

First detection of *European mountain ash ringspot-associated virus* in rowan trees in Scotland

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Received: 30 Oct 2012. Published: 10 Apr 2013. Keywords: RT-PCR, *Sorbus aucuparia*

Sorbus aucuparia (European mountain ash, rowan) is native to most parts of Europe. In the uplands of Scotland, rowan trees are associated with the native Caledonian pinewoods and are most abundant on the mountain and coastal fringes of the Northwest. Because of its hardiness, the European mountain ash is an important foundation species that fundamentally contributes to the biodiversity of woods (Raspe *et al.*, 2000). Virus-like symptoms on rowan trees such as chlorotic ringspots and leaf mottling were first described six decades ago and have also been reported to occur frequently in mountain ash trees in the United Kingdom (Cooper, 1993). In 2007 these symptoms were associated with *European mountain ash ringspot-associated virus* (EMARaV), the type-species of the newly created Genus *Emaravirus* (Mühlbach & Mielke-Ehret, 2011). During July 2011 chlorotic spots, ringspots, oak leaf line pattern and mottling (Fig. 1) were observed in leaves of 23 mountain ash trees in different geographic regions of Scotland. Trees with indicative symptoms were found in urban areas (Dunvegan, Inverness, Killin, Lawers) as well as in the countryside, growing as roadside trees, as understory in the woods, or in mountainous regions at higher altitudes.

To confirm the presence of EMARaV, symptom-bearing leaflets of *S. aucuparia* collected in four different locations of the coastal northwestern highlands were subject to total RNA isolation and tested for EMARaV by RT-PCR using RNA2- and RNA3-specific primer pairs according to Mielke *et al.* (2008). Amplicons of the expected size (300 bp, RNA2; 204 bp, RNA3) were generated from five out of six analysed samples. Fragments were bi-directionally sequenced and the resulting consensus sequences were subjected to a database search applying BLASTn. Analyses revealed highest sequence identities of the 300 bp fragment (96-97%) with the RNA2-encoded glycoprotein precursor of the EMARaV-type strain from Germany (GenBank Accession No. AY563041), and 97-99% identity of the 204 bp sequence with the 3' untranslated region (3' UTR) of vRNA3 of an isolate from Finland (EU885289), respectively. Obtained sequences were deposited in EMBL-Bank (HF536523-HF536532). In a neighbour-joining phylogenetic tree generated from the partial RNA2 encoding the putative glycoprotein precursor of EMARaV, the sequence variants obtained from Scottish trees formed a distinct cluster (Fig. 2). Comparison of the partial 3' UTR of the RNA3 encoding the nucleocapsid protein of the virus did not corroborate the separate grouping of Scottish EMARaV variants. Phylogenetic

analyses produced two clusters, one including four Scottish samples grouping with the single Russian and with three of the Finnish accessions (Kallinen *et al.*, 2009, Valkonen & Rännäli, 2010). One sequence variant obtained from Scotland clustered together with other EMARaV variants identified in Finnish rowan trees as well as sequences originating from locations in Germany, Sweden, and the Czech Republic stored in GenBank (DQ831831, FR751461, FR751462, HE819379-HE819388). However, clustering was not supported by bootstrap values above 700 (data not shown). EMARaV was never recorded in Great Britain. Here, we report the first detection of the virus from rowan exhibiting chlorotic ringspots in leaves of the Scottish council areas Perth and Kinross, Stirling, and Highland. The Plant Health Service of the Forestry Commission of Great Britain was informed about the presence of EMARaV in diseased rowan trees in Scotland.

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Figure 1

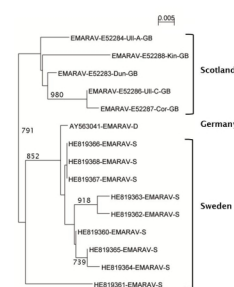


Figure 2

To cite this report: Robel J, Dieckmann L, von Bargaen S, Büttner C, 2013. First detection of *European mountain ash ringspot-associated virus* in rowan trees in Scotland. *New Disease Reports* **27**, 13. [http://dx.doi.org/10.5197/j.2044-0588.2013.027.013]

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