

A large-scale, multi-year virome survey in Belgium reveals potential phytosanitary risks through the first detection of five regulated non-quarantine pests (RNQPs).

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INTRODUCTION

Over the past decade, Belgian viticulture has undergone a remarkable transformation, expanding from only a handful of hectares to ~1,000 ha by 2024 (SPF Economie, 2025). This rapid rise, fueled by climate change and consumer enthusiasm, has resulted in a brand-new winemaking territory in Europe. Yet, with this growth comes uncertainty: which virus or viroid-based diseases may have been introduced with planting material or have already established and are causing disease? And what phytosanitary risks do they pose to Belgian vineyards in the long term?

The VITIBEL project, launched in May 2023, was designed to address these questions. Built on a unique partnership between scientists and growers, the project now delivers the first comprehensive overview of grapevine viruses and viroids in Belgium. By combining large-scale virome surveys based on high-throughput sequencing with targeted prevalence studies and continuous engagement with growers, VITIBEL goes beyond establishing an inventory: it fills a critical knowledge-gap and provides the foundation for understanding national and local epidemiological risks.

MATERIALS AND METHODS

In early summers 2023 and 2024, representative surveys were conducted, combining analysis on both asymptomatic pooled samples and individual symptomatic plants to establish the baseline virome of Belgian vineyards. It was accompanied by a grower's interview to collect metadata (cultivar, origin, year of planting and some other cultural and pest management practices). For all samples, leaf tissue was used as the biological matrix. Symptomatic samples underwent total RNA extraction, while asymptomatic pools were processed using an antibody-based dsRNA enrichment protocol (Blouin et al., 2023). Extracts were subjected to Illumina high-throughput sequencing (HTS), and viral detections were validated through RT-PCR and Sanger sequencing.

In 2025, prevalence surveys were conducted within selected parcels where regulated non-quarantine pests (RNQPs) for the European Union had been detected during the initial survey. Targeted viruses were *Nepovirus foliumflabelli* (GFLV), *Ampelovirus univitis* and *Ampelovirus trivitis* (GLRaV-1, -3), *Nepovirus arabis* (ArMV), and *Nepovirus rubi* (RpRSV). Sample size per vineyard was calculated using a binomial proportion approach, corrected for finite population size and design effect. Sampling followed a systematic grid across vines and rows, with individual leaves collected and pooled in groups of five. DAS-ELISA analyses are currently ongoing, and positive pools will be resolved by retesting individual vines. For GFLV, the final round of sampling is still in progress, and in addition neighboring plants of symptomatic vines are specifically targeted for testing. Soil samples will be collected and analysed for nematode detection and identification. Confirmed viral isolates will subsequently undergo Sanger sequencing for genetic diversity analysis.

RESULTS AND DISCUSSION

The combined 2023-2024 HTS campaigns provided the first comprehensive overview of grapevine viruses and viroids in Belgium. Across both years, 86 vineyards and three genetic collections were surveyed, covering all production areas in Belgium. Approximately 4,400 vines samples from more than 50 cultivars, different planting years and nursery origins were sampled and deep-sequenced. Illumina data revealed over 35 distinct viral and viroid species, spanning at least 8 families and 12 genera, which corresponds to a substantial proportion of the currently known global grapevine virus diversity. Twenty-one species were confirmed by RT-PCR and Sanger sequencing, while the remaining detections are under validation. Most correspond to first records of grapevine viruses and viroids in Belgium, highlighting both the diversity of the virome and the detection of five regulated non-quarantine viruses (GFLV, GLRaV-1, -3, ArMV, and RpRSV) that may threaten production. Most correspond to first records of grapevine viruses and viroids in Belgium.

Building on these findings, the 2025 prevalence survey was launched to estimate field prevalence of the five RNQPs. In total, 5,318 vines from 25 parcels were sampled across 16 vineyards and 3 genetic collections. Laboratory analyses are still in progress. Preliminary results confirmed independently the detection of the five RNQP viruses and enabled initial estimation of their prevalence and distribution in the different vineyards. These prevalence data, combined with the HTS-based virus inventory, will strengthen the phytosanitary baseline for Belgian viticulture and inform both growers and regulators on the magnitude of viral threats and open a question about future impact of new viral diseases for this emerging sector. In parallel, correlations between virus occurrence and vineyard metadata (cultivar, nursery origin, planting year, and management practices) are being explored to identify potential sources and pathways of virus introduction and spread within the country.

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