Virology Journal

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Grapevine Pinot gris virus spreads in infected vineyards: latent infections have no direct impact on grape production

Noemi Messmer[1,2], Patricia Bohnert[1], Lars Askani[1], Stefan Schumacher[1], Ralf T. Voegele[2] and René Fuchs[1*]

Abstract

Background Grapevine Pinot gris virus (GPGV) infects grapevines worldwide and causes symptoms such as chlorotic mottling and deformations on leaves, stunted shoots and short panicles, or none of these symptoms if it appears as latent infection. So far, the consequences of GPGV infections for winegrowers are difficult to assess since important information such as plant performance at different GPGV infection levels and symptom expression are not fully clarified.

Methods In order to investigate the course of GPGV spread, annual visual evaluations and ELISA tests were conducted over 3–4 consecutive years in four GPGV-infected vineyards in southern Germany: GEM, HEC, NIM, and REI. The program PATCHY was used to analyze spatial disease patterns. Sanger sequencing was used to determine virus isolates in vines at different GPGV infection levels, to test their respective influence on symptom expression. Yield and GrapeScan (FTIR) analyses were conducted to test the impact of different GPGV infection levels and isolates on fruit quantity and quality.

Results GPGV infections significantly increased in all four vineyards (GEM 22–32%, HEC 50–99%, NIM 83–90%, REI 56–76%) with significant spreading patterns across and along rows. Specific symptom progression patterns were not observed. According to our results, the virus isolate has an influence on whether symptoms develop during a GPGV infection. While yield analyses revealed that yield losses only occur in symptomatic vines and range from 13 to 96% depending on the severity of symptoms, latent infections have no impact on grape production. No relevant effects of GPGV infections on must quality were observed.

Conclusions Secondary spread of GPGV was observed in all vineyards monitored, indicating vector-borne transmission that is likely to be accelerated by human viticultural management. GPGV should be further monitored to prevent the accumulation of detrimental symptomatic isolates. The results of this study can be used to assess the risk of GPGV to viticulture and should be considered when developing management strategies against the virus.

Keywords Grapevine Pinot gris virus (GPGV), Secondary viral spread, GPGV isolates, Yield analysis, Must analysis, GrapeScan

*Correspondence:
René Fuchs
rene.fuchs@wbi.bwl.de
[1] Department of Biology, State Institute of Viticulture and Enology (WBI), Merzhauser Strasse 119, 79100 Freiburg, Germany
[2] Department of Phytopathology, University of Hohenheim, Otto-Sander-Strasse 5, 70593 Stuttgart, Germany

Background

Grapevine Pinot gris virus (GPGV) is a new *Trichovirus* on the rise. GPGV was first characterized in Italian vines showing stunted growth and deformed leaves with chlorotic mottling, subsequently referred to as Grapevine Leaf Mottling and Deformation (GLMD) disease [1]. In search for the responsible pathogen,

high-throughput sequencing (HTS) revealed the presence of a previously unknown viral sequence in symptomatic vines from now on named GPGV [2]. Since its identification, presence of GPGV was verified in over 30 wine-producing countries of all five continents in many different grapevine varieties [3–6].

The occurrence of the virus in vineyards around the world has raised many questions about the origin and transmission of the virus. Recent phylogenetic studies revealed that the origin of GPGV was in Asia (China/Japan) over 3,500 years ago [7–9]. GPGV entered Europe, probably through Germany, around the nineteenth century and eventually spread from there to other continents. Spatio-temporal analyses support the hypothesis, that the global spread of GPGV was primarily caused by human trade of infected plant material [9].

However, also secondary spreading events within vineyards are known [8, 10]. Under controlled conditions and in semi-field trials, the Eriophyid mite *Colomerus vitis* was verified to transmit GPGV on grapevine, which is to date the only known vector of GPGV [10–12]. However, GPGV infections in various herbaceous and woody plants such as *Silene latifolia*, *Chenopodium album* and *Fraxinus* sp. indicate the presence of at least one additional insect vector [13, 14].

Since not all infected vines show symptoms of GLMD, a direct correlation between the disease and GPGV was questioned. Hypotheses regarding the influence of viral titer or varying virulence of different GPGV isolates have been investigated as possible explanations for the different expression of GLMD symptoms. Polymorphisms in the 3′ region of the RNA coding for movement protein (MP), for instance, have been shown to increase virus titer, augment the accumulation of GPGV-derived small interfering RNAs and enhance GLMD symptom expression [15]. Furthermore, there is evidence that the coat protein (CP) of GPGV can suppress the plant's post-transcriptional gene silencing (PTGS) machinery, allowing GPGV to establish itself better in susceptible plants [15, 16] However, results of different research groups are not yet entirely consistent [15, 17–20]. Hence, the etiology of GLMD, the pathogenesis of GPGV and the virus-plant interaction have not yet been conclusively clarified.

Another uncertainty is the actual damage the virus can cause in vineyards. There are reports of general yield losses of approximately 50% for infected grapevines caused by lower numbers and weight of grapevine bunches [21]. However, these numbers only refer to symptomatic vines. Further information about the performance of latent infected vines is not described, so far. The lack of important information such as knowledge of all viral vector insects, insights into symptom expression, and plant performance at different GPGV infection levels, makes it difficult to assess the risk of GPGV infections in vineyards and to properly manage GPGV infections.

In the current study, the course of GPGV infection in four vineyards in south-west Germany was monitored over three to four years from 2018 to 2021. A potential correlation between symptom expression and different GPGV isolates was investigated. Furthermore, the impact of GPGV infections on yield and must composition was analyzed.

Methods

Vineyard selection

Distribution and impact of GPGV infections on grapevine were investigated over three and four consecutive years (2018–2021), respectively, in four vineyards. All vineyards were in commercial use during the trials and managed by four different winegrowers. The selected vineyards are located in the southern German winegrowing regions Baden (Hecklingen, Nimburg, Reicholzheim) and Wuerttemberg (Gemmrigheim) and will be referred to as HEC, NIM, REI and GEM hereafter (Table 1, Additional File 1). GEM was cultivated with *Vitis vinifera* cultivar (cv.) Pinot noir planted in 2017, HEC with cv. Gewurztraminer planted in 2010, NIM with cv. Pinot blanc planted in 1994, and REI with cv. Pinot meunier planted in 2012.

GEM and HEC were cultivated manually, consequently all pruning and harvesting operations were carried out by hand. Vines of REI were used for clonal selection and the

Table 1 Experimental sites

Vineyard	Location, region, sub-region	Planting year	Cultivar	Number of tested plants	Trial years
GEM	Gemmrigheim, Wuerttemberg, Unterland	2017	Pinot noir	459	2019–2021
HEC	Hecklingen, Baden, Breisgau	2010	Gewurztraminer	224	2018–2021
NIM	Nimburg, Baden, Kaiserstuhl	1994	Pinot blanc	478	2019–2021
REI	Reichholzheim, Baden, Tauberfranken	2012	Pinot meunier	384	2018–2020

All experiments described in this study were conducted in four vineyards in southern Germany. Details of the vineyards are listed above

vineyard was also managed manually, however, additional yield reduction was carried out by grape cluster division. In all three vineyards, the vines were trained in a trellis system in a flat arch. NIM was converted to mechanical pruning and harvesting during this study. Vines were trained in a flat arch until 2020 and then switched to a minimal pruning system in the trellis.

Disease assessment on vines

Visual assessment of GLMD symptoms

Each grapevine plant growing in one of the four vineyards was visually examined for GLMD symptoms at least once a year. Examinations were done before flowering during the phenological stages 53–57 (according to the BBCH scheme; [22]) and in September shortly before harvest (BBCH 89). Monthly evaluations were conducted in the vineyards HEC and NIM from BBCH 53 until BBCH 89.

Symptoms were assessed according to a four-level scale: 1: no symptoms; 2: symptoms at one single shoot; 3: symptoms like stunted growth and chlorotic leaves at multiple shoots while some parts of the plant might be asymptomatic; 4: severe symptoms with all shoots of the plant showing stunted growth and chlorotic leaves (Fig. 1). Evaluation was performed in three (REI: 2018–2020; GEM/NIM: 2019–2021) or four (HEC: 2018–2021) consecutive years. GPGV infected plants with varying degrees of GLMD symptoms were found in all four vineyards as well as latent infected plants without any symptoms and plants free of GPGV. The REI vineyard was partially uprooted after harvesting in 2020.

During visual assessment of GLMD symptoms, the presence of leaf erinea by *Colomerus vitis* was also observed but not systematically recorded.

Serological evaluation of GPGV infection

Vines were tested annually for GPGV using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with the equipment, protocols, and reagents of BIOREBA AG (Reinach, Switzerland). Positive and negative controls originated from the virus collection of the State Institute of Viticulture and Enology (WBI) Freiburg, Germany. Samples were counted positive if the absorbance value was twice the value of the negative control sample. Each sample was tested in two replicates. All samples were collected during times of the season when symptoms were not visible. In 2018, 1 g of wood samples collected in November were used as test material. From 2019 onwards, 1–2 freshly emerged shoots (BBCH 10–16) collected in early spring between April and May were used for the assays.

In the first vineyard testing year, each vine was also tested for the common grapevine viruses Arabis mosaic virus (ArMV), Grapevine fanleaf virus (GFLV), Grapevine fleck virus (GFkV) and Grapevine leafroll-associated virus 1 and -3 (GLRaV-1, -3).

Categorization of vines in GPGV infection levels

According to the visual and serological evaluations, each plant was annually assigned to a category describing the individual infection status for GPGV. In total, five categorial levels were differentiated (Table 2): 1: healthy plants (h), 2: latent infected plants (l), 3: slightly symptomatic plants (sls), 4: moderate symptomatic plants (ms), 5: severe symptomatic plants (svs).

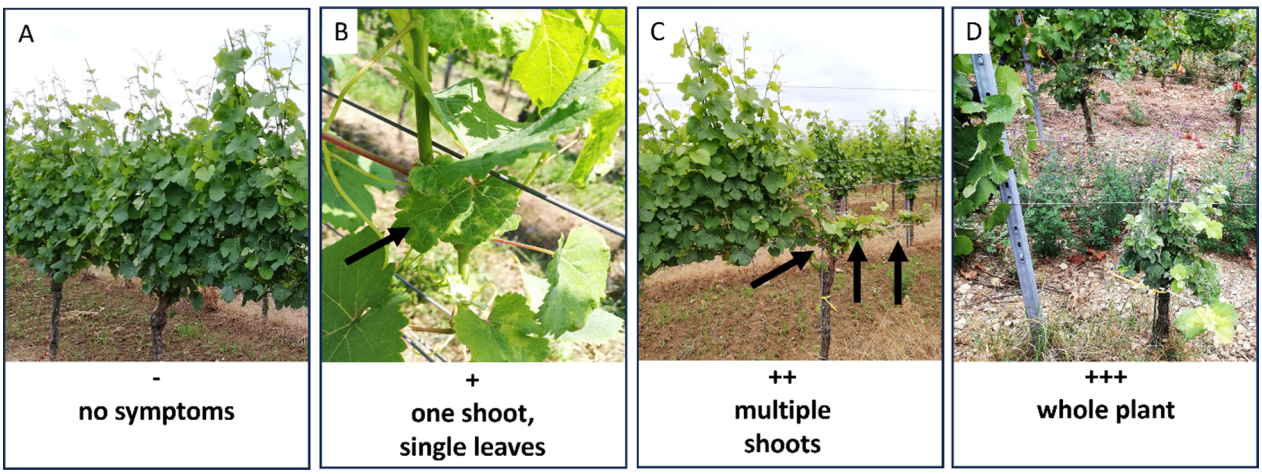

Fig. 1 Visual evaluation scale of GLMD symptoms. GLMD symptoms were assessed according to a four-level scale:—: no symptoms **A**; +: symptoms at one single shoot or single leaves **B**; ++: symptoms at multiple shoots **C**; +++: severe symptoms on the entire plant **D**. Arrows indicate GLMD symptoms

Table 2 GPGV infection levels

GPGV infection level	Healthy (h)	Latent (l)	Slightly symptomatic (sls)	Moderate symptomatic (ms)	Severe symptomatic (svs)
GPGV ELISA	–	+	+	+	+
GLMD symptoms	–	–	+	++	+++

Each plant was assigned to a GPGV infection level according to the results of ELISA and visual evaluation

Molecular genetic studies

Sample material

Based on the data of GPGV infection levels, several vines were selected from the HEC and NIM vineyards representing different infection groups during the observation period: L (latent): grapevines that were latently infected over the entire period (HEC-L/NIM-L); S (symptomatic): grapevines that showed symptoms over the entire period (HEC-S/NIM-S); IS (immediately symptomatic): grapevines that were healthy during the first two years followed by GPGV infection with immediate symptom expression in the infection year (HEC-IS/NIM-IS). Four vines per group were randomly collected and analyzed in June 2021. Four middle aged leaves with as few symptoms as possible were collected per vine from different shoots near the stem. 100 mg of leaf material were pooled in one sample and total RNA was isolated using the Universal RNA Kit (RoboKlon GmbH, Berlin, Germany) following the manufacturer's recommendations. RNA samples were stored at − 80 °C until further processing.

Isolate identification

5 µg RNA from leaf samples was transcribed into cDNA with Moloney Murine Leukemia Virus reverse transcriptase (M-MLV RT; Lucigen, LGC, Teddington, UK) and oligo$(dT)_{18}$ primer following the manufacturer's protocol. A GPGV specific PCR was performed using Det-primer (DetF 5′-TGGTCTGCAGCCAGGGGACA-3′; DetR 5′-TCACGACCGGCAGGGAAGGA-3′) amplifying a 588 bp long sequence of its movement and coat protein (MP/CP region) [17]. PCR was carried out in a final volume of 50 µL with 1 µL cDNA as template and 1 U proofreading S7 Fusion Polymerase (Mobydiag, Espoo, Finland). PCR settings were chosen as followed: initial denaturation at 95 °C for 3 min, followed by 35 cycles of 30 s at 95 °C, 30 s at 60 °C, 40 s at 72 °C and a final elongation step at 72 °C for 5 min. 5 µL of PCR reactions were loaded onto an 1% (w/v) agarose gel to verify the correct amplification size. The remaining PCR reaction was purified using the NucleoSpin® Gel and PCR Clean-up Kit (Macherey–Nagel GmbH & Co. KG, Düren, Germany). Quality of the purified product was measured using a NanoDrop™ One spectrophotometer (Thermo Fisher Scientific Inc, Waltham, MA, USA). Samples were sent for Sanger sequencing and aligned to single contigs. All sequences were deposited in GenBank (PP348014–PP348037). Alignment with reference genomes and the assignment into clades A, B and C [17, 19] was carried out using the ClustalW algorithm of MEGA X Version 10.1.8 [23]. The reference genomes with the following GenBank accession numbers were selected from NCBI for clade A: LN606703.1 (MOLA 6), KU845348.1 (PIA-G44), MH019203.1 (RQ25) and MH019204.1 (RQ30); for clade B: KU845367.1 (ORM-G40) and LN606705.1 (MOLA 14); for clade C: KU845372.1 (SUS-G49), LN606739.1 (ALA-P4) and FR877530.2 (ZA505-1A). A phylogenetic tree was constructed in MEGA X following the maximum likelihood method and Tamura-3 parameter model with a bootstrap of 2000.

qRT PCR assays

One step quantitative real time PCR (qRT PCR) assays were performed with the same RNA samples used for isolate identification. Assays were conducted using the SYBR GPGV set (Qualiplante SAS, Clapiers, France) following the manufacturer's instructions. Each sample was tested in triplet in three independent reactions. GPGV positive and negative control were provided by the qPCR kit. Additionally, a non-template control (NTC) was included. No internal grapevine standard was included, the virus load was not quantified. Assays were carried out using a CFX Opus Real-Time PCR System (Bio-Rad Laboratories, Hercules-CA, USA).

Yield analysis

A maximum of 20 plants per GPGV infection level (Table 2) were chosen for the yield analyses in 2019, 2020 and 2021. The sample size of symptomatic vines was partially reduced as their occurrence was limited (Figs. 5 and 6). Vines were randomly chosen each year on the basis of visual assessment in spring. Fruit from each vine was separately harvested and the number of bunches as well as total yielding weight per vine were determined.

Must analysis

For the analysis of grape must, the Fourier transformation infrared spectroscopy (FTIR) was used and analyzed with GrapeScan (Foss, Hillerød, Denmark). Two replicates of 100 individual berries were collected from different parts of the grape bunches in each vineyard and for each GPGV infection level from the same plants used for yield analyses. 100 berries represented one GrapeScan sample. Berries were pressed and the resulting juice was stored over night at 4 °C to allow settling of crude sediments. The next day, juice was centrifuged for 5 min

at 9000 rcf and subsequently automatically analyzed by GrapeScan for the following parameters: Density, Glucose, Fructose, Ratio Glucose:Fructose, Tartaric acid, Malic acid, Ratio Tartaric acid:Malic acid, total acidity and pH. The calibration settings of the software were based on 2017 vintage musts.

Screening for alternative GPGV hosts

Between 2019 and 2020 samples from the accompanying flora of vines in the vineyards were collected. Sampling and sample processing were conducted as described by Messmer et al. [24]. Briefly, samples were ground to a fine powder, total RNA was extracted, and cDNA was synthesized. PCR was performed using the Det primer pair [25] checking for GPGV infection and the Nad5 primer pair [26] to check for successful RNA extraction and cDNA synthesis. Amplicons were loaded on a 1% (w/v) agarose gel to verify the PCR success.

Statistical analysis

The PATCHY program was used to analyze spatial disease patterns in vineyards for hints for possible vine-to-vine spread [27]. In order to find unidimensional virus clustering along and across rows, respectively, ordinary runs analyses were conducted [28]. Thereby, PATCHY computes a simulated line through the whole vineyard along each plant and decides whether infected vines are randomly distributed or non-random. A non-random distribution may suggest an infection clustering. Additionally, PATCHY arranges the vineyards into grids of different sizes. Those grids are tested for possible gradients of infected vines, indicated by a significant correlation coefficient r^2. Tests were performed for individual as well as for concatenated rows.

Further data analyses were carried out with the statistical software R (Version 1.2.5001, Boston, MA, USA). Two-way analysis of variance (ANOVA) with subsequent Tukey HSD tests were used to compare yield and must results of various infection levels of grapevines with those of healthy grapevines. For all statistical tests an alpha level of 0.05 was chosen.

Results

Annual virus testing and visual symptom evaluation

The initial ELISA in each vineyard revealed high incidences of GPGV and only few infections with GLRaV-1 (GEM) and GFkV (all vineyards), most of them in GPGV negative plants. Therefore, only GPGV was tested for in further assays.

The annual serological tests revealed a steady increase in GPGV infected vines in all four vineyards (Fig. 2, Table 3). The increase of GPGV infections in GEM was slower than in the other vineyards tested, also the total GPGV incidence remained lower. In 2019 22.1% of the plants were positive for GPGV increasing to 33% in 2020 and decreasing to 31.9% in 2021. In HEC the percentage of GPGV-infected vines increases rapidly from 50% in 2018 to 79.5% in 2019 and to 86.6% in 2020. Grapevines in HEC were additionally tested in 2021 showing a GPGV incidence of 98.7%. In REI, the GPGV incidence increased from 55% over 71.5% up to 75.7% between 2019 and 2021. GPGV incidences in NIM were very high right from the beginning of testing. In 2019 83.1% of the plants were tested positive for GPGV, increasing to 92.3% in 2020 while 2021 GPGV-infected grapevines decreased to 89.5%.

If the results of the visual evaluation are also considered, it becomes clear that most GPGV infections were latent (Fig. 2, Table 3, Additional Files 2–5). In GEM, HEC and NIM the percentage of latent infected vines was higher than the percentage of symptomatic vines in every year. However, this was not the case in REI. Here, a massive increase in symptomatic vines from 31% (2018) to 49% (2019) and finally 52% (2020) was recorded. The number of latent infected vines remained constant with a percentage between 23 and 25%.

In HEC and NIM, three visual evaluations were conducted between BBCH 57 in June and BBCH 89 in August/September (Additional file 3 and 4). In both vineyards GLMD symptoms decreased over the season. Most symptoms were observed between June and July whereas in August/September many of these plants became symptomless.

To get an idea about possible clustering of GPGV infections within vineyards and certain spreading patterns, all vineyards were analyzed with the software PATCHY [27]. In GEM, a nonrandom distribution across the six observed rows of the vineyard was found only in 2019 (Table 3). A gradient from East to West was found in 2021 in the lower half of the vineyard (Fig. 3). In HEC non-random distributions of GPGV-infected vines were found in 2018 and 2019 along and across rows. In 2018 an additional gradient from West to East was found in the vineyard. Analyses for 2020 and 2021 could not be performed because GPGV incidences were already too high. In NIM non-random distributions were found along and across rows in 2019. Furthermore, a gradient from East to West was found for the entire left side of the vineyard up to row 6. In 2020 and 2021, GPGV incidences were too high for reliable analyses. In REI, nonrandom distributions of GPGV-infected vines were found in 2018. In 2019 and 2020, the analyses could not be performed due to the high GPGV incidences. No significant gradient was found in this vineyard.

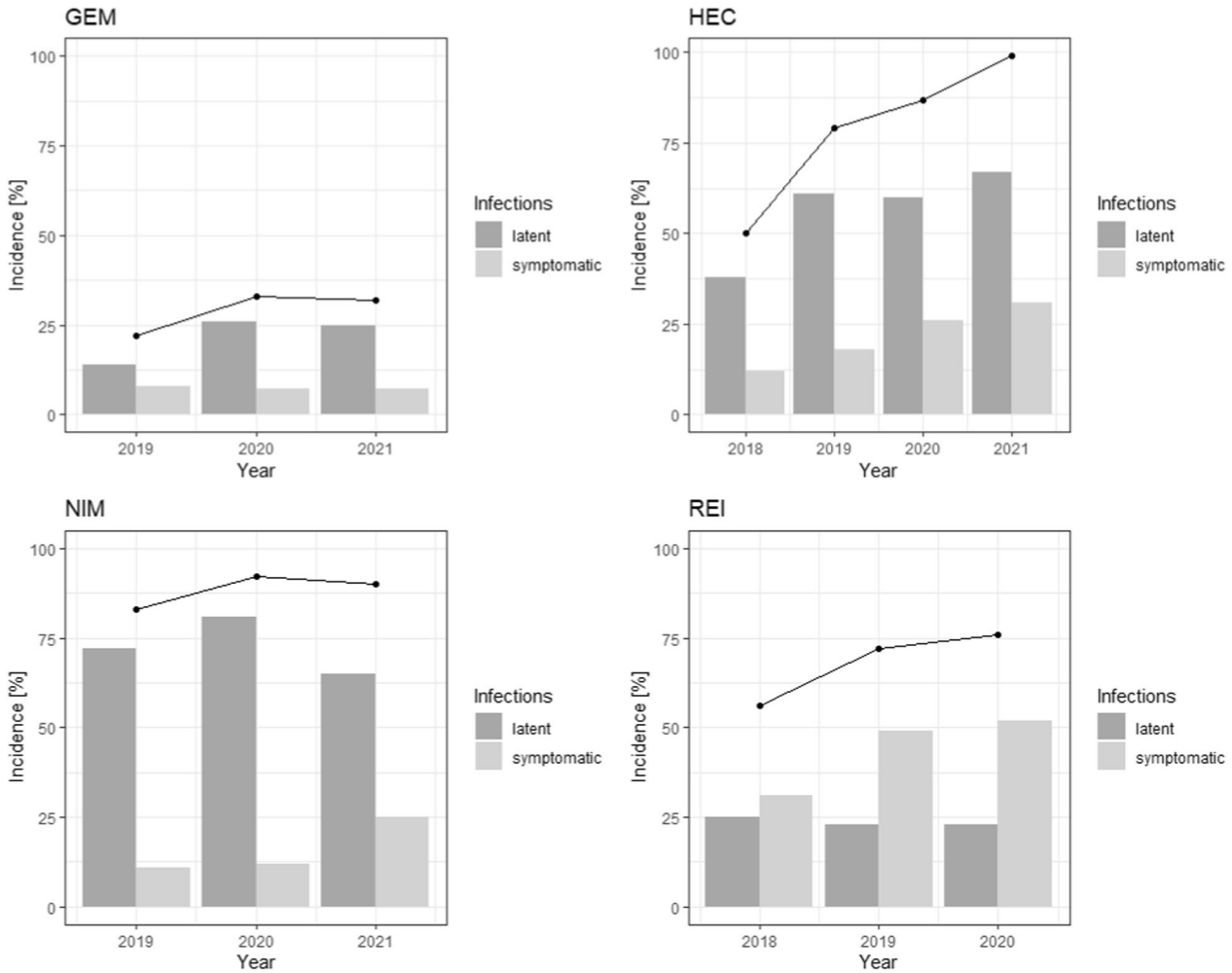

Fig. 2 GPGV progress curve. Shown are the disease progress curve of GPGV (black line) and the incidence of latent (dark grey bars) and symptomatic infections (light grey bars) in all four vineyards

GPGV detection and genetic GPGV isolates in infected grapevines

Based on the annual serological and visual evaluations, single grapevines from HEC and NIM were selected (Table 4). The aim was to identify differences between three groups of GPGV infection levels (latent (HEC-L/NIM-L), symptom (HEC-S/NIM-S), immediately symptomatic (HEC-IS/NIM-IS)) in GPGV isolates and to compare the detection efficacy of qPCR, PCR using Det-Primer pairs and ELISA.

Regarding the genetic diversity of GPGV samples from HEC and NIM, isolates from clades A (9/24) and C (14/24) were predominant (Fig. 4). Only one isolate, extracted from NIM-L4, clustered to clade B. As expected, most isolates in clade C originated from always symptomatic (S) and immediately symptomatic (IS) plants. However, also NIM-L1 and NIM-L2 were found in this group. In clade A, on the other hand, four out of nine isolates came from immediately symptomatic (HEC-IS1, 3 and 4) or always symptomatic (HEC-S3) vines. The other five isolates originated from latent infected vines (L), as expected. While most isolates from HEC were assigned to clade A (8/12), the isolates in NIM were mainly assigned to clade C (10/12).

GPGV was detected in all samples by using qPCR, PCR and ELISA (Table 4). Cycle threshold (Ct) values of plants from HEC ranged between 22.67 (HEC-IS 3, clade C) and 25.94 (HEC-S 3, clade C). Ct values from NIM vines were between 22.31 (NIM-IS 1, clade C) and 25.95 (NIM-L 4, clade B). The Det primer pair was able to amplify GPGV sequences in all samples. Also the ELISA results indicate the presence of GPGV in all samples. ELISA signals vary most between the four HEC-L samples, which were all infected with clade A isolates. In all other samples the signal strength was more or less homogeneous.

Table 3 GPGV incidences and spatial analysis within vineyards

Vineyard	Year	Total	Healthy	GPGV incidence		Latent	Symptomatic		Runs analysis				Gradient		
		[n]	[n]	[n]	[%]	[n]	[%]	[n]	[%]	Along rows	Across rows	Rows	Plants	r^2	Direction
GEM	2019	457	356	101	22.1	66	14	35	8	ns	***				
	2020		306	151	33.0	117	26	34	7	ns	ns				
	2021		311	146	31.9	113	25	33	7	ns	ns	1–6	1–39	0.70	Across rows
HEC	2018	224	112	112	50.0	86	38	26	12	***	***	1–9	1–31	0.73	Across rows
	2019		46	178	79.5	137	61	41	18	**	**				
	2020		30	194	86.6	135	60	59	26	–	–				
	2021		3	221	98.7	151	67	70	31	–	–				
NIM	2019	478	81	397	83.1	344	72	53	11	***	***	1–6	1–41	0.98	Across rows
	2020		37	441	92.3	385	81	56	12	–	–				
	2021		50	428	89.5	310	65	118	25	–	–				
REI	2018	375	166	209	55.7	93	25	116	31	***	***				
	2019		107	268	71.5	85	23	183	49	–	–				
	2020		91	284	75.7	88	23	196	52	–	–				

Listed are the number (n) of healthy, GPGV latent infected and GPGV symptomatic vines of all four vineyard observed in this study between the years 2018 and 2021. Runs analyses and the presence of infection gradients were calculated by PATCHY [27]

Tukey HSD test: *ns* not significant, ***p*-value < 0.01; ****p*-value < 0.001; – no relevant results due to too high GPGV incidences

r^2 = correlation coefficient

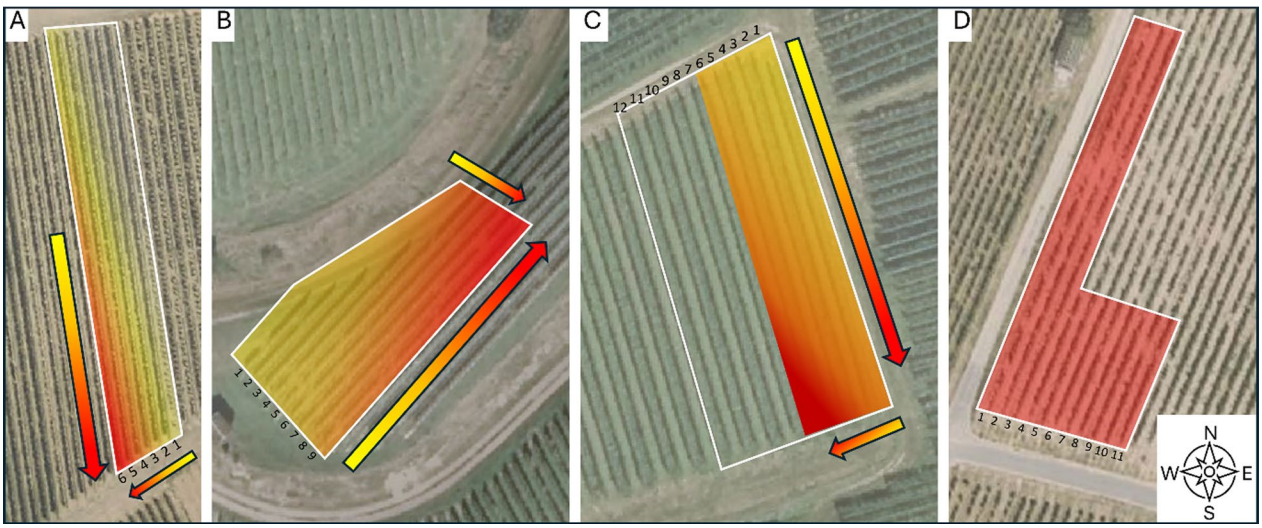

Fig. 3 GPGV Infection gradients within vineyards. Shown are GPGV infection gradients computed by PATCHY in GEM in 2021 (**A**); in HEC in 2018 (**B**); in NIM in 2019 (**C**). REI showed no significant gradient, but the incidence of GPGV infection was very high in all years (**D**)

Harvest quantity and quality of infected grapevines

In 2019 and 2020 yield and must analyses were conducted in all four vineyards. Single grapevines from each GPGV infection level were selected and separately harvested. The number of grape bunches as well as the yielding weight were recorded (Additional file 7). Multiple severe frost incidences during the 2021 vegetation period resulted in a reduced yielding in all four vineyards. Consequently, differences induced by virus infection were masked and the 2021 yield analysis was excluded from the study. In 2020 late frost was also present in REI, therefore only data from 2019 is shown for this vineyard.

Yield analysis

Yield analyses revealed similar results in all four vineyards in both years, 2019 and 2020 (Fig. 5).

Table 4 GPGV isolates and detection methods

Vineyard	Group	Sample	Plant	Infection level				Clade	Ct values	PCR Det primer	ELISA
				2018	2019	2020	2021				
HEC	Latent	HEC-L 1	7/8	l	l	l	l	A	25.02 ± 1.71	+	+++
		HEC-L 2	7/27	l	l	l	l	A	23.61 ± 1.67	+	++
		HEC-L 3	8/23	l	l	l	l	A	25.86 ± 1.1	+	+
		HEC-L 7	9/24	l	l	l	l	A	24.52 ± 0.74	+	+++
	Symptomatic	HEC-S 1	7/10	svs	ms	sls	ms	C	24.55 ± 2.55	+	+++
		HEC-S 2	7/18	svs	svs	sls	ms	C	24.24 ± 2.02	+	+++
		HEC-S 3	8/18	sls	ms	ms	svs	A	25.94 ± 1.91	+	+
		HEC-S 4	5/12	svs	l	sls	svs	C	24.44 ± 2.06	+	+++
	Immediately symptomatic	HEC-IS 1	4/12	h	h	ms	svs	A	23.62 ± 0.89	+	++
		HEC-IS 2	7/22	h	h	ms	svs	C	25.66 ± 1.64	+	++
		HEC-IS 3	8/7	h	h	ms	ms	A	22.67 ± 0.94	+	++++
		HEC-IS 4	9/31	h	h	h	ms	A	23.65 ± 1.09	+	++++
NIM	Latent	NIM-L 1	1/5		l	l	l	C	22.92 ± 0.38	+	++++
		NIM-L 2	3/40		l	l	l	C	24.41 ± 0.56	+	++
		NIM-L 3	4/13		l	l	l	A	22.68 ± 0.57	+	+++
		NIM-L 4	8/20		l	l	l	B	25.95 ± 0.70	+	+++
	Symptomatic	NIM-S 1	3/17		svs	ms	ms	C	23.12 ± 0.75	+	++++
		NIM-S 2	6/10		svs	svs	ms	C	22.99 ± 0.32	+	++++
		NIM-S 3	7/10		svs	svs	svs	C	23.42 ± 0.41	+	+++
		NIM-S 4	10/23		svs	ms	svs	C	22.49 ± 1.12	+	++++
	Immediately symptomatic	NIM-IS 1	2/28		h	h	sls	C	22.31 ± 0.70	+	+++
		NIM-IS 2	3/21		h	h	sls	C	22.53 ± 0.38	+	++++
		NIM-IS 3	10/20		h	h	sls	C	23.35 ± 0.47	+	+++
		NIM-IS 4	10/36		h	h	ms	C	24.35 ± 0.37	+	++++
		Positiv control							34.18 ± 0.55	+	
		Negative control							N/A	–	
		NTC							N/A	–	

Listed are samples from three different GPGV infection groups (latent (L), symptomatic (S), immediately symptomatic (IS)) from HEC and NIM vineyards, classified according their infection levels between 2018 and 2021 (Table 2), with corresponding results of isolate determination, quantitative real time PCR (qRT-PCR), PCR and ELISA. Isolates were determined by Sanger sequencing. Upper case letters correspond to the assignment of isolates into GPGV clades A–C [19]. Cycle threshold (Ct) values of qRT-PCR indicate higher virus titer e.g. virus load

ELISA results were ranked into five categories according to their absorbance values—: x < 100;+: 100 < x < 300;++: 300 < x < 800;+++: 800 < x < 1700; ++++: 1700 < x

NTC Non template control

N/A not applicable

Latent infected plants showed no yield loss compared to healthy control plants in six out of seven analyses (2019: HEC −18% [−0.6 kg]). On the contrary, latent infected plants produced slightly more fruit (+3%–+29% [+0.1–+0.7 kg]). However, the only statistically significant difference to non-infected plants was detected in 2020, when latent GPGV infected grapevines in HEC had a significantly higher yield. As soon as GLMD symptoms were visible on infected grapevines, yield decreased compared to healthy plants. The panicles of grapes were shortened compared to healthy and latent infected vines which resulted in smaller and more compact grape bunches (Additional file 8). In each analysis, the yield of strong symptomatic plants was significantly reduced (−56%–−96% [−1.35 kg–−4.8 kg]) compared to the healthy control. In five out of seven analyses, plants with moderate GLMD symptoms produced significantly less yield than healthy plants with average yield losses between −28 and −56% (−0.8 kg–−2.5 kg). Grapevines with slight GLMD symptoms were significantly different to healthy ones only in 2019 in the vineyards GEM and REI (−31% [−1.2 kg], and −68% [−1.0 kg], respectively). In all other analyses the average yield losses of this category ranged between −13% and −36% (−0.4 kg–−1.1 kg), without being statistically significant.

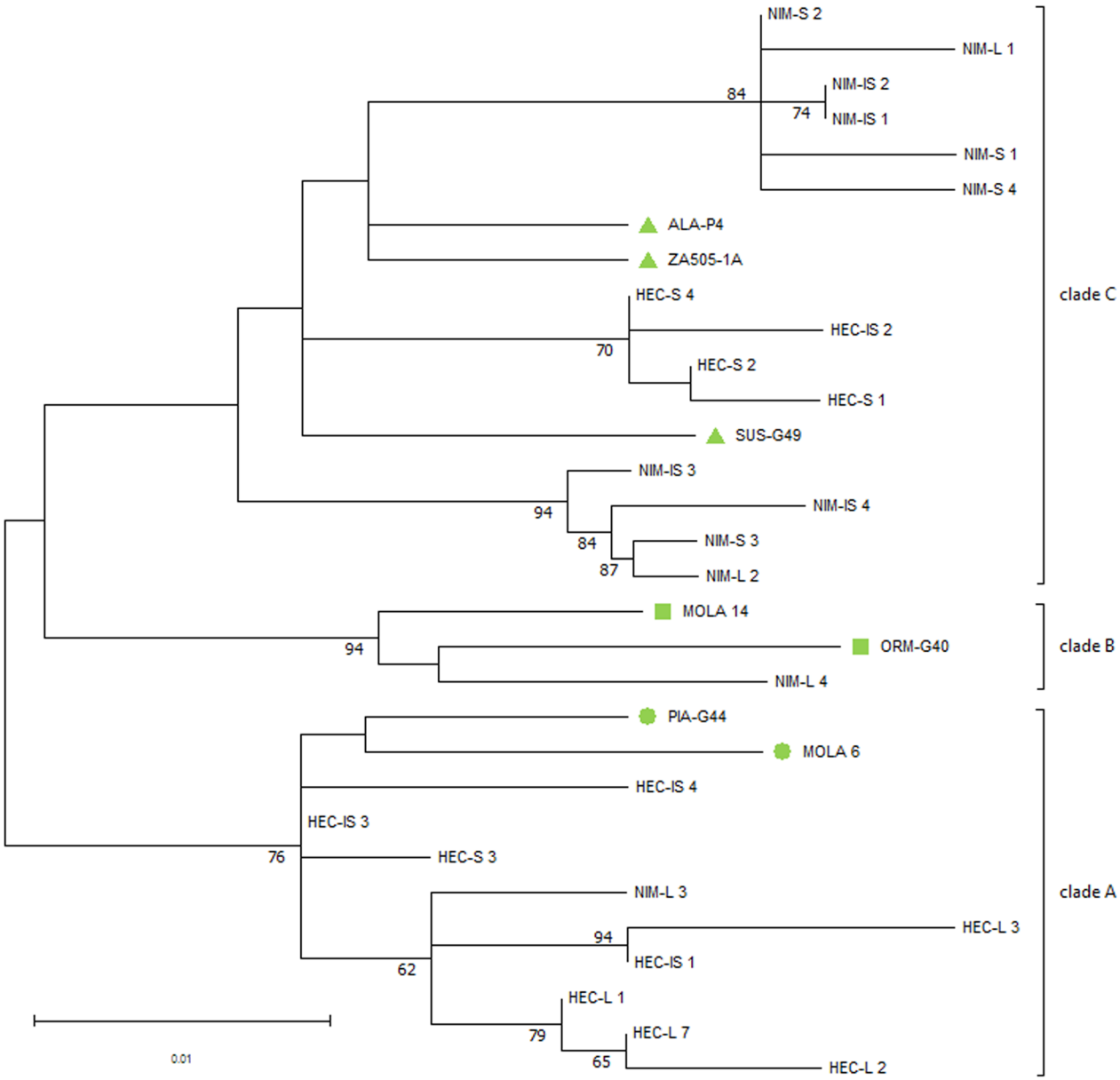

Fig. 4 Phylogeny of selected GPGV isolates from HEC and NIM vineyards. The tree was constructed by using the Maximum Likelihood method and Tamura-3 model of MEGA X software. The tree with the highest log likelihood (– 1364.92) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Reference isolates are tagged with green icons (clade A dots, clade B squares, clade C triangles). More details on the different samples are listed in Table 4

Similar patterns were observed concerning the average number of bunches per plant (Fig. 6). Less grape bunches (gbs) were counted on symptomatic plants compared to healthy vines at all sites and in both years. Significantly less bunches were always observed on severe symptomatic plants (– 40%–– 89%). Plants with moderate or slight GLMD symptoms showed significantly fewer gbs in three out of seven analyses compared to healthy vines (moderate (ms): GEM 2019 – 46%, HEC 2019 and 2020 – 47% and – 45%, REI 2019 – 37%; slight symptomatic (sls): GEM 2020 – 30%, HEC 2019 – 31%, REI 2019 – 54%). In five out of seven analyses, latent infected plants produced slightly more grape bunches than healthy plants, however, no significances were found (Fig. 6).

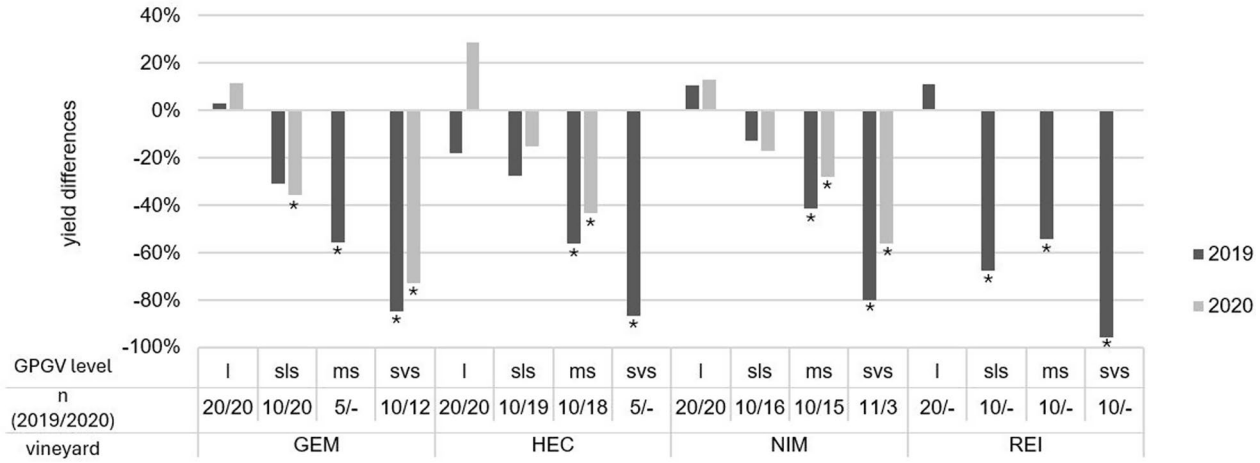

Fig. 5 GPGV symptomatic vines produce significantly less yield. The percentage yield difference between GPGV infected vines of different infection levels (l = latent; sls = slightly symptomatic; ms = moderate symptomatic; svs = severe symptomatic) and healthy vines without GPGV infections in 2019 and 2020 are shown. Latent GPGV infections have no negative effect on yield while symptomatic vines show drastic yield losses. Asterisks indicate significant yield differences compared to GPGV negative vines according to ANOVA and Tukey HSD tests

Must analysis

FTIR results of must samples showed only slight differences between GPGV infection level groups while only few of them were significantly different (Additional file 9). For instance, berries from vines with severe GLMD symptoms had minimal lower densities,

Table 5 GPGV has no influence on quality of berries

Vineyard	Category	Density		Ratio Glc.:Frc		Ratio TA:MA		Total acidity	
2019									
GEM	h	1.098 ± 0.003		1.02 ± 0.01		1.80 ± 0.11		11.12 ± 0.29	
	l	1.093 ± 0.001	ns	1.02 ± 0.01	ns	1.92 ± 0.18	ns	10.33 ± 0.50	ns
	sls	1.098 ± 0.001	ns	1.02 ± 0.01	ns	2.16 ± 0.20	*	9.78 ± 0.51	*
	ms	1.093 ± 0.004	ns	1.02 ± 0.00	ns	2.10 ± 0.18	*	9.40 ± 0.38	*
	svs	1.088 ± 0.004	**	1.00 ± 0.01	ns	1.70 ± 0.24	ns	10.30 ± 0.99	ns
HEC	h	1.095 ± 0.002		1.02 ± 0.01		1.38 ± 0.09		4.73 ± 0.17	
	l	1.093 ± 0.003	ns	1.00 ± 0.03	ns	1.62 ± 0.36	ns	4.63 ± 0.54	ns
	sls	1.096 ± 0.001	ns	1.00 ± 0.01	ns	1.38 ± 0.16	ns	4.42 ± 0.75	ns
	ms	1.094 ± 0.002	ns	1.00 ± 0.01	ns	1.38 ± 0.04	ns	3.88 ± 0.28	ns
	svs	1.089 ± 0.002	**	0.96 ± 0.04	*	1.75 ± 0.47	ns	3.77 ± 1.39	ns
NIM	h	1.102 ± 0.001		0.99 ± 0.01		1.99 ± 0.09		9.63 ± 0.39	
	l	1.102 ± 0.001	ns	0.97 ± 0.01	ns	2.03 ± 0.10	ns	9.28 ± 0.31	ns
	sls	1.100 ± 0.002	ns	0.99 ± 0.01	ns	2.03 ± 0.18	ns	9.52 ± 0.88	ns
	ms	1.099 ± 0.004	ns	0.97 ± 0.03	ns	2.21 ± 0.22	ns	9.05 ± 0.92	ns
	svs	1.094 ± 0.002	ns	0.97 ± 0.03	ns	1.96 ± 0.28	ns	9.53 ± 0.47	ns
REI	h	1.091 ± 0.004		1.00 ± 0.01		1.93 ± 0.15		6.77 ± 0.05	
	l	1.086 ± 0.001	*	0.98 ± 0.01	ns	2.16 ± 0.18	ns	6.60 ± 0.48	ns
	sls	1.091 ± 0.004	ns	0.99 ± 0.00	ns	1.87 ± 0.08	ns	7.05 ± 0.33	ns
	ms	1.091 ± 0.003	ns	0.98 ± 0.01	ns	1.96 ± 0.33	ns	6.83 ± 1.44	ns
	svs	1.088 ± 0.010	*	0.97 ± 0.01	*	1.63 ± 0.23	ns	9.12 ± 1.75	*

Displayed are a representative selection of GrapeScan results from 2019: Density (sugar content), ratio glucose:fructose (Glc.:Frc.), ratio tartaric acid:malic acid (TA:MA) and the total acidity. Total GrapeScan results are listed in Additional file 9. Juice from grapes of different infection categories were used as samples: healthy vines (h), latent infected vines (l), vines with slight GLMD symptoms (sls), vines with moderate GLMD symptoms (ms) and vines with severe GLMD symptoms (svs). Asterisks indicate significant differences to GPGV-negative vines according to ANOVA and Tukey HSD tests. Ns not significant; *p-value < 0.05; **p-value < 0.001

i.e. lower sugar contents, than berries from the other infection categories as well as slightly lower ratios in glucose:fructose (Glc.:Frc.) and tartaric acid:malic acid (TA:MA) in 2019 (Table 5). Juice samples from the other infected categories showed similar results as samples from healthy control plants. The total acidity values clearly reflect the differences between the vineyards, indicating environmental influences.

Alternative GPGV hosts

51 samples of the accompanying flora of the vineyards HEC, NIM and REI were collected and tested for GPGV infection via RT-PCR between 2019 and 2020. GPGV was not detected in any of the samples, even though some exhibited leaf mottling or stunted growth (Additional file 10).

Discussion

The aim of the present study was to investigate the spread of GPGV infections in vineyards and to find a possible correlation between GPGV isolates and the expression of GLMD symptoms. In addition, the consequences of GPGV infection on the yield in terms of fruit quality and quantity were to be analyzed.

The spread of GPGV infections in all four monitored vineyards was significant. An increase of approximately 10% GPGV infections per year was noticed in HEC from 2018 until 2021 (Fig. 2, Table 3). High increases of GPGV infected vines were also observed from 2018 to 2019 in REI (+15.8%) and from 2019 to 2020 in GEM (+10.9%) as well as in NIM (+9.2%). A specific pattern of disease progression in infected vines was not detected. Latent infected plants becoming symptomatic were observed in equal numbers as symptomatic plants becoming asymptomatic. However, GLMD symptoms decreased over the growing season in all trial years of this study, which is in line with other publications [18, 19]. GLMD symptom expression was correlated with the different GPGV isolates. The majority of isolates from always symptomatic and immediately symptomatic vines (12 of 16 plants) belonged to clade C, which is known for symptom expression. Most isolates from latent vines (5 of 8 plants) belonged to clade A, known for asymptomatic isolates (Fig. 4, Table 4) [19]. Yield analyses revealed that moderate and severe GLMD symptoms cause significant yield loss on grapevines which range between −28% and −96%, while latent GPGV infections have no influence on fruit production (Fig. 5). According to FTIR analyses, GPGV has only little influence on fruit quality, independent of symptom severity, without any relevance to vinification (Table 5, Additional file 9).

GPGV transmission by infected plant material was shown recently [29]. The observations of our study provide further evidence that additional vector dispersal occurs in vineyards [10, 18, 30]. In controlled settings and in semi-field trials, the Eriophyid mite *Colomerus vitis* has been shown to transmit GPGV on grapevines [12]. To date, *C. vitis* is the only known vector of GPGV. The mite consists of two morphs, the spring–summer morph (protogynes) and the more robust winter morph (deutogynes). While protogynes mainly migrate from leaf to leaf, deutogynes are able to travel with the wind over long distances [31]. Additional transmission of *C. vitis* by humans through management measures in the vineyard, such as pruning, is probable. The partial non-random

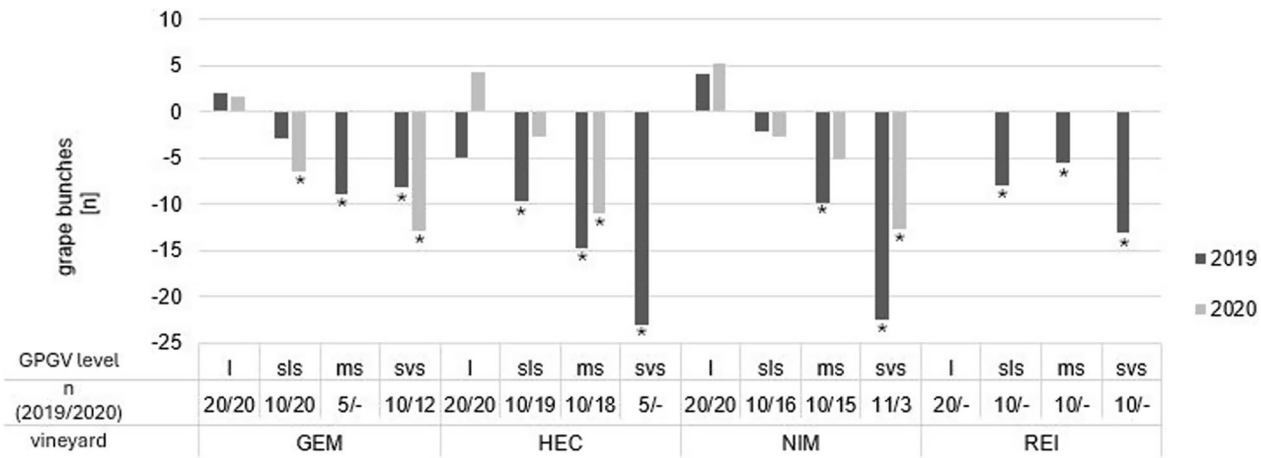

Fig. 6 GPGV symptomatic vines produce significantly fewer grape bunches. Differences in grape bunches produced between GPGV-infected vines of different infection levels (*I* latent, *sls* slightly symptomatic, *ms* moderate symptomatic, *svs* severe symptomatic) and vines without GPGV infections in 2019 and 2020 are shown. Latent GPGV infections have no negative impact on the number of grape bunches, while symptomatic vines have fewer grapes. Asterisks indicate significant differences to GPGV-negative vines according to ANOVA and Tukey HSD tests

distributions of GPGV infections in the vineyards, especially along rows, support such a hypothesis (Table 3). Analysis of GPGV spread in 14 vineyards in Italy resulted in a similar assumption [19]. The significant distributions across rows could be explained by wind dispersal. In fact, all vineyards except REI were planted downwind. All vineyards are regularly exposed to sporadic strong winds that may explain the infection gradients in GEM, HEC and NIM (Table 3).

The continuous increase of GPGV infected plants in HEC over years is consistent with observations in Italy [10], while the sudden increase of infections followed by constant values in GEM, NIM and REI corresponds to reports from France [8]. A sudden increase can possibly be explained by the population dynamics of the vector [8]. This can be caused, for example, by wind-induced migration of *C. vitis* from outside the vineyard or by the spread of the mite through canopy management. In all four vineyards of this study, *C. vitis* and its typical erinea were present on the leaves. However, a more detailed investigation of the dispersal behavior of the mite was not carried out in the vineyards. Thus, a possible transmission of *C. vitis* through canopy management can neither be confirmed nor excluded in the present study. GEM, HEC and REI are cultivated in a similar way (trellis training in a flat arch), while in NIM the vineyard was converted to minimal pruning. In the neighboring vineyards, no studies were carried out on the occurrence of the virus or the vector that would prove a wind-induced migration of the mite and thus of the disease. Since *C. vitis* is monophagous on grapevine, GPGV infections in various herbaceous plants as *Silene latifolia* and *Chenopodium album* indicate the presence of at least one additional vector insect [12, 14]. No alternative hosts with GPGV infections have been found in Germany, so far [24], including the vineyards of this study (Additional file 10). However, this does not exclude the presence of at least one additional vector. For example, the Fig mosaic virus is known to be transmitted by the fig bluster mite *Aceria ficus* and the fig wax scale *Ceroplastes rusci* [32]. Future research should address the question of further insect vectors in order to gain a better understanding of the spread of GPGV.

Focusing on GPGV infected vines only, great differences in the ratios of latent and symptomatic vines were found between the four vineyards monitored. Latent infected vines dominate in GEM, HEC and NIM while much more symptomatic vines were found in REI (Table 3). In HEC, NIM and REI symptomatic vines increased during the monitoring while no increase was observed in GEM (Fig. 2). Latent infected vines remained on an almost constant level in REI around 25%. In HEC latent vines increased from 2018 to 2021 (38–67%) while in GEM and NIM latent infected vines increased in the second and decreased in third year (14–26–25% and 72–81–65%). Decreasing numbers in 2021 in NIM and GEM can be explained by replanted vines (five vines in GEM, 13 vines in NIM) due to Esca and GPGV damages in 2020. However, we could not derive a common rule for symptom progression in infected vines over the 3–4 trial years.

In order to determine the influence of GPGV isolates on symptom expression, we selected vines with different symptom progressions and analyzed their GPGV isolates (Table 4). The analyses revealed a certain association towards symptom expression. Isolates extracted from latent plants mostly clustered into clade A while isolates extracted from always symptomatic and immediately symptomatic vines dominantly clustered into clade C. Admittedly, the correlation is not significant, and the general sample size was limited by the requirements for symptom progressions. However, the trend of isolate distributions in our study is in line with former studies stating a correlation between clade A isolates with asymptomatic infections and clade C isolates with symptomatic infections [17, 19]. Exceptions observed in this study can be explained by mixed infections with GPGV isolates which are difficult to detect by Sanger sequencing. Regardless of GPGV isolate or development of GLMD symptoms in the corresponding vine, GPGV was detected in all samples by the PCR methods using different primer pairs, and ELISA. That the virus load is not determining symptom expression, was published before [19, 20]. Fluctuations in virus load or signal strength in ELISA obtained during this study did not tend to be related to the virus isolate and the course of symptoms. Furthermore, no obvious differences in magnitude of Ct-values between different symptomatic samples were visible. In addition, no correlation of qPCR with ELISA signal strength was visible. However, robust statements about the viral load in the different samples are not possible, as qPCR was solely used for detection without an appropriate reference for virus quantification.

The reason for the discrepancies between detected isolates from clade B and C and the lack of symptom expression in three always latent infected vines in NIM are not clear. A possible explanation could be a cross-protection caused by a former infection with a less virulent GPGV isolate resulting in a certain priming effect as lately observed in Italy [10]. Thereby the plant's RNA-induced silencing complex (RISC), consisting of Argonaut peptides that bind small interfering RNA (siRNA) fragments of the virus, is activated and the invading virus is recognized more quickly [33]. It is probably due to this plant defense strategy why symptom expression decreases in plant parts developing later within the vegetation period

[34]. Virus titer studies at different points in the season could provide further evidence in this context. Contrary to this theory and in line with the evolutionary arms race between pathogen and host, there is also evidence that the CP protein of GPGV can suppress the plant's post-transcriptional gene silencing (PTGS) machinery, allowing GPGV to better establish itself in the plant [15, 16]. However, it is not clear whether this factors have an influence on the expression of symptoms or not, especially since virus titer seems to have no influence on symptom development. Another factor influencing symptomatology of GPGV in vines are nonsynonymous SNPs (nsSNPs) at the 3′-region of the MP protein [15]. To investigate their influence on symptom expression in more detail, more comprehensive sequencing studies would need to be conducted. Last but not least, a recent study has shown that German GPGV isolates are in the middle of a genetic selection phase [9]. Deleterious mutations and less-fit variants are sorted out while mainly regions in the movement protein are positively selected. Ultimately, this means that GPGV variants with high fitness and the ability to systematically infect are currently on the rise. The extent to which symptom expression is related to viral fitness is not yet clear. In our study, latent infected vines were predominant, however, an increase in the proportion of vines with symptoms was monitored in 3 out of 4 vineyards (Fig. 2). Furthermore, it seems very likely that other factors have an influence on the manifestation of symptoms. These may include the microclimate, nutrient supply, cultivation methods and even the different climates of the entire wine-growing regions.

Severe GLMD symptoms have only been reported in the three southern German wine-growing regions of Wuerttemberg, Baden, and Franconia. In the ten remaining German wine-growing regions no widespread GLMD symptoms are known so far, despite GPGV infections [24]. Interestingly, it has also been reported from France that GPGV infections are widespread in all wine-growing regions but severe GLMD symptoms only occur in the Champagne region [35]. Champagne as well as the three German regions are located on the same latitude, which is their most obvious common feature and leads to similar temperature conditions throughout the year. However, the French region Alsace is in direct neighborhood to Baden with similar grape varieties but only few reported GLMD symptoms [36]. Hence, climate conditions as well as regional circumstances might influence symptom expression locally. Results of this study suggest a certain influence of the isolate on symptom expression, which could affect the difference between both regions. Phylogenetic analyses of GPGV isolates show that isolates from the same sampling location often cluster together indicating an influence of the origin of the sample [8, 24]. Both could be an indicator for the important role of vectoral virus spread within vineyards. As mentioned above, some isolates might be able to better establish themselves in plants due to their more virulent properties. In consequence, these isolates would be taken up more frequently by suitable vector insects and can therefore accumulate in the vineyards and thus in the region. Thus, regional GLMD clusters might probably be a combination of the occurrence of symptom-causing isolates with high virulence characteristics and optimal conditions for vector insects. The Alsace region is significantly drier than Baden, making it less favorable for vector insects such as *C. vitis* (https://www.weatherbase.com). This could explain the significantly lower GLMD presence in Alsace than in Baden.

The current study confirms that the presence of GLMD symptoms has certain economic consequences for winegrowers. Regardless of location and year, yield of infected vines declined when symptoms appeared. Significant yield reductions were always noted in moderate and severely symptomatic vines compared to healthy vines (between −41% and −96% in 2019; between −28% and −56% in 2020) while latent vines tended to have even higher yields (between +3% and +29%) (Fig. 5 and Additional file 7). Differences regarding yield reduction due to symptom development between the four cultivar varieties were not visible. Gewurztraminer as well as Pinot blanc produced less bunches, however, it is not clear if this was due to the practices of the grower or caused by the virus. We had no data on the rootstocks or clones used, so we could not make any statements about their influence on GPGV infections. However, since all four vineyards were managed by four different winemakers and were cultivated with four different cultivars, it is very likely that the results of this study can be considered generally valid. Similar experiments were conducted in Champagne, France, where more moderate yield losses of 5–20% on symptomatic vines compared to asymptomatic vines were recorded [35]. Approximately 65% less yield on symptomatic vines were documented in Italian vineyards [21]. In all three studies, yield losses could be attributed to smaller and fewer grape bunches ((Fig. 6, Additional file 7 and 8), [21, 35]). The French and Italian authors also compared the sugar content and acidity of the juice, finding no significant differences [21, 35]. In our study, the must analyses revealed some significant differences between healthy, latent, and symptomatic grapes, although, in practice, none of these deviations would have a serious impact on vinification (Additional file 9). For instance, symptomatic grapes produced fruit with significant less sugar content, and significantly less Glucose:Fructose ratio and Tartaric:Malic acid ratio. These three parameters are typically used for ripeness

determination [37]. All must samples tested in this study were considered ripe according to reference values. The Glucose:Fructose ratio should range between 0.95 and 1.05 and the Tartaric:Malic acid ratio between 1 and 2, in fruit considered ripe. Tartaric:Malic acid ratios above 2 indicate high temperatures during the growing season which ultimately lead to low amounts of total acidity. This was mainly true for samples originating from HEC in 2020. The delay in sugar storage in berries from symptomatic vines can be attributed to the chlorotic and deformed leaves of those plants as well as the reduced canopy due to stunted growth. In addition, physiological and transcriptomic studies showed a significant reduction in photosynthesis rate in GLMD symptomatic grapevines triggered by a reprogramming of plant primary metabolism in favor of virus replication [38]. Based on the results, we can conclude that GPGV has no relevant influence on fruit quality, but that yield is significantly reduced as soon as GLMD symptoms appear. Latent infected vines have neither a significant influence on yield nor on must quality.

Conclusions

Based on the results of our study, several conclusions can be put forward:

1. A spread of GPGV was recordable in vineyards. Partial clustering and gradients of GPGV infections could be detected indicating vectorial transmission (active migration, wind-borne migration and/or human transmission of vectors).
2. The number of symptomatic vines increased in 3 out of 4 vineyards during the trial years.
3. GLMD symptoms showed a certain correlation with GPGV isolates. Most GPGV isolates from symptomatic vines were assigned to clade C, while most isolates from asymptomatic vines assigned to clade A.
4. GLMD symptoms have a negative influence on yield. Moderate and severe symptoms significantly reduce the yield. Yield losses are caused by smaller and less grape bunches.
5. Latent GPGV infections have no influence on yield.

GPGV infections have no influence on the quality of must and therefore on vinification, neither in its asymptomatic nor in its symptomatic form. The damage of the virus to viticulture depends on the severity of symptoms. Although, latent infected vines initially have no negative effects on viticulture, with the observed increase in symptomatic vines, this trend can quickly reverse. Therefore, the virus should continue to be monitored in order to prevent the more virulent isolates from spreading.

Abbreviations

ANOVA	Analysis of variance
cDNA	Complementary desoxyribonucleic acid
Ct	Cycle threshold
cv.	Cultivar
DAS-ELISA	Double antibody sandwich enzyme-linked immunosorbent assay
FTIR	Fourier transformation infrared
gbs	Grape bunches
GEM	Gemmrigheim (location of observed vineyard)
GLMD	Grapevine leaf mottling and deformation
GPGV	Grapevine Pinot gris virus
H/h	Healthy
HEC	Hecklingen (location of observed vineyard)
HTS	High-throughput sequencing
IS	Immediately symptomatic
L/l	Latent
ms	Moderate symptomatic
NIM	Nimburg (location of observed vineyard)
ns	Not symptomatic
nsSNP	Nonsynonymous single nucleotide polymorphism
NTC	Non template control
rcf	Relative centrifugal force
REI	Reicholzheim (location of observed vineyard)
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
PCR	Polymerase chain reaction
PTGS	Post-transcriptional gene silencing
siRNA	Small interfering ribonucleic acid
sls	Slightly symptomatic
svs	Severely symptomatic
qPCR	Quantitative polymerase chain reaction
qRT PCR	Quantitative real time polymerase chain reaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-024-02453-4.

Additional file 1.
Additional file 2.
Additional file 3.
Additional file 4.
Additional file 5.
Additional file 6.
Additional file 7.
Additional file 8.
Additional file 9.
Additional file 10.

Acknowledgements

Great thanks to the Knoll family, Mr. and Mrs. Guldenfels, Mathias Meier and Mr. Schlör for making their vineyards available to us and for their valuable support during harvest. We also thank Petra Ehrhardt for her excellent technical assistance as well as all WBI staff who supported us during visual evaluation, harvesting or other projects. We also thank Dr. Michael Maixner a lot for providing us with his statistical tool PATCHY.

Author contributions

Conceptualization, N.M., and R.F.; methodology, N.M., and R.F.; validation, N.M., R.F., and S.S.; formal analysis, N.M., and L.A; investigation, N.M.; resources, R.F.; data curation, N.M.; writing—original draft preparation, N.M.; writing—review and editing, R.F., S.S. and R.T.V.; visualization, N.M.; supervision, R.F. and R.T.V.; project administration, R.F.; funding acquisition, R.F.

Funding
This research was funded by the Forschungsring des Deutschen Weinbaus (FDW).

Availability of data and materials
Sequences from this study were submitted to GenBank and assigned the accession numbers PP348014–PP348037.

Declarations

Ethics approval and consent to participate
This article does not contain any studies with human or animal subjects performed by any of the authors.

Consent for publication
All authors have reviewed the manuscript and approved its submission.

Competing interests
The authors declare no competing interests.

Received: 30 April 2024 Accepted: 31 July 2024
Published online: 06 August 2024

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