

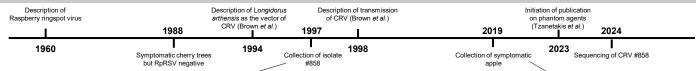
Chasing phantoms

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INTRODUCTION

Safe exchange of plant material relies on accurate diagnostic tools and up-to-date lists of regulated pathogens. However, some of these lists include obscure diseases, often reported only once in non-peer-reviewed publications such as conference proceedings. In a global effort to remove such agents from all regulatory lists, a recent publication identified over 120 agents/diseases for which essential information was lacking (Tzanetakis *et al.*, 2024) and referred them as phantoms. The preliminary list encompassed the cherry rosette virus (CRV). Nevertheless, an isolate of CRV was preserved at Agroscope, which subsequently prompted the present study.



ISOLATE #858

- <u>1997</u> Reception of a cherry tree from Central Switzerland (Arth, Schwyz)
- Tested positive for CRV by ELISA
- Transmission of the virus on herbaceous hosts and conservation of the isolate #858
- <u>2024</u> Genome sequencing from *N.occidentalis* inoculated from material frozen in 2007

#GENOMES

- The analysis of both genomes obtained from HTS revealed that they are of the same species.
- RNA 1 is ~7400 nts (86% ID) coding for one polyprotein of 2133 aa (93% ID)
- RNA 2 is ~6170 nts for #858, and ~5720 nts for #Malus (86% ID). The former codes for a 1685 aa and 1555 aa polyprotein, respectively (82% ID aa).
- The observed disparity in the size of the polyproteins 2 can be attributed to a 139amino-acid (AA) gap in #Malus located between positions 518 and 657 of #858.

Figure 4: Maximum ikelihood phylogenetic tree for the polyproteins encoded by RNA1 (replication block) of 42 members of the genus Nepovius, Fasthere on Galaxy (version 2.1.10) was used with the protein evolution model LG + CAT. Black circles on the branch indicate bootstrap support > 75%. The position of the two CRV isolates is highlighted in bold. Satsuma olwari virus (NP_SC056c1) was used to root the initial trees. Tree was manually



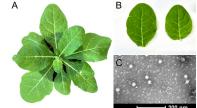
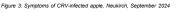
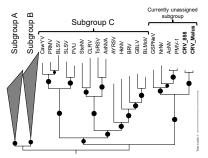


Figure 2: A: N.occidentalis infected with CRV #858 14dpi, B: Healthy (left) and symptoma leave (right) 14dpi; C: Particles observed on Transmission Electron Microscope (TEM)







ISOLATE #MALUS

- <u>2019</u> Reception of symptomatic apple from Nord Switzerland (Neukirch, Thurgau)
- Spherical particles observed by TEM
- New nepovirus sequence obtained by sequencing
- No transmission on herbaceous hosts yet, but successfully grafted on rootstocks

CURRENT PRESENCE OF CRV

- Two infected old cherry trees were found in the Arth region in 2024.
- Survey in the apple orchard where CRV was detected in 2019 showed 52% incidence (99/191).
- > Further survey work is to be conducted.
- No trace of CRV was found by datamining SRAs.
- Phylogenetic analyses show that for both polyproteins, CRV is in a monophylogenetic clade with four recently described nepoviruses, including green Sichuan pepper nepovirus. It is therefore pertinent to consider whether these viruses may form a fourth nepovirus subgroup.

CONCLUSION

With the sequences and plant material now available, this work confirms that cherry rosette virus is not a phantom pathogen. The virus and its vector, *Longidorus arthensis*, appear to be endemic to Switzerland. The absence of documented cases of CRV in cherry trees over the past two decades may be attributed to the observation that symptoms have been noted exclusively in high-stem trees, rather than in newer and more intensive plantations. The detection of CRV on apple, associated with symptoms, in one of the most important fruit-growing regions of Switzerland where the presence of the vector is known, will lead to further research.

References



Schweizerische Eidgenossenschaft Confédération suisse Confederazione Svizzera Confederaziun svizra Département fédéral de l'économie, de la formation et de la recherche DEFR Agroscope