Nandina domestica, a new host of Apple stem grooving virus and Alternanthera mosaic virus

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Abstract. An ornamental plant, *Nandina domestica*, has been found to be infected for the first time with *Apple stem grooving virus* (ASGV) in New Zealand and *Alternanthera mosaic virus* (AltMV) in Oklahoma, USA. ASGV may infect *N. domestica* latently and the symptoms observed in New Zealand are thought to be induced by the co-infecting *Cucumber mosaic virus* (CMV). Similarly, *Plantago asiatica mosaic virus* (PIAMV, syn. *Nandina mosaic virus*) was found to co-infect with AltMV in the USA, and therefore the observed symptoms may be induced by AltMV and/or PIAMV.

Nandina domestica (heavenly bamboo) is an ornamental shrub and is widely grown as a landscaping plant in many countries including New Zealand and the USA. This species has previously been found to be infected with *Cucumber mosaic virus* (CMV) in the USA (Barnett and Baxter 1973) and New Zealand (unpublished results), as well as *Plantago asiatica mosaic virus* (PIAMV, syn. *Nandina mosaic virus*) and *Nandina stem pitting virus* (NSPV, a tentative species) in the USA (Ahmed *et al.* 1983; Hughes *et al.* 2005).

In April 2004, a variety of symptoms including leaf mosaic and rolling (Fig. 1a) were observed on a clump of *N. domestica* plants in a public garden in Auckland, New Zealand. *Chenopodium amaranticolor* and *C. quinoa* inoculated with sap from symptomatic leaves developed small necrotic local lesions, followed by systemic epinasty on new growth (Fig. 1b, c). No systemic symptoms were observed from inoculated plants of *Phaseolus vulgaris* and *Vigna unguiculata*. Flexuous, filamentous virus particles ~650 nm long were observed by transmission

Fig. 1. *Apple stem grooving virus* and *Cucumber mosaic virus* (CMV) co-infecting *Nandina domestica* (a) and induced symptoms on inoculated *Chenopodium amaranticolor* (b) and *C. quinoa* (c). CMV was detected only in the inoculated lower leaves; these leaves had necrotic local lesions and are not shown in these pictures.

electron microscopy (TEM) of crude sap preparations from systemically infected *C. amaranticolor* and *C. quinoa*. Plants of *N. domestica* and the two inoculated indicator species containing virus particles tested positive using *Apple stem grooving virus* (ASGV, *Capillovirus*, *Flexiviridae*) polyclonal antiserum in double-antibody sandwich (DAS) – enzyme-linked immunosorbent assay (ELISA) (Loewe Biochemica GmbH, Sauerlach, Germany). Nucleic acid was extracted from leaves of plants of each of the three infected species with an RNeasy Plant Mini Kit (Qiagen, Melbourne, Vic., Australia) and then used in reverse transcription–polymerase chain reaction (RT–PCR) tests using ASGV-specific primers (MacKenzie *et al.* 1997). PCR products of the expected size (524 bp) were obtained, and an amplicon from *N. domestica* was cloned using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, CA, USA) and sequenced. The obtained consensus from four clones was deposited in the GenBank database under the accession reference GU126685. A BLAST search in GenBank showed 98% nucleotide sequence identities with several isolates of ASGV (GenBank accessions AB004063, D14455, D16681 and D16368). The ASGV-infected *N. domestica* plants were found to be co-infected with CMV using a virus-specific RT–PCR (Raj *et al.* 1998). Subsequent testing of an additional three plants from the site showed that *N. domestica* plants which were infected with ASGV alone were symptomless. This suggests that the symptoms observed in *N. domestica* were caused by CMV or a synergistic interaction between CMV and ASGV.

In May 2009, a further six samples of *N. domestica* with chlorotic ringspot and/or distortion symptoms (Fig. 2a) were collected from Oklahoma, USA. Sap from symptomatic leaves induced local and systemic necrotic or chlorotic symptoms on inoculated *C. amaranticolor* and *C. quinoa* (Fig. 2b, c). Filamentous particles of ~550 nm were observed by TEM from the *N. domestica* samples and the two inoculated species. However, these infected plants tested negative for ASGV using the ELISA and RT–PCR methods described above. PCR products of ~740 bp were obtained using generic potexvirus primers (van der Vlugt and Berendsen 2002). One amplicon from *N. domestica* was cloned and five clones were sequenced

in both directions. Two distinct sequences were recovered and BLAST searches showed that sequences had 93% (GenBank Accession number GU126686) and 95% nucleotide sequence identities with *Alternanthera mosaic virus* (AltMV, *Potexvirus*, *Flexiviridae*) (GenBank accession AY863024) and PIAMV (GenBank accession AY800279), respectively. In order to confirm the presence of AltMV in the *N. domestica* samples, forward (5'-CCACATCCAATCTCCTTCCA-3') and reverse (5'-AGAGTTGCTGGCGAAGTTGT-3') primers specific to AltMV were designed to amplify a 495 bp fragment in the coat protein gene of this virus. A single product of the expected size was obtained from all *N. domestica* samples and the symptomatic indicator plants. The *N. domestica* plants were shown to be co-infected with PIAMV using RT–PCR with novel PIAMV-specific primers (data not shown) and therefore it is not known whether the observed symptoms were induced by AltMV and/or PIAMV.

ASGV has been reported to infect several important horticultural crops, including *Actinidia*, *Citrus*, *Malus*, *Lilium*, *Prunus* and *Pyrus* (Clover *et al.* 2003). In New Zealand, some apple (*Malus domestica*) cultivars are commonly but latently infected with ASGV (Wood 1996). A BLAST Align comparison of the partial coat protein gene of the *Nandina* isolate of ASGV with an isolate from *M. domestica* collected in New Zealand (GenBank accession GU250886) showed 90% and 97% nucleotide and amino acid identities, respectively. These two isolates of ASGV also showed similar reactions in ELISA using an ASGV antiserum but were found to have different experimental host ranges. The *Malus* ASGV isolate induced symptoms on *C. quinoa*, *P. vulgaris* and *V. unguiculata*, but not on *C. amaranticolor* whereas the *Nandina* isolate induced symptoms on both *Chenopodium* species but not on *P. vulgaris* or *V. unguiculata*.

AltMV was first described in *Alternanthera pungens* in 1999 (Geering and Thomas 1999) and was subsequently reported in *Angelonia*, *Crossandra*, *Phlox*, *Portulaca*, *Scutellaria* and *Torenia* (Baker *et al.* 2006; Hammond *et al.* 2006; Duarte *et al.* 2008; Lockhart and Daughtrey 2008). This is the first report of ASGV and AltMV infecting *N. domestica*.

Fig. 2. *Alternanthera mosaic virus* (AltMV) and *Plantago asiatica mosaic virus* co-infecting *Nandina domestica* showing various symptoms (a) and necrotic local spots and systemic chlorotic spotting symptoms induced on mechanically-inoculated *C. amaranticolor* (b) and *C. quinoa* (c). All plants tested positive for both viruses except *C. amaranticolor* which only tested positive for AltMV.

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