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First Report of Ash Shoestring–Associated Virus Infecting Common Ash (*Fraxinus excelsior*) in Ireland

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Ash shoestring–associated virus (ASaV), a member of the species *Emaravirus fraxini* (*Fimoviridae*), was first reported in ash (*Fraxinus* spp.) trees exhibiting chlorotic ringspots, mottling and leaf deformation in Europe in 2021 (Gaskin et al. 2021). Since then, the virus has been detected in symptomatic trees of both common ash (*Fraxinus excelsior*) and manna ash (*Fraxinus ornus*) across Western and Central Europe (Nouri et al. 2025).

In autumn 2024, five young, grafted *F. excelsior* trees in two field trials located in counties Carlow and Kilkenny, Ireland, exhibited symptoms consistent with viral infection, including leaf deformation, chlorotic mottling and line patterns (Figure 1A,B). The four samples from County Carlow were derived from Irish plant material, while the sample from County Kilkenny was grafted using scion wood imported from Austria. RNA was extracted from diseased leaves using an RNeasy Plant Mini Kit (Qiagen, Germany). Illumina paired-end sequencing (150 bp) was carried out on libraries prepared following rRNA depletion, and data for all sequenced samples were deposited in the NCBI Sequence Read Archive (Bioproject PRJNA1274965).

Reads were quality-trimmed and filtered by alignment against the *F. excelsior* genome (Meger et al. 2024). Taxonomic profiling of the filtered reads was conducted using Kraken 2 (Wood et al. 2019), and viral reads were extracted for de novo assembly using metaSPAdes (Nurk et al. 2017). MEGABLAST searches of several assembled scaffolds from the five samples revealed high identity (>90%) with the ASaV genome. No other plant virus sequences were detected in these samples. Subsequently, filtered reads were used for reference-based genome assemblies in Geneious Prime using the longest sequences available in Genbank (Accession Nos. OU466875, OP501824, OU466882, OU466883, and OU466884) as a reference. Complete genomes were obtained for five new ASaV isolates (PV737465–PV737489).

Primers targeting highly conserved regions of ASaV RNA4, ASaV-F1 (5′-CCTGAGACAAGTGGAATGA-′) and ASaV-R1 (5′-CCTAAACCTTTCATCAATCACA-3′) were designed and used for validation by RT-PCR, employing the SuperScript III Reverse Transcriptase kit (Thermo Fisher Scientific, USA) and Classic++ Taq Polymerase Master Mix (Cytek Biosciences, USA), with an annealing temperature of 54°C. Amplification of the expected 309 bp fragment validated the presence of ASaV in all five samples. This diagnostic test also confirmed the presence of the virus in two additional *F. excelsior* plants sampled in spring 2025 from a glasshouse in County Dublin; one showing leaf deformation and mottling, originally from County Wicklow (Figure 1C), and one showing mosaic patterns, recently grafted using scion wood sourced from a mature tree in County Dublin (Figure 1D).

This study represents the first report of ASaV in Ireland and the first detection of the virus outside continental Europe. These findings suggest that the virus is more widespread in *F. excelsior* in Europe than previously recognised. Given that

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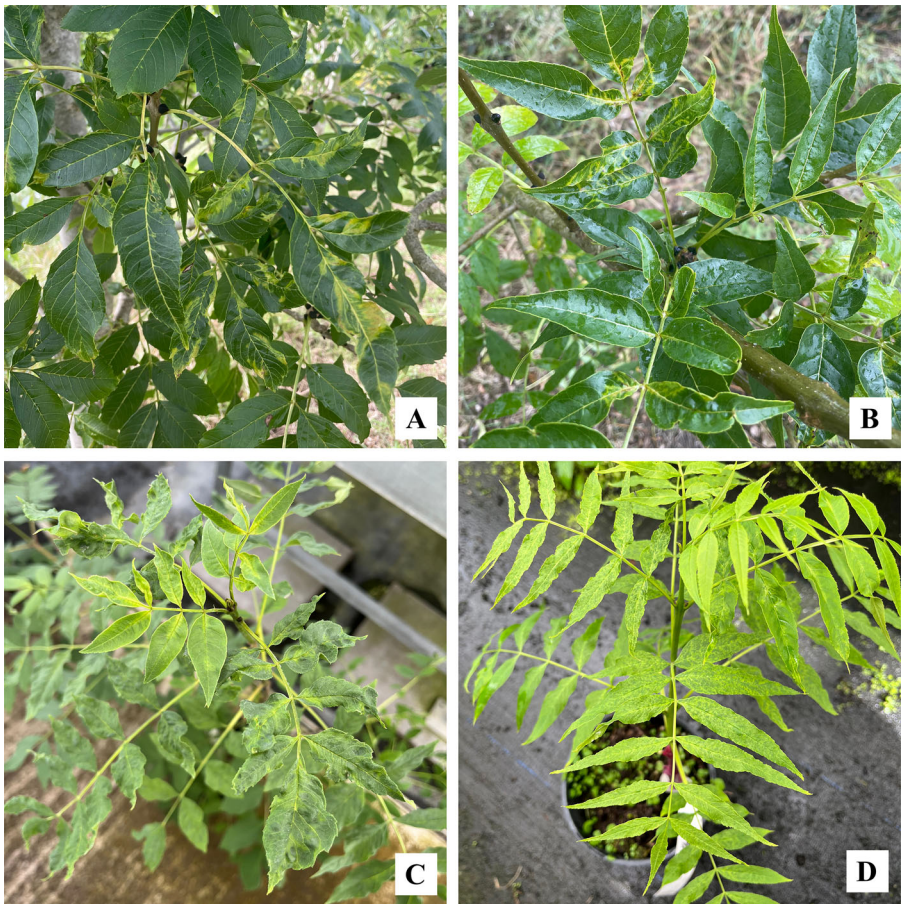


FIGURE 1 | *Fraxinus excelsior* exhibiting symptoms of infection associated with ash shoestring–associated virus in Ireland: (A) leaf deformation and chlorotic mottling and (B) chlorotic line patterns in field trial in County Carlow, and (C) leaf deformation and chlorotic mottling and (D) mosaic in glasshouse in County Dublin.

this species is already under severe threat from ash dieback caused by *Hymenoscyphus fraxineus*, the emergence of viral pathogens such as ASaV, now shown to be more widespread through high-throughput sequencing, warrants closer monitoring. Additionally, potential interactions between fungal and viral pathogens could exacerbate the impact on *F. excelsior* and warrant further investigation.

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