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

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Leafroll disease is one of the most important and widespread viral diseases of grapevines worldwide. It can affect *Vitis vinifera*, American grapes, French-American hybrids and rootstocks (Martelli, 1993). Leafroll causes significant yield losses (up to 30-50%) and delays fruit ripening. Reduced soluble solids and increased titratable acidity are also often reported. Berries of red-fruited cultivars may show pale coloring due to reduced skin anthocyanin pigments (Martelli, 1993). To date, ten different phloem-limited filamentous viruses identified as grapevine leafroll-associated viruses (GLRaVs) have been isolated and characterized from leafroll-infected grapevines (Abou Ghanem-Sabanadzovic et al. 2006; Martelli, 2006). All GLRaVs are readily transmitted by propagation and grafting. In addition, some of them (GLRaV-1, GLRaV- 3, GLRaV-5 and GLRaV-9) are transmitted by several species of mealybugs and soft scale insects (Gugerli, 2003). Prevalent viruses in leafroll-affected *V. vinifera* are GLRaV-1, GLRaV-2 and GLRaV-3 (Zimmermann et al. 1990).

Leafroll disease has been reported in American grapes (Wilcox et al. 1998) and *V. vinifera* (Hu et al. 1990) in New York, especially GLRaV-2 and GLRaV-3. Also, the full-length genomic sequence of the latter two viruses has been determined from New York isolates (Ling et al. 2004; Zhu et al., 1998). Admits these advances on disease occurrence and viral genome structure and expression, little is known on the incidence and prevalence of GLRaV-2 and GLRaV-3 in New York vineyards. 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. The objectives of our study were to assess the incidence of GLRaV-1, GLRaV-2 and GLRaV-3, examine the relative distribution and abundance of mealybugs and soft scale insect vectors.

A total of 95 vineyard blocks, including 80 of *V. vinifera* and 15 of French-American hybrids, were surveyed in the Finger Lakes region. Vineyard blocks were randomly selected without prior knowledge of their sanitary status. A 5 x 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. Leaf samples were collected from each quadrat, five 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 viruses by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). GLRaV-1, GLRaV-2 and/or GLRaV-3 were detected in nearly two thirds (68%, 65 of 95) of the vineyard blocks surveyed (Table 1). Virus incidence was low in 7% (7 of 95) of the blocks tested, moderate in 21% (20 of 95), and high to extremely high in 40% (38 of 95) of them (Table 1). The three target viruses were found in 10% (113 of 1,124), 3% (36 of 1,124) and 15% (173 of 1,124) of the samples tested, respectively. Mixed infection occurred in 3.6% (40 of 1,124) of the samples, essentially with GLRaV-1 and GLRaV-3 (2.5%, 28 of 1,124).

The presence of the three target viruses was confirmed in a few samples by reverse transcription-polymerase chain reaction (RT-PCR). 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). No DNA amplicon was obtained in total RNA from leaves of healthy vines, except the 183 nucleotide-long product for the *Vitis* sp. Rbcl gene used as internal control, as expected. Virus isolates were further characterized by sequencing DNA amplicons obtained by RT-PCR. Comparative sequence analysis indicated high nucleotide identities in the coat protein gene (86-99% for GLRaV-1 and 81-100% for GLRaV-2) and in the heat shock 70 homologue gene (90-99% for GLRaV-3) of New York isolates with other isolates from various geographic origin.

Two species of soft scales (*Parthenolecanium corni* and *Neopulvinaria innumerabilis*) and one species of mealybug (*Pseudococcus maritimus*) were tentatively identified in the majority of vineyards blocks surveyed (85%, 25 of 31). Their abundance in Finger Lakes vineyards is quite low (0.18 scale insects/min on the edge and 0.16 scale insects/min in the interior) for the most part. Groups of insects or individual instars were collected in vineyard blocks in which the occurrence of GLRaV-1 and GLRaV-3 was documented and assayed by RT-PCR to assess their viruliferous potential. Some of the *P. corni* and *N. innumerabilis* tested reacted positively to GLRaV-1. The viral nature of DNA products obtained from soft scales was confirmed by sequencing. These results provided direct evidence of the viruliferous status of soft scales in Finger Lakes vineyards.

Our research provided new insights into the incidence and distribution of GLRaV-1, GLRaV-2, and -3 in New York. The prevalence of the three major leafroll disease-associated viruses in Finger Lakes vineyards results likely from a poor sanitary status of planting materials, stressing the need to reinstate a certification program in New York. Our study also shed light on the distribution and abundance of mealybugs and soft scales, and potential spread of GLRaV-1 and GLRaV-3, suggesting the need for pest management strategies to be deployed for vectors of leafroll-associated viruses in Finger Lakes vineyards.

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Table 1. Incidence of GLRaV-1, GLRaV-2 and GLRaV-3 in Finger Lakes vineyard blocks.

	Vineyard blocks	
Virus incidence (%)[a] (category)	No. 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 the number of quadrats per vineyard block in which samples infected by GLRaV-1, GLRaV-2 and/or GLRaV-3 were detected by DAS-ELISA over the number of quadrats tested.