

## Grapevine virus E Detected in Ohio Vineyards

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### Summary

Winegrape production in Ohio is challenging due to unpredictable winter temperatures, late and/or early frosts, a short growing season, and a plethora of diseases that can reduce grape yield and quality. Viral diseases in vineyards can result in significantly reduced economic returns due to reduced yield or diminished fruit quality. From 2012 to 2016, surveys of major viruses in commercial winegrapes were conducted to obtain a comprehensive understanding of the prevalence of viral diseases in Ohio vineyards. To date, these surveys have shown that *Grape leafroll associated viruses* 2 (GLRaV-2) and 3 (GLRaV-3) and *Grapevine red blotch virus* (GRBV) are present in commercial vines across the state.<sup>1,2</sup> In this study, we report that *Grapevine virus E* (GVE) is also present in Ohio grapevines, further increasing our knowledge of the types of grapevine viruses present in the state.

Over 80 viruses can infect grapevines, more than any other perennial crop.<sup>3</sup> Grapevines can be infected with a single virus or with multiple viruses. In addition, synergistic interactions between grapevine viruses from different virus families have been reported.<sup>4</sup> Grapevine viruses are transmitted by insect, nematode, or eriophyid mite vectors, and all grapevine viruses can be spread from region to region through distribution of infected vegetative cuttings.<sup>5</sup> The impact of viruses on grapevines varies considerably, depending on incidence, distribution, presence of coinfections or synergistic interactions, and available control strategies. While some viruses do not cause symptoms and/or direct economic losses, others reduce plant growth, vigor, and berry quality and can even kill the vine.<sup>5</sup> Nonetheless, all viruses are important for maintaining clean stock programs and for the sanitary status of vineyards across the country.

GVE is a relatively new virus associated with grapevines worldwide. It was first described in 2018 in a Japanese table grape (*Vitis labrusca* cv. Aki Queen and Pione) and classified as a new member of the genus *Vitivirus*.<sup>6</sup> Since 2008, GVE has been reported in South Africa,<sup>7</sup> China,<sup>8</sup> Tunisia,<sup>9</sup> Washington State (USA),<sup>10</sup> Argentina,<sup>11</sup> and Greece.<sup>12</sup> The virus has been isolated from visibly healthy vines,<sup>6,10,11</sup> vines with stem pitting symptoms,<sup>6</sup> vines with leafroll and Shiraz disease symptoms,<sup>7</sup> and vines with rugose wood disease complex.<sup>13</sup> GVE can be transmitted nonpersistently by the Comstock mealybug (*Pseudococcus comstocki* Kuwanae),<sup>6</sup> and although mechanical transmission is not suspected,<sup>6</sup> the virus is likely spread from region to region through movement of infected vegetative cuttings. While the role of GVE in causing disease and displaying symptoms is unclear, it correlated with greater incidence and titer of other vitiviruses (i.e., *Grapevine virus A* and *Grapevine virus B*) and with increased disease severity in vines coinfecting with the economically important viruses GLRaV-2 and/or GLRaV-3.<sup>4,14,15</sup> Thus, the potential for increased severity of disease in vines coinfecting with GVE, GLRaV-2, and/or GLRaV-3 exists and must be explored further.

Monitoring for the presence of known and unknown viruses in high-value crops such as grape is essential to target and implement control measures for potentially devastating new or existing viral diseases. At the request of the

Ohio winegrape industry, we conducted a study to characterize the viruses infecting grapevines between 2012 and 2016. Grapevine leaves with virus-like symptoms, including leaf curling or thickening, interveinal leaf reddening, vine stunting, delayed fruit maturity, and/or red or yellow leaf discoloration (blotching), were collected from 40 vineyards across seven Ohio winegrape growing regions (Northeast, Lake Shore, Northwest, Canal Country, Central, Southwest, and Appalachian). Through high-throughput RNA sequencing (RNAseq), we discovered the near-complete sequence of a new GVE isolate in Ohio. High-throughput sequencing (HTS) technology has revolutionized the field of virology by allowing rapid, precise genetic and evolutionary analyses of viral populations in diverse sample types.<sup>16,17</sup> In grapevines, HTS allows assembly of complete genomes of infecting viruses.<sup>16,18</sup> In this study, we pooled collected grapevine leaves (n = 140) by growing region, generated seven RNAseq data sets, and retrieved six assembled sequences (contigs) that ranged in size from 677 to 2648 nucleotides (nt). These six contigs were compared to published viral sequences in the NIH genetic sequence database (GenBank) and shared sequence identity (73 to 91%) with a GVE isolate from South Africa (SA94, GU903012.1).<sup>7</sup> Overall, the two isolates share 77% nt level sequence identity. A nearly complete genomic sequence (7530 nt) of GVE (GVE-OH) was assembled from the contigs and the final sequence was deposited in GenBank under the accession number MK331711. Although the sequence is missing ~25 nt, the Ohio isolate is predicted to encode four proteins with amino acid sequences similar to those in the South African isolate.<sup>7</sup> These proteins are predicted to function in virion assembly (capsid protein, 95% amino acid [aa] sequence similarity), cell-to-cell movement (movement protein, 80%), and genome replication (replicase, 82%) processes within host cells. There is also an as-yet-uncharacterized protein (putative nucleic acid binding protein, 93%).

GVE-OH was traced back to two different vineyards (Lake Shore and Central growing regions) more than 100 miles apart, both of which were also positive for GLRaV-2 and -3.<sup>1</sup> This is the first report of GVE in Ohio vineyards and of GVE in winegrape production areas east of the Rocky Mountains. These results also confirm that co-infections of GVE with one or more GLRaV are occurring; this is not unexpected, as mixed viral infections in grapevines are common<sup>19</sup> and both viruses are transmitted by mealybugs and spread through the movement of propagation material. Because GVE was detected in two vineyards separated by more than 100 miles, GVE was likely introduced into these vineyards through propaga-

tive material, emphasizing the importance of surveys to monitor for early detection and management of infected vines and of planting certified, virus-tested planting material. In Ohio, widespread testing is recommended to confirm the distribution of GVE in the grapegrowing regions of the state, the primary method of dispersal, and whether or not there is a synergistic interaction occurring in vines with GVE, GLRaV-2, and -3 coinfections.

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