



A follow-up report regarding new hosts and distribution of *European mountain ash ringspot-associated virus* in *Sorbus* spp. in England

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Symptoms consistent with *European mountain ash ringspot-associated virus* (EMARaV), genus *Emaravirus*, have been historically observed across the UK on *Sorbus aucuparia* (rowan or European mountain ash) (Cooper, 1993). The virus was recently confirmed across Scotland (Robel *et al.*, 2013). EMARaV has also been detected by molecular methods in Scandinavia, Russia and the Czech Republic (Grimová *et al.*, 2015).

In England, there was an initial finding of EMARaV from rowan leaves exhibiting chlorotic ring and line-pattern symptoms (Fig. 1.) at the Yorkshire Arboretum, North Yorkshire, in July 2013. A site-specific survey of *Sorbus* spp. was conducted by Fera in July 2014. The virus was also included as a 'priority pest' in the Observatree citizen science project, with further symptomatic samples of *Sorbus* spp. submitted from sites across England.

To confirm the presence of the virus in these samples, total RNA was extracted and tested by RT-PCR (Mielke *et al.*, 2008). Amplicons of the expected size for RNA 2 (300 bp) and RNA 3 (204 bp) were sent for sequencing (Table 1). These were used to construct maximum likelihood (1000 replicates) phylogenetic trees including a representative selection of other EMARaV sequences available on GenBank.

EMARaV was confirmed at the Yorkshire Arboretum in *S. aucuparia* subsp. *sibirica*, and *S. glomerulata*, *S. rehderiana*, *S. sp. aff. scalaris* and *S. pteridophylla*, representing first host records for the latter four species. The virus was additionally detected from *S. aucuparia* from Crawley, West Sussex, Amotherby and Coneythorpe Bank (both North Yorkshire), and in *Sorbus* spp. from southwest London.

A neighbour joining phylogenetic tree generated from the partial RNA2 sequences obtained from the UK findings using MEGA6 (Fig. 2) confirmed the viruses as EMARaV. It also showed that the main outbreak in the Yorkshire Arboretum and Amotherby, c. 5 miles away, may have originated from the same source but that the original 2013 outbreak at the Yorkshire Arboretum, Coneythorpe Bank and London may be distinct from this outbreak and each other (approximately 2% sequence divergence). No RNA2 sequence was obtained from the Crawley sample. Similar results were obtained for RNA3 (data not presented).

Following initial findings in Scotland, a UK pest risk analysis was

conducted (Defra, 2013). These findings support the analysis's hypothesis that the virus is present in a broad range of *Sorbus* species and confirms that EMARaV is present in several different regions of England with the infections likely to be from multiple previous incursions.

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Figure 1



Figure 2

Location	Species	Fera or Yorkshire Arboretum reference	GenBank Accession No.
AM	<i>Sorbus aucuparia</i>	21511951 (RNA2)	MF576285
AM	<i>Sorbus aucuparia</i>	21511951 (RNA3)	MF576286
CB	<i>Sorbus aucuparia</i>	21520689 (RNA2)	MF576287
CB	<i>Sorbus aucuparia</i>	21520689 (RNA3)	MF576297
CR	<i>Sorbus aucuparia</i>	21512131 (RNA3)	MF576295
YA	<i>Sorbus aucuparia</i> subsp. <i>sibirica</i>	1986_33643 (RNA2)	MF576274
YA	<i>Sorbus aucuparia</i> subsp. <i>sibirica</i>	1986_33639 (RNA3)	MF576281
YA	<i>Sorbus aucuparia</i> subsp. <i>sibirica</i>	198633639 (RNA3)	MF576293
YA	<i>Sorbus glomerulata</i>	1999_70 (RNA2)	MF576276
YA	<i>Sorbus glomerulata</i>	199970 (RNA3)	MF576288
YA	<i>Sorbus glomerulata</i>	1999_71 (RNA2)	MF576282
YA	<i>Sorbus glomerulata</i>	199971 (RNA3)	MF576294
YA	<i>Sorbus glomerulata</i>	1999_72 (RNA2)	MF576279
YA	<i>Sorbus glomerulata</i>	199972 (RNA3)	MF576291
YA	<i>Sorbus pteridophylla</i>	2001_1188 (RNA2)	MF576277
YA	<i>Sorbus pteridophylla</i>	20011188 (RNA3)	MF576289
YA	<i>Sorbus rehderiana</i>	2006_4100 (RNA2)	MF576275
YA	<i>Sorbus rehderiana</i>	2006_4102 (RNA2)	MF576286
YA	<i>Sorbus rehderiana</i>	2006_4099 (RNA2)	MF576284
YA	<i>Sorbus rehderiana</i> var. <i>Cupeeae</i>	1999_80 (RNA2)	MF576283
YA	<i>Sorbus sp. aff. S. scalaris</i>	1991_10156 (RNA2)	MF576278
YA	<i>Sorbus sp. aff. S. scalaris</i>	199110156 (RNA3)	MF576290
YA	<i>Sorbus spp.</i>	21312037 (RNA2)	MF576280
YA	<i>Sorbus spp.</i>	21312037 (RNA3)	MF576292
SWL	<i>Sorbus spp.</i>	21620357 (RNA2)	MF576273

Figure 3

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