Annals of Applied Biology ISSN 0003-4746

RESEARCH ARTICLE

Incidence and prevalence of aphid-borne viruses infecting strawberry in Argentina

C. Luciani[1], M.G. Celli[1], A.K. Torrico[2], F. Asinari[2], E. Pozzi[1], A. Peña Malavera[3], D.S. Kirschbaum[4], M.C. Perotto[1,2] & V.C. Conci[1,2]

1 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) in Instituto de Patología Vegetal (IPAVE) of Instituto Nacional de Tecnología Agropecuaria (INTA), Córdoba, Argentina
2 Instituto de Patología Vegetal (IPAVE), Centro de Investigaciones Agropecuarias (CIAP) of Instituto Nacional de Tecnología Agropecuaria (INTA), Córdoba, Argentina
3 CONICET in Estadística y Biometría, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Ciudad Universitaria, Córdoba, Argentina
4 Estación Experimental Agropecuaria INTA Famaillá, Tucumán, Argentina

Keywords
Epidemiology; multiplex RT-PCR assay; sampling; *Strawberry crinkle virus*; *Strawberry mild yellow edge virus*; *Strawberry mottle virus*; Strawberry polerovirus 1; survey.

Correspondence
Dr V.C. Conci, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Instituto de Patología Vegetal (IPAVE) of Instituto Nacional de Tecnología Agropecuaria (INTA), Camino 60 Cuadras Km 5½, Córdoba X5020ICA, Argentina.
Email: conci.vilma@inta.gob.ar

Received: 27 November 2017; revised version accepted: 1 May 2018.

doi:10.1111/aab.12437

Abstract

The incidence and prevalence of strawberry viruses were determined in surveys of randomly selected strawberry plants grown in different regions of Argentina. In 2009 and 2010, 1034 plants from 28 fields and 1060 plants from 33 fields, respectively, were collected from Lules and Coronda. The samples were analysed by double-antibody sandwich enzyme-linked immunosorbent assay to detect *Strawberry mild yellow edge virus* (SMYEV). In 2014, 606 plants from 43 fields in Lules, Coronda and Mar del Plata were analysed by reverse transcription polymerase chain reaction with primers specific for SMYEV, *Strawberry crinkle virus* (SCV), *Strawberry mottle virus* (SMoV) and Strawberry polerovirus 1 (SPV1). The SMYEV incidence was 4–35%, while prevalence was 60–100%, depending on the year and region sampled. Meanwhile, SMoV and SPV1 incidences were 8–17%, and prevalences were 46–62%, depending on the virus and region sampled. SCV was observed relatively low (incidence was 0.5–8% and prevalence was 8–50%), although it was more abundant in Mar del Plata than in the other analysed regions. Spearman's correlation analysis indicated that SCV and SMYEV were correlated with disease symptoms ($P < 0.005$). A principal component analysis revealed a close relationship between SMYEV and SCV in Mar del Plata, in which the lowest temperatures were recorded. Interactions among viruses, regions and climatic conditions will need to be studied in greater detail. Accurately determining the incidence and prevalence of viruses in different regions will improve estimations of possible damages or yield decreases caused by viral infections during strawberry production.

Introduction

Strawberries are the most consumed berry worldwide. Argentina produces strawberries on approximately 1300 ha, with annual yields of about 45 500 t. Although strawberries are commercially grown in more than eight Argentine provinces, most of the production occurs in Tucumán (Lules region) and Santa Fe (Coronda region), where plantlets are set in the field in March/April and harvested between May and November/December. The third most important province is Buenos Aires, where Mar del Plata region is located. These three provinces account for more than 70% of the strawberries produced in Argentina (Kirschbaum *et al.*, 2017), where strawberries are mostly grown as an annual crop. However, in colder regions (e.g. Mar del Plata, Mendoza and Patagonia), strawberries are biennial or even triennial crops. In Mar del Plata, strawberry fields are set in February/March and berries are harvested between November and May over two consecutive years.

Viruses are one of the most important factors that decrease strawberry fruit quality and yield worldwide.

Thus, virus-free strawberry plants are necessary to maintain acceptable fruit production levels. Globally, strawberries are infected by more than 30 systemically transmitted pathogens, including several viruses transmitted by aphids, whiteflies, nematodes and oomycetes, among other vectors (Converse, 1987; Martin & Tzanetakis, 2006). The most important viruses affecting strawberry production are transmitted by aphids, including *Strawberry mild yellow edge virus* (SMYEV), *Strawberry crinkle virus* (SCV), *Strawberry mottle virus* (SMoV), *Strawberry vein banding virus* (SVBV), *Strawberry pseudo mild yellow edge virus* and *Strawberry latent C virus* (Converse, 1987; Maas, 1998). Strawberry viruses can be present in single or mixed infections and disease symptoms may include stunted growth, dwarfism, deformed leaves, chlorosis, and decreased fruit quality and yield. Most strawberry cultivars not show symptoms in single virus infections. Globally, SMYEV, SMoV, SCV and SVBV are considered the most important viruses for strawberry production (Converse, 1987; Maas, 1998).

Previous studies revealed that SMoV was the most widespread virus affecting strawberries and it has been responsible for considerable yield losses (Converse, 1987; Maas, 1998; Martin & Tzanetakis, 2006). SMoV is a *Secoviridae*, belong to unassigned genus, transmitted by aphids (i.e. *Chaetosiphon* spp. and *Aphis gossypii*) in a semipersistent manner (Mellor & Frazier, 1970; Converse, 1987; Maas, 1998; Martin & Tzanetakis, 2006).

Although SCV has been described as the most harmful strawberry virus, relatively few studies have focused on the corresponding infection rate. This virus is a cytorhabdovirus and is transmitted by *Chaetosiphon fragaefolii* and *Chaetosiphon jacobi* in a replicative, persistent manner (Converse, 1987; Schoen *et al.,* 2001; Martin & Tzanetakis, 2006). In South America, SCV has been detected in Brazil (Betti, 1980), Chile (Thompson *et al.,* 2003; Cabrera *et al.,* 2005) and Argentina (Perotto *et al.,* 2014). Meanwhile, SVBV has been detected relatively infrequently in regions where strawberries are produced (Converse, 1987; Martin & Tzanetakis, 2006).

Strawberry mild yellow edge disease caused by SMYEV was the first viral disease confirmed in strawberry plants and SMYEV is one of the most widely distributed aphid-borne viruses (Horne, 1922; Converse, 1987; Martin & Tzanetakis, 2006). *Chaetosiphon* spp. were reported as the principal vectors for persistent or circulative transmission of this virus (Prentice & Harris, 1946; Mellor & Fitzpatrick, 1951; Converse, 1987). Jelkmann *et al.* (1990) reported a potexvirus associated with Strawberry mild yellow edge disease. The infective cDNA clone from the potexvirus was capable of causing the disease, and the virus was subsequently named SMYEV.

A polerovirus (*Luteoviridae*) was recently detected in strawberry plants grown in Canada and has been tentatively named Strawberry polerovirus 1 (SPV1) (Xiang *et al.,* 2015). This virus, which has since been detected in Argentina and the USA, can occur in single or mixed infections with other aphid-borne viruses (Luciani *et al.,* 2016; Thekke-Veetil & Tzanetakis, 2016; Conci *et al.,* 2017).

Since 2008, numerous symptomatic strawberry plants in Argentina have been tested by a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for SMYEV and eight more strawberry viruses belonging to *Nepovirus*, *Necrovirus*, *Sadwavirus* and *Ilarvirus* genus. Additionally, molecular tests have been used to detect SPV1, SMoV, SCV, SVBV, *Beet pseudo-yellows virus* and *Tobacco streak virus*. So far, only four aphid-borne viruses have been detected (SMoV, SMYEV, SCV and SPV1; Nome & Yossen, 1980; Conci *et al.,* 2009; Perotto *et al.,* 2014; Luciani *et al.,* 2016; Conci *et al.,* 2017). The incidence and prevalence of each of these viruses in different regions of Argentina are currently unknown.

Chaetosiphon spp., *Aphis* spp., *Myzus* spp., *Macrosiphum rosae*, *Acyrthosiphon malvae*, *Amphorophora agathonica*, *Rhodobium porosum* and *Aulacorthum solani* have been described as vectors of strawberry viruses (Maas, 1998). In Argentina, *Aphis forbesi*, *A. gossypii*, *C. fragaefolii*, *Chaetosiphon minor, Chaetosiphon thomasi, Macrosiphum euphorbiae* and *Myzus persicae* have been detected in strawberry-producing regions (Dughetti *et al.,* 2017).

Determining the importance of a pathogen is essential to make the decision to establish disease management strategies. A necessary aspect to determine the importance is to know how much damage they produce and what is their incidence and prevalence in crops.

In this study, we screened strawberry plants from main production regions in Argentina for the presence of SMYEV, SMoV, SCV and SPV1 using DAS-ELISA, reverse transcription polymerase chain reaction (RT-PCR) and multiplex RT-PCR for the simultaneous detection of different viruses to estimate the incidence and prevalence of viruses and its relationship with environmental conditions.

Materials and methods

Virus detection

Collected strawberry leaves samples were tested for SMYEV by DAS-ELISA with antisera (BIOREBA, Latin-American SRL, Mar del Plata, Argentina) according to the manufacturer's specifications. The leaves were also analysed in a multiplex RT-PCR to detect SMYEV, SMoV and SCV, as well as in a standard RT-PCR to screen for the presence of SPV1.

Nucleic acids were extracted from strawberry plants using a modified cetyltrimethylammonium bromide method as described by Chang *et al.* (2007). Random primers and the MMLV high-performance reverse transcriptase (Epicentre, an illumina company, Cat. No RT80125K USA) were then used to generate cDNA for PCR amplifications. The multiplex RT-PCR was developed using YT1 and Y2 primers for SMYEV (861 bp amplicon; Thompson & Jelkmann, 2004), Cito2/for and Cito2/rev primers for SCV (687 bp amplicon; Perotto *et al.,* 2014) and D1 and D3 primers for SMoV (219 bp amplicon; Thompson *et al.,* 2003). The PCR mixture (12.5 μL final volume) contained 1 μL cDNA template, 1.25 μL 10× reaction buffer (Kapa Biosystems), 0.25 μM dNTP mixture, 0.05 μM YT1 and Y2 primers, 0.25 μM D1 and D3 primers, and 0.6 μM Cito2/for and Cito2/rev primers, and 0.5 U Taq DNA polymerase (Kapa). The PCR programme was as follows: 94°C for 2 min; 40 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 1 min; 72°C for 10 min (Conci *et al.,* 2017).

The RT-PCR used to detect SPV1 involved the Polero2Fw and Polero40Rv primers (1030 bp amplicon), which had been designed based on the polymerase gene (Luciani *et al.,* 2016). The PCR mixture (20 μL final volume) contained 1 μL cDNA template, 2 μL 10× reaction buffer (Kapa), 0.25 μM dNTP mixture, 0.5 μM Polero2Fw and Polero40Rv primers, and 1 U Taq DNA polymerase (Kapa). The PCR programme was as follows: 94°C for 2 min; 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; 72°C for 10 min. Healthy *Fragaria virginiana* clone UC-12 plants and strawberry plants infected with viruses (i.e. SMYEV, SMoV, SCV and SPV1) were used as negative and positive controls, respectively. The PCR products were separated by 2% agarose gel electrophoresis using tris acetate-ethylenediaminetetraacetic acid buffer, and then visualised under UV light after being stained with ethidium bromide.

Sample collection

Two surveys were conducted in this study. The first involved screening strawberry plants for SMYEV by DAS-ELISA. The survey was completed over two consecutive years (2009 and 2010) in the most important strawberry-producing regions in Argentina, namely Lules in Tucumán province (−26.9255515 S, −65.337851 W) and Coronda in Santa Fe province (−31.957895 S, −60.930951 W). During the second survey in 2014, strawberry plants were analysed for the presence of four viruses (i.e. SMYEV, SMoV, SCV and SPV1). Samples were collected in Lules, Coronda and Mar del Plata in Buenos Aires province (−38.018455 S, −57.583571 W) and then analysed by RT-PCR. Mar del Plata region was added in this study because it represents an important area for strawberry production in Argentina. A map indicating the locations of the sampling sites is provided in Fig. 1.

First survey

In 2009, 1034 plants from 28 fields (698 plants from Lules and 336 plants from Coronda) were analysed. Another 1060 plants from 33 fields (700 plants from Lules and 360 plants from Coronda) were tested in 2010. From each plant, one young fully expanded trifoliate leaf collected during the flowering and fruiting stage of the crop was analysed.

Strawberry fields were sampled using a random block design. Five to nine blocks per field were analysed, and between 30 and 60 leaves were collected per field, depending on the size of the field. Each block was 20 m long and comprised two wide rows of plants. Six leaves were collected along the block (two at the beginning, two in the middle and two at the end). Young leaves were collected from different plants, transported to the laboratory in a refrigerator with coolant bags, and stored in polyethylene bags at 4°C until analysed. The sampled cultivars were Camino Real, Camarosa, Festival, Albion and Fortuna.

Second survey

In 2014, strawberry leaves were sampled from 43 fields, which represented 8–10% of the total planted area in each region. A total of 606 samples were collected during the flowering and fruiting stage of the crop (208 from Lules, 285 from Coronda and 113 from Mar del Plata; Fig. 1). A random sample was taken every 10 m in the direction of the furrows and several furrows were tracked in each field. Between 10 and 30 leaves were collected per field, depending on field size. Leaf samples were stored in plastic bags and transported to the laboratory in a refrigerator with coolant bags. They were then stored at −75°C until analysed. The collected samples included Camino Real, Festival, Benicia, Splendour and San Andrea cultivars. Additionally, the symptomatic plants were counted in each examined field.

Data analyses

Virus prevalence was calculated as the percentage of fields with infected plants (Madden *et al.,* 2007). Virus incidence was calculated as the percentage of infected plants adjusted to a generalised linear mixed model under a binary distribution with a logit link function. Additionally, *a posteriori* Fisher's least significant difference (LSD) test

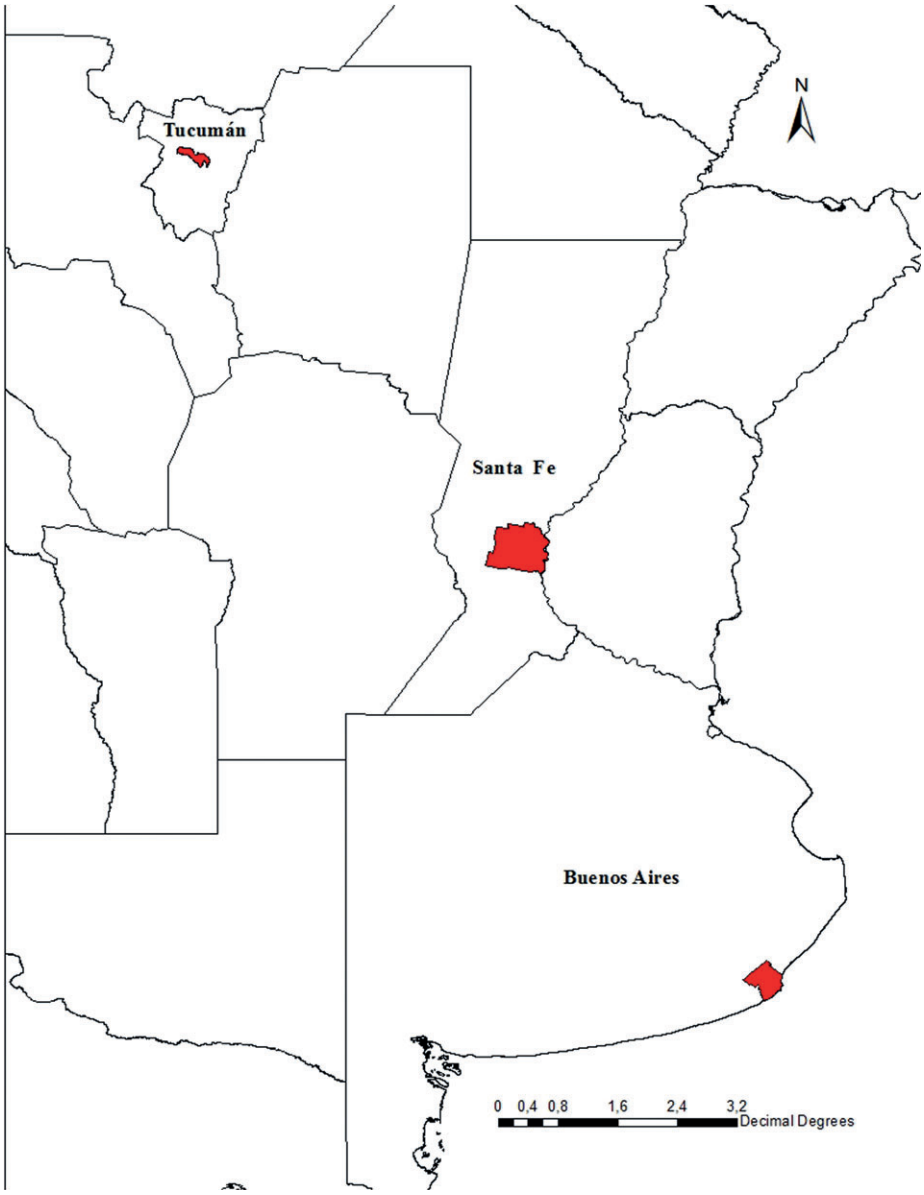

Figure 1 Section of Argentina map showing sampling regions. Lules in Tucumán province, Coronda in Santa Fe province and Mar del Plata in Buenos Aires province.

was used to compare data among regions, sampling years and cultivars. Subsequently, a general linear model was estimated for the following climatic parameters in each region: maximum (Tmax), minimum (Tmin) and mean (Tmean) temperatures as well as mean precipitation (PP). A biplot based on a principal component analysis was prepared for climatic variables and virus incidences using regions as the classification criteria. Associations between viruses, between viruses and symptomatic plants, and between viruses and climatic parameters were examined using Spearman's correlation coefficient test. All analyses were completed using the InfoStat program (version 2017) (Córdoba, Argentina, Di Rienzo *et al.*, 2017).

Results

Virus detection

The multiplex RT-PCR conducted on virus-infected strawberry plants amplified genomic fragments of the expected size for SMYEV, SCV and/or SMoV (Fig. 2). The RT-PCR for SPV1 detection also showed a band of the expected

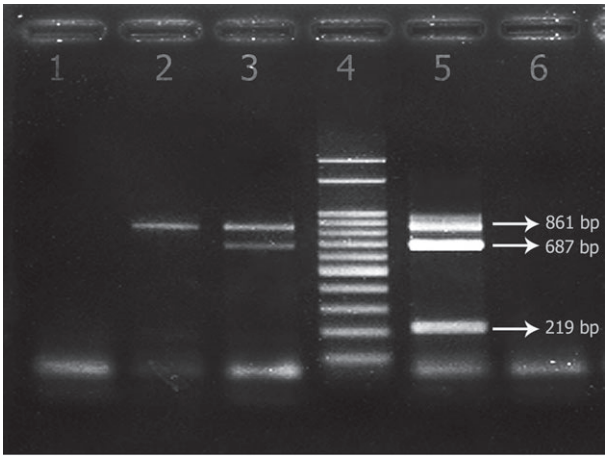

Figure 2 Agarose gel (2%) showing amplified fragments of expected sizes of *Strawberry mild yellow edge virus* (SMYEV, 861 bp), *Strawberry crinkle virus* (SCV, 687 bp) and *Strawberry mottle virus* (SMoV, 219 bp). Lane 1, PCR mix without sample used as negative control; lane 2, SMYEV-infected strawberry plant; lane 3, SMYEV- and SCV-infected strawberry plant; lane 4, molecular weight DNA ladder 100 bp (Embiotec); lane 5, SMYEV-, SCV- and SMoV-infected strawberry plant; lane 6, healthy strawberry plant used as negative control.

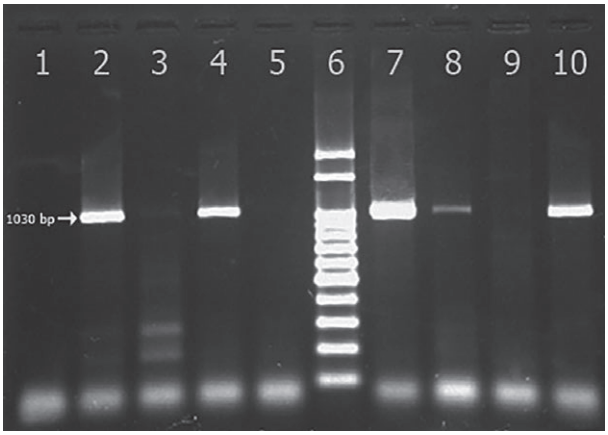

Figure 3 Agarose gel (2%) showing amplified fragments of expected sizes of Strawberry polerovirus 1 (SPV1, 1030 bp). Lane 1, healthy strawberry plant used as negative control; lanes 2–5, strawberry samples from Coronda; lane 6, molecular weight DNA ladder 100 bp (Embiotec); lanes 7–10 strawberry samples from Coronda.

size (Fig. 3). These tests were used to detect viruses in the samples collected in three regions.

Virus surveys

First survey

Significant differences in SMYEV incidence and prevalence were detected between the two analysed years ($P = 0.0001$). In 2009, the SMYEV incidence and prevalence were 3–4% and 60–65%, respectively, in Lules, Tucumán province and Coronda, Santa Fe province, with no significant differences between regions (Table 1). In 2010, the Lules region had a higher SMYEV incidence (22%) than the Coronda region (11%), while there was no significant difference in the virus prevalence (92% and 100%; Table 1).

In 2009 and 2010, 60% of the surveyed area consisted of the Camarosa cultivar, which was the only cultivar that was grown in both Lules and Coronda. The virus incidence for this cultivar was not significantly different between regions in both tested years ($P = 0.5131$, 2009 and $P = 0.3537$, 2010). When the cultivar and region were considered together, there were no significant differences between cultivars in the SMYEV incidence in 2009. In contrast, in 2010, there were significant differences between the SMYEV incidence of Albion and Camino Real and that of Fortuna, Festival, and Camarosa (Table 2).

Second survey

Four viruses were detected in all regions. The mean incidence of SMYEV in all studied regions was 20.5% (124 infected samples), while the incidences of SMoV (94 infected plants), SPV1 (72 infected plants) and SCV (14 infected plants) were 15.5%, 12%, and 2%, respectively, for the 606 analysed samples collected in the three regions.

The data revealed that SMYEV was the most frequently detected virus (17–35%, depending on the region). The highest SMYEV incidence was detected in Mar del Plata (35%). However, there were no significant differences in the SMYEV prevalence among the regions. The SMoV incidence was 15–17% and prevalence was 62–64% among regions and there were no significant differences. In contrast, the SPV1 incidence differed between Lules (8%) and Coronda (15%), while the SPV1 incidence in Mar del Plata was in between the values for the other two regions. The SPV1 prevalence was similar in the three regions. The SCV incidence was the lowest of all the analysed viruses (0.5–8%), but significant differences were observed among regions, with the highest incidence (8%) and prevalence (50%) recorded for Mar del Plata (Table 1).

The cultivars most affected by SMYEV were San Andreas (in Lules and Mar del Plata) and Camino Real in Coronda, with incidences between 31% and 35%. The lowest SMYEV incidence (3%) was observed for Camino Real in Lules. Relatively low SMYEV incidences were also observed for the Festival, Splendour and Benicia cultivars (4–19%) (Table 2). Of the plants infected by SMoV, San Andreas plants in Lules appeared to have

Table 1 Incidence and prevalence of SMYEV, SMoV, SPV1 and SCV

	SMYEV						SMoV		SPV1		SCV	
	2009		2010		2014		2014		2014		2014	
Regions	Incidence	Prevalence	Incidence	Prevalence	Incidence	Prevalence	Incidence	Prevalence	Incidence	Prevalence	Incidence	Prevalence
Lules	3.7a	65a	21.7a	100a	16.8b	69a	16.8a	62a	8.2b	46a	0.5b	8b
Coronda	3.3a	60a	10.6b	92a	17.2b	64a	14.7a	64a	15.1a	50a	1.4b	9b
Mar del Plata	–	–	–	–	35.4a	88a	15.0a	63a	10.6ab	50a	8.0a	50a
Total[a]	3.6	63	17.9	97	20.5	69.7	15.5	62.8	11.9	48.8	2.3	16.3

SCV, *Strawberry crinkle virus*; SMoV, *Strawberry mottle virus*; SMYEV, *Strawberry mild yellow edge virus*; SPV1, Strawberry polerovirus 1. Values with different letters are significantly different between regions based on a posterior comparison method of LSD Fisher ($P < 0.05$).
[a]Incidence and prevalence total was calculated from the total of analysed plants, not as an average of regions.

Table 2 Percentage incidence of SMYEV, SMoV, SPV1 and SCV per cultivar and region

	SMYEV			SMoV	SPV1	SCV
Cultivar–Region	2009	2010	2014	2014	2014	2014
Albion–Lules	3a	37a	–	–	–	–
Fortuna–Coronda	–	3c	–	–	–	–
Camarosa–Lules	4a	16c	–	–	–	–
Camarosa–Coronda	5a	17bc	–	–	–	–
Camino Real–Lules	–	24ab	3e	4a	9bcd	0a
Camino Real–Coronda	–	–	31ab	26a	29a	1a
Festival–Lules	–	–	7cde	0a	21ab	4a
Festival–Coronda	0a	5c	4de	4a	1d	0a
Splendour–Coronda	–	–	13cd	13a	3cd	5a
Benicia–Coronda	–	–	19bc	16a	24a	0a
San Andreas–Lules	–	–	33a	34a	3cd	0a
San Andreas–Mar del Plata	–	–	35a	15a	11bc	8a

SCV, *Strawberry crinkle virus*; SMoV, *Strawberry mottle virus*; SMYEV, *Strawberry mild yellow edge virus*; SPV1, Strawberry polerovirus 1. Values with different letters are significantly different between cultivar–region in the year based on a posterior comparison method LSD Fisher ($P < 0.05$).

the highest incidence (34%). Nevertheless, for this virus there were no significant differences in the incidences for all tested cultivars, regardless of location (Table 2). The cultivars most affected by SPV1 were Benicia and Camino Real in Coronda and Festival in Lules (21–29%). However, there were no significant differences between Festival and Camino Real in Lules and San Andreas in Mar del Plata. Additionally, the SPV1 incidence for Camino Real was significantly lower in Lules (9%) than in Coronda (29%). Moreover, the SPV1 incidence for Festival differed between Lules (21%) and Coronda (1%) (Table 2). Meanwhile, SCV was not detected in some cultivars in specific regions. San Andreas from Mar del Plata appeared to be the cultivar most affected by SCV (8%). However, there were no significant differences among the SCV incidences among cultivars and regions (Table 2).

Of the 606 analysed plants, 207 were infected (34%), of which 63%, 29%, 7% and 1% were infected by 1, 2, 3 and 4 viruses, respectively. The most frequent infections were caused by SMYEV (26%), SMoV (18%) and SPV1 (17%) alone or SMYEV + SMoV (16%). Infections involving SMYEV + SPV1 (7%) or SMYEV + SMoV + SPV1 (6%) occurred less frequently, while infections by the remaining viral combinations were relatively rare (Fig. 4).

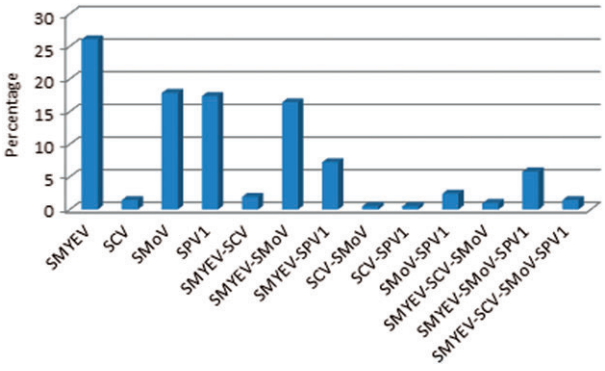

Figure 4 Percentage of strawberry plants infected with different virus combination in the second survey (2014). SCV, *Strawberry crinkle virus*; SMoV, *Strawberry mottle virus*; SMYEV, *Strawberry mild yellow edge virus*; SPV1, Strawberry polerovirus 1.

Table 3 Correlation analysis between viruses and virus with symptomatic plants

Variable(1)	Variable(2)	Spearman	*P*-value
SMYEV	SCV	0.17	<0.0001
SMYEV	SMoV	0.36	<0.0001
SMYEV	SPV1	0.19	<0.0001
SMoV	SCV	0.12	0.0042
SMoV	SPV1	0.12	0.0022
SCV	SPV1	0.08	0.051
Symptomatic plants	SMYEV	0.12	0.0026
Symptomatic plants	SCV	0.17	<0.0001
Symptomatic plants	SMoV	0.07	0.0852
Symptomatic plants	SPV1	0.02	0.6714
Tmax	SMYEV	−0.13	0.0011
Tmax	SCV	−0.15	0.0002
Tmax	SMoV	0.02	0.5881
Tmax	SPV1	−0.05	0.2084
Tmin	SMYEV	−0.13	0.0017
Tmin	SCV	−0.11	0.0052
Tmin	SMoV	−0.01	0.7496
Tmin	SPV1	0.08	0.0534
Tmean	SMYEV	−0.13	0.0017
Tmean	SCV	−0.11	0.0052
Tmean	SMoV	−0.01	0.7496
Tmean	SPV1	0.08	0.0534
PP	SMYEV	−0.02	0.6897
PP	SCV	0.01	0.8717
PP	SMoV	−0.02	0.5416
PP	SPV1	0.1	0.0173

SCV, *Strawberry crinkle virus*; SMoV, *Strawberry mottle virus*; SMYEV, *Strawberry mild yellow edge virus*; SPV1, Strawberry polerovirus 1. Spearman correlation analysed on the total number of plants ($n = 606$).

When the relationships among the four viruses were analysed by Spearman's correlation coefficient, a significant and positive correlation was observed between SMYEV and SMoV, SCV and SPV1 as well as between SMoV and SCV and SPV1 (Table 3). There were also significant and positive correlations between the number of symptomatic plants in each field and two viruses (SCV and SMYEV; Table 3). Significant negative correlations were observed between temperature parameters (Tmax, Tmin and Tmean) and specific viruses (SMYEV and SCV), while the PP was correlated only with SPV1 (Table 3), although the ρ values of the correlations were low.

Additionally, there were significant differences in the climatic parameters among the examined regions. Mar del Plata had lower Tmax (20.89), Tmin (7.27) and Tmean (13.55) values than Lules and Coronda (Tmax, 23.57 and 22.83; Tmin, 11.41 and 12.33; and Tmean, 17.15 and 17.22, respectively). However, there were no significant differences in the PP of different regions (1.22, 2.42 and 1.8 in Lules, Coronda and Mar del Plata, respectively).

A multivariate principal component analysis was completed and a biplot was constructed. Principal components 1 and 2 explained 64% and 36% of the data variability, respectively. SCV and SMYEV were positively related to Mar del Plata, but negatively related to temperatures. The SPV1 was related to Coronda and the PP, while SMoV was related to Lules and partially related to Tmax and Tmean (Fig. 5).

Discussion

Surveys of viral diseases are often conducted using serological assays such as ELISA because many plants can be tested simultaneously. Of the four strawberry viruses detected in Argentina, only SMYEV can be analysed by DAS-ELISA, probably because it is the only one that has sufficient concentration in the plant to be detected serologically. The other three viruses (SCV, SMoV and SPV1) must be analysed by more sensitive techniques. The RT-PCR represents the most reliable technique for virus detection because it is highly sensitive, even at low virus concentrations. Drawbacks to PCR-based diagnostic tests include the fact that they are relatively expensive and laborious, especially when analysing many samples. For this reason, a multiplex RT-PCR including three of the detected viruses was implemented. This facilitated the diagnosis of viruses in a high number of samples. Consequently, more sensitive and efficient diagnostic systems should be developed to simplify the detection of viruses.

Plants sampled in the three most important strawberry-producing regions in Argentina were infected by SMYEV, SMoV, SCV and SPV1, with varying infection rates depending on the virus, year and region. The first incidence and prevalence studies focused on SMYEV infections in large sample sizes, which was possible because a DAS-ELISA was used. Subsequent viral identifications, characterizations and diagnoses of viruses in Argentina revealed SMoV, SCV and SPV1 in addition to SMYEV in single or mixed infections (Conci *et al.,* 2009; Perotto *et al.,* 2014; Luciani *et al.,* 2016; Conci *et al.,* 2017). Additionally, molecular analyses of the four viruses detected in 606 randomly collected samples from three regions provided new details regarding viral incidence, prevalence and relationships. A multiplex RT-PCR facilitated the analysis of many samples. The results showed that the most abundant virus was SMYEV, followed by SMoV and SPV1. Additionally, SCV was detected with low frequency, but the incidence and prevalence values indicated that this virus was more abundant in Mar del Plata than in the other regions. Comparing our viral prevalence and incidence data with those in other studies conducted outside Argentina may be problematic because most of the previous studies involved relatively few samples as well as infection rates for symptomatic plants only. There have been few reports

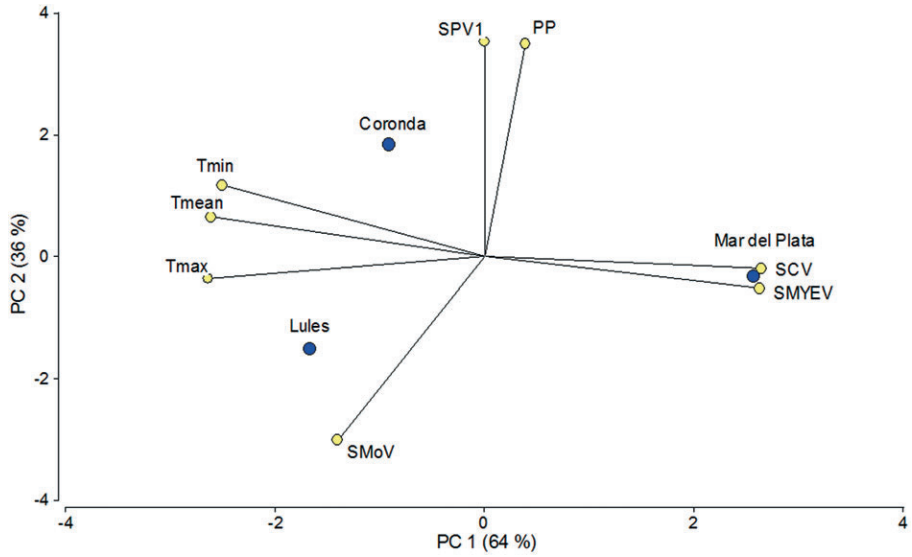

Figure 5 Principal components analysis between climatic conditions, sampled regions and viruses.

describing the incidence of SMoV, SPV1 and SCV in a large number of plants randomly sampled from different strawberry fields (Martin & Tzanetakis, 2013; Rojas *et al.*, 2013).

According to the DAS-ELISA results, 4–22% of the analysed plants were infected by SMYEV in 2009 and 2010. In 2014, when samples were analysed by RT-PCR, which is capable of detecting the viruses at relatively low concentrations, 17% of the plants were infected by SMYEV in Lules and Coronda, while 35% of the plants were infected in Mar del Plata. The significant differences in the virus incidences between years may have been due to several factors. For example, varying climatic conditions between years may have influenced the multiplication of the viral vectors, thereby affecting the number of infected plants. Moreover, there may have been differences in the efficiency of the methods used to control the viral vectors in the nurseries during different crop cycles. Furthermore, the certified plants arriving in Argentina may have differed regarding infection rates. Additionally, in Mar del Plata region the strawberry plant is frequently kept for 2 or 3 years and this is an important condition to increase the virus dispersion. Interestingly, this increase in the number of infected plants in Mar del Plata was only observed for SMYEV and SCV, but not for the other two viruses.

The widespread infection by SMYEV was not particularly noteworthy because this virus has been detected in many countries worldwide (Converse, 1987; Martin & Tzanetakis, 2006). Thus, it was unsurprising that the prevalence of SMYEV was high (60–100%), depending on the region and sampling year. In randomly collected samples from different North American regions, 167 of 464 (36%) strawberry plants were infected with SMYEV (Martin & Tzanetakis, 2013). Additionally, in the region formerly known as Yugoslavia, the SMYEV incidence was 20% (Dulic-Markovic *et al.*, 1998), while in China, SMYEV was detected in 53 of 93 (57%) samples collected in different strawberry-producing areas from 2005 to 2009 (Li & Yang, 2011). An earlier study in Chile indicated the SMYEV incidence was 66% in symptomatic plants (Cabrera *et al.*, 2005), while a more recent study involving 20 randomly selected plants per region reported a SMYEV incidence between 0% and 100% (Rojas *et al.*, 2013). In Argentina, SMYEV was first reported in 2009, when it was detected in the strawberry cultivar Camarosa grown in Tucumán province. The virus was present in 67% of symptomatic plants (8/12) and in 3% of asymptomatic plants (12/454) (Conci *et al.*, 2009).

When we study the pathogens that affect a crop, a very important aspect is to establish the damages caused by this pathogen to production. For this it is necessary to determine the percentage of affected plants, the incidence, and the number of lots in which the virus is present, the prevalence. These parameters are fundamental to estimate what will be the decrease in crop yield and fruit quality. This information contributes to establish strategies of crop management tending to decrease the incidence and prevalence of the pathogen. In a previous study, we concluded that SMYEV can reduce strawberry fruit production by 28–63% (Torrico *et al.*, 2017). Another study determined that the yield losses caused by this virus in strawberries vary between 0% and 30% (Martin & Tzanetakis, 2006). Therefore, the incidence and

prevalence of SMYEV observed in this study are probably causing a considerable decrease in the production of strawberries.

When the presence of SMYEV in cultivars and regions were analysed, significant differences were detected in the years in which the virus was present at relatively high levels (2010 and 2014). When the region was considered, significant differences were observed in the data for Camino Real plants grown in Lules and Coronda, but not for the other cultivars that were tested simultaneously in more than one region. Many strawberry cultivars exhibit a differential response to viral infections (Martin & Tzanetakis, 2006). Although there is no known resistance to SMYEV among *Fragaria* spp., most cultivars are tolerant to a single SMYEV infection. Meanwhile, susceptible genotypes exhibit dwarfism, marginal chlorosis, leaf distortion and decreased fruit size, while tolerant species are asymptomatic unless they are infected by a highly virulent strain or by more than one virus (Martin & Tzanetakis, 2006).

The second most abundant virus detected in this study was SMoV. There were no significant differences in the SMoV incidence and prevalence between regions or between cultivars. The average incidence and prevalence for this virus among the analysed regions were 15% and 63%, respectively. Although SMoV was reportedly the most widespread strawberry virus (Converse, 1987; Maas, 1998; Martin & Tzanetakis, 2006), similar to other strawberry viruses, there have been relatively few studies that evaluated its incidence and prevalence in randomly collected samples. There have been reports of a relatively high SMoV prevalence (>60%), but the SMoV incidence in Argentina has mostly been lower than that observed in other countries, with no significant differences among regions. In randomly collected samples from different parts of North America, 182 of 464 plants were infected by SMoV, with important differences among regions (8–69%) (Martin & Tzanetakis, 2013). In Chile, SMoV was detected in six of seven investigated regions (86%) (Rojas *et al.,* 2013). Furthermore, in seven strawberry-producing regions in the former Yugoslavia, the most frequently encountered strawberry virus was SMoV (24 of 33 fields; 73%) (Dulic-Markovic *et al.,* 1998).

With an incidence and prevalence of 2.3% and 16.3%, respectively, the most infrequently detected virus in Argentina was SCV. This observation is consistent with the results of previous studies conducted in other countries. In Yugoslavia, SCV was detected in only 3 of 33 analysed fields (Dulic-Markovic *et al.,* 1998), while in North America, only 50 of 464 plants were infected by SCV, with differences among regions (Martin & Tzanetakis, 2013). We also observed significant differences in the incidence and prevalence of SCV among regions beginning high in Mar del Plata (8% and 50%, respectively). The environmental conditions in the Mar del Plata region may be conducive to increasing the vector population or viral concentrations in plants and/or vectors. SCV is a rhabdovirus that is transmitted by aphids in a persistent-propagative manner, and the transmission time is influenced by temperature. Additionally, for the efficient transmission of SCV, the vector must remain viable for a sufficiently long period to enable the completion of the virus latency period (Krczal, 1982). Thus, the relatively long strawberry life cycle in the Mar del Plata region is likely favourable for the vector transmission of SCV.

Our analyses revealed a strong correlation between SCV levels and disease symptoms in the field ($P < 0.0001$). This is consistent with the results of other studies in which researchers concluded that SCV is probably the most harmful of the strawberry viruses, with some strains capable of inducing severe symptoms, mainly in mixed infections (Converse, 1987; Martin & Tzanetakis, 2006).

Of the analysed regions, Mar del Plata had the lowest temperatures (Tmax, 20.89°C; Tmin, 7.27°C and Tmean, 13.55°C), and the temperature parameters were negatively correlated with SMYEV and SCV. This is consistent with the lowest incidence values recorded for Lules and Coronda, which were associated with relatively high temperatures (Tmax, 23–24°C; Tmin, 11–12°C and Tmean, 17°C). This is in accordance with Frazier *et al.* (1965) that mentioned that SCV was inactivated in a high percentage of plants during summer temperatures reached a mean of 32 °C.

The third most important virus was SPV1, with an average incidence and prevalence of 12% and 49%, respectively. The SPV1 incidence varied significantly among regions and cultivars. Xiang *et al.* (2015) recently described this virus as a polerovirus (*Luteoviridae*). Additionally, there are no previous incidence reports, although a luteovirus has been detected in strawberry plants (Yoshikawa *et al.,* 1984; Converse, 1987). The fact that SMYEV was considered to be a luteovirus (Converse, 1987) before being identified as a potexvirus suggests SMYEV and a luteovirus may be related (Jelkmann *et al.,* 1990). Several years later, Xiang *et al.* (2015) detected a luteovirus in strawberry (SPV1) and observed that 87% of SMYEV-infected plants were also infected with SPV1. However, we did not detect a correlation between these two viruses. Instead, the most common combination observed in this study was SMoV–SMYEV (Fig. 4), which is not surprising considering these two viruses were the most frequently encountered. Spearman's correlation analyses indicated SMYEV was correlated with SCV, SMoV and SPV1 ($P < 0.0001$), although the ρ values were low. Additionally, the principal component analysis revealed that SMYEV was closely related to SCV in the

Mar del Plata region, but not to SMoV and SPV1. The potential interaction between SMYEV and SPV1 should be studied more extensively.

The principal component analysis also implied that SPV1 was closely related to the PP and Coronda where the PP was higher than Lules but not significantly different than Mar del Plata regions, then, this relation is not clear. However, in Spearman's analysis, also a relationship between PP and SPV1 was detected. This should be better studied since it is difficult to find an association between PP and viruses or viral vectors.

Considering the total of the infected plants detected (34%) the most frequent infections were caused by the viruses alone (63%). Assuming that in most of the strawberry cultivars symptoms are observed when the infections are mixed, it could be assumed that 37% of the total of infected plants showed symptoms of disease. It is known that strawberry plants with virus symptoms do not produce fruits, or very few (Maas, 1998) so these results could imply the losses caused by viruses in production.

Undoubtedly, another important factor influencing the distribution of these viruses is the presence of aphids. *Chaetosiphon* species, especially *C. fragaefolii*, are reportedly the main natural vectors of strawberry viruses (Converse, 1987), including SMYEV, SMoV, SCV and a strawberry luteovirus. Additionally, *C. fragaefolii* has been detected in Argentinian strawberry fields (Dughetti *et al.*, 2017). The abundance of this aphid in strawberry plants appears to be influenced by the cultivar, with Carmine, Diamante and Festival being associated with the highest aphid levels (Rondon & Cantliffe, 2004). This implies that aphids have preferences for some strawberry cultivars, which may increase the chances the preferred cultivars will be infected. This may explain, at least partially, some of the differences between cultivars observed in this study. However, the virus incidences of the Festival plants were not higher than those of the other cultivars. This point should be examined in greater detail in future studies.

Fluctuations in population size have been frequently reported for many strawberry aphid species (Bernardi *et al.*, 2013; Dughetti *et al.*, 2017). For example, in the case of *C. fragaefolii* in Argentina, significant changes in the population size have been observed during the strawberry lifecycle. These changes may be related to the agronomic practices used during strawberry production (Cédola & Greco, 2010). Changes in the aphid population may be particularly problematic because they cannot be detected until it is too late. A frequently used method to control aphid populations involves the regular application of insecticides based on an annual schedule. However, many strawberry growers have started to decrease the use of pesticides because of the associated costs and/or a desire to produce fruits that are free of chemical contaminants. The controlled use of pesticides is usually advised, but inefficient management of aphid populations, especially in the nursery, may increase aphid numbers and enhance the spread of viruses.

Viruses are widely distributed in all strawberry-growing areas worldwide. Their presence should be a cause for concern as they can be transmitted by the same vectors to produce mixed infections. The synergistic effects among different viral combinations may lead to considerable decreases in strawberry quality and yield, which are preceded by severe deformations of plants.

Acknowledgements

This work was supported by the Instituto Nacional de Tecnología Agropecuaria (INTA) of Argentina and the Agencia Nacional de Promoción Científica y Tecnológica of Argentina through a FONCyT grant. The authors thank the National Meteorological Service of Argentina and the weather and water information system of INTA for providing the daily values for the analysed climatic parameters. The authors also thank M.H. Sordo, N. Meneguzzi, S. Salazar, F. Fernandez, A.M. Borquez, E.G. Adlercreutz, R.J. Quiroga and M.E. Navarro for their assistance in collecting samples in different strawberry-producing regions. We thank Edanz Group (www.edanzediting.com/ac) for English edit. This paper does not represent any conflicts of interest.

References

Bernardi D., Araujo E.S., Zawadneak M.A.C., Botton M., Mogor A.F., Garcia M.S. (2013) Aphid species and population dynamics associated with strawberry. *Neotropical Entomology*, **42**, 628–633.

Betti J.A. (1980) Viroses do morangueiro e sue controle. *Fitopatologia Brasileira*, **6**, 217–218.

Cabrera M., Toro M., Muñoz M., Flores R., Baldera M., Bustos A., Lopez L., Martin R. (2005) Prospección de virus en plantas de *Fragaria x annanasa* y *Fragaria chiloensis* en la VIII región. Fitopatología, 40, 148.

Cédola C., Greco N. (2010) Presence of the aphid, *Chaetosiphon fragaefolii*, on strawberry in Argentina. *Journal of Insect Science*, **10**, 1–9.

Chang L., Zhang Z., Yang H., Li H., Dai H. (2007) Detection of strawberry RNA and DNA viruses by RT-PCR using total nucleic acid as a template. *Journal of Phytopathology*, **155**, 431–436. https://doi.org/10.1111/j.1439-0434.2007.01254.x.

Conci V.C., Torrico A.K., Cafrune E., Quevedo V., Baino O., Ramallo J.C., Borquez A.M., Mollinedo V.A., Agüero J.J., Kirschbaum D. (2009) First report of *Strawberry mild*

yellow edge virus in Argentina. *Acta Horticulturae*, **842**, 303–306.

Conci V.C., Luciani C.E., Merino M.C., Celli M.G., Perotto M.C., Torrico A.K., Pozzi A.E., Strumia G., Dughetti A.C., Asinari F., Conci L.R., Fernandez F.D., Salazar S.M., Meneguzzi N.G., Kirschbaum D.S. (2017) Advances in characterization and epidemiology of strawberry viruses and phytoplasmas in Argentina. *Acta Horticulturae*, **1156**, 801–809. https://doi.org/10.17660/ActaHortic.2017.1156.118.

Converse R.H. (1987) *Virus Diseases of Small Fruits*. Washington, DC, USA: USDA, Department of Agriculture, Agricultural Research Service.

Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M., Robledo C.W. (2017) *InfoStat Versión 2017*. Argentina: Grupo InfoStat, FCA, Universidad Nacional de Córdoba URL: http://www.infostat.com.ar.

Dughetti A.C., Kirshbaum D., Conci V.C. (2017) Áfidos trasmisores de virus detectados en cultivos de frutilla en Argentina. *Revista de Investigaciones Agropecuarias*, **43**, 36–50.

Dulic-Markovic I., Rankovic M., Converse R.H. (1998) Occurrence of strawberry viruses in Yugoslavia. In Proceedings of the Eighth International Symposium on Small Fruit Virus Diseases, Bethesda, MD, USA, Vol. 471, pp. 35–38. Ed. R.R. Martin.

Frazier N.W., Voth V., Bringhurst R.S. (1965) Inactivation of two strawberry viruses in plants grown in a natural high-temperature environment. *Phytopathology*, **55**, 1203–1205.

Horne W.T. (1922) Strawberry troubles. *California Agricultural Experiment Station Report*, **1921–22**, 122–123.

Jelkmann W., Martin R.R., Lesemann D.E., Vetten H.J., Skelton F. (1990) A new potexvirus associated with strawberry mild yellow edge disease. *Journal of General Virology*, **71**, 1251–1258.

Kirschbaum D.S., Vicente C.E., Cano-Torres M.A., Gambardella M., Veizaga-Pinto F.K., Antunes L.E.C. (2017) Strawberry in South America from the Caribbean to Patagonia. *Acta Horticulturae*, **1156**, 947–956.

Krczal H. (1982) Investigations of the biology of the strawberry aphid (*Chaetosiphon fragaefolli*), the most important vector of strawberry viruses in West Germany. *Acta Horticulturae*, **129**, 63–68.

Li L., Yang H. (2011) A new Chinese isolate of *Strawberry mild yellow edge virus* from *Fragaria pentaphylla*. *Journal of Plant Pathology*, **93**, S4.76.

Luciani C.E., Celli M.G., Merino M.C., Perotto M.C., Pozzi E., Conci V.C. (2016) First report of Strawberry polerovirus 1 in Argentina. *Plant Disease*, **100**, 1510. https://doi.org/10.1094/PDIS-10-15-1213-PDN.

Maas J.R. (1998) *Compendium of Strawberry Virus Diseases*. St Paul, MN, USA: APS Press.

Madden L.V., Hughes G., van den Bosch F. (2007) *The Study of Plant Disease Epidemics*. St Paul, MN, USA: APS Press.

Martin R.R., Tzanetakis I.E. (2006) Characterization and recent advances in detection of strawberry viruses. *Plant Disease*, **90**, 384–396.

Martin R.R., Tzanetakis I.E. (2013) High risk strawberry viruses by region in the United States and Canada: implications for certification, nurseries, and fruit production. *Plant Disease*, **97**, 1358–1362.

Mellor F.C., Fitzpatrick R.E. (1951) Studies of virus diseases of strawberries in British Columbia. II. The separation of the component viruses of yellows. *Canadian Journal of Botany*, **29**, 411–420.

Mellor F.C., Frazier N.W. (1970) *Strawberry mottle*. In *Virus Diseases of Small Fruits and Grapevines*, pp. 4–8. Ed. N.W. Frazier. Berkeley, CA, USA: University of California, Division of Agricultural Sciences.

Nome S.F., Yossen V. (1980) Identificación de virus de frutilla en Argentina. I. Virus del moteado de frutilla (*Strawberry mottle virus*). *Revista de Investigaciones Agropecuarias*, **15**, 245–258.

Perotto M.C., Luciani C., Celli M.G., Torrico A.K., Conci V.C. (2014) First report *of Strawberry crinkle virus* in Argentina. *New Disease Reports*, **30**, 5. https://doi.org/10.5197/j.2044-0588.2014.030.005.

Prentice I.W., Harris R.V. (1946) Resolution of strawberry virus complexes by means of the aphid vector *Capitophorus fragariae* Theo. *Annals of Applied Biology*, **33**, 50–53.

Rojas P., Almada R.D., Sandoval C., Keller K.E., Martin R.R., Caligari P.D.S. (2013) Occurrence of aphid-borne viruses in southernmost South American populations of *Fragaria chiloensis* spp. *chiloensis*. *Plant Pathology*, **62**, 428–435.

Rondon S.I., Cantliffe D.J. (2004) *Chaetosiphon fragaefolii* (Homoptera: Aphididae): a potential new pest in Florida? *Florida Entomologist*, **87**, 612–615.

Schoen C.D., Limpens W., Moller I., Groeneveld L., Klerks M.M., Lindner J.L. (2001) The complete genomic sequence of *Strawberry crinkle virus*, a member of the *Rhabdoviridae*. *Acta Horticulturae*, **656**, 45–50.

Thekke-Veetil T., Tzanetakis I.E. (2016) First report of Strawberry polerovirus-1 in strawberry in the United States. *Plant Disease*, **100**, 867. https://doi.org/10.1094/PDIS-09-15-1044-PDN.

Thompson J.R., Jelkmann W. (2004) Strain diversity and conserved genome elements in *Strawberry mild yellow edge virus*. *Archives of Virology*, **149**, 1897–1909. https://doi.org/10.1007/s00705-004-0353-4.

Thompson J.R., Wetzel S., Klerks M.M., Vašková D., Schoen S.D., Špak J., Jelkmann W. (2003) Multiplex RT-PCR detection of four aphid-borne strawberry viruses in *Fragaria* spp. in combination with a plant mRNA specific internal control. *Journal of Virological Methods*, **111**, 85–93.

Torrico A.K., Salazar S.M., Kirschbaum D.S., Conci V.C. (2017) Yield losses of asymptomatic strawberry plants infected with *Strawberry mild yellow edge virus*. *European Journal of Plant Pathology*, **150**, 983–990. https://doi.org/10.1007/s10658-017-1337-z.

Xiang Y., Bernardy M., Bhagwat B., Wiersma P.A., De Young R., Bouthillier M. (2015) The complete genome sequence of a new polerovirus in strawberry plants from eastern Canada showing strawberry decline symptoms. *Archives of Virology*, **160**, 553–556. https://doi.org/10.1007/s00705-014-2267-0.

Yoshikawa N., Ohki S.T., Kobatake H., Osaki T., Inouye T. (1984) Luteovirus-like particles in phloem tissue of *Strawberry mild yellow edge virus* infected plants. *Annals of the Phytopathological Society of Japan*, **50**, 659–663.