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Sugar beet yellowing viruses in Belgium: insights from two complementary field surveys

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Abstract

Sugar beet cultivation faces significant threats from aphid-transmitted yellowing viruses, revived because of the European ban on neonicotinoid insecticides. To support integrated management, this study investigated virus occurrence, alternative hosts, aphid vectors, and genetic diversity in Belgium. Between 2022 and 2024, two independent field surveys were conducted in Flanders and Wallonia. Sugar beets, surrounding vegetation, and aphids were screened using RT-qPCR, and selected positive beets were analyzed via total RNA Illumina sequencing. Beet mild yellowing virus (BMYV) was the most frequently detected virus across all years, followed by beet chlorosis virus (BChV) and beet yellows virus (BYV), whereas beet mosaic virus (BtMV) occurred sporadically. Detection frequencies varied among years and production areas, and mixed detections, particularly BMYV+BChV and BMYV+BYV, were common. Beet yellowing viruses were detected sporadically in surrounding vegetation, mainly during the sugar beet growing season, and none of the wild species maintained infection across the crop-free period. However, Beta vulgaris plants persisting in winter field margins showed high-titre BChV or BYV infections, highlighting a potential overwintering reservoir. Yellowing viruses were detected in less than 1% of screened aphids in both surveys, generally near the RT-qPCR cut-off and without independent confirmation. Sequence analyses indicated comparative limited genetic variation in BYV and BMYV, whereas BChV was more variable, without consistent clustering by Belgian production area or sampling year. Together, these findings provide baseline data to support integrated management of yellowing viruses in sugar beet.

Introduction

Sugar beet (*Beta vulgaris* L.) is an important arable crop worldwide. In Europe alone, approximately 1.6×10^6 hectares of land are dedicated to its cultivation (Hossain et al., 2021). In Belgium, the total growing area was 53,255 hectares with a production yield of 89.1 tonnes per hectare in 2022 (Statbel, 2023). The sugar beet cultivation, however, faces many threats from yellowing viruses. These viruses include beet yellows virus (BYV, *Closterovirus flavibetae*), beet mild yellowing virus (BMYV, *Polerovirus BMYV*), beet chlorosis virus (BChV, *Polerovirus BChV*), beet mosaic virus (BtMV, *Potyvirus betaceum*) and beet western yellows virus (BWYV, *Polerovirus BWYV*). Turnip yellows virus (TuYV, *Polerovirus TuYV*) is closely related to BWYV and other beet-polerovirus species (Yoshida & Tamada, 2019), yet is not commonly associated with sugar beet (Hauser et al., 2000). Yellowing viruses are transmitted by aphids, with the green peach aphid (*Myzus persicae* Sulzer) being considered as the primary vector. Other known, yet less efficient vectors, include the black bean aphid (*Aphis fabae* Scopoli), the potato aphid (*Macrosiphum euphorbiae* Thomas) and the shallot aphid (*Myzus ascalonicus* Doncaster), among others (Doncaster & Kassanis, 1946; Dusi & Peters, 1999; Limburg et al., 1997; Kirk et al., 1991; Kozlowska-Makulska et al., 2009). Yellowing viruses are transmitted through different modes. Poleroviruses are transmitted in a persistent, circulative and non-propagative manner (Reinbold et al., 2001). In contrast, both BYV and BtMV are non-circulative and non-propagative viruses, with BYV and BtMV being semi- and non-persistent, respectively (Sylvester, 1956). While aphids carrying persistently transmitted viruses (e.g. BMYV and BChV) remain viruliferous for a long period (days to months), non-circulative viruses are acquired and retained for shorter periods. Semi-persistent viruses (like BYV) are acquired and retained for minutes to hours, whereas non-persistent viruses (like BtMV) are acquired and lost within seconds to minutes (Brault et al., 2010; Ng & Falk, 2006).

Until recently, beet yellowing viruses were controlled by targeting the aphid vectors using neonicotinoid insecticides. Since their ban in Europe, the re-emergence of these viruses has caused substantial yield reductions and severe economic losses for the sugar beet industry (Dewar & Qi, 2021; Hossain et al., 2021). Among the yellowing viruses reported in Europe, BYV, BMYV and BChV are generally considered the principal viruses associated with virus yellows in sugar beet. BtMV is also part of this complex, but has been detected less consistently, with reported occurrence varying between countries and years (Hossain et al., 2021). Although BWYV is reported in Asia and was historically significant in the United States of America (Hossain et al., 2021; Stevens et al., 2005b; Wintermantel, 2005; Xiang et al., 2008), recent surveys suggest its current economic impact there is minimal (Chinnadurai et al., 2024). In the EU, it has only been officially reported in beet once, namely in Greece (Lotos et al., 2014). Yellowing viruses typically cause yellowing of the foliage, which can manifest in several ways: leaf mottling or mosaic, yellowing of leaf margins, (inter)veinal clearing and overall yellowing of the leaf surface. Other characteristic symptoms are malformation of the leaves, regrowth of heart leaves, stunting and necrosis (Hossain et al., 2021; Smith & Hallsworth, 1990; Wintermantel, 2005). This can lead to serious losses in terms of root yield, sugar content and sugar extractability. In cases of early infection, yield losses from BChV, BMYV and BWYV can reach 24%, 29% and 30%, respectively (Hossain et al., 2021; Smith & Hallsworth, 1990; Stevens et al., 2004; Wintermantel, 2005). BYV can cause even higher losses, up to 47% (Hossain et al., 2021; Smith & Hallsworth, 1990), while BtMV typically results in less than 10% loss (Wintermantel, 2005). Recent field surveys in Europe have confirmed the natural occurrence of mixed infections involving multiple yellowing viruses (Hossain et al., 2021; Mahillon et al., 2022). These infections can be of epidemiological concern, as co-infection can lead to increased virulence. Specific additive effects on yield loss have been documented for BYV when plants were co-infected with BWYV or BtMV (Kirk et al., 1991; Wintermantel, 2005). Other epidemiological concerns include wild plant species that can act as virus reservoirs, thereby enhancing the spread of yellowing viruses. Identifying these important reservoirs is a fundamental step in developing control strategies. While Beuve et al. (2008), Stevens et al. (1994) and Yoshida & Tamada (2019) previously assessed the host ranges of beet-poleroviruses and BYV, updating the knowledge of local host species remains a critical component for refining control strategies.

Genetic diversity studies are important for understanding the population dynamics and spread of sugar beet yellowing viruses. The principal viruses associated with beet yellowing disease have positive-sense, single-stranded RNA genomes, but differ substantially in genome organisation and size: BYV has a genome of approximately 15.5 kb, BtMV of approximately 9.6 kb, and beet poleroviruses of approximately 5.7 kb (Dolja, 2003; Nemchinov et al., 2004; Stevens et al., 2005b). Recent studies have investigated the genetic variability of these viruses using both complete and partial genome sequences. In Switzerland, Mahillon et al. (2022) generated near-complete genome data for five BYV, three BChV and three BMYV isolates and reported limited sequence variation within this dataset. Partial genome analyses, particularly those targeting the coat protein (CP) region, have also been widely used to compare beet polerovirus isolates and investigate their phylogenetic relationships (Kozlowska-Makulska et al., 2015; Mahillon et al., 2022). CP nucleotide sequences are available for many more isolates than complete genomes, which allows newly obtained isolates to be compared against a broad set of references, whereas

near-complete genomes offer higher resolution across the whole genome. Combining both types of data is therefore valuable, and expanding these datasets remains important for improving our understanding of yellowing virus diversity, phylogeography and the current situation in Belgium.

To develop a comprehensive, integrated and sustainable disease management strategy, it is crucial to improve our understanding of yellowing viruses and their aphid vectors. To contribute to this, we carried out two complementary field surveys in Belgium - one in Flanders and one in Wallonia - and placed the results in a European context by comparing them with published surveys from other countries. Our specific objectives were to identify the yellowing viruses circulating in Belgian sugar beet and characterise their relative detection frequency across seasons and production areas, to describe the frequency and composition of single and mixed infections among the sampled plants, to document the aphids collected in and around sugar beet fields and screen them for the presence of yellowing viruses, to assess whether beet-infecting viruses could be detected in the surrounding vegetation as potential reservoir hosts, and to characterise the genetic diversity and phylogenetic relationships of the three predominant viruses.

Materials and methods

Sampling strategy

Two separate field surveys were conducted, one in Flanders and one in Wallonia. They were designed independently and differed in several methodological aspects, including plant-selection criteria, sampling intensity and timing, and the molecular detection methods applied. Moreover, the surveys were not spatially exclusive, as some locations were only a few kilometres apart. The labels ‘Flanders survey’ and ‘Wallonia survey’ therefore refer to independently designed monitoring efforts rather than to a regional north–south comparison. Differences between the surveys, should be interpreted in light of their different designs rather than as evidence of spatial or epidemiological variation. Direct comparison is therefore limited for parameters potentially influenced by these methodological differences. Each survey is described separately below, with an overview of the respective sampling methodologies provided in Electronic Supplementary Material Online Resource 1.

In Flanders, field sampling was conducted annually from 2022 to 2024 at ten locations in important sugar beet-growing regions, namely the Polders, Ieper, Scheldevallei, Pajottenland, the region around Dijle and Ijse, and the fruit-growing region (Fig. 1). Three sampling timepoints were defined per season: aphid appearance – May/Jun (T1), 4–6 weeks after aphid appearance – Jun/Jul (T2), and the end of the sugar beet growing season – Sep/Oct (T3). Sugar beet plant sampling was conducted in all three years at the end of the season (T3). At each location a total of 15–20 sugar beet samples were collected. Each sugar beet sample consisted of two leaves collected from one individual plant. Sugar beet plants were preferentially selected based on virus-like symptoms. Symptoms considered during sampling included yellowing of or between the veins, yellowing along the leaf margin, complete leaf yellowing, yellowish or green mottling or mosaic, necrosis, regrowth of young leaves in the centre, stunting and leaf deformities. When disease pressure was low, mildly symptomatic and, in some cases, asymptomatic plants were also sampled to reach the target of 15–20 samples per field.

Surrounding vegetation was sampled in 2023 at timepoints T2 and T3 and in 2024 across all three timepoints (T1, T2, T3). These timepoints are in-season timepoints only, meaning that the sugar beet crop was present in the field. At each location and sampled timepoint, a total of 3–5 samples were collected from plants in the surrounding vegetation. Each surrounding-vegetation sample consisted of leaves pooled from three plants of the same species, with the number of leaves or leaflets collected depending on species-specific leaf size. At each timepoint, a new set of plants was selected for sampling, without specific revisitation of previously sampled individuals. Plant selection was not based on symptom expression and was intended to cover a broad range of surrounding plant species. However, the sampling was not entirely random, particularly in 2024, when species in which sugar beet yellowing viruses had been detected in 2023 were sampled more frequently. For all plant sample types, a 100 mg tissue subsample was excised in the laboratory from the leaves in each sampling bag for downstream processing. In total, 499 sugar beet plants and 209 pooled wild plant samples were analysed.

Aphid sampling was performed at timepoints T1 and T2 in 2023 and 2024, with a target of collecting 20 aphids per location at each sampling timepoint. In 2024, multiple collection methods were used, and aphid collection was balanced as much as possible among methods within this target number, depending on aphid availability. This target could not always be reached because aphid availability varied among locations, timepoints and collection methods. Yellow sticky plates (Biobest, Westerlo, Belgium) were used in both years. In 2023, four plates were placed in the sugar beet field and four in the surrounding vegetation at each location, whereas in 2024, two plates were placed in each vegetation type. In 2024, aphids were additionally collected by direct, non-destructive manual suction from plants using a pooter (Grootaert et al., 2010) and by applying glue spray on plant leaves. For manual suction collection, aphids were sampled from five aphid-infested plants in the sugar beet field and five in the surrounding vegetation, when available. For the glue spray method, leaves on eight plants within the sugar beet field and eight plants in the surrounding vegetation were sprayed with Soveurode Special Glue (Witasek, Kärnten, Austria), with the surrounding-vegetation plants selected to represent four different plant species. Yellow sticky plates and glue-sprayed leaves were retrieved after 24 h. In the laboratory, aphids were detached from the adhesive using Citrosol-Extra (ITM, Veldhoven, the Netherlands) and stored individually in 2 mL tubes at − 20°C for molecular identification and virus detection. Due to the limited availability of aphids at some sampling timepoints and locations, a total of 805 aphids were collected and analysed.

In Wallonia, the survey encompassed 32 annual locations within key production zones (Mettet, Nivelles, Tournai, and Waremme) from 2022 to 2024. Sugar beet samples were collected in 2023 and 2024, with individual plants sampled once per location towards the end of the growing season (Aug/Sep) (Fig. 1). At each location, six symptomatic and six asymptomatic sugar beet plants were sampled, with three leaves collected from each plant.

Wild plant species in the surrounding vegetation were surveyed over the full three-year period (2022–2024). Whole wild plants were collected and tested individually rather than pooled, as in the study in Flanders. Surrounding vegetation was sampled during the sugar beet growing season, and the same fields were revisited during the subsequent crop-free period. Growing-season sampling of wild plants occurred between late April and early September in 2022–2023, whereas crop-free follow-up sampling occurred between late January and mid-March of the following year. An additional growing-season survey was

conducted from mid-September to early November 2024. For virus analysis of plant sample, a 100 mg tissue subsample was excised from pooled-leaf tissue of a single plant in the laboratory for downstream processing. In total, 735 sugar beet plants and 1816 wild plants were analysed.

Aphids were collected in 2024 on a weekly basis (when present), from beet emergence (mid-April) until row closure (late June to mid-July, depending on the year). Sampling was performed at 32 field locations, with four additional fields included as experimental platforms for the project. Sampling was performed using yellow sticky traps and manual collection. Four yellow sticky traps were placed at each location weekly and exchanged for the previous set. Additionally, 40 sugar beet plants were sampled for aphids by randomly selecting 8 groups of 5 consecutive plants. Aphid identification was performed morphologically and focused on taxa that could be reliably distinguished within the applied taxonomic framework. In contrast to the Flanders survey, a targeted testing strategy was adopted in Wallonia. Due to the overwhelming field abundance of *A. fabae* and other black aphids, virological analysis were only performed on *M. persicae*, *M. ascalonicus*, *M. euphorbiae* as well as some unidentified aphid species. Specimens collected on sticky traps were detached using soft entomological tweezers without the use of a solvent. Subsequently, aphids were stored individually in 2 mL tubes at -20°C for molecular virus analysis. Sample sizes varied based on aphid availability per field and time point, with a total of 700 aphids analysed.

Nucleic acid extraction

In the study conducted in Flanders, all samples were bead-beaten in a TissueLyser II (Qiagen, Hilden, Germany) using 2 ml tubes, liquid nitrogen and ~ 5 mm steel beads (Simply Bearings, Rijswijk, Netherlands). The TissueLyser II was operated for 1 minute at 30 Hz. Leaf and aphid samples intended for virus detection by RT-qPCR or aphid molecular identification were processed using the KingFisher MagMAX™ Plant RNA/DNA Isolation Kit (Applied Biosystems, Massachusetts, USA) for RNA or simultaneous RNA and DNA extraction, respectively. Samples intended for total RNA Illumina sequencing were extracted using the Spectrum Plant Total RNA Kit (Merck, Darmstadt, Germany). In the survey conducted in Wallonia, virological diagnostics of weeds and aphids followed a two-step scheme. Samples were first screened by RT-qPCR following crude extraction. Plant material (100 mg) was homogenized by bead-beating (~ 5 mm steel bead, 1.5 min, 30 Hz; Retsch MM40), followed by the addition of 1 mL of extraction buffer (PBS, pH 7.4, supplemented with 0.05% Tween 20, 20 $g \cdot L^{-1}$ PVP, and 2 $g \cdot L^{-1}$ ovalbumin). After mixing and brief centrifugation, the supernatant was diluted 10-fold in water and used as RT-qPCR template. Single aphids were processed similarly using 5–10 glass beads (2 mm) in 20 µL TE buffer (10 mM Tris, 1 mM EDTA, pH 8). Positive samples were then subjected to total RNA extraction using the NucleoSpin RNA Plant and Fungi kit (Macherey-Nagel, Düren, Germany) for confirmatory RT-qPCR. For this second extraction, the starting material consisted of either resampled plant tissue that had been stored at -20°C or the crude TE extract from the corresponding aphid sample, likewise stored at -20°C. RNA from beet samples was extracted directly using this method.

Aphid identification

In the study conducted in Flanders, aphids were identified using COI PCR barcoding. PCR was performed using the generic primers LCO-1490 and HCO-2198 (Folmer et al., 1994), in combination with the FastStart *Taq* DNA Polymerase (5 U/µL; Merck, Darmstadt, Germany), according to the manufacturer's instructions, with a standard primer concentration of 0.2 µM. Thermocycling conditions consisted of 5 cycles of 94°C for 30 s, 45°C for 30 s and 72°C for 1 min, followed by 35 cycles of 94°C for 30 s, 51°C for 1 min and 72°C for 1 min. A final elongation step of 10 min at 72°C completed the run. PCR products were analysed on 1.5% agarose gel using Midori Green Direct (Nippon Gene Co., Ltd., Düren, Germany) as staining dye. The PCR product was Sanger sequenced by Macrogen (Amsterdam, the Netherlands). The sequences were processed with Bionumerics software (v8.1.; Applied Maths NV, Sint-Martens-Latem, Belgium) and analysed with BLASTn against the non-redundant nucleotide (nr/nt) database (Altschul et al., 1990). In the Walloon survey, molecular identification of aphid vectors was not performed. Instead, aphids were screened using a stereomicroscope morphologically and identified up to the most precise taxonomic rank.

RT-qPCR virus detection

In the Flanders study, yellowing viruses (BMYV, BChV, BYV, BtMV, BWYV and TuYV) were detected by RT-qPCR using the TaqPath 1-Step Multiplex Master Mix (No ROX; Applied Biosystems, Massachusetts, USA), according to the manufacturer's instructions. In the Walloon survey, yellowing viruses (BMYV, BChV, BYV, BtMV and BWYV) were detected by RT-qPCR using the One-Step Takyon Ultra Probe 4X MasterMix (Eurogentec, Seraing, Belgium), following the manufacturer's instructions. Primer and probe sequences for BMYV, BYV and BChV in the Flanders survey remain confidential, whereas the Wallonia survey utilized those described by Ruh et al. (2023). Detection of TuYV and BWYV was based on the assays described by Buxton-Kirk et al. (2021) and Hellin et al. (2026), respectively. A Cq cut-off of 35, established during assay validation, was used for each RT-qPCR assay, and any sample with a value at or below this threshold was considered positive.

Total RNA Illumina Sequencing

Sequence data were obtained for 31 samples, including 30 field samples from individual sugar beet plants (18 from Flanders and 12 from Wallonia) and one sugar beet plant derived from a BChV aphid culture (Electronic Supplementary Material, Online Resource 2). Plant extracts were processed individually and were not pooled across plants. Sequencing was outsourced to GenomeScan (Leiden, the Netherlands) and Biomarker Technologies (Münster, Germany) and carried out on an Illumina NovaSeq 6000 platform, generating 2 × 150 bp paired-end reads at a target depth of approximately 40 million read pairs per sample. All libraries underwent quality control and DNase treatment. For the GenomeScan libraries, total RNA was extracted using the Spectrum Plant Total RNA Kit (Sigma-Aldrich), and strand-specific libraries were prepared from ribosomal RNA-depleted RNA using the NEBNext Ultra II Directional RNA Library Prep Kit in combination with QIAseq FastSelect. For the Biomarker Technologies libraries, total RNA was extracted using the NucleoSpin RNA Plant and Fungi

Kit (Macherey-Nagel), and libraries were prepared from ribosomal RNA-depleted total RNA. A per-run summary of providers, platforms, flow cells, lanes, read configurations and library-preparation chemistries is given in Electronic Supplementary Material (Online Resource 3).

Bioinformatics

The bioinformatics pipeline was identical for all samples from both surveys. Sequence reads were quality-filtered and submitted to an in house pipeline using VirusDetect (Zheng et al., 2017) for plant virus detection and identification. After initial quality filtering and trimming using Cutadapt (Martin, 2011), the VirusDetect pipeline does a *de novo* assembly of the reads into contigs and subsequently identifies the contigs against a dedicated plant virus genome database. Confirmatory analyses were performed by uploading the reads to CZID (formerly called IDseq) (https://czid.org/version 7.0; Kalantar et al., 2020). For genome reconstruction of Belgian isolates, reads were mapped to reference sequences using BWA-MEM (v0.7.8-r455; Li & Durbin, 2009) with default parameters. SAM files were converted to BAM format and duplicate reads were marked using Picard tools (v1.113). Read mappings were visually inspected in Geneious Prime (v2025.0.2) and consensus sequences were generated using the QUASR pipeline (Watson et al., 2013). The sample metadata, raw read data and viral genome sequences generated in this study were deposited in the NCBI database under BioProject PRJNA1465690. Viral genome sequences were deposited at GenBank [PZ564691 to PZ564732]. A comprehensive list linking our internal sample IDs with their respective BioSample, SRA, and GenBank accession numbers is available in Electronic Supplementary Material (Online Resource 2). Following genome reconstruction, the coat protein (CP) nucleotide sequences were extracted for phylogenetic analysis. For the phylogenetic and pairwise identity analyses, sequences generated in this study were combined with sequences retrieved from public databases, including both nearly complete genome sequences and CP sequences. Phylogenetic trees were generated by first performing multiple sequence alignments with MAFFT (v7.505; Katoh & Standley, 2013). The best-fit nucleotide substitution models were determined using ModelTest (v0.1.7), and maximum likelihood (ML) trees were subsequently reconstructed using RAxML (v8.2.12). To assess the reliability of the internal branches, branch support was assessed using 1000 replicates of the standard Felsenstein bootstrap proportions (FBP). The resulting trees were visualized and annotated in iTOL (v7.5.1; Letunic & Bork, 2007). Pairwise identity matrices were generated in R v4.2.2 (running in RStudio Server v2022.07.2 build 576; R Core Team, 2024) using the following steps: 1) read fasta file in R with BioStrings package (v2.70.3; Pages et al., 2013), 2) align sequences using the AlignSeqs function from the DECIPHER package (v2.30.0; Wright, 2015), 3) calculate pairwise distances using the “shortest” method from DECIPHER’s DistanceMatrix function, 4) cluster the sequences based on their distances using hierarchical clustering with complete linkage, 5) visualize the distance or identity matrix in the clustering order in a heatmap with the heatmap.2 function of the gplots package (v3.1.3.1).

Statistical Analysis

Statistical analyses were conducted in R v.4.2.2 (running in RStudio Server v2022.07.2 build 576). To compare the virus detection frequency between symptomatic and asymptomatic sugar beet samples originating from the Walloon survey, a Fisher’s Exact Test was performed for each virus species separately and for the overall category of at least one virus detected. Differences were considered statistically significant at $p < 0.05$ (indicated as *), with further levels of significance denoted as ** ($p < 0.01$) and *** ($p < 0.001$). Non-significant results were indicated as 'ns'.

Results

Yellowing virus detections across survey years

The sugar beet analyses included 499 plants from the Flanders survey and 372 plants from the Wallonia survey, sampled towards the end of the growing season. Because the surveys differed in sampling design and molecular detection methods, virus detections are reported separately. BWYV was not detected in either survey and is therefore not considered further. TuYV was screened only in the Flanders survey and was excluded from the main figures and tables; the results are either presented in the supplementary material or described only briefly. Consequently, the results below focus on BMYV, BChV, BYV and BtMV.

BMYV was the most frequently detected virus in each year of both surveys, followed by BChV and BYV, whereas BtMV was detected only sporadically (Fig. 2). In the Flanders survey, BMYV was detected in 57.5% of sampled plants in 2022, 38.9% in 2023 and 32.7% in 2024. BChV was detected at similar frequencies across the three years, ranging from 14.7% to 17.5%, whereas BYV was detected most frequently in 2022 (19.5%) and only rarely thereafter (5.4% in 2023 and 2.0% in 2024). BtMV remained below 4% in each year. In the Wallonia survey, BMYV was detected in 78.3% of plants in 2023 and 51.9% in 2024, and BChV in 39.2% and 30.6%, respectively. BYV showed the opposite year-to-year pattern to the Flanders survey, being detected more often in 2024 (27.9%) than in 2023 (17.5%). BtMV was detected only in a single plant (Fig. 2).

Considered across years, BMYV detections were highest in the earliest survey-year available for each survey, decreasing from 2022 to 2024 in Flanders and from 2023 to 2024 in Wallonia. The year-to-year patterns of BChV and BYV were less consistent between the surveys: BChV varied little across the three Flemish years and was detected somewhat less often in 2024 than in 2023 in the Wallonia survey, while BYV was detected less frequently over time in the Flanders survey but more frequently in 2024 than in 2023 in the Wallonia survey (Fig. 2).

TuYV was detected in only 3 of the 499 plants from the Flanders survey, including two plants sampled in 2022 and one in 2024. All three detections occurred together with BMYV and had relatively high Cq values. TuYV was not detected as a single infection.

Single and mixed virus infections

At least one of the four viruses was detected in 60.3% of the investigated plants in the Flanders survey (301/499) and 80.4% in the Wallonia survey (299/372). Each positive plant was assigned to a detection profile according to the virus or combination of viruses detected (Fig. 3).

In the Flanders survey, most positive plants carried a single virus (242/301; 80.4%). BMYV alone was the most common profile (167 plants), whereas other single-virus detections were less frequent: BChV (49), BYV (22) and BtMV (4). In 59 plants, more than one virus was detected, corresponding to 19.6% of the

virus-positive plants (Fig. 3a). In the Wallonia survey, single detections were less predominant (163/299; 54.5%), and more than one virus was detected in 136 plants (45.5%). BMYV alone remained the most common single profile (114 plants), while BMYV+BChV was the most frequent mixed profile, detected in a substantial number of plants (76 plants) (Fig. 3b). The composition of the mixed detections also differed between the surveys. In the Flanders survey, BMYV+BChV (26 plants) and BMYV + BYV (23 plants) were the most frequent combinations and occurred at a similar frequency. In the Wallonia survey, BMYV+BChV clearly predominated (76 plants), followed by BMYV + BYV (30 plants). Detections of three viruses always involved BMYV, BChV and BYV, and were uncommon in the Flanders survey (2 plants) but more frequent in the Wallonia survey (23 plants). No plant was positive for all four viruses. In both surveys, BMYV was part of nearly every mixed detection (55 of 59 in the Flanders survey; 129 of 136 in the Wallonia survey) (Fig. 3).

Symptomatic vs asymptomatic

Within the Wallonia survey, at least one virus was detected in 80.4% of symptomatic plants, compared with 14.6% of asymptomatic plants (Fisher's exact test, $p < 0.001$; Fig. 4). When considered separately, BMYV, BChV and BYV were also detected significantly more frequently in symptomatic than in asymptomatic plants ($p < 0.001$ for each virus). BMYV had the highest detection frequencies, being detected in 65.3% of symptomatic plants and 7.4% of asymptomatic plants. BChV was detected in 34.9% and 1.4% of symptomatic and asymptomatic plants, respectively, while the corresponding frequencies for BYV were 22.6% and 6.6%. BtMV was detected in only one symptomatic plant and no asymptomatic plants, with no significant difference between the two groups ($p = 1.00$).

Variation among production areas

Geographic variation was assessed by production area for BMYV, BChV and BYV. BtMV was excluded from this comparison because it was detected only sporadically (Fig. 5).

BMYV was the most consistently detected virus and was found in every production area in every year for which plants were available. Its detection frequency nonetheless spanned a wide range, from 13% (Polders and Dijle and Ijse, 2024) to 72% (Ieper, 2023) across the Flemish areas, and from 22% (Mettet, 2024) to 92% (Waremme, 2023) across the Walloon areas, with the highest values recorded in Tournai and Waremme in 2023. BChV was also detected in every production area of both surveys over the study period, but not in every production area in every year, and generally at lower detection frequencies than BMYV. In the Flanders survey, BChV showed a relatively stable overall detection frequency across survey-years, but its distribution among production areas was heterogeneous, with low or no detection in some area-year combinations. In the Wallonia survey, BChV was detected in all sampled production areas in both years, with particularly high observed frequencies in Waremme in 2023 (65%). BYV was likewise detected in all sampled production areas over the study period, with the exception of Pajottenland in the Flanders survey, which was only sampled in 2024. Compared with BChV, BYV showed stronger year-to-year variation: in the Flanders survey, BYV was detected in several production areas in 2022 but was infrequent or absent in most production areas in 2023 and 2024, whereas in the Wallonia survey BYV remained recurrent and was more prominent in several production areas in 2024 (42% in Tournai) (Fig. 5).

Detections of beet-associated viruses in surrounding vegetation

As for the sugar beet samples, surrounding-vegetation results are reported separately for the Flanders and Wallonia surveys because of methodological differences between them. A comprehensive list of all wild plant species screened for sugar beet yellowing viruses is presented in Electronic Supplementary Material (Online Resource 4). TuYV findings, screened only in Flanders, are reported in the Electronic Supplementary Material (Online Resource 5).

In the Flanders survey, 25 of 209 pooled surrounding-vegetation samples tested positive for at least one beet virus, representing 11 plant species listed in Table 1. Detections were not evenly distributed among species. *Chenopodium album*, an annual species, accounted for the largest number of detections, with BYV detected in 7 of 30 pooled samples, BtMV in 4 samples, BMYV in 1 sample and BChV in 1 sample. The other beet virus detections in the Flanders survey were sporadic and generally involved single positive pooled samples per plant-virus combination, including detections in annual species such as *Echinochloa crus-galli*, *Lipandra polysperma*, *Persicaria maculosa*, *Polygonum aviculare* and *Senecio vulgaris*, as well as perennial species such as *Artemisia vulgaris*, *Cirsium arvense*, *Hieracium laevigatum*, *Plantago lanceolata* and *Plantago major*. Most of these sporadic detections had high Cq values close to the assay cut-off, consistent with low target concentrations and requiring cautious interpretation.

Table 1
Yellowing-virus detections in surrounding vegetation of Belgian sugar beet fields. The table presents plant species life cycle, the number of samples tested, the number of positive samples per detected virus, mean Cq (± SD), and the season/sampling date of the positive detection(s). Flemish survey: three plants pooled per sample; Walloon survey: individual plants. The main table shows viruses screened in both surveys (BYV, BMYV, BChV, BtMV and BWYV).

Plant species	Life cycle	No. tested	Virus	No. positive	Mean Cq (± SD)	Sampling window & date(s) of positive(s)
Flanders						
Artemisia vulgaris	Perennial	8	BtMV	1	34.8	growing season (2024-05-30)
Echinochloa crus-galli	Annual	3	BYV	1	34.1	growing season (2023-08-04)
Chenopodium album	Annual	30	BYV	7	33.2 (± 1.1)	growing / late season (2023-08-04–2024-09-25)
			BMYV	1	18.8	growing season (2024-08-10)
			BChV	1	29.5	late season (2024-09-25)
			BtMV	4	32.1 (± 2.3)	growing season (2024-06-04 to 2024-08-01)
Cirsium arvense	Perennial	21	BYV	1	34.2	growing season (2023-08-04)
			BMYV	1	33.46	growing season (2024-08-10)
			BtMV	1	34.7	growing season (2024-05-30)
Hieracium laevigatum	Perennial	1	BYV	1	32.6	growing season (2023-08-04)
Lipandra polysperma	Annual	2	BYV	1	34.3	late season (2023-09-11)
Plantago lanceolata	Perennial	5	BMYV	1	33.2	late season (2023-09-11)
Plantago major	Perennial	5	BMYV	1	34.6	late season (2024-09-27)
Persicaria maculosa	Annual	7	BYV	1	33.9	late season (2024-09-25)
Polygonum aviculare	Annual	5	BYV	1	34.6	growing season (2023-08-04)
Senecio vulgaris	Annual	9	BChV	1	34.1	late season (2024-09-25)
Wallonia						
Artemisia vulgaris	Perennial	66	BMYV	1	34.9	growing season (2022-06-28)
Chenopodium album	Annual	87	BMYV	2	30.6 (± 2.2)	growing / late season (2022-07-18 to 2023-09-06)
Cirsium sp.	—	15	BMYV	1	34.5	late season (2023-09-07)
			BYV	1	33.6	late season (2023-09-07)
Convolvulus sepium	Perennial	13	BtMV	1	34.2	late season (2024-10-22)
Fallopia convolvulus	Annual	14	BMYV	1	32.7	growing season (2022-07-18)
Geranium dissectum	Annual	20	BMYV	1	26.1	late season (2023-09-07)
Heracleum sphondylium	Biennial-Perennial	54	BMYV	1	31.2	growing season (2022-06-20)
Lysimachia arvensis	Annual	7	BMYV	1	31.3	growing season (2022-06-20)
Papaver rhoeas	Annual	23	BMYV	1	33.7	growing season (2022-07-18)
Plantago lanceolata	Perennial	26	BMYV	1	33.1	growing season (2022-06-20)
Polygonum aviculare	Annual	58	BMYV	3	30.3 (± 2.9)	emergence / growing season (2022-04-26 to 2022-07-12)
Sonchus arvensis	Perennial	20	BMYV	1	30.5	late season (2023-09-07)
Veronica persica	Annual	48	BMYV	2	31.9 (± 2.1)	growing season (2022-06-20 to 2022-07-18)
Beta vulgaris	Biennial	6	BChV	2	21.7 / 24.4	crop-free (winter) (2023-01-31 to 2023-02-02)
			BYV	2	15.4 / 12.2	crop-free (winter/early spring)

Plant species	Life cycle	No. tested	Virus	No. positive	Mean Cq (± SD)	Sampling window & date(s) of positive(s)
						(2023-02-14 to 2024-03-05)

In the Wallonia survey, Table 1 reports 18 positive individual wild-plant samples among 1816 analysed plants, representing 13 plant species. Most of these detections involved BMYV. *Polygonum aviculare*, an annual species, accounted for three BMYV-positive samples among 58 tested plants, while *Veronica persica*, also annual, accounted for two BMYV-positive samples among 48 tested plants. Single BMYV detections were reported for *Artemisia vulgaris*, *Chenopodium album*, an unidentified *Cirsium* species., *Fallopia convolvulus*, *Geranium dissectum*, *Heracleum sphondylium*, *Lysimachia arvensis*, *Papaver rhoeas*, *Sonchus arvensis* and *Plantago lanceolata*. BtMV and BYV were detected once in *Convolvulus sepium* and *Cirsium* sp., respectively. Every positive wild-plant sample, including those from perennial and biennial species, was collected during the growing season (late April to early October) and yielded Cq values close to the assay cut-off. No wild plant sampled during the crop-free winter/early-spring window tested positive. During that same crop-free window, yellowing viruses were detected in *Beta vulgaris* persisting in field margins, on four occasions between late January and early March (BChV and BYV), with low Cq values (12–24) indicating high-titre infection.

Virus detection in aphids

Aphids, potential virus vectors, were collected, identified and tested for the presence of beet viruses. The Walloon survey used a targeted testing and identification strategy. For this reason, species-level abundance patterns are presented only for the Flanders survey (Fig. 6). In this survey, COI barcode sequences suitable for aphid identification were obtained for 560 of the 805 collected individuals, representing 56 species. Among the identified aphids, *A. fabae* was the most frequently collected species (182/560; 32.5%), followed by *M. persicae* (44/560; 7.9%) and *Rhopalosiphum padi* (41/560; 7.3%) (Fig. 6). The number of aphids collected varied among sampling timepoints and among collection methods (Electronic Supplementary Material, Online Resource 6), with fewer aphids collected at T2 than at T1 in both surveyed years. Because sampling effort was not standardised across the yellow sticky plate, glue spray and manual suction methods, these counts describe the composition of the collected aphid dataset rather than method-specific capture efficiency or field abundance.

Aphids from both surveys were screened for yellowing viruses by RT-qPCR. Because the surveys differed methodologically, aphid-level detection frequencies were neither compared nor combined between Flanders and Wallonia. In Flanders, viral RNA was detected by RT-qPCR in 4 of the 560 aphids (0.7%), excluding TuYV detections (Table 2). BYV was detected in one *R. padi* and one *Capitophorus elaeagni* individual, BChV in one *A. fabae*, and BtMV in one *Tetraneura ulmi*. BMYV was not detected in any of the identified aphids. In the Wallonia survey, 700 aphids were analysed using a targeted testing strategy, and viral RNA was detected in five individuals (0.7%). BMYV was detected in one *M. persicae* individual and one unidentified green aphid, while BtMV was detected in three *M. persicae* individuals. Neither BChV nor BYV was detected in the tested aphids (Table 2). In both surveys, most virus detections in aphids corresponded to mean Cq values close to the assay cut-off, and no additional confirmation assay was performed. These positive RT-qPCR results should therefore be interpreted as molecular detections of target viral sequences rather than as direct evidence of infection, virus retention, or transmission potential.

Table 2
Aphids testing positive for beet yellowing viruses by RT-qPCR in the Flemish and Walloon surveys, with sampling region, date, vegetation type (sugar beet or surrounding vegetation), aphid species, collection method, detected virus and mean Cq value (Cq cut-off = 35). A positive RT-qPCR result should be interpreted as molecular detection of the target viral sequence, rather than direct evidence of infection, virus retention or transmission potential.

Region	Date	Vegetation	Aphid species	Collection method	Virus	Cq value
Flanders						
Polders	13/06/2023	Sugar beet	*Rhopalosiphum padi*	Sticky plate	BYV	33.1
Polders	13/06/2023	Sugar beet	*Capitophorus elaeagni*	Sticky plate	BYV	34.7
Pajottenland	17/07/2024	Surrounding vegetation	*Tetraneura ulmi*	Sticky plate	BtMV	31.6
Pajottenland	18/08/2024	Surrounding vegetation	*Aphis fabae*	Manual collection	BChV	33.9
Wallonia						
Tournai	7/05/2024	Sugar beet	Green aphid*	Sticky plate	BMYV	30.5
Waremme	3/06/2024	Sugar beet	*Myzus persicae*	Manual collection	BtMV	33.7
Experimental field	17/06/2024	Sugar beet	*Myzus persicae*	Sticky plate	BtMV	32.6
Mettet	25/06/2024	Sugar beet	*Myzus persicae*	Manual collection	BtMV	33.0
Tournai	8/07/2024	Sugar beet	*Myzus persicae*	Manual collection	BMYV	32.2
* Could not be determined to species level						

TuYV, which was screened only in the Flanders survey, was detected by RT-qPCR in 38 identified aphids across multiple taxa. Of these, 32 were collected within the sugar beet field and six in the surrounding vegetation, and all were winged (Electronic Supplementary Material, Online Resource 7).

Genetic diversity of the three predominant beet viruses

An overview of the samples from which viral sequences were reconstructed, and corresponding accessions, are provided in Online Resource 2.

Twelve complete BYV genomes were reconstructed from the RNA-seq data of selected samples and compared to a representative subset of publicly available BYV sequences. Genetic diversity analysis indicated that their genomes are highly conserved. CP pairwise identities ranged from 88.89% to 100% (mean 98.24%) (Online Resource 8 – Fig. 1), while full genomes shared between 94.16% and 100% identity (mean 98.57%) (Fig. 7). Phylogenetic analysis of the CP gene (Online Resource 8 – Fig. 1) produced poorly defined branching structures, characterized by short branch lengths (scale = 0.01) and low bootstrap support across potential clades. A notable exception was isolate NC_001598.1 (Ukraine, 1994), which exhibited distinct divergence. Full-genome phylogenetic analysis (Fig. 7) showed slightly improved segregation, with isolates grouping into multiple highly-supported clades, alongside three isolates clustering separately, including NC_001598.1. In both the CP and full-genome phylogenies, Belgian BYV isolates were widely distributed across all observed clusters, indicating a lack of geographic structuring. Clustering could also not be linked to collection date.

Similarly, 17 BMYV genomes were reconstructed and compared to publicly available genomes. Sequence analysis demonstrated limited genetic diversity, comparable to that of BYV. Pairwise identities for the CP ranged from 96.0% to 100% (mean 98.13%) (Online Resource 8 – Fig. 2), while full genomes shared between 95.41% and 99.98% identity (mean 97.42%) (Fig. 8). Phylogenetic analysis of the CP sequences (Online Resource 8 – Fig. 2) revealed some clade formation (tree scale = 0.01), however, bootstrap support was very weak. In the similarity matrix, Wallonia_3663 (Mettet, 2024) showed the lowest identity, sharing only 96.4% with Flanders_114. In the phylogenetic tree, however, Wallonia_711 (Waremme, 2022) clustered further away from the other Belgian isolates than Wallonia_3663 did. In comparison to CP, full-genome phylogenetic analysis (Fig. 8) yielded a tree with high bootstrap support but also short genetic distances (tree scale = 0.01), lacking deep clade divisions. In this analysis, the divergence of Wallonia_3663 was less pronounced. Similar to BYV, the Belgian BMYV isolates did not segregate by geographic region, nor by collection year, although the foreign sequences were clearly separated with high bootstrap support.

In case of BChV 13 genomes were reconstructed and compared to publicly available genomes. This virus exhibited the highest degree of within-species genetic diversity among the three viral species evaluated in this study. Pairwise sequence identities for the CP gene ranged from 92.43% to 100% (mean 96.3%) (Online Resource 8 – Fig. 3), while full-genome identities ranged from 89.09% to 99.76% (mean 96.61%) (Fig. 9). Phylogenetic analysis of the CP gene (Online Resource 8 – Fig. 3) revealed some clade formation, with several terminal nodes showing moderate bootstrap support and visible branch lengths (tree scale = 0.01). This clustering was clearly visible in the pairwise identity matrix. Similarly, full-genome phylogenetic analysis (Fig. 9) robustly resolved the BChV population into major, well-supported clades (bootstraps 99–100), with three additional isolates branching independently. In line with the patterns observed for BYV and BMYV, Belgian BChV isolates formed several separate highly supported clusters, but showed no clear within-country geographic clustering.

Discussion

This study combined two independently designed surveys for beet viruses conducted in the principal sugar beet production areas of Belgium. Although the surveys differed in many methodological aspects, their findings provide complementary insights into virus occurrence. The proportion of sampled plants in which at least one virus was detected differed between the surveys, reaching 60.3% in Flanders and 80.4% in Wallonia. This difference should not be interpreted as evidence of a higher occurrence of virus infection in Walloon fields, because the surveys differed in many methodological aspects, including plant-selection criteria. In Wallonia, six symptomatic plants were selected per location, whereas the Flemish survey included mildly symptomatic or asymptomatic plants when insufficient symptomatic plants were available to reach the target of 15–20 samples per location. This occurred particularly in 2023 and 2024, when disease pressure was lower, likely contributing to the lower proportion of virus-positive samples in the Flemish dataset. Both surveys nevertheless identified the same principal viruses in Belgian sugar beet. BMYV was the most frequently detected virus in every survey-year, while BChV and BYV were detected recurrently but at lower frequencies. By contrast, BtMV was detected only sporadically, while BWYV was not detected. This general pattern is consistent with recent European monitoring, which identified BMYV, BChV and BYV as the principal viruses associated with virus yellows, whereas BtMV was detected infrequently (Hossain et al., 2021). Nevertheless, the relative representation of the three principal viruses varies among countries and years. For example, Hossain et al. (2021) reported that, in 2019, BYV was particularly frequent in samples from Spain, whereas poleroviruses were more frequently detected in samples from France, the Netherlands, and Belgium. In a recent survey of symptomatic beet leaves from the UK, BChV was more frequently detected than BMYV across the three surveyed years, while BYV occurrence varied markedly between years (Harder et al., 2026). Similarly, BChV and BYV predominated among the virus-positive samples examined in western Switzerland in 2020, whereas only two BMYV-positive samples were reported (Mahillon et al., 2022). Temporal variation was also evident within the Belgian surveys, with annual detection patterns differing among viruses: BMYV declined over time in both surveys, whereas BChV was stable in Flanders but declined in Wallonia, and BYV declined in Flanders but increased in Wallonia. These contrasting patterns indicate that temporal changes within the virus-yellows complex are variable and do not reflect a uniform change in overall virus occurrence. Interannual variation in yellowing-virus epidemics can be influenced by factors such as early-season vector abundance, sowing date, weather and landscape composition, as recently demonstrated in a dedicated epidemiological study in Flanders (Rojas-Preciado et al., 2025). In terms of geographic distribution: BMYV, BChV and BYV were detected across nearly all monitored production areas, indicating that the principal yellowing viruses were broadly distributed throughout the Belgian sugar beet-growing regions rather than restricted to a limited part of the country. The only exception was the absence of BYV detections in Pajottenland, although this production area was sampled in one year only and the result should therefore not be interpreted as evidence that BYV is absent from the area. Despite the widespread occurrence of yellowing viruses, virus-detection frequencies differed among production areas and years, and areas with comparatively frequent detections in one year did not consistently retain that pattern in subsequent years. The results therefore point to substantial local and temporal heterogeneity rather than stable geographic clustering of yellowing-virus detections. Comparable spatial variation has been reported at a broader European scale, with the relative representation of BMYV, BChV and BYV differing among countries, production regions and years (Stevens et al., 2005a; Hossain et al., 2021).

At the individual-plant level, viruses occurred either alone or in combination. Although single-virus detections predominated, mixed detections were common and mainly involved BMYV, BChV and BYV. BMYV+BChV and BMYV + BYV were the principal two-virus profiles, while all triple detections comprised these

three viruses. Mixed detections involving these viruses have also been reported in the UK and Switzerland, indicating that their co-occurrence is not specific to Belgium (Harder et al., 2026; Mahillon et al., 2022). The relative representation of the two principal combinations differed between the Belgian surveys, but whether this reflects epidemiological variation or differences in sampling and diagnostic methodology cannot be determined from the present data.

The comparison between symptomatic and asymptomatic plants demonstrates the practical value of symptom-guided sampling. At least one virus was detected in 80.4% of symptomatic plants, indicating that symptom-based selection strongly enriched the survey for virus-positive plants. Visual symptoms can therefore serve as a useful field indicator of virus presence and help target plants for diagnostic testing. At the same time, this association was far from absolute in either direction. At least one virus was also detected in 14.6% of asymptomatic plants, while 19.6% of symptomatic plants tested negative for all four viruses examined. Yellowing symptoms in sugar beet can result from other causes than viruses, including nutrient or water stress, feeding damage by capsid bugs, fungal vascular diseases and herbicide injury (Hossain et al., 2021). Symptom-based sampling therefore provides an efficient approach for detecting and characterising viruses in visibly affected crops, but it does not capture all virus-positive plants.

Genomic analysis of complete genomes and coat protein (CP) sequences showed that BYV and BMYV were relatively conserved, whereas BChV displayed greater within-species variation. This pattern was observed in both the complete-genome and CP datasets, with BChV showing lower mean pairwise identity than BMYV and BYV. These findings differ slightly from those of the Swiss survey, which reported limited variability for all three viruses based on complete genomes, as well as for the CP nucleotide sequences of BYV and BChV (not analysed for BMYV). Such discrepancies may stem from differences in sample size and the specific genomic targets analysed. The recent increase in available (full-genome) sequence data now allows for a more comprehensive analysis. Furthermore, while our study utilized full CP sequences to capture broader variation, the Swiss study relied on a shorter fragment (Mahillon et al., 2022). The greater variation observed for BChV is consistent with recent evidence that several distinct BChV lineages circulate in Europe (Ibrahim et al., 2026). Ibrahim et al. (2026) identified three lineages using the larger CP-readthrough domain and detected putative recombination events in this region and in a near-complete genome. These findings illustrate the complex evolutionary history of BChV, although they do not directly explain the variation observed in the present dataset. None of the three viruses showed consistent clustering by Belgian production area or sampling year. Belgian isolates occurred in several clades together with isolates from other European countries, providing no evidence for geographically distinct Belgian populations. Ibrahim et al. (2026) reported a comparable pattern for Czech BChV isolates, with different lineages occurring both across and within sampling locations, and proposed multiple introductions as one possible explanation. However, testing this hypothesis would require a more extensive survey and sequencing programme.

The potential role of surrounding vegetation as a reservoir for beet yellowing viruses requires careful consideration. According to epidemiological frameworks for multihost pathogens (Haydon et al., 2002; Viana et al., 2014), confirming a true reservoir requires demonstrating not only natural infection in a non-target host, but also the host's ability to permanently maintain the pathogen across crop-free periods and host the identical viral isolate to the target crop. By screening wild plant species for yellowing viruses during both the growing season and the crop-free period, our study addresses only part of these criteria. Overall, beet yellowing viruses were only rarely detected in the surrounding vegetation. Most detections were sporadic and had high Cq values, suggesting low amounts of viral RNA and warranting cautious interpretation, particularly for unexpected host species. *C. album* was an exception in Flanders, where repeated detections of several beet-associated viruses, particularly BYV, provide stronger evidence of genuine infection. In a previous study, experimental work showed that *C. album* was susceptible to BYV, but not to BMYV, under the conditions tested (Stevens et al., 1994). Natural infection with BYV is therefore biologically plausible. Nevertheless, because *C. album* is an annual species occurring mainly during the sugar beet growing season, its infection is more likely to reflect secondary spread or spillover from infected sugar beet fields than an overwintering reservoir role. Several perennial species also tested positive, but these detections generally had high Cq values. Moreover, although no crop-free winter sampling was performed in the Flanders survey, the Wallonia survey did include winter and early-spring sampling, but none of the wild plant species sampled during this period tested positive. Thus, persistence of infection across the period when sugar beet was absent was not demonstrated for any of the wild species examined. The similarly low detection of yellowing viruses in surrounding vegetation in the Flanders and Wallonia surveys is particularly relevant because the surveys differed substantially in sampling intensity, pooling strategy, plant selection and molecular detection methods. These differences prevent direct quantitative comparison but also indicate that the consistently low detection was not dependent on a single sampling or diagnostic approach. Thus, the methodological differences do not weaken but rather support the conclusion that yellowing viruses were uncommon in surrounding vegetation. However, the low frequency of natural detections does not necessarily imply that surrounding plant species are unsuitable hosts. Stevens et al. (1994) observed that although several weeds can be experimentally infected with beet yellowing viruses, these viruses may occur only rarely in weeds under field conditions. The viruses also differ in host range: BYV has a comparatively narrow one, with *Beta* species as its principal sources, whereas BMYV can overwinter in *Beta* species and in several common arable weeds (Stevens et al., 2005a). Borgolte & Varrelmann (2026) experimentally infected 13 of 25 tested cover crop and flower strip plants with BMYV and demonstrated under field conditions that aphids from selected BMYV-infected plants could transmit the virus to sugar beet. However, infection success also varied among species and between greenhouse and field experiments, illustrating that experimental susceptibility and transmission potential do not necessarily translate into epidemiological importance under natural conditions. This distinction between host susceptibility and epidemiological importance is important when assessing potential reservoirs. In this context, persisting *Beta vulgaris* plants in field margins deserve particular attention. Their high-titre detections of BChV and BYV during the crop-free period indicate that they could contribute to virus overwintering. Among the surrounding vegetation examined, they were the only plants found to meet the first two reservoir criteria, as they carried beet-infecting viruses and persisted in the absence of the crop. Their possible role as overwintering virus sources is supported by findings from France, where beet volunteers, regrowth and other surviving cultivated-beet material were frequently associated with beet yellowing viruses. Experimental evidence further indicates that overwintering virus hosts may serve as green bridges when they support transmission back to sugar beet (Nio et al., 2025).

The aphid surveys showed that the collected aphid community comprised 56 identified species, suggesting the availability of a taxonomically diverse set of potential virus vectors. *Aphis fabae* was the most frequently collected species, followed by *Myzus persicae* and *Rhopalosiphum padi*. The prominence of the first two species is epidemiologically relevant because *M. persicae* is an established vector of BMYV, BChV, BYV and BtMV, while *A. fabae* can efficiently transmit BYV (Dusi & Peters, 1999; Limburg et al., 1997; Kozłowska-Makulska et al., 2009). Their frequent occurrence confirms that important vector species were widely present in the surveyed sugar beet agroecosystems. However, despite their abundance, beet-associated viruses were detected in very few

aphids. Both the Flemish and Walloon surveys detected beet yellowing viruses in less than one percent of aphids. As with the surrounding-vegetation surveys, the similar findings across two independently designed surveys using different methodologies strengthen the conclusion that the low detection frequency reflects the sampled aphid populations rather than being specific to a particular sampling or diagnostic approach. When viral RNA was detected in aphids, it was generally associated with high Cq values close to the diagnostic cut-off of 35. Because the analysis included neither reference aphids fed on known virus-infected plants and subsequently transferred to non-host plants for gut clearing nor independent confirmatory assays, these findings require cautious interpretation. A positive RT-qPCR result indicates the presence of viral RNA but does not demonstrate retention of transmissible virus or vector competence. This is particularly important when interpreting unexpected detections in non-established vectors, such as BYV in *R. padi*, BChV in *A. fabae*, and TuYV in monocot-feeding aphids of the genera *Sitobion*, *Metopolophium* and *Rhopalosiphum*. For beet poleroviruses, Kozłowska-Makulska et al. (2009) similarly detected viral RNA in aphids that transmitted BMYV or BChV weakly or not at all, demonstrating that molecular detection and successful transmission are not equivalent.

The low overall detection frequency was accompanied by a mismatch between the viruses detected in aphids and those predominating in sugar beet. In our study, BMYV was the most frequently detected virus in the crop but was rarely detected in aphids, whereas TuYV was detected only sporadically in sugar beet but accounted for most aphid detections. Earlier UK monitoring of trapped *M. persicae* in sugar beet fields likewise detected BMYV less often than TuYV, formerly described as BWYV (0–8% vs 24–67%; Stevens et al., 1995). The comparatively high frequency of TuYV is likely driven by its exceptionally broad host range, which spans more than 20 plant families and includes common weeds and overwintering crops such as oilseed rape (Stevens et al., 2008; Slavíková et al., 2022). Although most TuYV-positive aphids were collected within the sugar beet field rather than in the surrounding vegetation (32 versus 6 of 38), all were winged and may therefore have originated from or moved through other parts of the agricultural landscape. Consequently, the viruses detected in field-collected aphids probably reflect the wider landscape-level virus pool rather than the virus composition observed in sugar beet. Observations from the UK further illustrate that virus-detection frequency in aphids does not necessarily correspond directly with subsequent disease development in sugar beet. Following the withdrawal of neonicotinoid seed treatments, fewer than 0.4% of trapped aphids tested positive for yellowing viruses in 2019 and approximately 0.4% in 2020, whereas national crop incidence increased from 1.8% to 38.1% between the two years (Dewar & Qi, 2021). The severe epidemic in 2020 coincided with exceptionally early and abundant aphid migration following a mild winter, indicating that disease development depends not only on the proportion of virus-positive aphids, but also on aphid abundance and arrival time. Thus, aphid screening may provide information on viruses circulating in the wider agricultural landscape, but neither the composition nor the frequency of viruses detected in aphids should be interpreted as a direct reflection of the viruses predominating in sugar beet or the subsequent extent of disease in the crop. At the same time, low frequencies of viral RNA detection in aphids do not necessarily indicate that aphids are of limited epidemiological importance. A point-in-time molecular survey captures only a small fraction of the aphids involved in transmission over an entire season, and substantial virus spread may occur even when viral RNA is detected in relatively few sampled aphids. Aphid population dynamics further support this possibility, as rapid local colonization by wingless individuals can be followed by the production of winged forms that enable wider dispersal (Fereres & Moreno, 2009; Kirk et al., 1991; Mehrparvar et al., 2013; Van Munster, 2020). Repeated cycles of local colonization and dispersal may therefore contribute to the broad distribution of yellowing viruses over space and time, despite low viral RNA detection frequencies in aphids at any individual sampling time.

In conclusion, the two Belgian surveys consistently identified BMYV, BChV and BYV as the principal viruses associated with virus yellows. Their detection frequencies varied across years and production areas, and mixed infections were common. BChV showed greater genetic variation than BMYV and BYV in the analysed datasets, while none of the three viruses displayed consistent geographic or temporal clustering. Beet-associated viruses were detected sporadically in surrounding vegetation, and no wild species was shown to maintain infection throughout the crop-free period. In contrast, four high-titre detections in *Beta vulgaris* from field margins suggest that these plants may serve as overwintering virus sources and warrant further investigation. From a practical management perspective, this supports the recommendation that farmers should ensure that no sugar beet plants survive during the crop-free period. While vector species such as *M. persicae* and *A. fabae* were widespread in the fields, the low detection of yellowing viruses in these aphids highlights the difficulty of directly linking vector abundance to transmission potential. Together, these findings provide a basis for managing virus yellows in sugar beet.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Data availability

The data supporting the findings of this study are available within the article and its Electronic Supplementary Material. Raw high-throughput sequencing data generated in this study were deposited in the NCBI Short Read Archive (SRA) under BioProject accession PRJNA1465690, with individual samples available under BioSample accessions SAMN59752496–SAMN59752526 and SRA accessions SRR39148104–SRR39148083. Viral genome sequences generated in this study were deposited in GenBank under accession numbers PZ564691–PZ564732. A comprehensive correspondence table linking internal sample identifiers to the BioSample, SRA and GenBank accession numbers is provided in the Electronic Supplementary Material (Online Resource 2)

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Figures

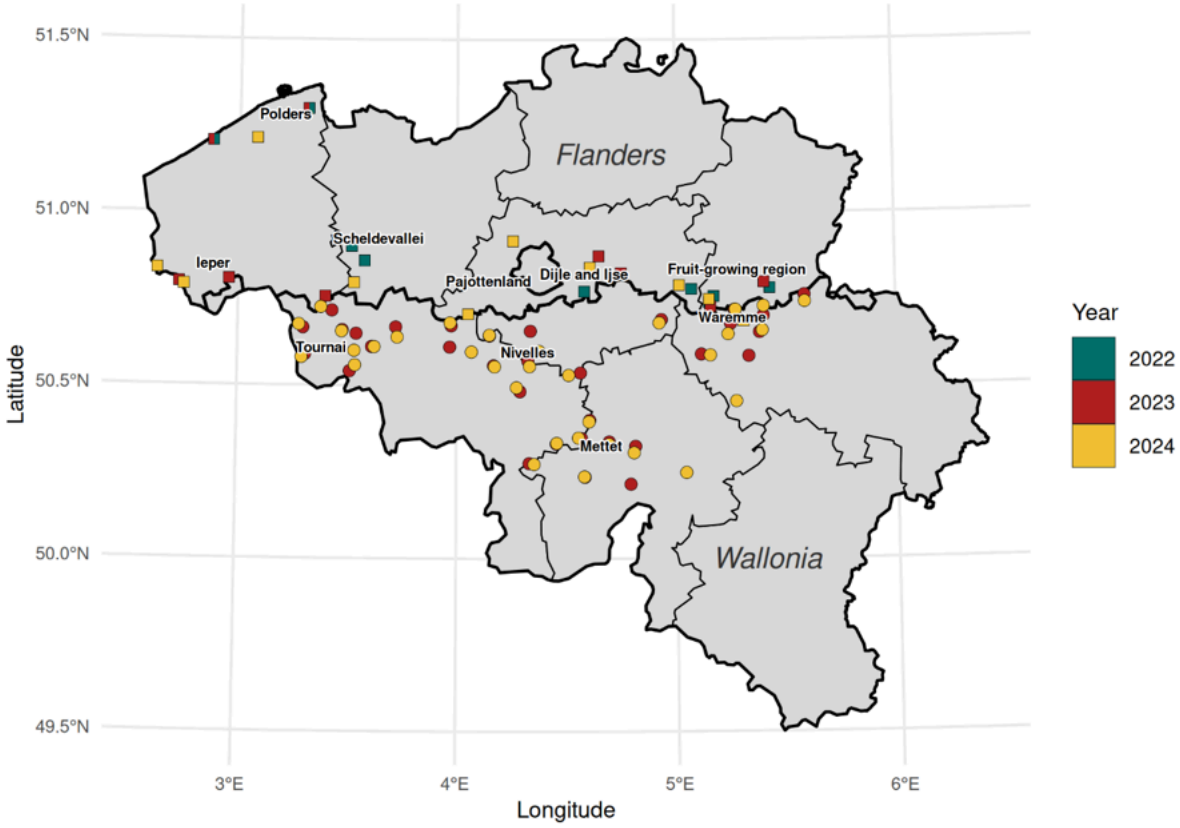


Figure 1

Sampling locations in the Flanders and Wallonia surveys, indicated by squares and circles, respectively.

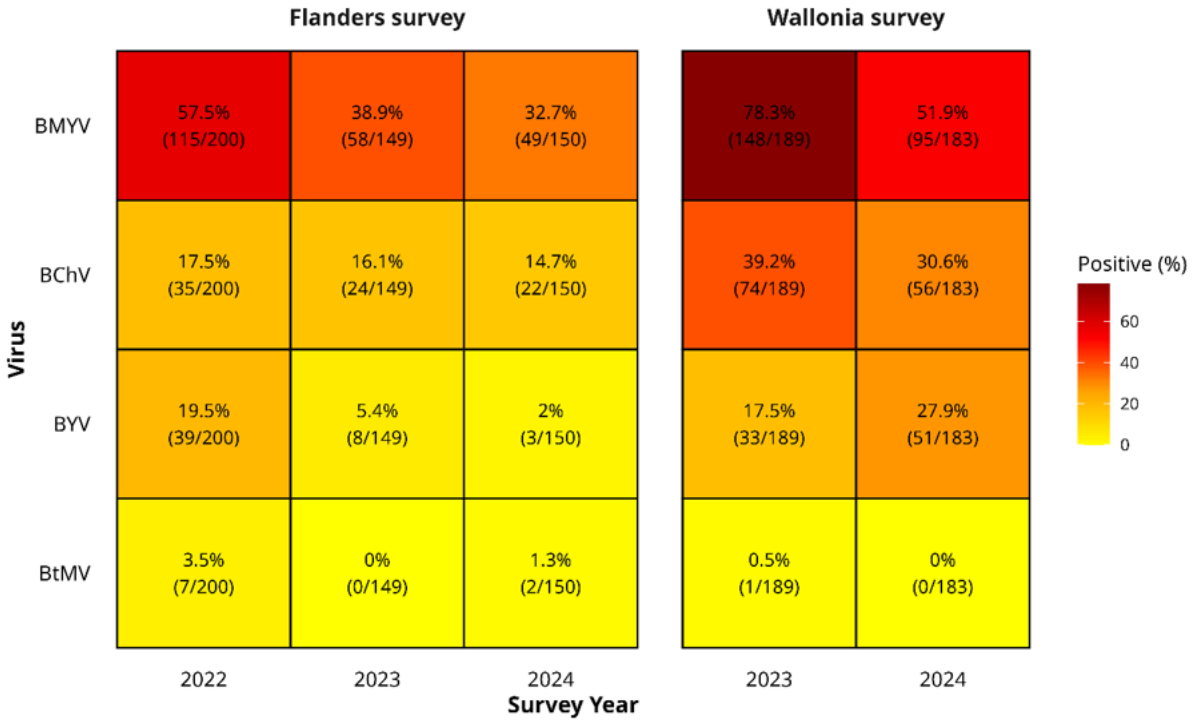


Figure 2

Virus-specific detection frequencies in symptom-selected end-of-season sugar beet samples by survey and year. Values indicate the percentage of sampled plants in which each virus was detected, with the corresponding number of positive samples and total number tested shown in each cell. Samples with mixed-virus detections contribute to more than one virus row, therefore, percentages across viruses are not additive. Values describe observed detection frequencies within the sampled plants and should not be interpreted as estimates of field prevalence.

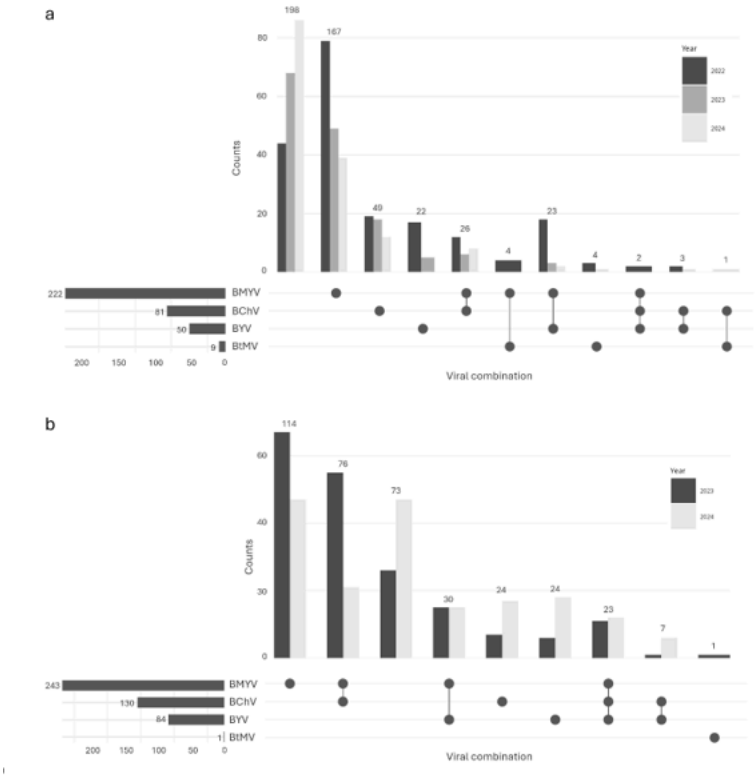


Figure 3

UpSet plots showing single- and mixed-virus detection profiles in end-of-season sugar beet samples collected through symptom-guided sampling in the Flanders survey (a) and symptom-based sampling in the Wallonia survey (b). Horizontal bars indicate the total number of samples in which each virus was detected across all included years and production areas. The intersection matrix identifies the virus or virus combination corresponding to each detection profile, with a single and connected dots indicating single and mixed-virus detections, respectively. Columns without dots represent samples in which none of the target viruses were detected. Vertical bars show the number of samples assigned to each detection profile, subdivided by sampling year as indicated in the legend; total counts across years are shown above the vertical bars.

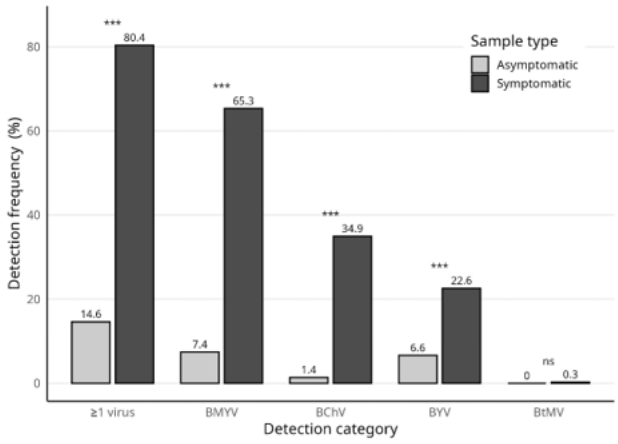


Figure 4

Detection frequencies of four virus species in symptomatic and asymptomatic sugar beet samples from the Wallonia survey. Bars represent the percentage of samples testing positive for each virus. Dark gray bars indicate symptomatic samples and light gray bars indicate asymptomatic samples. Statistical significance was determined using Fisher's Exact Test (ns: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

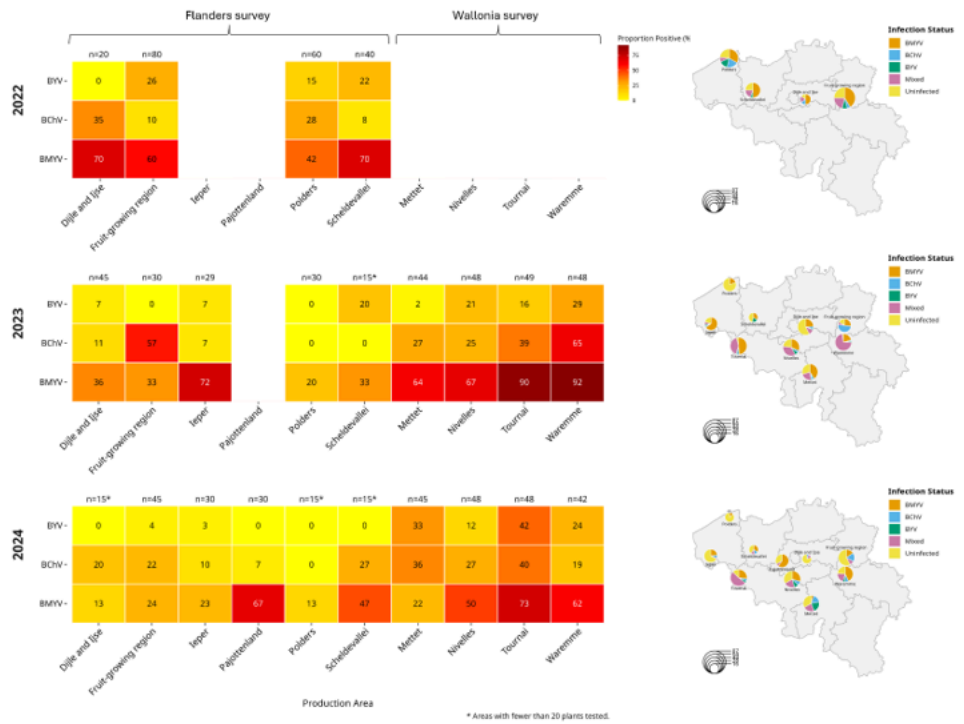


Figure 5

Geographic distribution of the three principal yellowing viruses detected in end-of-season sugar beet samples collected through symptom-guided sampling in the Flanders survey and symptom-based sampling in the Wallonia survey. (Left): Heatmaps showing the percentage of sampled plants in which BMYV, BChV or BYV was detected, presented by production area and year, with n indicating the number of sampled plants. (Right) Map of Belgium with pie charts indicating the proportions of BMYV, BChV, BYV, mixed infections and uninfected samples. The size of each pie chart is scaled to the total sample size per region across all locations presented per year.

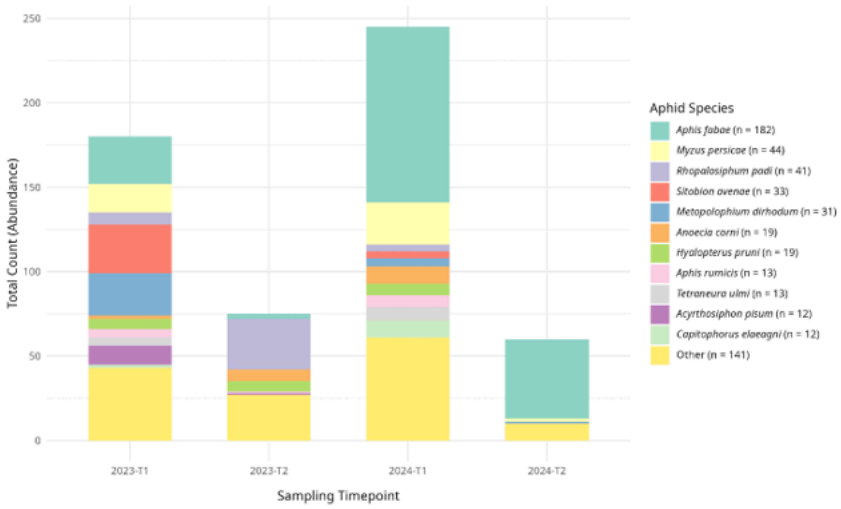


Figure 6

Aphid abundance per timepoint for Flanders, showing the aphid counts of the ten most frequently detected species. The counts reflect the total number of aphids collected across all sampling methodologies.

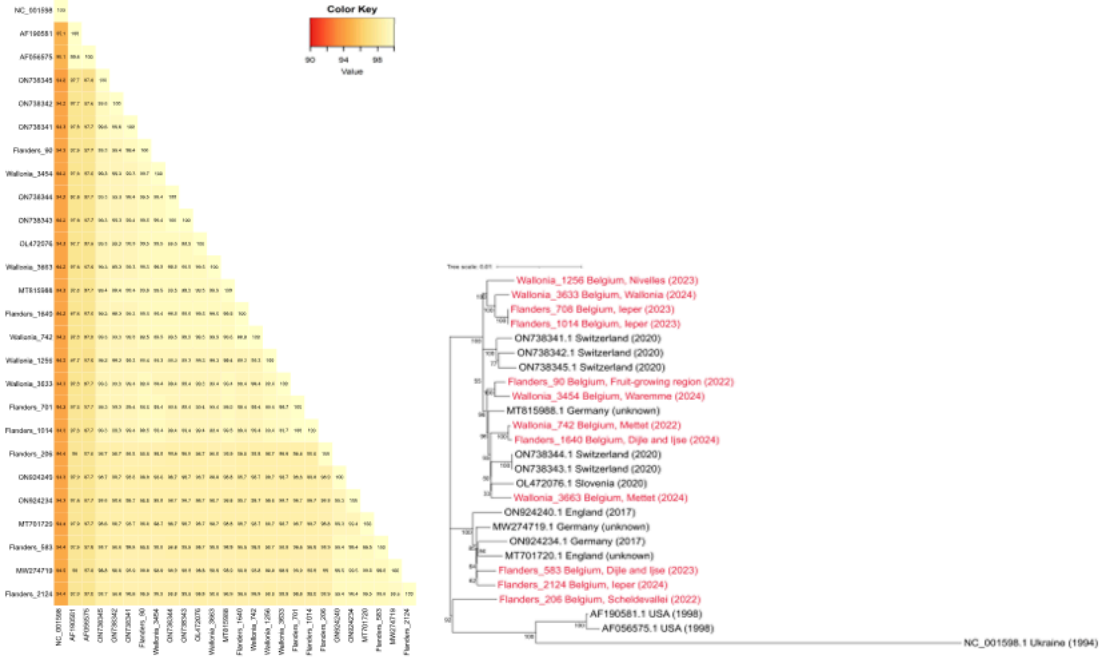


Figure 7

Pairwise identity matrix (left) and phylogenetic tree (right) based on full-genome sequences of Belgian BYV isolates and isolates retrieved from public databases. Belgian isolates are highlighted in red. The phylogenetic tree was constructed using the GTR+G4 nucleotide substitution model. Branch support was assessed using 1,000 bootstrap replicates. Bootstrap support values are indicated at the nodes. Belgian isolates are highlighted in red.

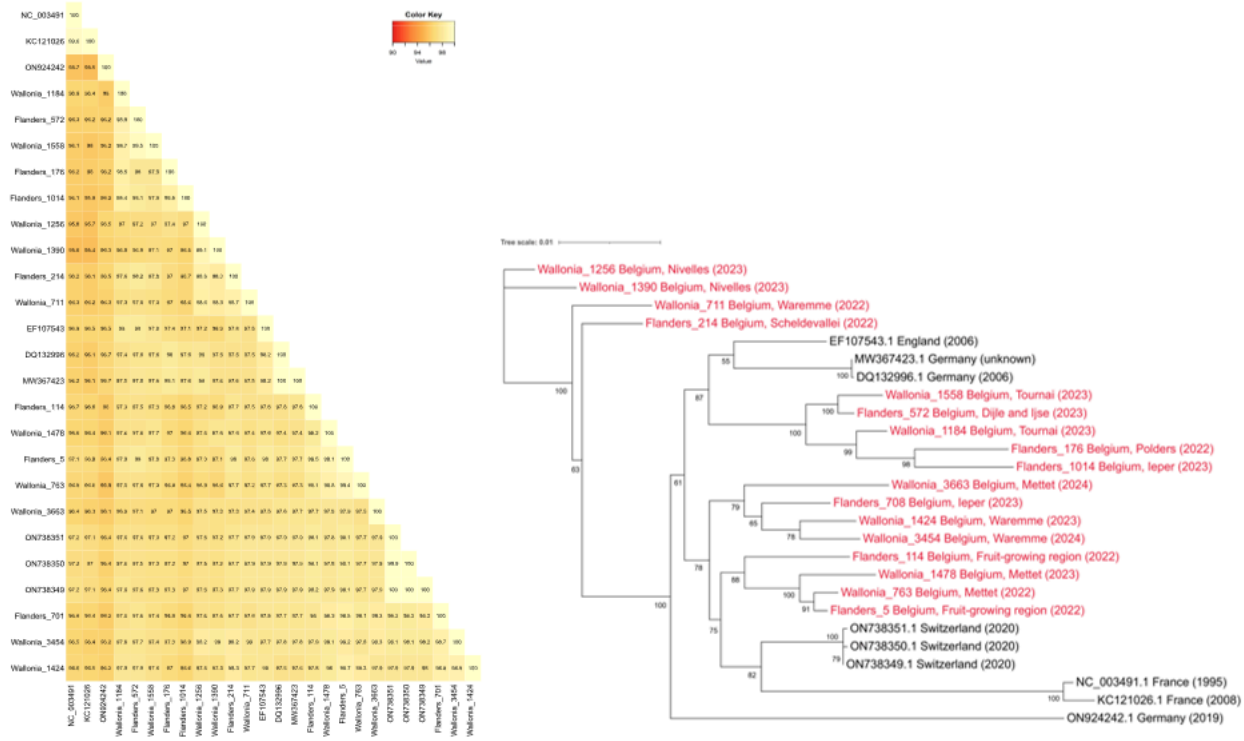


Figure 8

Pairwise identity matrix (left) and phylogenetic tree (right) based on full-genome sequences of Belgian BMYV isolates and isolates retrieved from public databases. Belgian isolates are highlighted in red. The phylogenetic tree was constructed using the HKY+I+G4 nucleotide substitution model. Branch support was assessed using 1,000 bootstrap replicates. Bootstrap support values are indicated at the nodes. Belgian isolates are highlighted in red.

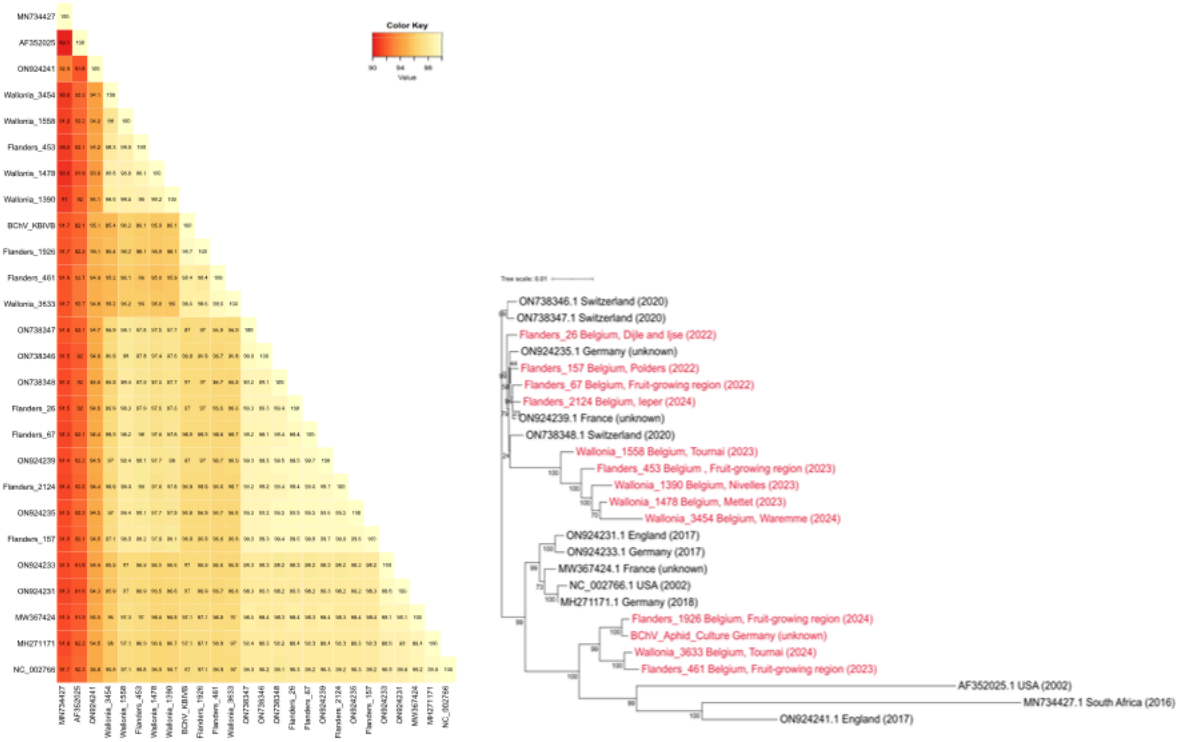


Figure 9

Pairwise identity matrix (left) and phylogenetic tree (right) based on full-genome sequences of Belgian BChV isolates and isolates retrieved from public databases. Belgian isolates are highlighted in red. The phylogenetic tree was constructed using the GTR+I+G4 nucleotide substitution model. Branch support

was assessed using 1,000 bootstrap replicates. Bootstrap support values are indicated at the nodes. Belgian isolates are highlighted in red.

Supplementary Files

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