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NEW DISEASE REPORT

First reports of Apple luteovirus 1, Apple rubodvirus 1 and Apple hammerhead viroid infecting apples in Belgium

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For a pilot study on germplasm collections, leaves were collected from six apple trees (*Malus domestica*) numbered Q9, Q27, Q35, Q37, Q39 and Q41 in a Belgian experimental orchard (CRA-W) in June 2019 and June 2020. At the time of sampling and during subsequent visits, no viral symptoms were observed on the trees. However, *Apple luteovirus 1* (ALV-1) has been identified in trees with Rapid apple decline, *Apple rubodvirus 1* (ARWV-1) is associated with apple rubbery wood disease, and *Apple hammerhead viroid* (AHVd) infection presents variable symptoms including swelling or limb flattening.

Double-stranded RNA (dsRNA) was extracted from all trees except Q9, reverse-transcribed and amplified (RT-PCR) for high-throughput sequencing following the protocol described by Marais et al. (2018). Total RNA was extracted from tree Q9 using the RNeasy Plant Mini Kit (Qiagen, Germany). Library preparation was performed at GIGA (University of Liege, Belgium) with the TruSeq Total RNA Library Preparation Kit (Illumina, USA) for the total RNA sample, and with the NEBNext Ultra II DNA library prep kit (New England BioLabs, USA) for the dsRNA samples. The Illumina Novaseq platform (2 × 150 nt) was used for sequencing, reads were assembled using SPAdes, and viral contigs were identified by BLASTn against the NCBI database. In Q9, three contigs of 7,210 nt (GenBank Accession No. OK398019), 1,606 nt (OK398020) and 1,004 nt (OK398021) showed high identity with the three genome segments of ARWV-1: 98.8%, 98.2%, and 98.2% identity; and 99.9%, 99.6%, and 99.5% coverage with isolates 982-11 segment L (NC_055390), 4342–5 segment M (MF062137), and 1148-13 segment S (MF062132), respectively. Four contigs of 2,629 nt (OK424912), 717 nt (OK424913), 407 nt, and 243 nt showed high identity to ALV-1 isolate PA8 (96.8%; 96.5%; 97.7%; and 93.2% identity with NC_040680, covering 67% of the genome) in Q39. In Q39, another contig (OK398018) of 433 nt was identified as AHVd with 94.7% identity and 100% coverage of isolate SD17_3-3 (MK188692). AHVd was also detected in all other samples. To confirm these detections, RNA extracted from original trees and eight surrounding trees of Q9 and Q39 were tested by RT-PCR, with MangoTaqTM DNA Polymerase (Bioline, Meridian Bioscience, UK) and primers ARWaV-1L3639F - ARWaV-1L4058R for ARWV-1 (Rott et al., 2018), ALuDetF6-ALuDetR6 for ALV-1 (Liu et al., 2018), and AHVd-88F-AHVd-331R for AHVd (Serra et al., 2018). Q9 tested positive for ARWV-1 and Q39 tested positive for ALV-1. All original trees were positive for AHVd by RT-PCR. PCR products of ARWV-1 (Q9), ALV-1 (Q39), and AHVd (Q37) were sequenced by Sanger sequencing at Macrogen Europe, confirming the presence of ARWV-1 (OK216005, 98.4% nt identity to NC_055390), ALV-1 (OK216004, 96.9% nt identity NC_040680), and AHVd (OK216006, 96.3% nt identity to MK188692).

In conclusion, this is the first report of ARWV-1, ALV-1, and AHVd in Belgium. The trees showed no visible symptoms of viral infection, suggesting that the symptoms associated with these viruses and viroid are likely to be variable or latent across different cultivars and environments.

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