

First report of *Carrot red leaf virus*-associated RNA co-infecting carrot with *Carrot red leaf virus* and *Carrot mottle mimic virus* to cause carrot motley dwarf disease in New Zealand

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Abstract. Carrot plants with severe leaf reddening and stunting symptoms caused by carrot motley dwarf disease were observed in the South Island of New Zealand. *Carrot red leaf virus* (CtRLV), *Carrot mottle mimic virus* and CtRLV-associated RNA (CtRLVaRNA) were shown to be the components of the virus complex causing the disease. CtRLVaRNA is reported for the first time in New Zealand.

Carrot motley dwarf (CMD) disease causes sporadic but severe losses in early carrot (*Daucus carota*) crops under cool climatic conditions (Watson and Falk 1994). CMD disease has recently been described as being caused by a complex of three viruses: *Carrot red leaf virus* (CtRLV), CtRLV-associated RNA (CtRLVaRNA), and either one of two umbraviruses, *Carrot mottle virus* (CMoV) or *Carrot mottle mimic virus* (CMoMV) (Watson *et al.* 1998). In New Zealand, CMD disease has been observed since 1954 and was thought to be associated with CtRLV and/or CMoV (Pennycook 1989), or CtRLV and CMoMV (Gibbs *et al.* 1996).

In August 2007, carrot plants with severe leaf reddening and stunting symptoms (Fig. 1) were collected from a farm in the Canterbury region, in the South Island of New Zealand. Herbaceous indicator plants (*Chenopodium amaranticolor*, *C. quinoa*, *Nicotiana clevelandii*, *N. tabacum* and *Phaseolus vulgaris*) were inoculated with sap from diseased leaves and maintained in a glasshouse at a mean temperature of 18°C. Systemic chlorotic spotting and distortion symptoms were observed on *N. clevelandii* 14 days after inoculation. The other indicator species did not develop symptoms.

Nucleic acid was extracted from leaves of symptomatic carrot and *N. clevelandii* plants with an RNeasy Plant Mini Kit (Qiagen, Melbourne, Australia). The isolated RNA was used for cDNA synthesis using the SuperScript II RNase H-Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Polymerase chain reaction (PCR) was done in a 20- μ L volume reaction containing 2 μ L cDNA, 10 μ L GoTaq Green Master Mix (Promega, Madison, WI, USA) and 0.25 μ mol each of the forward and reverse primers. PCR products of the expected size (~740 bp and 430 bp) were obtained from both *D. carota* and *N. clevelandii* plants using a published CtRLVa-RNA-specific primer pair (CtRLVaRNA-1/CtRLVaRNA-2; Vercruyssen *et al.* 2000), and novel CMoMV

specific primers (forward 5'-AAAGGGCAAGGTATGAGCAA-3', reverse 5'-TGAGCAGGTCCACAAACTCC-3'), respectively. An amplicon of 211 bp was obtained from *D. carota* using a CtRLV-specific primer pair (CtRLV-1/CtRLV-2; Vercruyssen *et al.* 2000) but not from the inoculated plants of *N. clevelandii*. The PCR products were sequenced directly. Basic Local Alignment Search Tool (BLAST) searches in GenBank showed that the 740, 430 and 211 bp nucleotide sequences had 95%, 97% and 92–97% identities with CtRLVaRNA (GenBank Accession number AF020616), CMoMV (GenBank Accession number U57305) and CtRLV (GenBank Accession numbers AY695933, AY325503 and AY325505), respectively.

In order to determine if the second umbravirus, CMoV, was also co-infecting the symptomatic carrots, a PCR was performed using the umbravirus-specific primers (Umbr-NNS/Umbr-FDQH) reported by Vercruyssen *et al.* (2000). A single product of 545 bp was obtained from the *D. carota* plants. The amplicon was cloned using TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) and five clones were sequenced. The sequences were identical and had 96% identities with CMoMV (GenBank Accession number U57305), confirming that the sample was infected with CMoMV, not CMoV.

Initial reports of CMD disease in New Zealand suggested that the disease was caused by CtRLV and/or CMoV (Pennycook 1989). More recently, Gibbs *et al.* (1996) concluded that the disease was caused by CtRLV and/or either CMoV (in the UK and Morocco) or CMoMV (in New Zealand). This report supports the hypothesis that CMD disease in New Zealand is caused by a combination of CtRLV, CMoMV and CtRLVaRNA. CtRLVaRNA has been reported previously to be the third component of the CMD disease complex and is always present in CMD-affected plants in California, USA (Watson *et al.* 1998) and the UK (Morton *et al.* 2003). This is the first report of



Fig. 1. *Daucus carota* plants showing severe leaf reddening and stunting symptoms typical of carrot motley dwarf disease.

CtRLVaRNA in New Zealand. The study found no evidence for the presence of CMoV in the samples studied but further research is required to clarify whether the virus is present in New Zealand.

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