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Yield losses of asymptomatic strawberry plants infected with *Strawberry mild yellow edge virus*

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Abstract After successive vegetative propagation cycles, strawberry (*Fragaria* × *ananassa* Duch.) plants often accumulate multiple virus species that result in viral symptoms and losses in yield and quality. However, strawberry plants infected by a single virus species usually remain asymptomatic with unknown effects on fruit production and quality. In this context, the effect of *Strawberry mild yellow edge virus* (SMYEV) on fruit production was studied in strawberry plants, cultivar Camarosa, over two years. Asymptomatic SMYEV-infected plants showed a significant reduction in total and marketable fruit number and weight compared with healthy plants. These reductions ranged between 28% and 63%, depending on the parameter measured and the production cycle. Fluctuations in SMYEV concentration in the plants was detected throughout the crop cycle, suggesting that samples for virus diagnosis should be taken when the plant has the highest virus concentration; in this study, this occurred at the end of the crop cycle. These results show that analyzing symptomless strawberry plants should be part of a virus disease management plant and an important component to control the quantitative and qualitative impacts of SMYEV on strawberry yield.

Keywords *Fragaria x ananassa* · Strawberry viruses · Fruit losses · Berry

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Introduction

Strawberries (*Fragaria x ananassa* Duch.) are grown worldwide in approximately 80 countries (FAO 2016), including tropical, subtropical, and temperate regions (Hummer 2008).

Among strawberry pathogens, viruses are responsible for significant crop losses caused by reductions in plant growth, fruit yield, and runner plant production. As systemic pathogens, viruses are hard to control in strawberry because these plants are vegetatively propagated to obtain the runner plants used for fruit production. This clonal propagation mechanism means that viruses are transmitted from diseased mother plants to daughter plants. Some strawberry viruses can also be transmitted by pollen and/or vectors such as aphids, thrips, nematodes, and fungi (Converse 1987; Maas 1998; Martin and Tzanetakis 2006).

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More than 30 viruses, phytoplasmas, and other systemic pathogens have been reported in strawberry (Converse 1987; Martin and Tzanetakis 2006; Fernández et al. 2013, 2015). An interesting feature of this pathosystem is that, in most strawberry cultivars, single virus infections show no symptoms; however, when a mixture of viruses infect strawberry plants symptoms and significant yield losses as well as reduction of plant growth and vigor are often observed (Barritt and Loo 1973; Bolton 1974; Babovic 1976; Converse 1987; Maas 1998; Martin and Tzanetakis 2006).

The main aphid-transmitted strawberry viruses are *Strawberry mottle virus* (SMoV), *Strawberry crinkle virus* (SCV), *Strawberry vein banding virus* (SVBV), *Strawberry mild yellow edge virus* (SMYEV), *Strawberry chlorotic fleck virus*, *Strawberry latent C virus,* and Strawberry polerovirus 1 (SPV1); these have been recorded in most strawberry production regions worldwide (Lamprecht and Jelkmann 1997; Mráz et al. 1998; Thompson and Jelkmann 2003; Klerks et al. 2004; Hanzlíková-Vašková et al. 2006; Martin and Tzanetakis 2006; Xiang et al. 2015). In Argentina, SMYEV, SCV, SMoV, and SPV1 have been detected in the most important production regions (Nome and Yossen 1980; Conci et al. 2009; Perotto et al. 2014; Luciani et al. 2016; Conci et al. 2017).

SMYEV was tentatively reported as a luteovirus (Converse 1987) before potexvirus particles were associated with this disease (Jelkmann et al. 1990; Lamprecht and Jelkmann 1997). This is one of the most common viruses in strawberry worldwide; SMYEV has been reported in all countries studied across Asia, Oceania, Europe, Africa, North America, and South America (Hepp and Martin 1992; Maas 1998; Conci et al. 2009; Cho et al. 2011; Ma et al. 2015).

A potexvirus identified as SMYEV has been detected in strawberry plants of the cultivar Camarosa in Argentina using a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and with immunocapture semi-nested reverse-transcription polymerase chain reaction (RT-PCR) with specific primers (Conci et al. 2009). Pathogen identity was confirmed by amplification, cloning, and comparison of the genomic sequence of the viral capsid protein gene with other sequences published in GenBank (Torrico et al. 2016).

The economic importance of SMYEV as a plant pathogen is related to its synergistic effects in mixed viral infections that can result in yield losses of up to 80% (Converse 1987; Martin and Tzanetakis 2006; Cho et al. 2011). Plants infected with a mixture of viruses often show a loss of vigor and severe symptoms including dwarfing, leaf distortion, size reduction, mottling and suffer severe yield losses (Converse 1987; Maas 1998). However, little information is available regarding the specific effects of SMYEV in single infections on strawberry yields because such plants are often asymptomatic. This is an important issue as it may represent a serious threat in terms of fruit production.

The lack of information regarding the effect of single strawberry viruses on yield is partly due to difficulties in obtaining single virus infections as the aphid-transmitted viruses are all vectored by the same aphid. This paper reports the effects of SMYEV on fruit production in asymptomatic strawberry plants and describes the changes in SMYEV concentration observed during the crop cycle.

Materials and methods

Two groups of 'Camarosa' strawberry plants, one healthy and one naturally infected by SMYEV but asymptomatic, were compared in two different field experiments. Experiment I was performed over two crop cycles while Experiment II was performed over a single growing season.

In both experiments, fresh dug, leafless, bare-rooted 'Camarosa' plants were planted at the Instituto Nacional de Tecnología Agropecuaria (INTA), at the Estación Experimental Agropecuaria Famaillá (27° 03′ S, 65° 25′ W, 363-m altitude) in Tucumán, Argentina. Plants were established in offset two-row beds covered with black polyethylene and filled with mulch (0.50 m wide × 0.30 m high) using an in-row plant spacing of 0.23 m (69,500 plants ha^{-1}).

Before planting, 48 kg ha^{-1} N and 123 kg ha^{-1} P_2O_5 fertilizer were applied to the beds. Additional N (144 kg ha^{-1}, K (303 kg ha^{-1} K_2O), P (57 kg ha^{-1} P_2O_5), Ca (125 kg ha^{-1} CaO), and Mg (25.5 kg ha^{-1} MgO) were applied during the growing season by fertigation twice per week. Irrigation was applied three to four times per week. Insecticides were applied to prevent aphid infestations.

Fruit were harvested once or twice per week as they matured and were classified as being marketable or non-marketable. Marketable fruit were ≥3/4 red, ≥10 g weight, and free from malformations, rots, and other diseases.

Experiment I-1 (2009 growing season) At the end of the 2008 strawberry season (December), leaf samples of

asymptomatic plants were collected in the experimental field for virus testing. Different tests were used depending on the target virus. SMYEV, *Arabis mosaic virus*, *Tomato ringspot virus*, *Tomato black ring virus*, *Strawberry latent ringspot virus*, *Raspberry ringspot virus* and *Apple mosaic virus*, were analyzed using DAS-ELISA with specific antisera (BIOREBA SRL Latin America) according to the manufacturer's specifications. The presence of SCV was analyzed by RT-nested PCR; the presence of SMoV and SVBV by RT-PCR using specific primers and methods described by Posthuma et al. (2002) and Chang et al. (2007), respectively.

For DAS-ELISA, young strawberry leaves were homogenized 1:5 (wt/vol) in extraction buffer (phosphate buffer +0.05% Tween-20 + 2% polyvinylpyrrolidone +2% nonfat dried milk). 200 μL of reagents were added per well at each step of the ELISA process and absorbance values at 405 nm were recorded with a Dynex MRX II spectrophotometer (DYNEX. Technologies, VA, USA). Samples with absorbance values more than twice as high as the average of five healthy controls, evaluated in the same ELISA plate, were considered positive for SMYEV. Strawberry plants previously identified as infected with the virus were used as positive controls of SMYEV infection (Conci et al. 2009). The BIOREBA SRL Latin America virus controls were used in testing for the other viruses. In each ELISA plate, five healthy control plants were used including *Fragaria vesca* var. *semperflorens* (Duch.) 'Alpine', a complex hybrid of *F. vesca, F. chiloensis and F. virginiana* 'UC-5', and a *F. virginiana* 'UC-12' virus indicator plant belonging to INTA's *Instituto de Patología Vegetal* plant stock.

Thirty-six SMYEV-infected and 15 SMYEV-negative plants (called healthy plants in these experiments) that were also free of the other tested viruses were used for propagation. The infected and healthy plants were tagged and kept in an aphid proof greenhouse under controlled conditions to avoid virus vectors. During the summer, these plants produced runner plants that were individually rooted in plastic pots to maintain clonal identity. Insecticides were used in order to maintain control of possible virus vectors. Daughter plants were separated from mother plants and planted in the field for fruit production evaluation on 4 May 2009. These plants were analyzed for viruses using the methodology described above, resulting in the identification of a total of 167 SMYEV-infected plants and 48 healthy plants. The experimental design was completely randomized and each plant was labeled. Fruits were harvested from July 1 to November 13, once or twice per week, as they matured. Plants were tested for the presence of SMYEV at different times during the season on June 8, August 24, and November 13, and the mean monthly temperature was evaluated.

Experiment I-2 (2010 growing season) Plants from experiment I-1 were maintained in the field and tested for viruses by DAS-ELISA and RT-PCR as described above. Healthy and SMYEV- infected plants were used in this experiment. The planting was established on April 27, and 16 SMYEV-infected plants and eight healthy plants were planted. The experimental design was completely randomized and each plant was tagged. Fruit from these plants were harvested from September 5 to November 25, once or twice a week, as they matured. Plants were tested for the presence of SMYEV at different times during the season on June 15, August 10, October 13, and November 25, and the mean monthly temperature was evaluated.

Experiment II (2010 growing season) On May 11, another experimental plot of 'Camarosa' plants was established. During the growing season, 350 asymptomatic plants belonging to a lot planted with certified plants were tagged and leaf samples from these plants were collected for DAS-ELISA and RT-PCR virus testing as described for experiment I. A total of 34 plants, infected only with SMYEV (asymptomatic SMYEV-infected plants) and 33 healthy plants were individually tagged and used in this study. Another group of 11 plants showing dwarfism, mottling, and leaf deformation also were chosen for virus detection tests and the plants with a mixture of viruses were used in this assay. Fruit from these plants were harvested from September 5 to November 25, once or twice a week as they matured, and were tested for SMYEV at different times during the season on August 10, October 13, and November 25, and the mean monthly temperature was evaluated.

Statistical analysis Weekly records of fruit number and weight on a per plant basis were compared with the absorbance values obtained for each infected plant at the different SMYEV sampling dates. Data were subjected to analysis of variance (ANOVA) and Fisher's Least Significant Difference test to compare treatments (healthy and diseased plants), using *InfoStat* statistical software (Di Rienzo et al. 2012).

Additionally, an estimation of the SMYEV concentration in the last part of the cycle was calculated using

the absorbance values obtained for each plant in November. Values were divided into three categories of absorbance: H, high; M, medium; and L, low, with the 33rd percentile marking the first division and the 66th percentile marking the second division.

An ANOVA and Fisher's test were performed for each harvest parameter evaluated.

To compare the virus concentrations obtained at different months throughout the crop cycle, a relative concentration value was calculated. This value was obtained by dividing the absorbance of each sample by the average of the five healthy controls tested on the same ELISA plate, plus twice the standard deviation of the healthy controls (Conci et al. 2002).

Results

Experiment I-1 Asymptomatic SMYEV-infected plants produced a lower total fruit number (28%) than healthy plants and showed a reduction of 36% in total fruit weight. Marketable fruit number and weight were reduced by 38% and 40%, respectively. The number of malformed and rotten fruit was not statistically different between infected and non-infected plants (Table 1).

To analyze the impact of infection intensity on fruit production, diseased plants were classified into three categories according to virus absorbance value ranges: high (2.22 to 4.00), medium (1.72 to 2.21), and low (0.29 to 1.71). All the studied parameters, number of marketable fruit, weight of marketable fruit, total fruit number and total fruit weight per plant were significantly higher in plants with the lowest absorbance values (Table 2).

The mean relative concentration of SMYEV over three or four months in each assay was obtained and variations in virus concentration were observed during crop cycles. In experiment I-1 the highest value was detected in November at the end of the cycle (Table 3).

Experiment I-2 Since many of the plants from experiment I-1 that were kept for a second production season became infected by other viruses (SMoV or SCV, the other viruses tested were never detected) or died from different causes, therefore the experiment I-2 was conducted with fewer plants than experiment I-1. While only a small number of plants survived to the end of experiment I-2 (16 infected and 8 healthy plants) important yield differences were still detected (Table 1). SMYEV-infected plants showed a statistically significant reduction in the total number of fruit per plant (63%). Although the rest of the parameters tested showed the same trend, the differences between healthy and infected plants (>30% decrease) were not statistically significant (Table 1).

The limited number of plants in each category (high, medium, and low virus concentration) meant that the effect of estimated SMYEV concentration on yield components was not calculated in assay I-2. The highest relative virus concentration throughout the crop cycle occurred in November at the end of the season (Table 3).

Experiment II The plants infected with other viruses (SMoV or SCV, the other viruses tested were never detected), or destroyed for different causes (fungi, insects, etc.) during the trial were eliminated, resulting in final group sizes of 19 asymptomatic SMYEV-infected plant and 27 healthy plants.

The yield loss of symptomatic plants infected with a mixture of viruses compared with healthy plants was significant with reductions of between 78 and 99%, depending on the parameter examined, when compared with the healthy plants.

Yield losses were also observed when the asymptomatic SMYEV-infected plants were compared with healthy plants, but only the reduction in the total number of fruit produced per plant was significant (33%) (Table 1).

To analyze the impact of infection intensity on fruit production, diseased plants were classified into three categories according to virus absorbance values: high (2.60 to 3.10), medium (1.98 to 2.59) and low (0.12 to 1.97). While the differences among categories were not statistically significant, the highest yield was obtained from plants with the lowest virus concentration (Table 2). The estimated SMYEV concentration throughout the crop cycle was highest in October and November (Table 3).

Discussion

The results presented here indicate that a single infection with SMYEV affects the quantity and weight of the fruit produced by strawberry plants, even though symptoms are not observed. The results also suggest that this virus does not alter fruit shape or the susceptibility of the fruit to fungal or bacterial pathogens.

Table 1 Effect of SMYEV infection and a mixture of viruses on strawberry yield (Experiments I-1, I-2 and II)

Experiments	Treatments	Number of marketable fruit per plant	Weight of marketable fruit per plant (g)	Number of malformed fruit per plant	Number of rotted fruit per plant	Total fruit number per plant	Total fruit weight per plant (g)
I-1	Healthy plants	8.38 a	117.77 a	2.67 a	1.33 a	25.44 a	207.35 a
I-1	Asymptomatic SMYEV infected plants	5.15 b	70.28 b	2.80 a	1.24 a	18.31 b	133.11 b
I-1	Yield loss of asymptomatic SMYEV infected plants	38%	40%			28%	36%
I-2	Healthy plants	19.80 a	266.40 a	1.60 a	2.00 a	94.20 a	466.40 a
I-2	Asymptomatic SMYEV infected plants	12.63 a	178.5 a	1.13 a	1.88 a	35.25 b	298.00 a
I-2	Yield loss of asymptomatic SMYEV infected plants					63%	
II	Healthy plants	13.93 a	229.96 a	3.48 a	2.44 a	45.44 a	326.78 a
II	Asymptomatic SMYEV infected plants	14.53 a	214.74 a	3.26 a	2.42 a	30.42 b	282.37 a
II	Symtomatic plants	2.64 b	35.45 b	1.64 a	0.18 b	9.91 c	3.82 b
II	Yield loss of asymptomatic SMYEV infected plants					33%	
II	Yield loss of symptomatic plants	81%	85%			78%	99%

Values followed by different letters were significantly different (LSD Fisher test, $p \leq 0.05$)

A significant reduction in multiple yield components was observed in asymptomatic SMYEV-infected plants even when the infections occurred the previous year. In the first year of the experiment (I-1), statistically significant changes in yield losses were recorded for all the parameters studied, with reductions between healthy and infected fruit ranging from between 26 and 40%, depending on the parameter studied. In the second year of the experiment (I-2) yield losses also were observed although only the reduction in the total fruit number (63%) was statistically significant. This may be the result of the smaller number of plants used in the second year because of plant losses between assay years, or caused by other factors such as root pests.

In experiment II, yield reductions were also observed, with the total fruit number decreasing by 33% in SMYEV-infected plants compared with healthy plants. While differences were detected in the other parameters tested they were not statistically significant. These differences between experiment I and II may be because the infected plants used in experiment I were obtained from mother plants SMYEV infected in previous crop cycles and then propagated to obtain plants for the experiment I, while in experiment II the infected plants were selected from a field, and it is probably that the infected plants were recently infected with SMYEV during this fruit production season. Therefore, we propose that in experiment II the plants were recently infected, which resulted in a less severe impact on fruit production. This suggests that SMYEV infection does not affect plant productivity in the first fruiting cycle. This behavior was also observed in other cultivated species, with

Table 2 Effect of SMYEV concentration on yield components of asymptomatic SMYEV-infected plants (Experiments I-1 and II)

Category of plants for virus concentration	Number of marketable fruit per plant		Weight of marketable fruit per plant (g)		Total fruit number per plant		Total fruit weight per plant (g)	
Experiments	I-1	II	I-1	II	I-1	II	I-1	II
High	3.59^{a}	11.86^{a}	47.77^{a}	164.00^{a}	14.23^{a}	30.57^{a}	95.71^{a}	213.29^{a}
Medium	4.56^{a}	14.00^{a}	61.33^{a}	188.40^{a}	16.35^{a}	30.80^{a}	118.16^{a}	279.60^{a}
Low	7.33^{b}	17.57^{a}	102.15^{b}	284.29^{a}	24.42^{b}	30.00^{a}	186.13^{b}	353.43^{a}
Probability (p-value)	0.0014	03	0.0012	006	0.0003	09	0.0002	011

Values followed by different letters were significantly different (LSD Fisher test, $p \leq 0.05$)

Table 3 SMYEV relative concentration at different time of the strawberry crop cycle

Date	Experiment I-1				Experiment I-2				Experiment II			
	Mean	n	S.E.	T	Mean	n	S.E.	T	Mean	n	S.E.	T
June	28.20 a	166	4.51	11.9	29.93 b	16	3.19	12.3	–	–	–	12.3
August	76.03 b	61	7.44	15.3	11.91 a	11	3.85	12.6	16.58 a	21	3.18	12.6
October	–	–	–	22.2	38.13 b	9	4.26	19.8	37.37 c	19	3.34	19.8
November	161.14 c	121	5.28	25.2	60.47 c	8	4.04	23.3	27.63 b	19	3.34	23.3

References: Mean values followed by different letters were significantly different among date (LSD Fisher test, $p \leq 0.05$); n, number of plant; S.E., standard error; T, mean temperature (°C)

Conci et al. (2003) reporting that virus-infected garlic plants experienced a progressive loss in bulb weight after successive crop cycles. Lot et al. (1998) also observed greater losses in garlic production when the plants went through more than one crop cycle of infection compared to newly infected plants. Studies with another strawberry virus, SCV, also showed that no significant effects on yield were observed in the first year of infection, but were observed in the second and the third years, compared to healthy controls (Babovic 1976).

Our results showed that strawberry runner plants produced by plants that were infected with SMYEV during the previous fruit production cycle had a significant reduction in yield despite being asymptomatic for the disease. Contrary to Barritt and Loo (1973), who reported that single infections of strawberry plants with SMYEV or SMoV did not significantly affect plant yield, we observed yield losses of up to 40% in experiment I-1 and 63% in I-2 in plants infected with SMYEV. These differences may be because Barritt and Loo (1973) used healthy plants grafted to SMYEV infected plants to propagate the runner plants that were used for the assay. This is similar to experiment II of our study, where the plants were infected and tested in the same cycle and the virus was found to have little effect on yield, similar to the results of Barritt and Loo (1973). When the effects of the virus in experiment II were analyzed according to virus concentration ranges, however, most of the fruit production parameters were found to be higher when the virus concentration was lower.

Our results suggest that a lack of symptoms in an infected plant does not mean that SMYEV will not have a negative impact on crop yields, consistent with studies conducted in other species (Gibson et al. 1997; Hane and Hamm 1999; Liu et al. 2003; Chinestra et al. 2010). Additionally, in infected plants, a negative correlation between virus concentration in the plant and strawberry production was detected, indicating that plants with lower virus DAS-ELISA absorbance levels had higher yield. This suggests a relationship between the initial quantity of inoculum and crop yield, as has been previously described by Barritt and Loo (1973) for strawberries, Coutts et al. (2009) for peas, and Ramkat et al. (2008) for tomatoes.

In both experiments SMYEV concentration increased towards the end of the crop cycle coinciding with the increase of mean temperatures, suggesting that would be the best time to take samples for virus detection. More generally, this study shows the importance of characterizing different virus/host interactions to help identify the best sampling time for more reliable virus analyses. The significant fluctuations in strawberry plant SMYEV concentration throughout the crop cycle could be affecting the results of virus diagnosis tests. Infected plants with a low virus concentration at one point in the crop cycle could give absorbance readings below the detection limit for DAS-ELISA or other diagnostic techniques, resulting in a false negative for viral infection. Fluctuation in virus concentration throughout the crop cycle has also been observed in other crops. This has been determined for *Leek yellow stripe virus in garlic* (Conci et al. 2002), *Prunus necrotic ringspot virus* in cherry and peach (Dal Zotto et al. 1999; Mink 1980), *Cowpea chlorotic mottle virus* in soybeans (Bijaisoradat and Kuhn 1985), *Barley yellow dwarf virus* in barley (Leclercq-Le Quillec et al. 2000), among other.

We also showed the effect of multiple virus infections on strawberry plants, with yield reductions of between 78 and 99% observed depending on the parameter examined. This dramatic impact on fruit production has been previously reported in a number of studies examining strawberry viruses (Converse 1987; Maas 1998; Martin

and Tzanetakis 2006). While we did not follow the dynamics of aphid populations in these assays, mixed infections do generally occur in the field because of the high levels of aphids present. A number of aphid species involved in the transmission of strawberry viruses have been reported on strawberry crops in Argentina including *Aphis forbesi* Weed, *A. gossypii* Glover, *Chaetosiphon fragaefolii* (Cockerell), *C. minor* (Forbes), *C. thomasi* Hille Ris Lambers, and *Myzus persicae* (Sulzer) (Ortego 1997; Kirschbaum and Hancock 2000; Delfino 2004; Delfino et al. 2007; Dughetti et al. 2017).

The effects of SMYEV infection on strawberry yield in asymptomatic plants may be significant in regions where the strawberry aphid (*C. fragaefolii*) is present, as silent spreading of the disease might occur with negative impacts on the strawberry industry. These aphids have previously shown the greatest affinity for the Camarosa and Candonga (also called Sabrosa) strawberry cultivars (Bernardi et al. 2012). Taken together, these results highlight the importance of using virus-free strawberry plants for commercial fruit production (Converse 1987; Maas 1998; Martin and Tzanetakis 2006).

Our results show that, even in asymptomatic plants, single virus infection of SMYEV affects strawberry fruit production; highlighting the need for testing of plants material rather than relying on visual symptoms.

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Compliance with ethical standards Authors confirm that this manuscript has not been published elsewhere and is not under consideration by another journal. All authors have approved the manuscript and agree with submission to this Journal.

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