

NEW DISEASE REPORT

First report of raspberry leaf blotch virus on wild raspberry in Belgium, highlighting the presence of multiple RNA segment variants

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KEYWORDS

Emaravirus idaeobati, nanopore, RLBV, *Rubus*



FIGURE 1 Symptoms of leaf blotch and vein banding associated with raspberry leaf blotch virus infection on wild raspberry. Some symptoms may result from other uninvestigated biotic or abiotic factors.

Raspberry leaf blotch virus (RLBV; *Emaravirus idaeobati*) is an emaravirus causing severe damage to raspberry (*Rubus idaeus*), transmitted by the eriophyid mite *Phyllocoptes gracilis*. First reported in the UK (McGavin et al., 2012), it has since been detected in France (Marais et al., unpublished; MK433584.1), Finland, and the Balkan countries (Rehanek et al., 2022). In August 2024, plants from a wild raspberry shrub showing leaf yellowing (Fig. 1) were collected in Sechery, Belgium. After RNA extraction (Botermans et al., 2013), infection was confirmed using generic emaravirus primers targeting RNA1 (Elbeaino et al., 2013). Sequencing the expected c. 360 bp amplicon showed 93.3% identity with RLBV RefSeq (Genbank Acc. No. PV684701). No

amplification was observed in an asymptomatic wild *Rubus* species growing farther away.

A full-length RT-PCR was performed with PDAP213 primers (Di Bello et al., 2015), targeting the conserved termini of all emaravirus RNAs (Rehanek et al., 2022), using Q5 polymerase (NEB, USA). The amplicon mix was purified and sequenced using Oxford Nanopore sequencing (Eurofins, Luxembourg). Bioinformatic analyses were conducted using two workflows publicly available on the EU Galaxy server ("Vir-O-Novo" for *de novo* assembly and "Vir-O-Map" for polishing and annotation). Briefly, quality-filtered reads were assembled *de novo* with CAP3 and screened via tBLASTx against emaravirus RefSeq entries (GenBank, Oct 2024). RLBV-matching contigs were edited in UGENE, aligned with MiniMap2, polished with medaka, and coding sequences were predicted using orfipy. This resulted in the recovery of all RLBV RNA sequences, except RNA1, which were submitted to GenBank (Table 1).

Two distinct variants of RNAs 3 to 8 were identified: variant "a" closer to the RLBV RefSeq, and a more divergent "b" variant, generally showing higher sequencing depth (except for RNA 5). The lowest amino acid identity with the RefSeq was observed for RNA3b (nucleocapsid) and RNA4b (movement protein). These differences were confirmed by RT-PCR using variant-specific primers. For RNA3a, primers from McGavin et al. (2012) were used, whereas new primers were designed for RNA3b (RLBV315-3F: 5'-ACTAAGCAAAGTAGTTGCATTATTC-3' / RLBV315-3R: 5'-CAGAGCAGGATCCAGCAACA-3'), RNA4a (RLBVref-4-F: 5'-GCAATGATCTCCTTGGGAAGTA-3' / RLBVref-4-R:

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**TABLE 1** Sequencing depth of raspberry leaf blotch virus RNA contigs and analysis of amino acid sequence similarity.

RNA	variant	length (nt)	mean read depth	aa identity vs RefSeq (%)	aa identity a vs b (%)	GenBank Acc. No.
2	a	2109	308.4	99.5		PV684688
3	a	1151	8.4	99.7	96.2	PV684689
	b	1351	168.5	95.9		PV684690
4	a	1616	8.9	99.1	93.3	PV684691
	b	1612	157.1	94.2		PV684692
5	a	1692	79.9	99.6	97.5	PV684693
	b	1692	10	97.7		PV684694
6	a	1069	54.1	98.9	97.3	PV684695
	b	1079	132.7	98.4		PV684696
7	a	1064	221.3	98.9	96.8	PV684697
	b	1063	396.7	97.3		PV684698
8	a	1100	8.8	99.4	98.7	PV684699
	b	1276	32.8	97.9		PV684700

5'-AGCACTAAGCTCAAGACAGATC-3'), and RNA4b (RLBV315-4-F: 5'-TCTCTTCCTTAATCTTCCTGAGTTC-3' / RLBV315-4-R: 5'-TTGG GAATCTGGAGCTGGC-3'). Both variants of RNA3 and RNA4 were subsequently detected in other RNA extracts from individual leaves from diseased plants, but not from a healthy raspberry control, indicating either a mixed infection or ongoing RLBV evolution within its perennial host. Co-occurring variants of individual RNAs have been reported for other emaraviruses (Rehanek et al. 2022).

This is the first report of RLBV in Belgium. Although unknown to the raspberry-growing sector in Belgium, the virus likely occurs elsewhere in the country and may have spread unnoticed. Given its impact, raspberry growers should take precautions when sourcing new plants and avoid introducing viruliferous mites.

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