Discovering new viruses in raspberry crops

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Although great care is taken to ensure that raspberry plants that are propagated for commercial sale are virus-free, the perennial growth habit of the plant, which may be cultivated for ten years or more, means that it is exposed to virus diseases in the field for a considerable time allowing for a continual build up of infection. When this is combined with the modern approach of growing the plants under a protective tunnel, which increases both temperature and humidity around the plant, there is ample opportunity for new viruses or new combinations of viruses to move into the crop.

Under experimental conditions, a few raspberry viruses, e.g. *Raspberry bushy dwarf virus* and *Raspberry ringspot virus*, can also infect herbaceous plants and this has made it possible fairly easily to isolate and characterise them. Many other viruses, e.g. *Raspberry leaf spot virus* (RLSV), *Black raspberry necrosis virus* (BRNV) and *Raspberry vein chlorosis virus* (RVCV) are restricted to raspberry plants and are present only at very low levels. This has made them very difficult to study and indeed until very recently all three were only characterised by the apparent symptoms they cause when infecting particular raspberry varieties. Work done in the USA and at SCRI has now provided genome sequence information for BRNV and RLSV, allowing PCR-based diagnostic tests to be developed. The identity of RVCV still remains elusive.

Figure 1 Leaf symptoms of a plant infected with RGLBV and leaf and bud mite.

To obtain further sequence information for raspberry viruses we have been analysing double-stranded (ds) RNA isolated from plants grown in plantations in Fife and Tayside that were showing symptoms of infection such as leaf curling and chlorosis. Production of dsRNA is a characteristic of virus infection, and it can be purified by binding to cellulose powder, allowing it to be concentrated and separated from plant-derived nucleic acids. The dsRNA is then reverse-transcribed into cDNA using random primers and either cloned directly or PCR amplified before cloning. Using this approach we have identified three new viruses, one of which we have completely sequenced.

One virus is most closely related to White clover cryptic virus 1, which is a member of the Alphacryptovirus genus and has a genome comprising two dsRNA molecules. An unusual feature of these viruses is that they are transmitted by infected ovule and pollen to the developing seed but otherwise have no cell-to-cell or systemic movement in the plant and are not graft transmitted.

The second virus has only two known relatives, Wheat mosaic virus and Pigeonpea sterility mosaic virus. These two viruses are rather difficult to work with and have not been characterised in much detail, however, they are thought to have multiple genome segments comprised of negative strand RNA. This means that the virus RNAs present inside the virus particle cannot be translated directly into viral proteins, and so an amount of viral polymerase protein must be included within the particle to initiate replication when the virus infects a new host plant. The wheat and pigeonpea viruses are

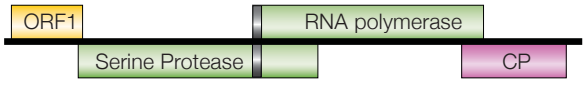

Figure 2 Genome diagram of RuCMV.

transmitted by eriophyid mites, and we suspect that the raspberry virus, which we have named Raspberry green leaf blotch virus (RGLBV), is spread by leaf and bud mite, a pest that is of growing importance to the raspberry industry.

The third new virus, that we have named Rubus chlorotic mottle virus (RuCMV), is a member of the Sobemovirus genus. This virus has a small, positive-strand RNA genome that is encapsidated in spherical particles. We have obtained the complete sequence for this virus, showing that it encodes four major proteins: ORF1, a pathogenicity protein; ORF2, a cysteine protease; ORF3, an RNA polymerase that is expressed by frameshifting as a C-terminal fusion to ORF2 protein; ORF4, the coat protein. We have constructed a cDNA clone of RuCMV from which we can produce synthetic RNA transcripts that are fully infectious when inoculated to herbaceous plants. This confirms that our derived sequence is authentic, and will allow us to do in-depth reverse-genetics studies to understand the molecular details of RuCMV biology.