Survey for the Three Major Leafroll Disease-Associated Viruses in Finger Lakes Vineyards in New York

M. Fuchs, Department of Plant Pathology and Plant-Microbe Biology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456; **T. E. Martinson**, Department of Horticultural Sciences, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456; **G. M. Loeb**, Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456; and **H. C. Hoch**, Department of Plant Pathology and Plant-Microbe Biology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456

ABSTRACT

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Vineyards in the Finger Lakes region in New York were surveyed for the three major viruses associated with leafroll disease, i.e., *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 2* (GLRaV-2), and *Grapevine leafroll-associated virus 3* (GLRaV-3). Target viruses were detected in nearly two-thirds (68%, 65 of 95) of the vineyard blocks surveyed by enzyme-linked immunosorbent assay. Single infections by GLRaV-1, GLRaV-2, and GLRaV-3 occurred in 10% (113 of 1,124), 3% (36 of 1,124), and 15% (173 of 1,124) of the samples tested, respectively, whereas mixed infections affected 3.6% (40 of 1,124) of them, essentially with GLRaV-1 and GLRaV-3 (2.5%, 28 of 1,124). Presence of the target viruses was confirmed in selected samples by reverse transcription-polymerase chain reaction and sequencing. Comparative analysis indicated moderate to high nucleotide sequence identities in the second diverged copy of the GLRaV-1 coat protein gene (81.0 to 86.7%), GLRaV-2 coat protein gene (87.6 to 99.2%), and GLRaV-3 heat shock protein 70 homologue gene (91.5 to 98.3%) of New York isolates with corresponding virus reference strains. The prevalence of the three major leafroll disease-associated viruses in Finger Lakes vineyards results likely from poor sanitary status of planting materials, stressing the need to reinstate a certification program in New York.

Leafroll disease is an important and widespread viral disease of grapevines. It occurs in all the major grape-growing regions of the world and can affect *Vitis* spp., interspecific hybrids, and rootstocks (8,15,25,27). In late summer and fall, older leaves of leafroll-affected vines are cupped with principal veins often remaining green. Symptoms are usually conspicuous in red-fruited cultivars of *V. vinifera*, with interveinal leaf tissue becoming distinctly red in addition to cupping. In white-fruited *V. vinifera* cultivars, symptoms are less pronounced, consisting of a slight chlorosis in addition to cupping. *V. labrusca*, interspecific hybrids, and rootstocks usually are symptomless (15,25,27). Leafroll causes significant yield losses (up to 30 to 50%) and delays fruit ripening. Reduced soluble solids and increased titratable acidity often are reported in fruit juice. Berries of red-fruited cultivars may show pale

coloring due to reduced skin anthocyanin pigments (8,15,25,27).

To date, 10 different phloem-limited filamentous viruses identified as grapevine leafroll-associated viruses (GLRaVs) have been isolated and characterized from leafroll-infected grapevines (1,26,27). These viruses are unrelated serologically and belong to the family *Closteroviridae*. All GLRaVs are readily transmitted by propagation and grafting, and some of them (*Grapevine leafroll-associated virus 1* [GLRaV-1], *Grapevine leafroll-associated virus 3* [GLRaV-3], *Grapevine leafroll-associated virus 5* [GLRaV-5], and *Grapevine leafroll-associated virus 9* [GLRaV-9]) are also transmitted by several species of mealybugs and soft scale insects (17,27,32). GLRaV-1, *Grapevine leafroll-associated virus 2* (GLRaV-2), and GLRaV-3 are often prevalent in *V. vinifera* affected by leafroll disease (13,39).

The wine and grape industry in New York ranks third behind California and Washington, providing 2.6% of U.S. grape production (3). There are four major grape growing areas in New York: Lake Erie and the Niagara escarpment, Finger Lakes, Hudson River, and Long Island (Fig. 1). Three-fourths of the grape production in New York is for juice and other products,

and one-fourth is for wine production. Juice is produced mainly from *V. labrusca*, whereas wines are produced from *V. vinifera*, *V. labrusca*, and interspecific hybrids. Most *V. vinifera* in New York are grown in Long Island and the Finger Lakes region.

The occurrence of leafroll disease has been reported in *V. vinifera* (18) and *V. labrusca* (37) in New York. Also, the full-length genomic sequences of GLRaV-2 and GLRaV-3 have been determined from New York isolates (22,23,29,38). Amidst advances on disease presence and viral genome structure and expression, little is known on the incidence and prevalence of GLRaV-2 and GLRaV-3 in vineyards in New York. Similarly, no information is available on the presence of GLRaV-1 in New York vineyards. Therefore, we examined the status of these three major leafroll-associated viruses in Finger Lakes vineyards in New York. Here we report on the prevalence of GLRaV-1, GLRaV-2, and GLRaV-3 in the majority of vineyard blocks of *V. vinifera* and interspecific hybrids that were surveyed. The implications of our findings will be discussed in terms of the need to reinstate a grape certification program in New York for improving the sanitary status of planting materials.

MATERIALS AND METHODS

Vineyards and leaf sample collection. Ninety-five vineyard blocks of *V. vinifera* and interspecific hybrids were surveyed in this study. A 4 × 5 quadrat sampling strategy with a stratified regular quadrat distribution was used to collect leaf samples from the lower vine canopy in late August through early October of 2006. Leaf samples were collected from each quadrat, four quadrats per row, every five rows, for a maximum of 20 quadrats per vineyard block. Composite samples of three leaves per vine and five vines per quadrat, making a total of 15 leaves per sample, were collected and further tested for the presence of the three target viruses, i.e., GLRaV-1, GLRaV-2, and GLRaV-3.

Virus detection by double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA). GLRaV-1, GLRaV-2, and GLRaV-3 were detected by DAS-ELISA in leaf extracts with specific

Corresponding author: M. Fuchs
E-mail: mf13@cornell.edu

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antibodies (Bioreba, Reinach, Switzerland). A portion of 15 stacked leaves was torn (0.5 g) and ground in 200 mM Tris-HCl, pH 8.2, 140 mM NaCl, 2% polyvinylpyrrolidone 40, and 0.05% Tween 20 (35,39) at a 1:10 ratio (wt/vol) using a semi-automated ball-bearing HOMEX tissue homogenizer (Bioreba). Test conditions were according to the manufacturer's instructions (Bioreba). Substrate (*p*-nitrophenylphosphate) hydrolysis was recorded at 405 nm with an absorbance BioTek ELx808 microplate reader (BioTek, Winoski, VT). Samples were considered positive if their optical density (OD_{405nm}) readings were at least twice those of healthy controls.

Prior to the survey, the detection thresholds of GLRaV-1, GLRaV-2, and GLRaV-3 in DAS-ELISA were determined by testing serial dilutions of virus-infected leaf extracts prepared in leaf extracts from healthy vines to validate the sample collection strategy. Detection threshold experiments were replicated three times for each virus with different infected vines.

Virus detection by reverse transcription-polymerase chain reaction (RT-PCR). Some of the grapevine leaf samples

that tested positive for GLRaV-1, GLRaV-2, and GLRaV-3 in DAS-ELISA were further assayed by RT-PCR. Fresh leaf tissue (100 mg) from single leaves was placed in a 2.0-ml microfuge tube, dipped in liquid nitrogen, and disrupted with a TissueLyser homogenizer (Qiagen, Valencia, CA) for 2 min at 30 MHz in the presence of one stainless steel bead (5 mm diameter). Total RNA was extracted from homogenized material using the RNeasy Mini Plant kit (Qiagen).

A segment of the second diverged copy of the coat protein (CPd2) gene of GLRaV-1 (open reading frame 6), coat protein (CP) gene of GLRaV-2 (open reading frame 6), and heat shock protein 70 homologue (HSP70h) gene of GLRaV-3 (open reading frame 4) was characterized by RT-PCR from total RNA. Primers reported previously for GLRaV-1 (28), GLRaV-2 (4), and GLRaV-3 (28) were used. In addition, a primer pair specific to the ribulose 1,5-bisphosphate carboxylase chloroplast gene (RbcL) of *Vitis vinifera* (NC_007957.1) was designed (forward primer 5' TACTTG AATGCTACTGCAG 3' and reverse primer 5' CTGCATGCATTGCACGATC 3') and used as internal control to amplify the

corresponding mRNA in standard and multiplex RT-PCR. The *Vitis* sp. RbcL primers were designed based on RbcL primers of *Prunus persica* (GenBank accession no. AF206813) (31). Alternatively, primers to the *Vitis* 18S ribosomal RNA were used as internal control in RT-PCR assays (14).

One-step RT-PCR was carried out using the Access System (Promega, Madison, WI) with *Avian myeloblastosis virus* RTase, *Tfl* DNA polymerase, and 50 pmoles of specific primers in a 50- μ l final volume according to the manufacturer's protocol. Single-tube RT-PCR used a 45-min heating step at 45°C and a 2-min heating step at 94°C, followed by 45 cycles of 1 min melting at 94°C, 1 min annealing at 50°C, and 2 min elongation at 68°C, with a final extension of 7 min at 68°C. The reaction products of standard and multiplex assays were resolved by electrophoresis in 1.5 and 2% agarose gels, respectively, in 90 mM Tris-borate, 2 mM EDTA, pH 8.0, and subsequently visualized under UV light after staining with ethidium bromide.

Viral sequence determination and analysis. DNA amplicons obtained by RT-PCR were extracted from agarose gels with the QIAquick Gel Extraction kit (Qiagen) and sequenced bidirectionally using the Big Dye Terminator kit, AmpliTaq-FS DNA polymerase, and an Applied Biosystem Automated 3730xl DNA Analyzer at the DNA Sequencing facility at Cornell University in Ithaca, NY. Sequences were analyzed and compared using the DNASTAR Lasergene v7.2 software package. Viral strains used as reference in comparative sequence analysis were GLRaV-1 strain BL4 from *V. vinifera* cv. Sultana (GenBank accession no. AF195822) (12), GLRaV-2 strain PN from *V. vinifera* cv. Pinot noir (GenBank accession no. AF039204) (38), and GLRaV-3 strain NY-1 from *V. vinifera* cv. Pinot noir (GenBank accession no. AF037268) (22,23). The program CLUSTAL W was used for alignment of nucleotide sequences (33). Phylogenetic relationships were determined with the neighbor-joining method (30).

RESULTS

Ninety-five vineyard blocks from 25 grape growers were surveyed for GLRaV-1, GLRaV-2, and GLRaV-3 in the Finger Lakes region in New York (Fig. 1). Vineyard blocks were randomly selected without prior knowledge of their sanitary status. Eighty vineyard blocks were established with *V. vinifera* and 15 with interspecific hybrids (Table 1). They spanned from the western side of Keuka Lake to the western shores of Cayuga Lake, including both sides of Seneca Lake (Fig. 1). The area surveyed covered about 2,500 km².

Prior to the survey, the detection thresholds of GLRaV-1, GLRaV-2, and GLRaV-3 in DAS-ELISA were assessed in order to

Fig. 1. Map of the four major grape growing areas in New York State with the location of 25 farms in the Finger Lakes region for which vineyard blocks were surveyed for the three major viruses associated with grapevine leafroll disease.

validate our sample collection strategy. GLRaV-1, GLRaV-2, and GLRaV-3 were detected in 1/1,280, 1/320, and 1/640 dilutions of leaf extracts with absorbance values ranging from 0.179 to 0.194, 0.126 to 0.198, and 0.138 to 0.177, respectively, after 4 h of substrate hydrolysis. Healthy controls had absorbance values of 0.011 to 0.014. These dilution endpoints were equivalent to one infected leaf in 72, 54, and 64 healthy leaves for GLRaV-1, GLRaV-2, and GLRaV-3, respectively. These data indicated that the three target viruses could confidently be detected if one virus-infected leaf was mixed with 14 healthy leaves. Therefore, composite samples consisting of 15 leaves were collected from five vines and processed by DAS-ELISA.

Of the 95 vineyard blocks surveyed, nearly two-thirds (68%, 65 of 95) had at least one of the three target viruses, as shown by DAS-ELISA (Table 1). Virus incidence was slightly higher in blocks of *V. vinifera* (71%, 55 of 80) than in blocks of interspecific hybrids (63%, 10 of 15) (Table 1). A good relationship was evident between leafroll disease symptom expression and virus detection in blocks of *V. vinifera*, especially those of red-berried cultivars (data not shown). Our results were consistent with a prevalence of GLRaV-1, GLRaV-2, and/or GLRaV-3 in the majority of Finger Lakes vineyards surveyed.

Virus incidence as percentage of quadrats infected per vineyard block was high to extremely high in the majority of blocks surveyed (40%, 38 of 95), while a moderate and low incidence was found in 21% (20 of 95) and 7% (7 of 95) of them, respectively (Table 2). Of the 1,124 samples tested (pooled 15 leaves from a quadrat of five vines), 362 (32%) were positive for at least one of the three target viruses, including 173 (15%) for GLRaV-3, 113 (10%) for GLRaV-1, and 36 (3%) for GLRaV-2 (Table 3). Single infection occurred in 29% (322 of 1,124) of the samples tested and mixed infection in 3.6% (40 of 1,124), essentially with GLRaV-1 and GLRaV-3 (Table 3). The remaining 762 (68%) samples tested were negative for GLRaV-1, GLRaV-2, and GLRaV-3. DAS-ELISA data were also consistent with a similar distribution of target viruses in *V. vinifera* and interspecific hybrids, with GLRaV-1 and GLRaV-3 being the most prevalent both in single and mixed infections (data not shown).

RT-PCR was used to confirm the presence of the three target viruses in selected samples (Fig. 2). DNA products of the expected size were obtained in total RNA from grapevine leaves infected with GLRaV-1 (401 bp), GLRaV-2 (515 bp), and GLRaV-3 (546 bp). In addition, mixed virus infection was shown in single vines, confirming DAS-ELISA data obtained with leaf material pooled from several

Table 1. Detection of *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 2* (GLRaV-2), and *Grapevine leafroll-associated virus 3* (GLRaV-3) in vineyard blocks in the Finger Lakes region in New York

Genotype/cultivar	Number of vineyard blocks		% Infection
	Infected ^a	Tested	
<i>Vitis vinifera</i>			
Cabernet Franc	9	15	60
Cabernet Sauvignon	4	4	100
Carmine	0	1	0
Chardonnay	7	10	70
Gewurztraminer	1	1	100
Lemberger	7	10	70
Merlot	4	5	80
Pinot gris	1	2	50
Pinot noir	14	20	70
Riesling	5	9	56
Rkatsiteli	2	2	100
Sangiovese	1	1	100
Subtotal	55	80	71
Interspecific hybrids			
Chambourcin	0	1	0
De Chaunac	1	1	100
Landot noir	1	1	100
Noiret	0	1	0
Rougeon	1	1	100
Seyval	3	4	75
Vignoles	4	6	67
Subtotal	10	15	63
Total	65	95	68

^a Data represent number of vineyard blocks for which samples tested positive in double antibody sandwich–enzyme-linked immunosorbent assay for one of the three target viruses, GLRaV-1, GLRaV-2, and/or GLRaV-3.

Table 2. Incidence of *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 2* (GLRaV-2), and *Grapevine leafroll-associated virus 3* (GLRaV-3) in Finger Lakes vineyard blocks

Virus incidence (%) ^a (category)	Number of vineyard blocks	
	Tested	Infected (%)
0 (none)	30	32
1-10 (low)	7	7
11-20 (moderate)	20	21
21-50 (high)	20	21
51-90 (very high)	14	15
91-100 (extremely high)	4	4
Total	95	100

^a Data represent number of quadrats per vineyard block in which samples infected by GLRaV-1, GLRaV-2, and/or GLRaV-3 were detected by double antibody sandwich–enzyme-linked immunosorbent assay over the number of quadrats tested. Number of quadrats tested per block ranged from 4 to 20 depending on size of the vineyard block surveyed.

Table 3. Rates of single and mixed infections by *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 2* (GLRaV-2), and/or *Grapevine leafroll-associated virus 3* (GLRaV-3) in grapevine leaf samples (pooled 15 leaves from a five-vine quadrat) from Finger Lakes vineyards

Virus combination	Virus-infected samples	
	Infected/tested ^a	Percent
No infection	762/1,124	68
Single infection		
GLRaV-1	113/1,124	10
GLRaV-2	36/1,124	3
GLRaV-3	173/1,124	15
Subtotal	322/1,124	29
Multiple infection		
GLRaV-1 + GLRaV-2	8/1,124	0.7
GLRaV-1 + GLRaV-3	28/1,124	2.5
GLRaV-2 + GLRaV-3	3/1,124	0.3
GLRaV-1 + GLRaV-2 + GLRaV-3	1/1,124	0.1
Subtotal	40/1,124	3.6

^a Infected samples were detected by double antibody sandwich–enzyme-linked immunosorbent assay.

vines (Fig. 2, lanes 4 to 7). No viral DNA amplicon was obtained in total RNA from leaves of healthy vines, except a 183-nucleotide-long RT-PCR product for the *Vitis* sp. Rbcl gene (Fig. 2).

DNA amplicons obtained by RT-PCR for three to five isolates of GLRaV-1, GLRaV-2, and GLRaV-3 each were sequenced directly. Nucleic acid sequences determined in this study were made available in GenBank as accession numbers FJ195739 to FJ195749. Sequence analysis confirmed the respective viral nature of RT-PCR products and indicated relatively high homologies of virus isolates characterized in this study and reference strains. Identities of 81.0 to 86.7% and 77.8 to 84.6% in the partial CPd2 gene of the type strain of GLRaV-1 (12), 87.6 to 99.2% and 92.6 to 97.8% in the partial CP gene of the type strain of GLRaV-2 (37), and 91.5 to 98.3% and 92.9 to 98.1% in the partial HSP70h gene of the type strain of GLRaV-3 (22,23) were obtained at the nucleotide and amino acid levels, respectively.

Multiple sequence alignments indicated that three GLRaV-1, three GLRaV-2, and five GLRaV-3 isolates from New York and four GLRaV-1, 40 GLRaV-2, and 25 GLRaV-3 isolates from other geographic origins (Table 4) had 79.5 to 99%, 67.8 to 100%, and 74.1 to 100% identity at the nucleotide level, respectively; and 72.1 to 98.5%, 71.3 to 100%, and 85.9 to 100% identity at the amino acid level, respectively, in the CPd2 (GLRaV-1), CP (GLRaV-2), and HSP70h (GLRaV-3) genes, respectively.

Phylogenetic relationships among viral isolates were inferred using the neighbor-joining method for a 205-nt portion of the GLRaV-1 CPd2 gene, a 345-nt fragment of the GLRaV-2 CP gene, and a 468-nt segment of the GLRaV-3 HSP70h gene (Fig. 3). The seven GLRaV-1 isolates clustered into three groups (Fig. 3A), the 43 GLRaV-2 isolates into six groups (Fig. 3B), and the 30 GLRaV-3 isolates into five groups (Fig. 3C). The same grouping of virus isolates was obtained with phylograms obtained from deduced amino acid sequences (68 aa of the GLRaV-1 CPd2, 115 aa of the GLRaV-2 CP, and 156 aa of the GLRaV-3 HSP70h) (data not shown).

The three GLRaV-1 isolates from New York clustered into two of the three phylogroups, including one with two isolates from *V. vinifera* cv. Lemberger (Fig. 3A). The three GLRaV-2 isolates from New York clustered into the two predominant groups PN with isolates from Italy, Brazil, and the People's Republic of China, and H4 with isolates from Italy and Brazil (Fig. 3B). The five GLRaV-3 isolates from New York clustered into (i) the predominant group NY-1 with isolates from Europe, the Middle East, and the People's Republic of China, (ii) group GP18 with isolates from Austria, South Africa, and Syria, and (iii)

group MT48-2 with isolates from Italy (Fig. 3C). Phylogenetic analyses further inferred no grouping of genetic variants based on *Vitis* sp. or geographical origin, except maybe for GLRaV-1 isolates from *V. vinifera* cv. Lemberger. However, the

limited number of GLRaV-1 isolates for which sequence information is available in the CPd2 gene precludes any definite conclusion on an association between genetic variability and geographical origin or host. More sequence information would be

Fig. 2. Agarose gel analysis of DNA amplicons obtained by multiplex reverse transcription-polymerase chain reaction from total RNA of grapevine leaves infected by *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 2* (GLRaV-2), and *Grapevine leafroll-associated virus 3* (GLRaV-3), and primer pairs specific to each virus and to the *Vitis* sp. Rbcl gene. Lane H: healthy *V. vinifera* cv. Pinot noir; lane 1: *V. vinifera* cv. Lemberger infected by GLRaV-1; lane 2: *V. vinifera* cv. Chardonnay infected by GLRaV-2; lane 3: *V. vinifera* cv. Cabernet franc infected by GLRaV-3; lane 4: *V. vinifera* cv. Lemberger infected by GLRaV-1 and GLRaV-2; lane 5: *V. vinifera* cv. Riesling infected by GLRaV-1 and GLRaV-3; lane 6: *V. vinifera* cv. Pinot noir infected by GLRaV-2 and GLRaV-3; and lane 7: *V. vinifera* cv. Chardonnay infected by GLRaV-1, GLRaV-2, and GLRaV-3. Lanes M: 100 bp DNA size standard (Promega, Madison, WI). DNA products were resolved by electrophoresis on 2% agarose gels.

Table 4. Virus isolates used in this study to determine phylogenetic relationships

Virus	Isolate	Cultivar	Country	GenBank accession no.
GLRaV-1	B4L	Sultana	Australia	AF195822
	Cf	Cabernet franc	P.R. China	EF583823
	17NM1	Neo Muscat	Japan	AB222849
	21KY1	Kyoho	Japan	AB222850
	Cs	Cabernet sauvignon	United States	FJ195739
	Lem1	Lemberger	United States	FJ195740
	Lem2	Lemberger	United States	FJ195741
	PN	Pinot noir	United States	AF039204
	PnTM	Pinot noir	United States	FJ195742
	Ch	Chardonnay	United States	FJ195743
GLRaV-2	Cf	Cabernet franc	United States	FJ195744
	Rei	Regina	Italy	DQ314604
	L/I	Isabel	Italy	EU053126
	IT	Italia	Brazil	EU204910
	SE	Seibel	Brazil	EU204909
	Sg	Sangiovese	Italy	DQ314607
	Red1	Red Globe	Italy	DQ314602
	MuM	Muskat moransky	Italy	DQ314594
	Mon	Montepulciano	Italy	DQ314593
	Arv2	Arvino	Italy	DQ314590
	Tempr	Tempranillo	Italy	DQ314608
	Prim	Primitivo	Italy	DQ314601
	Shandong	Unknown	P.R. China	AY842932
	Isp	Isabella neva	Italy	DQ314591
	NA1	Negro amoro	Italy	DQ314595
	NA2	Negro amoro	Italy	DQ314596
	Red2	Red Globe	Italy	DQ314603
	SLIO	Unknown	P.R. China	DQ911147
	Tuc	Tuccanese	Italy	DQ314609
	110R	110R	Italy	DQ314587
	93/955	LN33	South Africa	AY842932
	Ner	Nera	Italy	DQ314598
	Pol1	Pollera nera	Italy	DQ314599
	Arv1	Arvino	Italy	DQ314588
	Can	Cannonao	Italy	DQ314589
	NerC	Nerello calabrese	Italy	DQ314597
	Pol2	Pollera nera	Italy	DQ314600
	M/C	Concord	Brazil	EU053125
	MH	Muscat Hamburg	Brazil	EU204911

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needed to ascertain the phylogenetic relationships among GLRaV-1 isolates.

DISCUSSION

We report here the occurrence and prevalence of the three major viruses associated with grapevine leafroll disease in Finger Lakes vineyards in New York. The very high incidence of GLRaV-1, GLRaV-2, and GLRaV-3 in the majority of vineyard blocks surveyed indicates a poor sanitary status of planting materials. There is little doubt that the use of uncertified graftwood and/or rootstocks is the main, if not the single, reason for the widespread distribution of the three target viruses in the vineyard blocks surveyed. Vector-mediated transmission cannot account for such an extensive virus distribution over the 2,500 km² area surveyed. In recent years (2001 to 2006), the acreage of *V. vinifera* increased by 38% in the Finger Lakes region (36). As a result of many new plantings, there has been a 9% increase in *V. vinifera* production in New York, while the tonnage of *V. labrusca* and interspecific hybrids decreased by 6% (3). Our survey indicates that the sanitary status of vine stocks used to establish new vineyards or

the mother plants from which they derive received little oversight, stressing the need to reinstate a grapevine certification program in New York to improve the sanitary status of planting stocks.

Our study is the first to report the occurrence of GLRaV-1 in Finger Lakes vineyards. It also provides further evidence on the incidence of GLRaV-2 and GLRaV-3 in New York (18,22,37,38). It is not too surprising to have found GLRaV-1, because this virus is prevalent in leafroll-affected *V. vinifera* along with GLRaV-2 and GLRaV-3 (13,37), and mixed viral infections are common in grapevines (7,10,13,20). Target viruses were distributed similarly in *V. vinifera* and interspecific hybrids, indicating that the host genotype has limited, if any, effect on infection rates.

The presence of GLRaV-1, GLRaV-2, and GLRaV-3 was determined by DAS-ELISA using specific antibodies. It was further ascertained in selected samples by RT-PCR using specific viral primers and a primer pair specific to the *Vitis* Rbc1 mRNA gene used as internal control. In a previous study, another set of primers was successfully used to amplify an 844-

nucleotide-long *Vitis* 18S ribosomal RNA as internal control in RT-PCR assays for the simultaneous detection of nine grapevine viruses (14). The Rbc1 genes of *V. vinifera* (complete sequence NC_007957.1) and *Prunus persica* (partial sequence AF2066813) have 95.6% nucleotide identity (M. Fuchs, *unpublished*). This high homology enabled us to design primers based on those used as internal control for virus detection by RT-PCR in *Prunus* (31). The use of primers specific to the Rbc1 gene mRNA allowed us to determine the effectiveness of RT-PCR and assess directly the quality of total RNA from grapevine leaves without electrophoresis on denaturing gels, among other techniques.

Analyses of partial gene sequences revealed a close relationship of GLRaV-1, GLRaV-2, and GLRaV-3 isolates from New York and other isolates from various geographical origins. These data are consistent with the fact that transmission of these viruses occurs predominantly through uncontrolled exchange and propagation of budwood material. Furthermore, we found no relationship between genetic variability of the target viruses and geographic origin or host genotype. These data confirm previous observations for GLRaV-1 (21,24), GLRaV-2 (2,5,6,29), and GLRaV-3 (19,34).

The fact that GLRaV-1 and GLRaV-3 can be transmitted between and within vineyard blocks by soft scale and mealybug species (17,25,27,32) emphasizes the need to assess whether indigenous insect populations can act as vectors in Finger Lakes vineyards. Spread of leafroll-associated viruses can be rapid, especially for GLRaV-3, resulting in new vineyard infection within a few years (9). Also, a single mealybug nymph is capable of infecting a healthy grapevine with GLRaV-3 (11). Since mealybugs and soft scales transmit GLRaV-3 in Australia, France, Israel, Italy, Greece, New Zealand, South Africa, Spain, Tunisia, and the United States, including California and Washington (16,17,27,28), it is conceivable that GLRaV-3 might spread similarly in New York. The same assumption holds true for GLRaV-1. Experiments to assess the transmissibility of GLRaV-1 and GLRaV-3 by soft scales and mealybugs in Finger Lakes vineyards are under way. These studies are critical to determine if pest management strategies need to be deployed for vectors of leafroll-associated viruses or if a certification program should be effective in controlling these viruses.

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Table 4. (continued from previous page)

Virus	Isolate	Cultivar	Country	GenBank accession no.
GLRaV-2 (cont.)	R1	Riesling	Brazil	EU204912
	H4	<i>V. rupestris</i> St George	Italy	AY697863
	RG2	Red Globe	Italy	DQ314606
	RG	Red Globe	USA	AF314061
	RG1	Red Globe	Italy	DQ314605
	PG11	Petit Meslier	France	EF012720
	PV44	Chardonnay	France	EF012718
	BD	Dan Mariano	Italy	DQ286725
	PV124	Negro Amoro	France	EF012717
	PV20	Savagnin	France	EF012721
	Leo	Leopold III	Italy	DQ314592
	NY1	Pinot noir	United States	AF039204
GLRaV-3	C3	Fujihami	R.P. China	AJ748514
	SY2-7	Homos	Syria	AJ748517
	MT48-1	LN33	Italy	AJ748518
	C4-1	Unknown	United States	DQ780888
	CfL	Cabernet franc	United States	FJ195745
	Ch	Chardonnay	United States	FJ195746
	Ries	Riesling	United States	FJ195747
	Mer	Merlot	United States	FJ195748
	CfW	Cabernet franc	United States	FJ195749
	C6-1	Beta	P.R. China	DQ780890
	AUSG5-5	Unknown	Austria	AJ748512
	SY2-2	Homos	Syria	AJ748515
	IL1	Hillmanis	Israel	AJ748524
	MT48-4	LN33	Italy	AJ748521
	N1-1	Unknown	United States	DQ780891
	USA-6	Shermann	United States	AJ748523
	C1-1	Unknown	United States	DQ780885
	C3-1	Fujihami	P.R. China	DQ780887
	C2-1	Beta	P.R. China	DQ780886
	AUSG5-4	Unknown	Austria	AJ748511
	Tu32	Unknown	Tunisia	AJ748522
	C5-1	Beta	P.R. China	DQ780889
	SY2-4	Homos	Syria	AJ748516
	AUSG5-6	Unknown	Austria	AJ748513
	GP18	Cabernet sauvignon	South Africa	EU259806
	AUSG5-2	Unknown	Austria	AJ748510
	MT48-2	LN33	Italy	AJ748519
	MT48-3	LN33	Italy	AJ748520
	NZ-1	Unknown	New Zealand	EF508151

Fig. 3. Phylogenetic trees reconstructed from partial nucleotide sequences of **A**, CP2d gene of *Grapevine leafroll-associated virus 1* (GLRaV-1), **B**, CP gene of *Grapevine leafroll-associated virus 2* (GLRaV-2), and **C**, HSP70h gene of *Grapevine leafroll-associated virus 3* (GLRaV-3) isolates by the neighbor-joining method with 1,000 bootstrap replicates. Branch length represents phylogenetic distances determined with distance matrices of nucleotide sequences. Numbers above critical branches are significant bootstrap values (>75%). Scale bar represents a relative genetic distance of 0.01. Representative isolates of each phylogroup are indicated as well as the country of origin for each sequence accession. GLRaV isolates sequenced in this study are indicated by stars.

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