

Raspberry leaf blotch virus, a new virus isolated from raspberry (*Rubus idaeus*)

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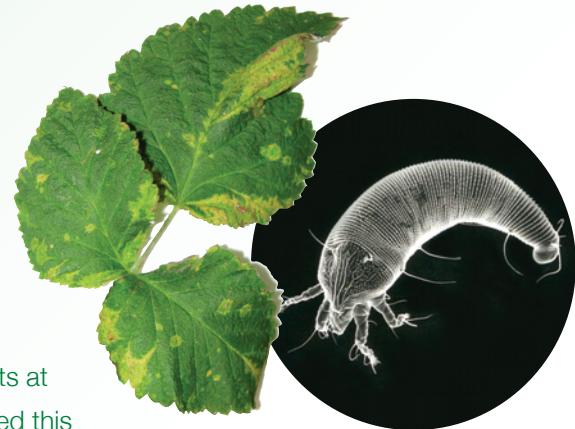
A severe disease occurred in a raspberry crop grown under protective tunnels in Fife, Scotland. The plants displayed strong symptoms of leaf chlorosis, distortion and patchy necrosis combined with a thinning leading to death of lateral branches and subsequent loss of fruit yield. Another clear symptom was a sectoring of the leaf where smooth, apparently hairless patches appeared on the underside of leaves.

However, cloning and sequencing of randomly-amplified cDNA derived from double-stranded RNA extracted from these plants identified a clone with homology to the nucleocapsid gene of wheat mosaic virus (WMV), a virus that was previously referred to as High Plains virus or maize red stripe virus. Fortunately, and unusually for a raspberry virus, we were able to infect *Nicotiana benthamiana* plants by mechanical inoculation with buffered-extracts of the symptomatic raspberry plants. Ds-RNA was extracted from the infected *N. benthamiana* plants, and randomly-primed and amplified cDNA was analysed by 454 sequencing.

The sequencing study has identified four viral RNAs of c. 7200 nt, 2136nt, 1675nt and 1363nt. The predicted translation products of these RNAs have some homologies with viruses in the proposed *Emaravirus* genus, which are segmented, single-stranded negative-strand RNA viruses. The type member of the genus is European mountain ash ringspot-associated virus (EMARAV), and recently the sequence of a related virus, Fig mosaic virus (FMV), was also reported. Partial nucleocapsid sequences show that WMV and Pigeonpea sterility mosaic virus (PPSMV) are probably also members of this genus.

The Raspberry leaf blotch virus (RLBV) RNAs each encode a single protein: RNA1 encodes the RdRP (c. 270kDa); RNA2 encodes a glycoprotein (75kDa); RNA3 encodes the nucleocapsid (32kDa); RNA 4 encodes an unidentified (P4) protein (42kDa). Homology searches using BLASTP show that the proteins of RLBV most closely resemble those of EMARAV and FMV, although shared sequence identity between these viruses is very low.

In the earlier study of tayberry leaf blotch disorder it was found that the infection was not transmitted by grafting from symptomatic plants to new, healthy plants, and that treatment of infected plants with a systemic insecticide reduced the extent of symptoms in subsequent years. Future work will aim to understand how RLBV is transmitted to plants, and to develop a reverse-genetics system for this virus which will allow us to study the life cycle of the virus in greater detail.



Entomologists at SCRI identified this disease as the leaf blotch disorder that had previously been observed in tayberry (a blackberry x raspberry hybrid) and which was associated with the eriophyid mite (*Phyllocoptes gracilis*)¹. Experiments done at that time suggested that there was not a virus associated with this disease.

