New Disease Reports

First report of *Prunus necrotic ringspot virus* and Mulberry cryptic virus 1 in mulberry (*Morus alba*) in the United Kingdom

A. Skelton [1]*, A. Fowkes [1], I. Adams [1], A. Buxton-Kirk [1], V. Harju [1], S. Forde [1], R. Ward [1], M. Kelly [1], P. Barber [2] and A. Fox [1]

[1] *Fera Science Ltd., Sand Hutton, York YO41 1LZ, UK*; [2] *Animal and Plant Health Agency, Sand Hutton, York, YO41 1LZ, UK*

*E-mail: anna.skelton@fera.co.uk

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In September 2017, a sample of mulberry leaf (*Morus alba* cv. Capsrum) was submitted from a nursery in Worcestershire, to Fera Science Ltd. Over 100 trees were affected with a chlorotic oak leaf line pattern on the fully developed leaves which was consistent with viral infection (Fig. 1).

The sample was tested by ELISA for *Arabis mosaic virus, Raspberry ringspot virus, Tomato black ring virus* (TBRV), *Tomato spotted wilt virus* (all antisera provided by DSMZ, Germany), *Impatiens necrotic spot virus, Strawberry latent ringspot virus,* TBRV (Bioreba, Switzerland) and *Cucumber mosaic virus* (CMV) (Agdia, USA). Healthy control mulberry was not available, so a composite of several plants was used, including tomato and *Nicotiana tabacum*. ELISA testing was negative except for a positive reaction for CMV. To confirm the CMV positive result, the mulberry was inoculated onto *Chenopodium quinoa*, *N. glutinosa*, *N. hesperis, N. occidentalis* P1 and *N. tabacum*. Twenty-one days post inoculation no virus symptoms were seen. The sample was also tested by real-time PCR for CMV (Table 1), CMV was not detected. After the initial testing asymptomatic mulberry leaves were also tested by ELISA for CMV and again a positive ELISA reaction was obtained.

To further investigate the virus infection status, the sample was screened using an Illumina MiSeq as described by Adams *et al.* (2014). From these data the presence of *Prunus necrotic ringspot virus* (PNRSV, genus *Ilarvirus*, family *Bromoviridae*) and Mulberry cryptic virus 1 (suggested acronym MuCV1, tentative member of the family *Partitiviridae*) was inferred. The sequences were submitted to GenBank, Accession Nos. MH282499 and MH282498, respectively. No CMV sequences were detected. Considered with the real-time PCR and sap inoculation results, the CMV ELISA reaction was an erroneous result. Given the likely cross reaction of mulberry leaf homogenate with the CMV antisera, mulberry may be a problematic host for ELISA testing. To confirm the PNRSV finding the sample was tested by RT-PCR using primers C and D (Sanchez-Navarro *et al*, 1997) and the resulting PCR product was sent for sequencing. PNRSV was confirmed (MH282500).

Cryptic viruses generally induce no or only very mild symptoms (Boccardo *et al*, 1987), therefore the oak leaf-like pattern seen may be caused by PNRSV, or by a synergistic effect with Mulberry cryptic virus 1. This is the first report of PNRSV in mulberry. As the only other report of Mulberry cryptic virus 1 is an incidental detection from mulberry in Fayetteville, Arkansas, USA (GU145316.1) (I. Tzanetakis, pers. comm.), this is the first report of MuCV1 in Europe.

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References

Adams IP, Skelton A, Macarthur R, Hodges T, Hinds H, Flint L, Nath PD, Boonham N, Fox A, 2014. *Carrot yellow leaf virus* is associated with carrot internal necrosis. *PLoS ONE* **9**, e109125. http://dx.doi.org/10.1371/journal.pone.0109125

Boccardo G, Lisa V, Luisoni E, Milne RG, 1987. Cryptic plant viruses. *Advances in Virus Research* **32**, 171-214. http://dx.doi.org/10.1016/S0065-3527(08)60477-7

Sanchez-Navarro JA, Reusken CB, Bol JF, Pallas V, 1997. Replication of alfalfa mosaic virus RNA 3 with movement and coat protein genes replaced by corresponding genes of Prunus necrotic ringspot ilarvirus. *Journal of General Virology* **78**, 3171-3176. http://dx.doi.org/10.1099/0022-1317-78-12-3171

Figure 1

Table 1. Primer and probe sequences of *Cucumber mosaic virus* real time PCR (TaqMan) assay.

Primer/Probe	Sequence 5′-3′
CMV Forward	GCTTGTTTCGCGCATTCAA
CMV Reverse I	GAGGCAGRAACTTTACGRACTGT
CMV Reverse II	TGAAGGTACTTTCCGAACTGTAACC
CMV Probe	[FAM] TTAATCCTTTGCCGAAATTTGATTCTACCGTGTG [TAMRA]

Figure 2

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