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Short communication

Differentiation between two potyviruses in *Alstroemeria*

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Abstract. *Alstroemeria* mosaic virus (AlMV) is one of the viruses known to occur in *Alstroemeria* spp. Its detection in DAS-ELISA needed improvement. The often simultaneous presence of a second potyvirus has been mentioned by various authors. The recently detected virus in *Alstroemeria*, tentatively named *Alstroemeria* streak virus [AlSV; Wong, 1992] was multiplied in indicator plants and had a host range similar to that of AlMV, although the symptoms in these hosts were less severe. Both viruses reacted with antisera prepared in the Netherlands and in Great Britain to AlMV-isolates purified from infected *Alstroemeria* plants, and from *Nicotiana clevelandii*, respectively. Where AlSV occurs separately, distinction from AlMV is possible by its negative reaction with potyvirus group-specific monoclonal antibodies.

In the last decade the ornamental crop *Alstroemeria* has gained interest all over the world. Most of the starting material is produced in the Netherlands. The crop has been multiplied vegetatively for many years, so viruses occurring in the crop have also been multiplied. Several viruses have been described [Hakkaart and Versluijs, 1985; Phillips and Brunt, 1986; Bellardi et al., 1992]. Of these, *Alstroemeria* mosaic virus [AlMV; Brunt and Phillips, 1981] was thought to be the most frequently occurring one. It is correlated with decrease in quality and number of flower-stems [Van Zaayen et al., 1992]. A double antibody sandwich (DAS-)ELISA with a polyclonal antiserum [Maat, 1983, 1989] has been used in the Netherlands for several years to detect AlMV. The virus can be eliminated by meristem culture [Hakkaart and Versluijs, 1985, 1988]. A successful application of the latter, however, is dependent on a reliable detection method for the virus. Certification of *Alstroemeria* plants by the General Netherlands Inspection Service for Ornamental Crops (NAKS) also requires reliable detection methods for viruses. As there were doubts about the

reliability of the DAS-ELISA for detection of AlMV, a study was started to improve the detection method [De Blank et al., 1994].

Preliminary results indicated an erratic distribution of the virus in *Alstroemeria* plants, as was shown by comparison of immunosorbent electron microscopy (ISEM) and ELISA, and ISEM-studies revealed the presence of other potyviruses. In all investigated plants showing flower colour breaking, a potyvirus was present, which was usually accompanied by AlMV [De Blank et al., 1994]. Other potyviruses have earlier been noticed in *Alstroemeria* by Hakkaart and Versluijs [1985], J. Dijkstra [pers. comm., 1985] and by Bellardi et al. [1992].

Wong [1992] reported the correlation of a potyvirus with streak symptoms of *Alstroemeria* leaves in a glasshouse in Ithaca, USA. Inoculated test plants (i.e., *Nicotiana benthamiana*, *N. clevelandii*, *N. glutinosa*, *Chenopodium amaranticolor* and *C. quinoa*; Wong, pers. comm., 1992) did not produce symptoms. Although naturally infected *Alstroemeria* plants only yielded a small amount of virus, Wong [1992] was able to perform some purification trials and observed both pinwheel and laminated inclusions in the cytoplasm of leaf tissues. He found no reactions with an antiserum to AlMV and with Agdia's Potyvirus group test, but a positive reaction with a polyclonal potyvirus group-specific antiserum provided by D.D. Shukla. This virus was tentatively named *Alstroemeria* streak virus [AlSV; Wong, 1992].

Three virus-free *Alstroemeria* plantlets grown from meristems, and three different varieties (i.e., 37B, Japan and P1) of *N. occidentalis* (one plant of each) were inoculated with 6 μg of purified AlSV, kindly provided by S.M. Wong, in 1 ml of cold 0.05 M potassium phosphate buffer, pH 7.2. Carborundum, 400 mesh, was used as an abrasive. The plants were kept in a glasshouse at 20–22 °C with 12 h light. After two weeks, only the variety Japan (*N. occidentalis*) showed round chlorotic spots c. 0.6 cm in diameter. When such spots were punched out, chopped in water with a razor blade [De Blank et al., 1994] and samples viewed in an electron microscope (Philips CM12) after negative staining with 2% (w/v) sodium phosphotungstate, pH 6.5, potyvirus-like particles were observed.

Subinoculation of part of a leaf with chlorotic spots of *N. occidentalis* Japan, crushed in 10 ml of cold buffer, onto a range of test plants resulted in various symptoms (Table 1). Particles resembling a potyvirus were always found in crude preparations from symptomatic leaves. The symptoms resembled, but were not equal to and were certainly weaker than, symptoms obtained after inoculation of sap from various *Alstroemeria* plants infected with AlMV and, possibly, a second potyvirus, on indicator plants [De Blank et al., 1994]. The symptoms caused by AlSV also differed from those obtained by inoculation of a range of test plants with sap from *N. clevelandii*-leaves, infected with an AlMV-isolate, kindly provided by A.A. Brunt (Fig. 1). In all three cases, however, a similar host range was involved and only minor differences in symptoms were observed.

Table 1. Symptom development after inoculation of sap from *Nicotiana hesperis* 67A with *Alstroemeria* streak virus on indicator plants

Indicator plant	Symptoms
Chenopodium amaranticolor	L_c
Hyoscyamus niger	L_c
Nicotiana benthamiana	L_c, S_c
N. clevelandii	L_c, S_c
N. glutinosa	0
N. hesperis 67A	L_c, S_{cn}
N. occidentalis 37B	S_c
N. occidentalis Japan	L_c, S_c
N. occidentalis P1	L_c, S_{cn}
Tetragonia expansa	L_c

L = local; S = systemic; c = chlorotic; n = necrotic; 0 = no symptoms.

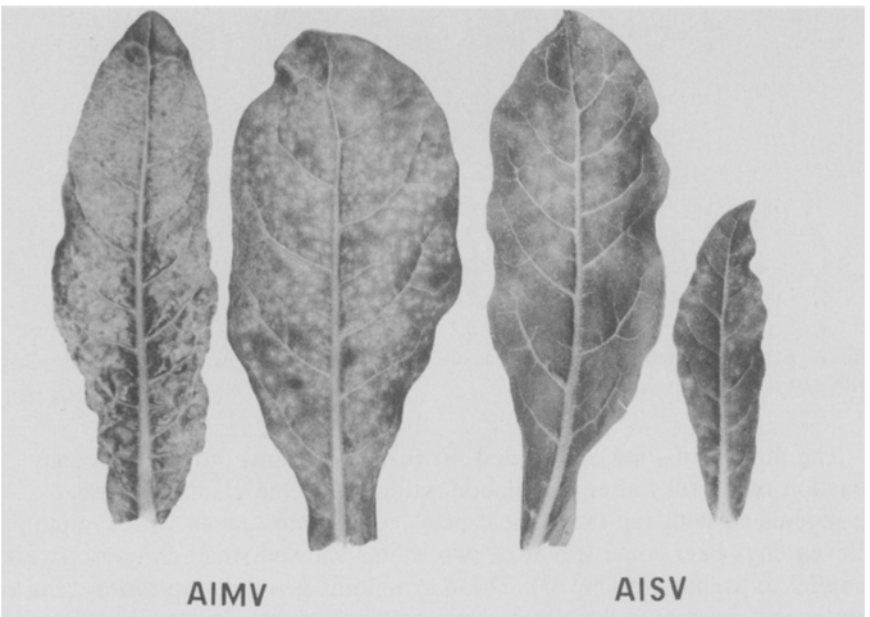

Fig. 1. Different symptoms on leaves of *Nicotiana occidentalis* P1 after inoculation with an *Alstroemeria* mosaic virus-isolate from England (left), respectively *Alstroemeria* streak virus (right).

N. hesperis 67A (IPO-DLO), showing local chlorotic lesions six days after inoculation with AlSV, followed by systemic chlorotic rings (Fig. 2), which became necrotic two to three weeks later, was considered to be the best plant for virus multiplication and purification experiments.

Fig. 2. Systemic chlorotic rings on a leaf of *Nicotiana hesperis* 67A, from a plant inoculated with *Alstroemeria* streak virus.

The three virus-free, inoculated *Alstroemeria* plants did not show any reaction two weeks after inoculation with AlSV. One plant was then re-inoculated with sap from a leaf of *N. occidentalis* Japan with symptoms. Eleven days later some leaves of two shoots showed small chlorotic streaks parallel to the midrib (Fig. 3). These symptoms gradually spread over most leaves and resembled the streak symptoms on leaves of naturally infected *Alstroemeria* plants provided by R.K. Horst, Ithaca, USA. Streaked leaves yielded potyvirus-like particles in electron microscopy.

Decoration experiments in the electron microscope [Milne and Luisoni, 1977] and experiments with DAS-ELISA showed that AlSV reacted strongly with the commonly used antisera to AlMV. An antiserum to AlMV provided by A.A. Brunt also gave a positive reaction with AlSV in decoration trials. Although AlMV reacted with the monoclonal potyvirus group-specific antibodies, sap from leaves infected with AlSV did not show any reaction, as had already been mentioned by Wong [1992].

Fig. 3. Leaves from an *Alstroemeria* plant, which was inoculated with *Alstroemeria* streak virus, showing chlorotic streaks parallel to the midrib.

Our conclusion is that virus-infected *Alstroemeria* plants may contain AlMV and/or AlSV, which can both be detected with the antisera to AlMV applied in our experiments. These antisera have been prepared from infected plants of *Alstroemeria* [Maat, 1983, 1989] or *N. clevelandii* [Phillips and Brunt, 1986], which apparently contained both viruses. Where AlSV occurs separately, distinction from AlMV is possible by its negative reaction to the monoclonal potyvirus group specific antiserum. Since this was discovered, AlSV has often been detected alone in *Alstroemeria* leaf samples. The separate occurrence and detection of AlSV should facilitate the preparation of a specific antiserum and an improved ELISA for both potyviruses.

Preliminary decoration experiments with sap from virus-infected *Alstroemeria* plants using antisera to freesia mosaic virus (FreMV), *Ornithogalum* mosaic virus (OrMV) and a range of other bulbviruses [De

Blank et al., 1994] showed very close relationships between AlSV and a number of these viruses, for instance FreMV. Freeze-dried leaves from the same *Alstroemeria* plant that was used by Bellardi et al. [1992] were tested by us in an ELISA, which in the Netherlands is applied for routine detection of FreMV. The result of this test was negative. A positive reaction in ELISA, however, was obtained with antiserum to AlMV.

The decoration with antiserum to OrMV indicated, as did earlier ISEM-studies with antiserum to AlMV [De Blank et al., 1994], that a third potyvirus may be present.

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