Although extremely particular virus-vector interactions are necessary, the insect vector serves just as a route for the nonpropagative viruses to transfer between plant hosts. After a molt, the vector keeps all circulative viruses (Gray and Banerjee 1999).

Plant viruses are spread noncirculatively by major insect vector taxa, such as nematodes and whiteflies, leafhoppers, and aphids (Gray and Banerjee 1999). The Rubus yellow net virus, a badnavirus was suggested to be spread in a semipersistent manner, because during the transmission access period, the virus was found in the heads, bodies, and headless bodies of aphids. (Jones et al. 2002). Similarly, GVCV was detected in the stylet and body of grapevine aphids, which may indicate a semipersistent or circulative, nonpropagative transmission mode (Uhls et al. 2021).

Some groups of insect vectors have high transmission efficiency of some viruses. For instance, the efficiency of papaya ringspot virus (PRSV) transmission by three aphid vectors (Aphis gossypii, A. craccivora, and Myzus persicae) was investigated in a single-aphid inoculation study. Results revealed that the transmission efficiency was 53%, 38%, and 56% respectively (Kalleshwaraswamy and Krishna Kumar 2008). However, when five aphids were employed per plant, transmission efficiency was 100% in all three species (Kalleshwaraswamy and Krishna Kumar 2008). Also, in a vector transmission experiment on taro plants by the mealybug, Pseudococcus solomonensis that had been reared on TaBV-infected taro plants, a 53% (30/51) transmission efficiency was recorded (Macanawai et al. 2005). Other groups of insect vectors have low transmission efficiency. For example, in a greenhouse study, grapevine
aphids were starved and placed onto GVCV-infected leaves for 48 hours acquisition access period. After acquiring GVCV, 10 grapevine aphids were placed on each GVCV-free Chardonel vine in the greenhouse for 48 hours inoculation access period and eliminated afterward by spraying with insecticide (Safer Insect Killing Soap). After 60 days of post-inoculation, GVCV was detected in 28% (10/36) of the Chardonel vines tested (Qiu, unpublished data). This result shows that grapevine aphids have relatively low GVCV transmission efficiency.

**Vertical Transmission of Plant Viruses**

Vegetative propagation from virus-infected plant material, planting seeds with infected embryos, or parental pollen fertilizing its own offspring plants are all examples of vertical transmission (Hull 2014, Jones 2018). It is proposed that the effectiveness of seed transmission would depend on (i) the virus's capacity to invade gametic tissues, which would be correlated with virus multiplication in reproductive organs; (ii) the virus's capacity to reach gametic tissues, which would be determined by the speed of within-host movement; (iii) the plants' capacity to produce progeny upon infection and (iv) the gamete and embryo's potential to thrive amidst the viral presence (Cobos et al. 2019; Hull 2014; Lipsitch et al. 1996; Maule and Wang 1996; Sastry 2013). Organisms both large and small, including plant viruses, are constantly looking for new ways to survive in the face of abiotic and biotic stresses. A key determinant of a pathogen's fitness is its transmission efficiency. More than 25% of plant viruses can be transmitted from parents to progeny through seeds (Sastry 2013; Simmons and Munkvold 2014). When hosts and/or vectors are not accessible, seed infection gives the virus the ability to persist for prolonged amounts of time (years), because several seed-transmitted viruses could survive inside the seed as long as it is viable (Sastry 2013; Simmons and Munkvold 2014). Many species' seeds
take a while to germinate, even in favorable conditions (Chichaghare et al. 2021). Any seed can only grow if it is viable, after its dormancy is over, and when the right conditions exist (Chichaghare et al. 2021). Seed dormancy is the temporary inability of a viable seed to germinate under optimal conditions (Bewley 1997). Common causes of seed dormancy include a hard seed coat, embryo dormancy, an underdeveloped embryo, and the presence of chemical inhibitors. For seeds to germinate, dormancy must be broken or overcome either naturally through activities like wildfires, animal gut movements, and rainfall or artificially through processes like mechanical scarification, chemical scarification, soaking in cold or hot water, after-ripening, and stratification (Bareke 2018). To stimulate germination in water-impermeable seed coats, mechanical scarification, chemical scarification, and soaking in boiling or hot water can be used.

A dicotyledonous seed like grape is made of a seed coat, endosperm, and embryo. The seed coat protects the seed from mechanical damage and unfavorable environmental conditions. The endosperm provides food for the developing embryo. The embryo consists of the cotyledon, radicle and plumule. The radicle develops to form the root system of the plant while the plumule forms the shoot system. The cotyledon is the seed leaf that provides nourishment to the developing plant.

Seed-borne viruses are plant viruses present inside or on the seed coat's surface and will not be transmitted to the progenies. On the other hand, seed-transmitted viruses (vertical transmission) can be transmitted from parents to the next generation. The epidemiological significance of virus seed spread would be negligible in grapevines considering they are mostly propagated vegetatively. But, wild vines serve as virus reservoirs and as food for the vectors, and that places a high priority on seed transmission (Digiaro et al. 2017; Murant and Taylor 1965).
Seeds of native plants serve as reservoirs and primary inoculum of viruses where they contribute significantly to the evolution and spread of these plant viruses. Vectors and agents of dispersal such as birds, rodents, and human activities help distribute these seed-containing viruses to near and far areas sufficient to create an epidemic (Pagán 2022). A virus enters the seed either by invading gametes early before fertilization or directly invading the embryo after fertilization through maternal tissues like the suspensor (Hull 2014). In the case of tobanoviruses, seed transmission is achieved when the contaminated external seed coat encounters the microlesions developed on germinating progenies (Dombrovsky and Smith 2017; Hull 2014). To prevent viral infections, plants have developed antiviral barriers made of callose, a polysaccharide forming a wall in the shoot apical meristem making it difficult for viruses to reach the reproductive organs of the plant (Bradamanete et al. 2021). In addition, virus infected plant cells can communicate through plasmodesmata connections and signal healthy cells to induce apoptosis (programmed-cell death) to prevent the spread of a viral infection (Bradamanete et al. 2021). Plants can also produce small interfering RNA to disrupt the replication of invading viruses leading to low viral titers that are insufficient to cause a disease (Guo et al. 2019). A virus that overcomes all these hindrances of the plant can be vertically transmitted.

Caulimoviridae and Badnaviruses

The Caulimoviridae family (plant pararetroviruses) comprises eleven genera with two distinct virion morphological features, bacilliform, and isometric particles. The bacilliform particles include members of Badnavirus (68 species) and Tungrovirus (one species) while the isometric particles include members of the Vacciniviruses (one species), Caulimoviruses (12 species), Soymovirus (one species), Solendovirus (two species), Cavemovirus (three species),
Petuvirus (one species), and Rosadnavirus (one species) (Bhat et al. 2016; Ishwara Bhat et al. 2023). There are no morphological particles for the Ruflodivirus and Dioscovirus genera (Ishwara Bhat et al. 2023). Like retroviruses, all pararetroviruses have a double-stranded DNA genome and replicate through an intermediary RNA. Contrary to retroviruses, pararetrovirus genome integration does not take place during their typical replication cycle (Bhat et al. 2016). Rather, they build up in the host nucleus as minichromosomes. The diverse species of badnaviruses in different crops produce economic losses that range from 10% to 90% (Bhat et al. 2016). Depending on the species, badnavirus virions have a diameter of about 30 nm and a length that ranges from 120 to 150 nm (Bhat et al. 2016).

Badnaviruses have at least three open reading frames (ORFs) that are assumed to have been translated from an RNA transcript longer than the genome (Bhat et al. 2016). Depending on the species, badnaviruses' ORF I contain 399 to 927 bp. In ComYMV, ORF I is 602 bp long and produces a 23 kD polypeptide that has been shown to be associated with a particular virion (Cheng et al. 1996). The smallest ORF, with a range of 312 to 561 bp, is the ORF II (Bhat et al. 2016). Its product was recognized as a nucleic acid-binding protein in Cacao swollen shoot virus (Jacquot et al. 1996), and for ComYMV, it was demonstrated that it also bound to the virus capsid (Medberry et al. 1990; Cheng et al. 1996). The longest ORF, measuring between 5100 and 6000 bp, is ORF III (Bhat et al. 2016). It encodes a polyprotein that cleaves into four to five products, including a putative protein for cell-to-cell movement, aspartate protease, reverse transcriptase, RNase H, and capsid protein (Hagen et al. 1993; Hohn et al. 1997). Aspartic protease is responsible for cutting this polyprotein into its constituent functional subunits (Hohn et al. 1997).
Both monocots and dicots have been known to be infected by badnaviruses, though most of the species have a restricted host range (Bhat et al. 2016). Overall, badnavirus symptoms vary depending on the host, the host's cultivars, the virus species, and the environmental factors. Symptoms are typically mild to moderate. They include deformed leaves, reduced internode length, and stunting of plants due to chlorotic mottle or necrotic streaks. For most plants infected with badnaviruses, the diseased plants' lack of symptoms and the masking of symptoms during specific times of the year are typical. When plants experience abiotic stress, such as temperature changes and nutrient depletion, symptoms reappear and become more severe.

Badnaviruses have relaxed circular DNA double strands with lengths ranging from 7.2 to 9.2 kb (Bhat et al. 2016). Those are signs of the beginnings of reverse transcription-based plus- and minus-strand DNA synthesis (Hohn and Rothnie 2013). The nucleus receives the genomic DNA that is wrapped within the virus capsid, where the discontinuities are fixed and mini chromosomes are created by joining the covalently closed, supercoiled DNA with histone proteins (Hull 2002). A promoter and a polyadenylation signal can be found in an intergenomic region that is 631 to 1177 bp long (Bhat et al. 2016). Then, using host-encoded DNA-dependent RNA polymerase II, mini-chromosomal DNA is transcribed into a terminally redundant RNA that serves as a pregenome and polycistronic messenger RNA (Bhat et al. 2016). Redundancy is produced by the circular DNA's polyadenylation signal being overlooked by the RNA polymerase at first and then being identified at second contact (Hull 2002). In the final stage of replication, the reverse transcriptase converts pregenomic RNA back to dsDNA. tRNA\text{met} primes the synthesis of single-stranded, (-) sense DNA, and purine-rich cleavage products left over from RNase H digestion of the pregenomic RNA prime the synthesis of (+) sense DNA (Hohn and Rothnie 2013; Medberry et al. 1990).
Most badnaviruses infect perennial hosts that are vegetatively propagated. While several species of mealybugs are responsible for the secondary or horizontal spread of the majority of badnavirus species, aphids are primarily responsible for the semi-persistent transmission of Rubus yellow net virus (RYNV), Gooseberry vein banding associated virus (GVBaV), and Spiraea yellow leaf spot virus (SYLSV) (Bhat et al. 2016).

Badnaviruses, including SPPV, SCBV, PYMoV, TaBV, KTSV, ComYMoV, BSV, and CSSV, have been found to be transmitted through seeds at various rates (Balan et al. 2022; Daniels et al. 1995; Deeshma and Bhat 2014; Hareesh and Bhat 2010; Hearon and Locker 1984; Medberry et al. 1990; Macanawai et al. 2005; Quainoo et al. 2008; Zhao et al. 2020). Studies on seed transmission in CSSV revealed that the virus was present in the testa, cotyledon, and embryo. The CSSV infection was confirmed in every seedling that came from infected seeds (Quainoo et al. 2008). Both self-pollinated and cross-pollinated taro plants were found to transmit TaBV through seed (Macanawai et al. 2005).

Questions Awaiting Answers

Grapevine aphids were found to be the transmission vector of GVCV where about 40% of single grapevine aphids carry GVCV (Uhls et al. 2021), but the presence of a virus in a vector does not automatically lead to transmission. Under greenhouse conditions, grapevine aphids transmitted GVCV to Chardonel grapevine cultivar (28%) after 60 days post grapevine aphid transmission (Qiu, unpublished data) but the natural transmission efficiency of GVCV by grapevine aphids remains unknown. GVCV was found in 34% of Ampelopsis cordata sampled (Petersen et al. 2019), 10% of wild grapevines sampled (Beach et al. 2017), and 8% of cultivated grapevines sampled (Schoelz et al. 2021). About 25% of plant viruses are vertically transmitted.
from parents to their progenies. In addition, some members of the genus badnavirus have been reported to be seed-transmitted (Balan et al. 2022; Deeshma and Bhat 2014). Whether GVCV, a member of the genus badnavirus, can also be vertically transmitted by seeds is yet to be known.

Apart from its spread by a vector (horizontal transmission), can GVCV be transmitted through other routes like seeds (vertical transmission)?
METHODS

Synopsis

To investigate the efficiency of grapevine aphids in the natural transmission of GVCV, colonies of grapevine aphids, leaf tissues of host plants, and leaf tissues of Chardonel grape cultivar one year after grapevine aphids fed on them were collected.

Also, to test whether GVCV can be transmitted vertically through seeds, seeds were collected from GVCV-infected and GVCV-free (control) vines of A. cordata and Chardonel. Seeds were germinated and the cotyledons and true leaves of seedlings were collected. Following DNA extraction, a PCR assay was used to detect GVCV in all samples.

Collection of Seeds from A. cordata and Chardonel Grapevines

Seeds of A. cordata from unknown status and known GVCV-infected vines were collected in Springfield, MO. Also, seeds of Chardonel grape cultivar from known GVCV-infected and GVCV-free (control) vines were collected from a vineyard at Mountain Grove, MO. Seeds were brought to the lab and kept in a refrigerator at 4 °C.

Collection of Grapevine Aphid Colonies and Leaf Tissues of Host Plants

Grapevine aphids from wild Vitis and A. cordata plants and the leaf tissues of their host plants at native sites were collected from Mountain Grove, Springfield, and Augusta, Missouri in June, and July of 2021. The grapevine aphids and their corresponding leaf tissues of host plants were placed in a ziplock bag soaked with paper towel and brought to the laboratory in a cooler containing ice. Grapevine aphids were preserved in 80% ethanol in 2.0 ml of polypropylene tube
and stored at –20 °C. The leaf tissues were wrapped in aluminium foil and stored at –80 °C till DNA extraction after weighing about 100 mg of each sample.

To determine the efficiency of grapevine aphids in transmitting GVCV, five individual grapevine aphids of each colony were placed on sixty-eight Chardonel grapevines for forty-eight hours under greenhouse conditions and the grapevine aphids were eliminated afterwards with insecticide. Chardonel grapevine cultivar was selected because it is susceptible to GVCV, and it is the first grape cultivar from which GVCV was discovered. Leaf tissues were collected from each Chardonel grapevine after one year and tested for the presence of GVCV.

**Detection of GVCV in Leaf Tissues of Host Plants and Chardonel Grapevines, One Year Post Transmission**

To investigate whether GVCV was present in the host plants, Chardonel grapevines, one year post transmission, about 100 mg of leaf tissues were weighed and DNA was extracted from them using Synergy 2.0 plant DNA extraction kit following the manufacturer's instructions with slight modifications. After homogenizing the leaf tissues in the extraction buffer, 500 µl of cetyltrimethylammonium bromide (CTAB) was added to the mixture and incubated in a water bath at 60 °C for 30 minutes with brief vortexing every 10 minutes. The DNA was eluted with 50 µl of autoclaved distilled water. A nanodrop spectrophotometer was used to check the quality of the DNA after which DNA samples were diluted to 15 ng/µl.

Two sets of GVCV-specific primers 5044F-5387R and 963F-1634R (Table 1) were used to detect GVCV and to prevent false negative results. 5044F-5387R primers amplified a 344 bp portion and 963F-1634R amplified a 672 bp portion of GVCV. 16S forward and 16S reverse primers (Table 1) were used to amplify a 105 bp fragment of the 16S ribosomal RNA gene of the
plant to verify the quality of the DNA. For polymerase chain amplification, a master mix was prepared consisting of 1.25 unit of Taq polymerase, 0.4 µM each of forward and reverse primers, 0.28 mM dNTPs, 5X buffer, autoclaved water and 1µl of DNA was added to make a total volume of 25 µl. Samples were placed in a thermocycler. The thermocycling conditions using GVCV-specific primers 5044F-5387R were a one-minute initial denaturation at 95°C followed by 35 cycles of 95°C for 15 s, 58 °C for 15 s and 72°C for 25 s, and then a final extension of 72°C for 7 minutes. Also, the thermocycling conditions using GVCV-specific primers 963F-1634R were a one-minute initial denaturation at 95°C followed by 35 cycles of 95°C for 15 s, 60 °C for 30 s and 72°C for 30 s, and then a final extension of 72°C for 7 minutes. PCR products were loaded on a 1% agarose gel and visualized under UV after electrophoresis.

**Detection of GVCV in Grapevine Aphids**

To investigate whether GVCV was present in the grapevine aphids, DNA was extracted from groups of ten grapevine aphids of each remaining colony following the manufacturer’s protocol with slight modifications. The modifications include grinding the grapevine aphids using a sealed 1000 µl pipette tip in a 2.0 ml of polypropylene tube instead of a mortar and pestle and eluting the DNA with 30 µl of autoclaved distilled water instead of 50 µl. The concentration and quality of the DNA extracted was measured by a nanodrop spectrophotometer. PCR was used to detect and visualize GVCV in grapevine aphids, like the previous description in the host plants above with slight modifications. Concentrated grapevine aphid DNA in 1 µl was used as a template. EFF and EFR primers (Table 1) were used to amplify a 200 bp portion of the grapevine aphid elongation factor 1-α (EF1) (Accession KC897260) to verify the quality of the DNA extracted.
Table 1. List of primers used

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5’ to 3’)</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>16SF</td>
<td>TGCTTAACACATGCAAGTCGGA</td>
<td><em>A. cordata</em> and Chardonel</td>
</tr>
<tr>
<td>16SR</td>
<td>AGCCGTTTCCAGCTGGTGTTC</td>
<td><em>A. cordata</em> and Chardonel</td>
</tr>
<tr>
<td>963F</td>
<td>TCCATCACAGATCTAACGGCA</td>
<td>GVCV</td>
</tr>
<tr>
<td>1634R</td>
<td>CAAGGTAGCGGCCACCGAG</td>
<td>GVCV</td>
</tr>
<tr>
<td>5044F</td>
<td>ATTCCAGCCTCTTGCAG</td>
<td>GVCV</td>
</tr>
<tr>
<td>5387R</td>
<td>TCATTCCCTGCGAGGATCAT</td>
<td>GVCV</td>
</tr>
<tr>
<td>EFF</td>
<td>GGCTCTCCGTCCTCCACTCC</td>
<td><em>Aphis illinoisensis</em></td>
</tr>
<tr>
<td>EFR</td>
<td>TGGTGATGTGGCAGGTCG</td>
<td><em>Aphis illinoisensis</em></td>
</tr>
</tbody>
</table>

Detection of GVCV in *A. cordata* and Chardonel Seeds

True seed transmission is likely to be achieved when a virus is present in the embryo of the seed and not the seed coat (Bradamante et al. 2021). To this end, the seeds were soaked in 98% sulphuric acid for 30 minutes to soften the seed coats after which the seed coats were removed with a scalpel knife. The remaining portion of the seed (endosperm and embryo) were soaked in 10% sodium hypochlorite (bleach) for 10 minutes to denature any external viral DNA. Seeds were rinsed in deionized water. The detection of GVCV in single seeds (endosperm and embryo) was achieved by following the protocol as described previously in the detection of GVCV in host plants.

Detection of GVCV in Seedlings

To determine the seed transmission of GVCV, the seeds needed to be germinated. After storing randomly collected *A. cordata* seeds in the refrigerator (cold stratification) at 4 °C for six months, the seeds were removed and subjected to chemical and hot water treatments to determine the best seed dormancy breaking method to enhance seed germination (Table 2). For
hot water treatment, seeds were soaked in water at 25, 50 and, 100 °C for two treatment times, 30 and 60 minutes. For chemical treatment, seeds were immersed in 50% hydrochloric acid (HCl), 50 and 98% sulphuric acid (H\textsubscript{2}SO\textsubscript{4}) for two treatment times, 30 and 60 minutes. Twenty seeds of equal sizes were used for each treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of germinated seeds</th>
<th>% germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water at 25 °C for 30 minutes</td>
<td>0/20</td>
<td>0%</td>
</tr>
<tr>
<td>Water at 25 °C for 60 minutes</td>
<td>0/20</td>
<td>0%</td>
</tr>
<tr>
<td>Water at 50 °C for 30 minutes</td>
<td>0/20</td>
<td>0%</td>
</tr>
<tr>
<td>Water at 50 °C for 60 minutes</td>
<td>0/20</td>
<td>0%</td>
</tr>
<tr>
<td>Water at 100 °C for 30 minutes</td>
<td>0/20</td>
<td>0%</td>
</tr>
<tr>
<td>Water at 100 °C for 60 minutes</td>
<td>0/20</td>
<td>0%</td>
</tr>
<tr>
<td>50% HCl for 30 minutes</td>
<td>0/20</td>
<td>0%</td>
</tr>
<tr>
<td>50% HCl for 60 minutes</td>
<td>0/20</td>
<td>0%</td>
</tr>
<tr>
<td>50% H\textsubscript{2}SO\textsubscript{4} for 30 minutes</td>
<td>0/20</td>
<td>0%</td>
</tr>
<tr>
<td>50% H\textsubscript{2}SO\textsubscript{4} for 60 minutes</td>
<td>0/20</td>
<td>0%</td>
</tr>
<tr>
<td>98% H\textsubscript{2}SO\textsubscript{4} for 30 minutes</td>
<td>0/20</td>
<td>0%</td>
</tr>
<tr>
<td>98% H\textsubscript{2}SO\textsubscript{4} for 60 minutes</td>
<td>11/20</td>
<td>55%</td>
</tr>
</tbody>
</table>

Seeds were then rinsed in autoclaved water and soaked in 10% sodium hypochlorite for 10 minutes to prevent fungal growth. Seeds were placed in moist paper towels and kept in a growing chamber and monitored for 15 days. Germinating seeds were transplanted to rubber containers containing soil. To detect GVCV in seedlings, DNA was extracted and the presence of GVCV detected in the cotyledons and true leaves as previously described in host plants above.
RESULTS

Grapevine Aphids have Low GVCV Natural Transmission Efficiency

About 40% of grapevine aphids on wild plants in native sites carry GVCV and it takes a few seconds for grapevine aphids to transmit a virus to their host (Uhls et al. 2021). However, the presence of GVCV in its vector does not mean an automatic transmission to a new host. What is the natural transmission efficiency of GVCV by grapevine aphids?

To investigate the natural transmission efficiency of GVCV by grapevine aphids, grapevine aphid colonies and the leaf tissues of their host plants were collected at their native sites from Mountain Grove, Springfield, and Augusta, Missouri in June, and July of 2021. The grapevine aphid colonies and their native host vines were tested by PCR assay to know if GVCV was present in them. I detected GVCV in 15% of the native host plants from which the grapevine aphids were collected (Table 3), and 87% of the grapevine aphid colonies collected contained GVCV (Fig. 2A; Table 3). Groups of five grapevine aphids were then placed on sixty-eight Chardonel grapevines for forty-eight hours under greenhouse conditions and the grapevine aphids were eliminated afterwards with insecticide. I detected GVCV in just 3% of Chardonel grapevines, one year post transmission (Fig. 2B; Table 3).

<table>
<thead>
<tr>
<th>Sample tested</th>
<th>Total</th>
<th>GVCV positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host plant</td>
<td>40</td>
<td>6</td>
<td>15%</td>
</tr>
<tr>
<td>Grapevine aphid colonies</td>
<td>38</td>
<td>33</td>
<td>87%</td>
</tr>
<tr>
<td>Chardonel grapevine</td>
<td>68</td>
<td>2</td>
<td>3%</td>
</tr>
</tbody>
</table>
Fig. 2. Detecting GVCV in grapevine aphid colonies and Chardonel grapevines by PCR assay. A, GVCV detection in grapevine aphid colonies. L is a DNA ladder, + sign is GVCV positive samples, - sign is GVCV negative samples, P is positive control and N is negative control. B, GVCV detection in Chardonel grapevines one year after grapevine aphids fed on them. 16S ribosomal RNA primers amplified a 105 bp of the plant gene and GVCV specific primers amplified a 344bp fragment of the GVCV genome. EF primers amplified a 200 bp portion of the grapevine aphid elongation factor 1-α to verify the quality of the aphid DNA extracted.

GVCV is Present in Chardonel and *A. cordata* Seeds

More than 25% of plant viruses can be vertically transmitted from parents to progeny through seeds (Cobos et al. 2019; Sastry 2013; Simmons and Munkvold 2014). Interestingly, some members of the genus badnavirus to which GVCV belongs have been reported to be seed transmitted (Balan et al. 2022; Deeshma and Bhat 2014; Hareesh and Bhat 2010), but whether GVCV is seed transmitted or not remains unknown. In addition, GVCV is present in about 34% of *Ampelopsis cordata* (Petersen et al. 2019) but whether this spread is solely due to grapevine aphids or if seed transmission contributes a part remains unknown.
To determine whether GVCV is present in seeds, I collected seeds from known GVCV-infected and GVCV-free vines from Springfield in August, 2021 and Mountain Grove, Missouri in September of 2022. True seed transmission is likely to be achieved when a virus is present in the embryo of the seed and not the seed coat (Bradamante et al. 2021). To this end, I soaked the seeds in 98% sulphuric acid for 30 minutes to soften the seed coats after which the seed coats were removed with a scalpel knife. I detected GVCV in 18, 78 and 75% of randomly collected seeds of A. cordata, infected Chordonel and infected A. cordata (Fig. 3A, B and C; Table 4), respectively, but not in seeds of GVCV-free Chordonel (Fig. 3D; Table 4).

![PCR assay for detecting GVCV in Chordonel and A. cordata seeds. A, Detecting GVCV in randomly collected seeds of A. cordata. L is a DNA ladder, + sign is GVCV positive samples, - sign is GVCV negative samples, P is positive control and N is negative control. B, GVCV detection in seeds of GVCV-infected Chordonel. C, GVCV detection in seeds of GVCV-infected A. cordata. D, Detecting GVCV in seeds of GVCV-free Chordonel (control).](image)

**GVCV is not Seed Transmitted**

True seed transmission is achieved when the virus is transmitted from seeds to cotyledons and the true leaves of seedlings. I subjected randomly collected A. cordata seeds to chemical and hot water treatments to determine the best seed dormancy-breaking method to enhance seed
germination. Treating seeds with 98% sulphuric acid for 60 minutes is the only seed dormancy-breaking technique that resulted in germination by 15 days (Table 2). After 15 days, 55% (11/20) seeds germinated. I later treated the other seeds which did not germinate using the other seed dormancy breaking techniques, with 98% sulphuric acid for 60 minutes and germinated them. Seeds of GVCV-free Chardonel, GVCV-infected Chardonel and *A. cordata* were also treated with 98% sulphuric acid for 60 minutes after three months of cold stratification to enhance germination. A total of 6, 30 and 51 of GVCV-free Chardonel, GVCV-infected Chardonel and randomly collected *A. cordata* seeds were germinated respectively. However, there was no germination of GVCV-infected *A. cordata* seeds.

I then detected the presence of GVCV in the cotyledon and true leaves of the seedlings at three-leaf stage. GVCV was absent in the true leaves (Fig. 4A, C and E: Table 4) and cotyledons (Fig. 4B, D and F: Table 4) of randomly collected *A. cordata*, GVCV-infected and GVCV-free Chardonel seedlings.

<table>
<thead>
<tr>
<th>Sample tested</th>
<th>Total</th>
<th>GVCV positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed of randomly collected <em>A. cordata</em></td>
<td>102</td>
<td>18</td>
<td>18%</td>
</tr>
<tr>
<td>Cotyledons of seedlings</td>
<td>46</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>True leaves of seedlings</td>
<td>51</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Seeds of GVCV-infected <em>A. cordata</em></td>
<td>60</td>
<td>45</td>
<td>75%</td>
</tr>
<tr>
<td>Seeds of GVCV-infected Chardonel</td>
<td>68</td>
<td>53</td>
<td>78%</td>
</tr>
<tr>
<td>Cotyledons of seedlings</td>
<td>30</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>True leaves of seedlings</td>
<td>30</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Seeds of GVCV-free Chardonel (control)</td>
<td>18</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Cotyledons of seedlings</td>
<td>6</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>True leaves of seedlings</td>
<td>6</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>
Fig. 4. PCR assay for detecting GVCV in seedlings of Chardonel and A. cordata vines. A and B, Detecting GVCV in true leaves and cotyledons of randomly collected A. cordata, respectively. L is a DNA ladder, + sign is GVCV positive samples, - sign is GVCV negative samples, P is positive control and N is negative control. C and D, Detecting GVCV in true leaves and cotyledons of GVCV-infected Chardonel, respectively. E and F, Detecting GVCV in true leaves and cotyledons of GVCV-free Chardonel (control), respectively.
DISCUSSION

Grape cultivation in the Midwest region of the United States is seriously threatened by GVCV (Qiu et al. 2007; Zhang et al. 2011). This virus has caused the removal of seven commercial vineyards since its discovery in 2004 (Qiu and Schoelz 2017; Zhang et al. 2011). About 34% of *Ampelopsis cordata* wild vines are infected with GVCV and serve as a primary inoculum for the spread of the virus by grapevine aphids to commercial vineyards (Petersen et al. 2019). Though GVCV is present in about 40% of single grapevine aphids, that does not guarantee transmission to a new host (Uhls et al. 2021). In a greenhouse study, after feeding on GVCV-infected leaves, grapevine aphids achieved a transmission efficiency of 28% (10/36) (Qiu, unpublished data). However, the natural transmission efficiency of GVCV by grapevine aphids is unknown. I asked two questions in this study. 1) What is the natural transmission efficiency of GVCV by grapevine aphids? 2) Can GVCV be transmitted vertically via seeds? Answers to these two questions will not only add to the body of knowledge known about the virus but will most importantly help control the spread of this virus to reduce the economic loss to the grape and wine industry. Though the sample sizes are small, the findings provide key information to address the study questions.

I detected GVCV in 87% of the grapevine aphid colonies collected. This number is a bit higher when compared to the 79% of GVCV detected in grapevine aphid colonies previously (Uhls et al. 2021). Differences could be due to the sample size. I tested a total of 38 grapevine aphid colonies as opposed to the 70 tested by Uhls et al. 2021. However, only 15% of the native host plants from which the grapevine aphids were collected tested positive for GVCV. This means that most of the grapevine aphid colonies that tested positive for GVCV were collected
from GVCV-negative native host plants. It was reported that an insect vector for a plant virus can change how it selects hosts after acquiring the virus (Ingwell et al. 2012). For instance, the aphid *Rhopalosiphum padi* preferred noninfected wheat plants after acquiring Barley yellow dwarf virus (BYDV) during *in vitro* feeding, but noninfective aphids preferred BYDV-infected plants (Ingwell et al. 2012). As noninfective vector preference for infected plants would enhance acquisition and infective vector preference for noninfected hosts will increase transmission, this behavioral modification facilitates the spread of a virus (Ingwell et al. 2012).

The natural transmission efficiency of grapevine aphids was 3% (Table 3). This shows that the natural transmission efficiency of GVCV by grapevine aphids may be low since the presence of GVCV in its vector does not always result in transmission to a new host. This result is similar to the low 3% sweet potato chlorotic stunt virus transmission efficiency in sweet potato by the vector, and the 3.1% sweet potato leaf curl virus by the whitefly *Bemisia tabaci* recorded (Andreason et al. 2021; Valverde et al. 2004). GVCV was found in 34% of *A. cordata* sampled (Petersen et al. 2019), 10% of wild grapevines sampled (Beach et al. 2017), and 8% of cultivated grapevines sampled (Schoelz et al. 2021). It was proposed that the lower prevalence of GVCV in vineyards may be attributable to the seasonal management of insect pests in vineyards, which significantly lowers aphid population size and decreases possibilities for GVCV spread and infection in vineyards (Uhls et al. 2021). Also, GVCV infects mainly white berried-cultivars as opposed to red-berried cultivars like Norton and Chamboucin which are resistant to the virus (Qiu et al. 2020; Schoelz et al. 2021). Another reason for the low spread of GVCV in vineyards, according to this study, is the low natural transmission efficiency of GVCV by grapevine aphids. However, vineyards have a large population of grapevine aphids that can still spread GVCV, though inefficiently. A reason for the low natural transmission efficiency could be the small
number of grapevine aphids placed on the Chardonel vines. For example, with 50 whiteflies per test plant, high transmission efficiency of Sweet potato chlorotic stunt virus was achieved, whereas with 10 whiteflies, there were fewer than 15% of plants infected (Cohen et al. 1992). Also, in Israel, groups of 50 whiteflies per test plant were shown to have significant rates of *Ipomoea* crinkle leaf curl virus (ICLCV) transmission (50–60% of plants infected), compared to 20–30% for groups of 10 whiteflies per test plant (Cohen et al. 1997). The developmental stage of the grapevine aphids and acquisition access periods may also contribute to the low natural transmission efficiency of GVCV by the grapevine aphids (Uhls et al. 2021). With aphids that transmit viruses at semipersistent mode, like grapevine aphids, prolonged feeding on infected vines enhances their transmission efficiency (Palacios et al. 2002; Valverde et al. 2004). A future study will involve placing 20 and 50 grapevine aphids onto the leaves of Chardonel vines in the greenhouse and detecting the presence of GVCV after one year.

In a seed transmission experiment, I first investigated whether GVCV is present in seeds and detected GVCV in 18%, of seeds of randomly collected *A. cordata*, 78 and 75% GVCV-infected Chardonel and GVCV-infected *A. cordata* respectively. To test whether GVCV is seed transmitted, I subjected seeds to chemical and hot water treatments to determine the best seed dormancy-breaking method to enhance germination. Treatment of seeds with 98% sulphuric acid for 60 minutes resulted in 55% germination (Table 2). This result is not surprising because 98% sulphuric acid treatment for 60 minutes is reported to be efficient in breaking seed dormancy (Ali et al. 2011). A total of 6, 30 and 51 of GVCV-free Chardonel, GVCV-infected Chardonel and randomly collected *A. cordata* seeds were germinated. However, there was no germination of GVCV-infected *A. cordata* seeds. This is because fungi invaded the seeds, leading to the decay of the seeds and prevented germination. Another reason could be that seed embryo might not be
well developed. GVCV was absent in the cotyledon and true leaves of randomly collected A. cordata and GVCV-infected Chardonel seedlings. The presence of GVCV in seeds but not in the true leaves and cotyledons of seedlings shows that GVCV may be seed-borne but not seed-transmitted. This result is contrary to other research which demonstrated that some members of the genus badnavirus can effectively be spread through seeds at various transmission rates (Balan et al. 2022; Deeshma and Bhat 2014). For instance, studies on seed transmission in CSSV revealed that the virus was present in the testa, cotyledon, and embryo. The CSSV infection was confirmed in every seedling that came from infected seeds (Quainoo et al. 2008). Both self-pollinated and cross-pollinated taro plants were found to transmit TaBV through seed (Macanawai et al. 2005).

The transmission of seed-borne viruses to plants grown from those seeds does not always occur (Bhat and Rhao 2020). That viruses can be seed-borne and not seed-transmitted has been reported among members of the begomovirus genus including sweet potato leaf curl virus (Andreason et al. 2021), tomato leaf curl yellow virus (Pérez-Padilla et al. 2020), tomato yellow leaf curl sardinia virus (Tabein et al. 2021) and tomato leaf curl New Delhi virus (Fortes et al. 2022). To prevent viral infections, plants have developed antiviral barriers made of callose, a polysaccharide forming a wall making it difficult for viruses to reach the embryo (Allard 1916; Bradamanete et al. 2021). In addition, virus infected plant cells can communicate through plasmodesmata connections and signal healthy cells to induce apoptosis (programmed cell death) to prevent the spread of a viral infection (Bradamanete et al. 2021). Plants can also produce small interfering RNA to disrupt the replication of invading viruses leading to low viral titers that are insufficient to cause a disease (Guo et al. 2019). These reasons could explain why GVCV is seed-borne but not seed-transmitted. For viruses that are retained by seed but not seed-
transmitted and do not infect progeny plants, there are no epidemiological effects. However, if seed transmission occurs, it has epidemiological implications, first by allowing for long-distance viral transmission via commercial seed trading, and second by creating primary infection sites for secondary propagation in viral epidemics. (Campbell and Madden 1990; Maule and Wang 1996). The low natural transmission efficiency of GVCV by grapevine aphids as well as the lack of seed transmission explains in part why GVCV is endemic to the Midwest region of the United States specifically in Missouri, Arkansas, Indiana, Illinois and Tennessee and has not spread to other grape cultivating areas. Also, the absence of vertical seed transmission and the low natural transmission efficiency partly explains why GVCV was found in 8% of composite samples of grapevines in Missouri vineyards surveyed (Schoelz et al. 2021).

In conclusion, grapevine aphids have low GVCV natural transmission efficiency and GVCV is seed-borne but not seed-transmitted. This information is key to designing strategies to control the spread of the virus. In addition to breeding GVCV-resistant grape cultivars with superior wine qualities, planting tested virus-free grapevines, controlling grapevine aphids in vineyards and removal of wild vines which can serve as primary inoculum around vineyards are key to preventing the spread of GVCV in vineyards.
LITERATURE CITED


type strain of *Tomato yellow leaf curl virus* in tomato plants. Phytopathology 110:121–129.


