

## Detection and Frequency of Lily Viruses in Argentina

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### ABSTRACT

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In a survey of lily growing fields in various regions of Argentina, three viruses, *Lily symptomless virus* (LSV), *Lily motile virus* (LMOV), and *Cucumber mosaic virus* (CMV), were found in Longiflorum, Asiatic, Oriental, Longiflorum × Asiatic (LA), and Oriental × Trumpet (OT) hybrids. The areas surveyed were between latitude 26° 56' S and 43° 03' S, and longitude 65° 21' W and 71° 29' W. Virus detection was performed by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using polyclonal antiserum. In infected samples, viruses detected in decreasing order were LSV (60.5%), LMOV (51.0%), and CMV (28.7%) present in single or mixed infections. Virus infection varied among tested hybrids from 36.0% (Oriental Montecristo) to 94.7% (*Lilium longiflorum* Avita) in 2006 and from 38.9% (OT Yelloween) to 82.1% (LO Triumphator) in 2007, with an overall incidence of 64.1 and 70.7% in 2006 and 2007, respectively. A variation in virus incidence among localities was also observed. The highest virus incidence (89.6 and 87.6% in 2006 and 2007, respectively) was observed in Bahía Blanca (38° 44' S, 62° 16' W). The lowest virus incidences, detected in Trevelin (43° 03' S, 71° 29' W) and in Malargüe (35° 28' S, 69° 35' W), were 47.4 and 48.6% in 2006 and 2007, respectively. Moreover, a different distribution of each virus was observed between localities. The high occurrence of viruses infecting lily crops in Argentina could be due to both the use of infected bulbs for propagation and the lack of preventive virus vector control measures.

The genus *Lilium*, family Liliaceae, includes plants known as lilies or liliaceae. Lilies are in demand in the floriculture industry both as cut and potted flowers, and by 2002 there were more than 4,500 cultivated varieties (6). Commercially important groups of lily include Easter lily (*Lilium longiflorum*), Asiatic and Oriental hybrids, and recently, the intergroup hybrids Longiflorum × Asiatic (LA), Oriental × Trumpet (OT), Longiflorum × Oriental (LO), and Oriental × Asiatic (OA). Although lilies can be grown from seed, commercial lilies are generally propagated vegetatively by scaling, i.e., by the production of adventitious bulblets from scales detached from the mother bulb (10,28).

One of the main limiting factors in cultivation of lilies is their susceptibility to virus diseases. The most common viruses infecting lily are *Lily symptomless virus*

(LSV; genus *Carlavirus*), *Lily mottle virus* (LMOV; genus *Potyvirus*), and *Cucumber mosaic virus* (CMV; genus *Cucumovirus*) (15). These viruses are transmitted from one generation to another through vegetative propagation. They are also transmitted by aphids in a nonpersistent manner. These viruses affect the quality and yield of cut flowers, resulting in economic loss (20,27).

Viral infections in liliaceae often cause developmental abnormalities such as growth reduction, smaller flowers, and lower bulb yield (15,25). The symptoms in infected lilies differ in severity according to the type and strain of virus, susceptibility of the cultivar, and growing conditions. The reduction in bulb yield may differ among cultivars (4). Experimental data on the effect of LSV indicated 0 to 20% reduction in bulb yield in different cultivars. LMOV may cause greater yield reductions than LSV (4).

LSV usually causes no or mild symptoms under favorable growing conditions (11). Qualitatively, the appearance of infected plants in the field is mostly regarded as acceptable, apart from the premature senescence at the end of the growing season. Only occasionally, leaves show mild

vein clearing, plants show some stunting, and bulb yield is reduced in sensitive cultivars. When producing cut flowers in the greenhouse, symptoms become more evident, particularly under unfavorable growing conditions. The vase life of flowers is reduced by LSV (4). Co-infections of LSV, LMOV, and CMV can cause more severe symptoms than single infections (11,21). LSV has been reported in Asia, Australia, Europe, and the United States (2).

LMOV may produce vein clearing, leaf mottle, leaf mosaic, chlorotic and yellow streaking, and leaf curling symptoms. Some cultivars may show color breaking, and malformations and asymmetry of the flowers. The buds and flowers may drop prematurely. The vase life of flowers from virus-infected plants is reduced (1,14,22). LMOV has been reported in Asia, Australia, Europe, and the United States (3).

Symptoms of CMV include a light green and yellow discoloration along the veins, leaf mosaic, and ring-spot-like yellow and brownish-necrotic spots on the leaves. Leaves may show curling, twisting, and yellowing, whereas the flower buds may be twisted and malformed, and flowers of some cultivars show color breaking (17). CMV has been reported in many countries in Asia and Europe and also in the United States (2).

Other viruses have been reported for lily in only some countries, e.g., *Arabidopsis mosaic virus* (ArMV) in the Netherlands (7); *Broad bean wilt virus* (BBWV), *Tomato ringspot virus* (ToMRSV), and *Lily mild mosaic virus* (LMMV) in Korea (22); *Citrus tatter leaf virus* (CTLV) in Japan (18); *Tomato ringspot nepovirus* (ToRSV) in Italy (9); *Lily virus X* (LVX) in the UK (33) and in the Netherlands (24); and *Strawberry latent ringspot virus* (SLRV) and *Tobacco rattle virus* in Italy (9,35) and in the Netherlands (7,13).

Lily cut flower production in Argentina is mainly located close to the urban centers, i.e., Buenos Aires, Santa Fe, Tucumán, Córdoba, and Mendoza provinces, in addition to regions with agroclimatic advantages, such as northwest of Corrientes Province (Fig. 1). However, their cultivation is expanding. Lily bulb production is scarce and the flower bulb industry

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is supplied mainly from the Netherlands. The most important bulb production region is northwest of Chubut Province, and small-scale growers have recently begun production in the south of Buenos Aires Province and Malargüe (Fig. 1). Bulbs from these regions are sold to cut flower producers elsewhere. Bulb growers multiply bulbs of old hybrids and incorporate new ones from the Netherlands. In addition, some cut flower producers from Tucumán, Mendoza, and Corrientes save and multiply their own bulbs.

For bulb production, Oriental hybrids are mainly cultivated in Chubut and the other groups are produced everywhere. All types of hybrids are cultivated for the cut flower market irrespective of the production region.

Knowledge of the incidence of the principle viruses in lily crops is a prerequisite if production of high-quality bulbs is to be achieved. This is the first study to investigate the distribution of LSV, LMoV, and CMV in lily bulb production areas of Argentina.

## MATERIALS AND METHODS

**Survey.** Virus incidence was studied in lily crops in the following locations of Argentina: Lules (26° 56' S, 65° 21' W), Tucumán Province; 2: Mendoza (32° 53' S, 68° 49' W) and Malargüe (35° 28' S, 69° 35' W), Mendoza Province; Bahía Blanca (38° 44' S, 62° 16' W) and Hilario Ascasubi (39° 22' S, 62° 38' W), Buenos Aires Province; Epuyen (42° 14' S, 71° 22' W) and Trevelin (43° 03' S, 71° 29' W), Chubut Province (Fig. 1). The study was carried out during the years 2006 and 2007, and bulbs sampled each year in each location were not necessarily of the same origin. In the first year, bulb production locations studied were Bahía Blanca, Hilario Ascasubi, Epuyen, and Trevelin. In the second year, all indicated production sites were included.

Hybrids sampled in the year 2006 were *Lilium longiflorum* Avita, LA Fangio, and the Asiatic Navona in Hilario Ascasubi, Bahía Blanca, Epuyen, and Trevelin; and the Oriental Montecristo in Epuyen and Trevelin.

Hybrids evaluated in 2007 were *Lilium longiflorum* Avita; LA Fangio and Royal Respect; Asiatic Navona and Nello; the Orientals Dorgdogne and Expression, LO Triumphator and OT Yelloween. At each location, plants of one to nine hybrids were sampled.

Forty to 60 bulbs of each hybrid were selected at random from different sites and shipped to Bahía Blanca. They were disinfected in a solution of carbendazim (Carbendaglex CS 50%, Gleba) 0.1% a.i., captan (Captan Tomen WP 80%, Cheminova) 0.16% a.i., and carbofuran (Furadan CS 48%, FMC) 0.05% a.i. for 15 min, and then stored in boxes containing sphagnum peat moss at 4°C for 60 to 96 days. On 20

July 2006 and 16 August 2007, bulbs were planted in an aphid-free greenhouse in Bahía Blanca. After emergence and about 60 days after planting, plants were sprayed with a solution of imidacloprid (Confidor CS 35%, Bayer) 0.035% a.i., using a CO<sub>2</sub>-pressurized backpack sprayer with a 8004 flat-fan nozzle at a rate of 409 liters ha<sup>-1</sup>. Symptoms were recorded at the preflowering stage—80 to 100 days after planting depending on the hybrid—from visible flower buds to colored buds. Leaves were sampled from the upper quart of the plant and tested by double-antibody sandwich

enzyme-linked immunosorbent assay (DAS-ELISA).

In samples belonging to the hybrid *L. longiflorum* Avita in 2006, and in a subset of plants belonging to hybrids LA Fangio and LO Triumphator in 2007, infection detected by DAS-ELISA was compared with plant symptomatology.

In 2006, virus incidence was measured in leaves of *L. longiflorum* Avita and in 2007 in leaves and scales coming from bulbs of the hybrid LA Fangio, both imported from the Netherlands destined for cut flower production. In both years, leaves



**Fig. 1.** Map of Argentina showing locations (1 to 7) where lily bulbs were collected. 1: Lules (26° 56' S, 65° 21' W), Tucumán Province; 2: Mendoza (32° 53' S, 68° 49' W) and 3: Malargüe (35° 28' S, 69° 35' W), Mendoza Province; 4: Bahía Blanca (38° 44' S, 62° 16' W) and 5: Hilario Ascasubi (39° 22' S, 62° 38' W), Buenos Aires Province; 6: Epuyen (42° 14' S, 71° 22' W) and 7: Trevelin (43° 03' S, 71° 29' W), Chubut Province. Circles indicate regions of lily flower production. Provinces names are in italics.

were sampled from the upper quart of the plant at preflowering stage. In 2007, two scales from each bulb were taken before planting and were tested by DAS-ELISA. Then, leaves of the derived plants were tested at the preflowering stage.

**Serological assay.** Virus detection was carried out by DAS-ELISA according to the general protocol described by Clark and Adams (12). Commercial ELISA kits against LSV, LMoV, and CMV were purchased from BQ Support (Lisse, The Netherlands). Positive and negative controls, IgGs, and alkaline phosphatase-conjugated IgGs were used at dilutions recommended by the manufacturers.

Polystyrene microplate wells (Dynatech Immulon, Chantilly, VA) were coated with 150  $\mu\text{l}$  of 1 mg  $\text{ml}^{-1}$  anti-LSV, anti-LMoV, or anti-CMV IgG diluted in carbonate-bicarbonate coating buffer (34 mM  $\text{NaHCO}_3$ , 15 mM  $\text{Na}_2\text{CO}_3$ , and 3 mM  $\text{NaN}_3$ , pH 9.6) at a final concentration of 1  $\mu\text{g ml}^{-1}$ , 1  $\mu\text{g ml}^{-1}$ , and 2  $\mu\text{g ml}^{-1}$ , respectively. The

plate wells filled with coating solution were stored at 4°C for 24 h. For extracting plant material, young fully expanded leaves of lily plants at the preflowering stage were collected. Additionally, bulb scales of Fangio hybrid from the Netherlands were tested. Extracts of leaves and scales were obtained using a roller press (Pollahne, Meku, Germany) and extraction buffer (0.13 M  $\text{NaCl}$ , 0.11 M  $\text{Na}_2\text{HPO}_4$ , 3 mM  $\text{NaN}_3$ , 15 mM  $\text{Na}_2\text{SO}_3$ , and 0.2% Tween 20, pH 8.3) as above in a 1:4 wt/vol ratio. The resulting extract was considered the antigen solution. Two positive and two negative controls (BQ Support, Lisse) were used per plate. The positive control came from infected tissues and the negative from healthy plants. Moreover, two wells per plate were filled with extraction buffer for blank reaction. Plates were washed three times with distilled water at 3-min intervals each, then 160  $\mu\text{l}$  of plant extract was added to each well and incubated overnight at 4°C. After washing the plates three

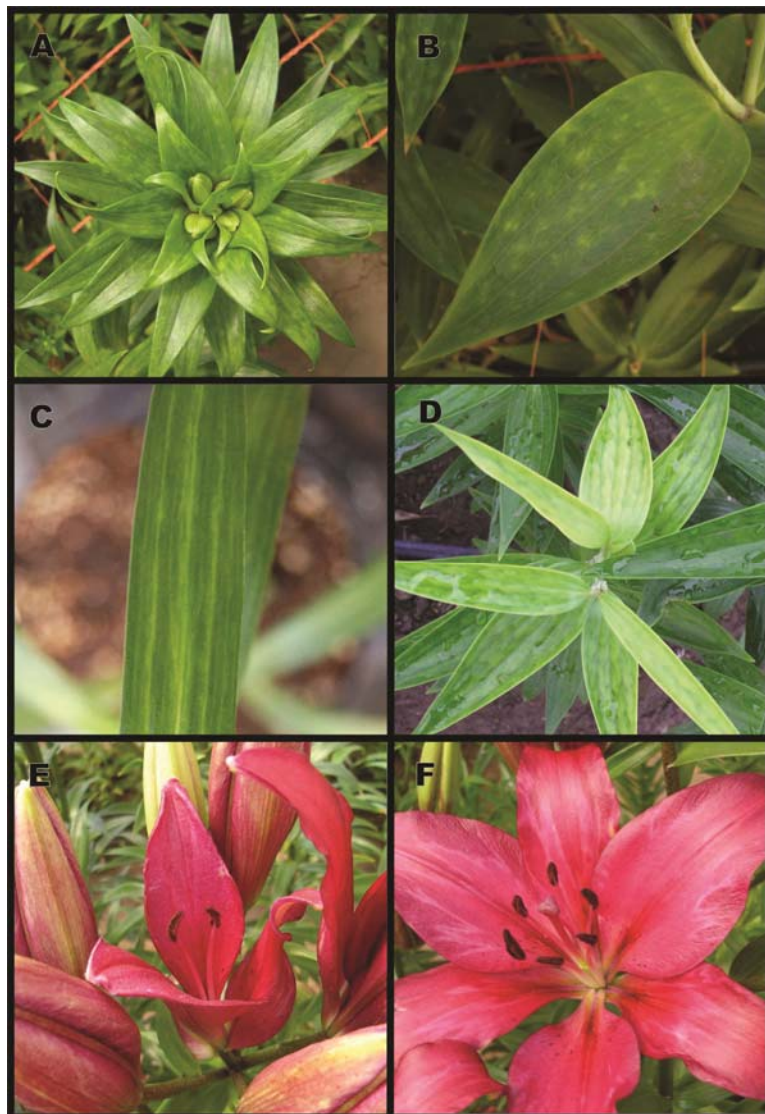
times at 3-min intervals each, 150  $\mu\text{l}$  of 1 mg  $\text{ml}^{-1}$  alkaline phosphatase-conjugated IG (BQ Support, Lisse) against LSV, LMoV, and CMV diluted in conjugate buffer (containing 0.13 M  $\text{NaCl}$ , 1.8 mM  $\text{KH}_2\text{PO}_4$ , 20 mM  $\text{Na}_2\text{HPO}_4$ , 3 mM  $\text{NaN}_3$ , 0.2% Tween 20, and 5 g  $\text{liter}^{-1}$  skimmed milk, pH 7.4) was added and incubated 2 h at 37°C. Enzymatic conjugate final concentration for LSV, LMoV, and CMV detection were 1  $\mu\text{g ml}^{-1}$ , 1  $\mu\text{g ml}^{-1}$ , and 0.5  $\mu\text{g ml}^{-1}$ , respectively. Wells were washed and 150  $\mu\text{l}$  of alkaline phosphatase substrate (0.5 mg  $\text{ml}^{-1}$  *p*-nitrophenyl phosphate [Sigma-Aldrich, Buenos Aires, Argentina] diluted in buffer [1 mM  $\text{MgCl}_2$ , 3 mM  $\text{NaN}_3$ , 9.7% diethanolamine, pH 9.6]) was added to the plate wells and incubated for 2 h at 37°C. Absorbance at 405 nm was measured using a digital microplate absorbance reader (Sunrise, Tecan, Austria). Samples were considered positive when their absorbance values were greater than three times the mean of the healthy extract absorbance values.

**Statistical analysis.** The Pearson's chi-square test was used to determine if virus incidence varied between sampling locations within each hybrid and year, and to compare virus incidence between different hybrids from the same location within the same year. This test was also used to compare the prevalence of each virus between hybrids and localities within a year. The Fisher's exact test was used when the expected values were below 5 in more than 20% of the cells of the contingency table. Data were analyzed using InfoStat software (Córdoba, Argentina).

## RESULTS

**Symptoms of viral infection.** Different symptoms were observed in the hybrids (Fig. 2). Leaf mosaic was observed in the hybrids Avita (12.3%), Navona (23.7%), Fangio (3.4%), and Triumphator (73.4%). Royal Respect had a mild mosaic. In Avita, vein chlorosis was observed (57.9%). In Dordogne, virus infection was detected but viral symptoms were not evident in leaves. Interveneal chlorosis was detected but this symptom is usually associated with iron deficiency and it is quite differentiated from the ones caused by viral infection (Fig. 2A–D). In Expression, leaf mottle (4%) was observed. In Nello and Yelloween, no symptoms were detected in leaves. The main symptoms observed in flowers were: aborted buds in Navona (32.4%), Nello (17.6%), Fangio (2.5%), Royal Respect (3.5%), Dordogne (10.5%), Expression (1.3%), Triumphator (11.2%), and Yelloween (4.2%); malformation of flowers in Navona (1.8%), Nello (3.4%), Fangio (0.8%), Dordogne (8.4%), Expression (7.6%), Triumphator (7%), and Yelloween (0.8%); and flower color breaking in Fangio (61%). Stunting was observed infrequently.

**Relationship between symptoms and viral infection.** In symptomatic plants of



**Fig. 2.** Symptoms observed in lily hybrids. **A**, Leaf mosaic in Triumphator. **B**, Leaf mottle in Expression. **C**, Vein chlorosis in Avita. **D**, Interveneal chlorosis due to iron deficiency in Dordogne. **E**, Flower malformation in Fangio. **F**, Flower color breaking in Fangio.

hybrids Avita, Fangio, and Triumphator, different proportions of the indexed viruses were found (Table 1). The proportion of symptomatic plants was 66.7% in Avita, 68.6% in Fangio, and 80.5% in Triumphator, whereas the infection detected by DAS-ELISA was 94.7, 76.9, and 82.1%, respectively.

In Avita, a high proportion of plants showing vein clearing was positive only for LSV. In Fangio, most of the symptoms were caused by LMoV and by mixed infections of LSV with LMoV. In Triumphator, the highest percentage of symptomatic plants showed mixed infections of all three viruses. More than one symptom per plant was observed in Avita (3.5%), Fangio (0.8%), and Triumphator (10.6%).

In Avita all symptomatic plants were positive by DAS-ELISA, while in Fangio and Triumphator, some of the symptomatic plants were negative. Symptoms included leaf mosaic, reduced height, leaf mottle, flower color breaking, aborted buds, or malformation in flowers (Table 1). Furthermore, some infected plants were found to be symptomless. The virus-infected asymptomatic plants presented different proportions of viruses: 94% LSV and 6% CMV in Avita; 50% LSV, 42% LMoV, and 8% CMV in Fangio; and 30% LSV, 60% LMoV, and 10% CMV in Triumphator.

**Virus incidence in imported bulbs.** Bulbs coming from the Netherlands were

infected with the three viruses LSV, LMoV, and CMV, and infection was detected both in scales and leaves, in different proportions (Table 2). No viral symptoms were observed. In hybrid Fangio, the percentage of infection with LSV and CMV was homogeneous among scales and leaves collected from the same plant ( $P > 0.05$ ), while a different infection of LMoV among scales and leaves was observed ( $P < 0.01$ ). Co-infections of LSV and LMoV were detected in bulb scales (2.3%) and leaves (2.5%) of hybrid Fangio. In hybrid Avita, co-infection of LSV and CMV was detected (3%).

**Virus incidence and distribution.** In 2006, virus incidence was uniform among hybrids in Bahía Blanca and Hilario Ascasubi ( $P > 0.05$ ). In Trevelin and Epuen, the incidence was highest in cv. Avita. The

highest virus incidence was detected in Bahía Blanca and the lowest in Trevelin (Table 3).

The percentage of viral infection in Avita and Montecristo was homogeneous among localities, while the percentage of viral infection in Fangio and Navona varied among locations. The hybrid that showed the highest incidence of infected plants was Avita, and the one that presented the lowest proportion of infected plants overall was Montecristo (Table 3).

In 2007, a different virus incidence among hybrids was found in all localities except in Mendoza, in which all hybrids had similar incidence of viral infection (Table 3). The total virus incidence was not homogeneous between localities. The highest virus incidence was detected in Bahía Blanca and the lowest in Malargüe

**Table 2.** *Lily symptomless virus* (LSV), *Lily mottle virus* (LMoV), and *Cucumber mosaic virus* (CMV) identified in bulbs from the Netherlands and the resulting greenhouse-grown plants

Hybrids	Plant part	No. of samples	Viruses <sup>y</sup>		
			LSV	LMoV	CMV
Fangio	Bulb scales	44	6 (13.6) <sup>z</sup>	2 (4.5)	1 (2.3)
	Leaves	40	5 (13.0)	14 (35.0)	0 (0.0)
Avita	Leaves	99	19 (19.2)	3 (3.0)	7 (7.0)

<sup>y</sup> Identification of viruses was based on serological reactions (DAS-ELISA: double-antibody sandwich enzyme-linked immunosorbent assay). Samples were considered positive when their absorbance values were greater than three times the mean of the healthy extract absorbance values.

<sup>z</sup> Total number of samples and percent virus incidence (in parentheses) infected with each virus in bulb scales or leaves.

**Table 1.** Symptoms and associated infection of *Lilium* cultivars Avita, Fangio, and Triumphator with *Lily symptomless virus* (LSV), *Lily mottle virus* (LMoV), and *Cucumber mosaic virus* (CMV) in Argentina

Hybrid	Symptoms	No. of symptomatic plants <sup>x</sup>	No. of DAS-ELISA positive plants (%) <sup>w</sup>						DAS-ELISA negative symptomatic plants (%)	
			LSV	LMoV	CMV	LSV LMoV	LMoV CMV	LSV CMV		
Avita 57 <sup>z</sup>	Vein clearing	33	25 <sup>y</sup> (75.8)	–	–	4 (12.1)	–	–	4 (12.1)	–
	Leaf mosaic	7	2 (28.6)	1 (14.3)	–	2 (28.6)	–	–	2 (28.6)	–
Fangio 118	Flower color breaking	72	–	34 (47.2)	–	34 (47.2)	–	–	–	4 (5.5)
	Leaf mosaic	4	–	2 (50.0)	–	2 (50.0)	–	–	–	–
	Bud abortion	3	–	1 (33.3)	–	1 (33.3)	–	–	–	1 (33.3)
	Flower malformation	1	–	1 (100)	–	–	–	–	–	–
	Dwarf plants	2	–	–	–	2 (100)	–	–	–	–
Triumphator 113	Leaf mosaic	83	10 (12.0)	14 (16.9)	2 (2.4)	20 (24.0)	4 (4.8)	5 (6.0)	26 (31.3)	2 (2.4)
	Leaf mottle	1	–	–	–	–	–	–	1 (100)	–
	Bud abortion	9	–	1 (11.1)	–	1 (11.1)	1 (11.1)	1 (11.1)	5 (55.5)	–
	Flower malformation	8	–	–	–	1 (12.5)	1 (12.5)	1 (12.5)	4 (50.0)	1 (12.5)
	Dwarf plants	2	–	–	–	–	–	–	2 (100)	–
Total		225	37 (16.4)	54 (24.0)	2 (0.9)	67 (29.8)	6 (2.7)	7 (3.1)	44 (19.5)	8 (3.6)

<sup>w</sup> Identification of LSV, LMoV, and CMV was based on serological reactions (DAS-ELISA: double-antibody sandwich enzyme-linked immunosorbent assay). Samples were considered positive when their absorbance values were greater than three times the mean of the healthy extract absorbance values.

<sup>x</sup> Number of plants with different symptoms per hybrid.

<sup>y</sup> Number of infected samples and percent virus incidence (in parentheses), differentiated in single and mixed infections.

<sup>z</sup> Total number of plants per hybrid.

and Hilario Ascasubi. Furthermore, different virus incidence in each hybrid among localities was also observed ( $P < 0.05$ ). Considering all the localities, a different incidence of viruses between hybrids was found ( $P < 0.0001$ ). The hybrid showing the highest proportion of infected plants was Triumphator and the one presenting the lowest proportion was Yelloween (Table 3).

Of the 1,168 samples of lily hybrids belonging to different locations in Argentina, 815 contained single or mixed infections of LSV, LMoV, and CMV (Table 4). LSV was the most common virus (60.5% of

samples) followed by LMoV (51.0%) and CMV (28.7%). Of the virus-infected plants, the proportion infected with each virus was not homogeneous between hybrids ( $P < 0.0001$ ). The highest proportions of LSV (92.7%), LMoV (91.0%), and CMV (50.7%) were detected in hybrids Expression, Navona, and Nello, respectively. The lowest proportion of LSV (9.5%), LMoV (0%), and CMV (4.8%) were observed in hybrids Dordogne, Royal Respect, and Yelloween, respectively.

The number of plants infected with each virus in the different locations during 2006

and 2007 is presented in Table 5. In both cases, the most prevalent virus was LSV (84.4 and 57.8%), then LMoV (43.1 and 51.2%), and finally CMV (14.7 and 30.7%). The proportion of plants infected with LSV, LMoV, and CMV calculated over the number of infected plants in each locality was not homogeneous among localities in 2006 ( $P < 0.05$ ) and in 2007 ( $P < 0.001$ ).

In 2006, the highest proportion of LMoV (69.2%) and CMV (26.9%) was observed in Bahía Blanca, and LSV (100%) in Hilario Ascasubi. The lowest

**Table 3.** Proportion of lily plants<sup>v</sup> infected with *Lily symptomless virus* (LSV), *Lily mottle virus* (LMoV), and *Cucumber mosaic virus* (CMV) in four and seven locations in Argentina, in 2006 and 2007, respectively

Year	Hybrid	Region/location (province)							Total <sup>w</sup>
		North	Central				South		
		Tucumán	Mendoza	Buenos Aires		Chubut			
	Lules	Mendoza	Malargüe	Hilario Ascasubi	Bahía Blanca	Trevelin	Epuyen		
2006	Avita	– <sup>x</sup>	–	–	9 (81.8)	10 (90.9)	10 (100) A	25 (100) A	54 (94.7) A
	Navona	–	–	–	5 (62.5) a	9 (90.0) a	1 (10.0) B b <sup>y</sup>	11 (55.0) B a	26 (54.2) B
	Montecristo	–	–	–	–	–	3 (37.5) B	6 (35.3) B	9 (36.0) B
	Fangio	–	–	–	4 (100) a	7 (87.5) a	4 (40.0) B b	5 (27.8) B b	20 (50.0) B
	Total 2006 <sup>z</sup>	–	–	–	18 (78.3) a	26 (89.6) a	18 (47.4) b	47 (58.7) b	109 (64.1)
2007	Avita	22 (100) A a	7 (70.0) b	–	13 (76.5) A b	22 (100) A a	11 (50.0) B b	20 (86.9) A a	95 (81.9) A
	Navona	–	–	5 (62.5) B a	10 (41.7) AB a	25 (96.1) A b	24 (100) A b	21 (84.0) A b	85 (79.4) A
	Nello	–	7 (58.3) a	–	7 (36.8) B a	23 (85.2) A b	22 (84.6) A b	8 (100) A b	67 (72.8) A
	Fangio	–	6 (75.0) a	2 (20.0) A b	7 (70.0) A c	38 (95.0) A a	32 (80.0) A a	28 (71.8) A c	113 (76.9) A
	Royal Respect	3 (50.0) B a	19 (76.0) b	4 (40.0) A a	22 (53.6) AB a	34 (85.0) A b	16 (40.0) B a	25 (62.5) B a	123 (60.9) B
	Expression	–	–	–	1 (9.1) C a	–	16 (64.0) A b	–	41 (67.2) B
	Dordogne	–	–	–	–	20 (83.3) A a	11 (44.0) B b	11 (44.0) B b	42 (56.7) B
	Triumphator	2 (20.0) B a	4 (80.0) b	7 (77.8) B b	–	39 (97.5) A b	31 (77.5) A b	36 (87.8) A b	119 (82.1) A
	Yelloween	–	8 (88.9) a	–	–	4 (26.7) B b	3 (20.0) B b	6 (40.0) B b	21 (38.9) C
	Total 2007	27 (71.0) b	51 (73.9) b	18 (48.6) c	60 (49.2) c	205 (87.6) a	166 (64.6) b	179 (74.3) b	706 (70.7)

<sup>v</sup> Virus detection was based on serological reactions (DAS-ELISA: double-antibody sandwich enzyme-linked immunosorbent assay). Samples were considered positive when their absorbance values were greater than three times the mean of the healthy extract absorbance values.

<sup>w</sup> In total column, the statistically significant variation among hybrids was informed with different capital letters.

<sup>x</sup> Hybrid not surveyed in the locality.

<sup>y</sup> Number and percentage (in parentheses) of plants infected with viruses. Numbers followed by the same letter are not different at  $P < 0.05$  using a Pearson's chi-square test or Fisher's exact test with expected value  $< 5$ . Capital letters indicate differences between hybrids for a location within 1 year (columns). Lowercase letters indicate differences between locations for the same hybrid (rows) within 1 year.

<sup>z</sup> In total rows, the statistically significant variation among localities was informed with different lowercase letters.

**Table 4.** Frequency of detection of *Lily symptomless virus* (LSV), *Lily mottle virus* (LMoV), and *Cucumber mosaic virus* (CMV) in four and seven locations in Argentina, in 2006 and 2007, respectively

Year	Hybrid	No. of samples	No. of infected plants	No. of DAS-ELISA positive plants (%) <sup>y</sup>						
				LSV	LMoV	CMV	LSV LMoV	LMoV CMV	LSV CMV	
2006	Avita	57	54	38 <sup>z</sup> (70.4)	1 (1.8)	–	9 (16.7)	–	1 (1.8)	5 (9.2)
	Navona	48	26	6 (23.1)	9 (34.6)	–	8 (30.8)	1 (3.8)	–	2 (7.7)
	Montecristo	25	9	5 (55.6)	–	2 (22.2)	1 (11.1)	–	1 (11.1)	–
	Fangio	40	20	7 (35.0)	1 (5.0)	2 (10.0)	7 (35.0)	–	–	3 (15.0)
	Total 2006	170	109	56 (51.4)	11 (10.1)	4 (3.7)	25 (22.9)	1 (0.9)	2 (1.8)	10 (9.2)
2007	Avita	116	95	22 (23.1)	28 (29.5)	10 (10.5)	8 (8.4)	10 (10.5)	13 (13.7)	4 (4.2)
	Navona	107	85	–	49 (57.6)	4 (4.7)	10 (11.8)	14 (16.5)	–	8 (9.4)
	Nello	92	67	27 (40.3)	6 (8.9)	16 (23.9)	–	–	18 (26.8)	–
	Fangio	147	113	4 (3.5)	53 (46.9)	2 (1.8)	54 (47.8)	–	–	–
	Royal Respect	202	123	63 (51.2)	–	33 (26.8)	–	–	27 (21.9)	–
	Expression	61	41	33 (80.5)	1 (2.4)	2 (4.9)	1 (2.4)	–	4 (9.7)	–
	Dordogne	74	42	2 (4.8)	30 (71.4)	4 (9.5)	2 (4.8)	4 (9.5)	–	–
	Triumphator	145	119	19 (15.9)	28 (23.5)	4 (3.4)	29 (24.4)	6 (5.0)	12 (10.1)	21 (17.6)
	Yelloween	54	21	17 (80.9)	1 (4.8)	1 (4.8)	2 (9.5)	–	–	–
	Total 2007	998	706	187 (26.5)	196 (27.8)	76 (10.8)	106 (15.0)	34 (4.8)	74 (10.5)	33 (4.7)

<sup>y</sup> Identification of LSV, LMoV, and CMV was based on serological reactions (DAS-ELISA: double-antibody sandwich enzyme-linked immunosorbent assay). Samples were considered positive when their absorbance values were greater than three times the mean of the healthy extract absorbance values.

<sup>z</sup> Number of infected samples differentiated in single and mixed infections and percentage of viruses (in parentheses) calculated over the number of infected plants.

proportion of LSV (72.2%) and LMoV (27.7%) was observed in Trevelin, and CMV (2.1%) in Epuyen (Table 5).

In 2007, the highest proportion of LMoV (57.6%) and CMV (46.4%) was found in Bahía Blanca, and LSV (88.9%) in Lules. The lowest proportion of LSV (30%) was found in Hilario Ascasubi, of CMV in Epuyen (15%), and of LMoV (13.7%) in Mendoza (Table 5).

## DISCUSSION

Lily bulb crops in Argentina have a high percentage of virus-infected plants. Almost all hybrids in all locations were infected with LSV, LMoV, and CMV. We found that LSV was the most widespread virus, but when present alone, it causes either no or mild symptomatology depending on the sensitivity of the cultivar. The second most widespread virus was LMoV followed by CMV.

Lilies are important ornamental plants worldwide. Economic value is reduced when they are infected with viruses, especially in mixed infections, as a result of reduction of plant quality, including flower abortion (4). All lily varieties tested showed similar symptoms to that previously described for other species and hybrids of genus *Lilium* (11,15,30,32). The negative results in DAS-ELISA for samples that showed typical virus symptomatology could be due to the presence of other viruses (not assayed), to other pathogenic agents, or even physiological and/or nutritional disorders that could affect lily crops.

It was reported that LSV, when present alone, could be asymptomatic (11). We found some asymptomatic plants infected

with LMoV or CMV. This could be due to low viral concentration, which produces no visible symptoms but can be detected by DAS-ELISA.

In 2006 and 2007, a variation in viral incidence between hybrids from the same locality and between localities for a specific hybrid was observed. This indicates that both the heterogeneous viral infection among hybrids and the differential infection with viruses in the sites of production affect the incidence of viruses in the studied hybrids.

Lily hybrids show differential response to the different virus (8,23) and different susceptibility and sensitivity to viral disease (4,14). Some species are resistant to viral disease (14), such as cultivars of Asiatic lily hybrids which show resistance to disease caused by LMoV (31). A significant difference in virus infection was detected in some of the assayed hybrids in specific localities, but these differences do not hold constant among localities. Further studies (i.e., inoculation trials) are needed to determine a possible differential response of these hybrids to viral infection.

In this study, DAS-ELISA proved to be a reliable technique for virus indexing in lily, in good agreement with the results of Sharma et al. (32). Bulb tests provide insights about the primary sources of inoculum that will be introduced in an area if these bulbs are used (6). Tests for LSV and CMV detection perform well on bulbs, but the detection of LMoV in many varieties of lily bulbs is not reliable because its concentration may be too low (6,16,19). LMoV detection in *L. longiflorum* could be improved through storage of bulb scales in polyethylene bags for 2 or 3 weeks at

20°C under white fluorescent light for 12 to 16 h/day (31). Furthermore, it could be detected by more sensitive methods such as reverse transcription-polymerase chain reaction (RT-PCR) or immunocapture (IC)-RT-PCR (29).

Bulbs coming from the Netherlands for flower production were infected with viruses in a proportion slightly higher than the accepted percentage of viral infection for stock II in bulb production. The European and Mediterranean Plant Protection Organization in the certification program for lily bulb production accepts up to 10% of LSV and 1% of LMoV infection in bulbs from the propagation stock II (34). Since bulbs for cut flowers are produced from propagation stock ones, a slightly higher proportion of infected bulbs for cut flower production is acceptable. However, if these bulbs were used for propagation, the viruses will multiply and will be transmitted to newly formed bulbs during their cultivation. In Argentina, farmers grow previously cultivated bulbs and propagate bulbs imported from the Netherlands for flower production; consequently, it is highly probable that a proportion of them are infected at the beginning of bulb production.

Aphids that are common vectors of lily viruses, *Mysus persicae*, *Macrosiphum euphorbiae*, and *Aphid fabae*, have been reported in Argentina (26), and they were noted or reported in all the studied areas. A program for control of vectors is not a common practice among farmers. Those facts can explain the high level of virus infection of lily crops. Hence, in order to avoid the spreading of virus infection, it is important that the starting material for bulb

**Table 5.** Proportion of lily plants infected with *Lily symptomless virus* (LSV), *Lily mottle virus* (LMoV), and *Cucumber mosaic virus* (CMV) growing in four and seven locations in Argentina, in 2006 and 2007, respectively

Year	Region/ location	No. of samples	No. of infected plants	No. of DAS-ELISA positive plants (%) <sup>y</sup>						
				LSV	LMoV	CMV	LSV LMoV	LMoV CMV	LSV CMV	LSV LMoV CMV
2006	<b>Central</b> Buenos Aires Province									
	Bahía Blanca	29	26	7 <sup>z</sup> (26.9)	4 (15.4)	–	8 (30.8)	–	1 (3.8)	6 (23.1)
	Hilario Ascasubi	23	18	8 (44.4)	–	–	8 (44.4)	–	–	2 (11.1)
	<b>South</b> Chubut Province									
	Trevelin	38	18	8 (44.4)	1 (5.5)	4 (22.2)	3 (16.7)	–	1 (5.5)	1 (5.5)
Epuyen	80	47	33 (79.2)	7 (14.9)	–	6 (12.8)	1 (2.1)	–	–	
Total 2006	170	109	56 (51.4)	12 (11.0)	4 (3.7)	25 (22.9)	1 (0.9)	2 (1.8)	9 (8.2)	
2007	<b>North</b> Tucumán Province									
	Lules	38	27	13 (48.1)	3 (11.1)	–	3 (11.1)	–	5 (18.5)	3 (11.1)
	<b>Central</b> Mendoza Province									
	Mendoza	69	51	23 (45.1)	1 (2.0)	16 (31.4)	5 (9.8)	1 (2.0)	5 (9.8)	–
	Malargüe	37	18	4 (22.2)	7 (38.9)	4 (22.2)	3 (16.7)	–	–	–
	<b>Central</b> Buenos Aires Province									
	Bahía Blanca	234	205	28 (13.6)	30 (14.6)	17 (8.3)	52 (25.4)	10 (4.9)	42 (20.5)	26 (12.7)
	Hilario Ascasubi	122	60	14 (23.3)	32 (53.3)	9 (15.0)	–	1 (1.7)	4 (6.7)	–
	<b>South</b> Chubut Province									
	Trevelin	257	166	35 (21.1)	67 (40.4)	23 (13.8)	18 (10.8)	7 (4.2)	13 (7.8)	3 (1.8)
Epuyen	241	179	71 (39.7)	55 (30.7)	7 (3.9)	25 (14.0)	8 (4.5)	11 (6.1)	2 (1.1)	
Total 2007	998	706	188 (26.6)	195 (27.6)	76 (10.8)	106 (15.0)	27 (3.8)	80 (11.3)	34 (4.8)	

<sup>y</sup> Identification of LSV, LMoV, and CMV was based on serological reactions (DAS-ELISA: double-antibody sandwich enzyme-linked immunosorbent assay). Samples were considered positive when their absorbance values were greater than three times the mean of the healthy extract absorbance values.

<sup>z</sup> Number of infected samples differentiated in single and mixed infections and percentage of viruses (in parentheses) calculated over the number of infected plants.

production be virus-free and an aphid control preventive program be implemented by spraying periodically with mineral oil combined with pyrethroid insecticides, as recommended by Asjes and Blom-Barnhoorn (5).

When dealing with different regions and locations, a significant variation in virus incidence was observed in some of the studied hybrids. This could be due to variety resistance as well as vector pressure on crops and access to inoculum. The north (Tucumán) and central-east (Bahía Blanca) production areas of Argentina have warm summer climate, which is not only appropriate for horticultural production, but also favorable for aphid proliferation and virus dissemination. In south production areas, tulip and different species of the family Liliaceae which could act as LMoV and LSV reservoirs are common in gardens or they are cultivated in small plots. To implement a certification program for production of virus-free bulbs, it is necessary to cultivate them in isolated areas free of related species that act as hosts of lily virus, as the case of strawberry in the south and potato in different regions of the country. This would be possible for lily close to the actual bulb production regions, due to the presence of uncultivated valleys in west Chubut Province, Ventania Hills in Buenos Aires Province, or Tafi del Valle in Tucumán Province, as well as Malargüe in Mendoza Province.

This is the first report of the incidence of lily viruses in lily crops for bulb production in different regions and locations of Argentina. In the areas under study, a high incidence of viruses was detected. The reasons why lily virus infection rate is so high could be due to the introduction of virus-infected bulbs by farmers, the re-use of infected bulbs in conventional propagation, and the lack of appropriate virus vector control measures. Hence, it is relevant to select the production site for commercial production of lily bulbs considering virus occurrence, and to use virus-free propagation material and extreme precautions to avoid virus transmission.

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