Synergistic Interaction of *Sweet potato chlorotic stunt virus* (*Crinivirus*) with Carla-, Cucumo-, Ipomo-, and Potyviruses Infecting Sweet Potato

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ABSTRACT

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Co-infection of *Sweet potato chlorotic stunt virus* (SPCSV, genus *Crinivirus*) with *Sweet potato feathery mottle virus* (SPFMV, genus *Potyvirus*) results in sweet potato virus disease (SPVD), a synergistic disease that is widely distributed in the sweet potato (*Ipomoea batatas*) growing regions of the world. Since both SPCSV and SPFMV are common and often detected as part of multiple co-infections of severely diseased plants, the occurrence of synergistic interactions with other viruses was investigated. Data from this study show that SPCSV, but not SPFMV, can cause synergistic diseases in sweet potato with all viruses tested, including members of the genus *Potyvirus* (*Sweet potato latent virus, Sweet potato mild speckling virus*), *Ipomovirus* (*Sweet potato mild mottle virus*), *Cucumovirus* (*Cucumber mosaic virus*), and putative members of the genus *Carlavirus* (Sweet potato chlorotic fleck virus and C-6 virus). The synergism was expressed as an increase in the severity of symptoms, virus accumulation, viral movement in plants, and as an effect on yield of storage roots. The presence of a third different virus in plants affected with SPVD increased the severity of symptoms even further compared with SPVD alone. There was a positive correlation between increase in virus accumulation and symptom expression in double and triple SPCSV-associated co-infections. The epidemiological implications of the results are discussed.

Additional keywords: cultivar decline, virus interaction, yield reduction

Viral co-infection is the simultaneous infection by distinct viruses or by different strains of one virus in the same host. It is commonly seen in natural conditions (7,35). When co-infections occur, viruses may or may not interact. No interaction or neutralism allows viruses to replicate, accumulate, and be transmitted without influencing each other, but co-infecting viruses may interact in either an antagonistic or a synergistic way (49). If infection or accumulation of one of the involved viruses or strains is completely or partially prevented or reduced, the antagonism takes the name of cross protection. This may occur either with strains of the same virus or with closely related viruses (6,35). In a similar manner, viral interference or concurrent protection may occur when viruses are unrelated (27).

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Synergism refers to a situation where one virus affects a co-infecting virus, allowing an increase in its accumulation in the plant by facilitating its replication (10,19,52) or movement to tissues that otherwise it could not invade (5). Synergism usually causes more severe symptoms in hosts than those caused by each single infection (35), and it is the cause of several extremely severe diseases in crops (10,23,40,42). Mechanisms of synergism have been broadly studied in different host–pathogen systems. At present, several researchers have proved that certain proteins considered responsible for the occurrence of synergisms are also capable of suppressing posttranscriptional gene silencing (PTGS) at different levels, suggesting a connection between the phenomena (2,28,30).

Currently, more than 20 viruses infect sweet potato (*Ipomoea batatas*) (32). Unlike *Sweet potato leaf curl virus* (SPLCV, genus *Begomovirus*) (12) and *Sweet potato chlorotic stunt virus* (SPCSV, genus *Crinivirus*) (21), most single infections cause mild or no symptoms, and consequently, no significant yield reduction is observed. Due to the lack of symptoms and the difficulties in detecting them directly in sweet potato (9,18,21), most single viral infections are often unrecognized by growers, who subsequently spread the viruses through propagation of infected vines. This situation has led to reports of large numbers of viral co-infections from almost every sweet potato producing region (15,21,22,36,44,47). Only a few of the synergisms that cause great negative impact on yield have been studied. The most important is sweet potato virus disease (SPVD), caused by a synergistic interaction of SPCSV and *Sweet potato feathery mottle virus* (SPFMV, genus *Potyvirus*). This interaction has become the major virus constraint for sweet potato production worldwide (32,44,47). However, sweet potato chlorotic dwarf (SPCD) (SPCSV+SPFMV+ *Sweet potato mild speckling virus* [SPMSV, genus *Potyvirus*]) (15), camote kulot (several viruses) (45), and the sweet potato severe mosaic disease (SPSMD) (SPCSV+*Sweet potato mild mottle virus* [SPMMV, genus *Ipomovirus*]) (39) have also been reported as synergistic diseases. These diseases cause very severe mosaic, chlorosis, stunting, and leaf reduction and deformation symptoms that lead to yield reductions often exceeding 70% (21,45).

Considering the broad geographic distribution of SPCSV and SPFMV, an investigation was conducted on the possible synergistic interaction between them and other viruses often found in sweet potato (4,12–15,38). This paper reports that co-infection of SPCSV with viruses belonging to the genera *Potyvirus*, *Carlavirus* (putative members), *Cucumovirus*, and *Ipomovirus* can result in synergistic diseases and that they severely affect sweet potato yield under field conditions.

MATERIALS AND METHODS

Plant material and virus isolates. Six sweet potato cultivars from different countries were selected. Paramonguino, Jonathan, and Costanero are commonly grown in the north, central, and south regions of Peru, respectively. Morada-INTA is the main cultivar in Argentina. Jewel and TIB-8 are North American and Nigerian cultivars, respectively. These cultivars, excepting Jewel and Paramonguino, have been used before on viral synergistic studies (15,21,44,47). Plantlets of those cultivars, determined to be virus-free by indexing them through grafting twice to *I. setosa* Ker. with subsequent serological tests (hereafter referred to as "healthy"), were provided by the International Potato Center (CIP, Lima, Peru). They were grown in plastic pots containing an autoclaved mix-

ture of soil, sand, and peat moss (equal volumes) and maintained at 25°C and 70% relative humidity (RH) in a vector-proof greenhouse.

Virus isolates were from CIP's collection. SPFMV-RC (Russet crack strain), *Sweet potato latent virus* (SPLV, genus *Potyvirus*), SPMSV, SPMMV, *Cucumber mosaic virus* (CMV, genus *Cucumovirus*), and Sweet potato chlorotic fleck virus (SPCFV, putative member in genus *Carlavirus*) isolates were maintained on *I. nil* (L.) Roth by mechanical infection, whereas SPCSV-(M2-47) and C-6 virus (a putative carlavirus; 32) isolates were maintained on *I. setosa* by side-graft-inoculation. CMV was previously isolated from *Arracacia xanthorrhiza* Bancroft by mechanical inoculation to *Nicotiana glutinosa* L.

Virus inoculations. Five 15-day-old plants were inoculated with single and multiple viruses by side-grafting. For double and triple infections, plants were grafted, first with scions of plants containing SPFMV, SPCSV, or SPFMV+SPCSV, 1 week before grafting them again with scions of plants containing SPLV, SPMSV, SPMMV, CMV, SPCFV, or C-6. To avoid contamination, groups of infected plants were maintained in separate rooms in the greenhouse. Insect vectors were controlled with yellow traps and periodic sprays of insecticide. Symptoms were recorded 15, 21, 35, and 50 days after inoculation (dai) during January to March 2004, in plants grown at 10,000 to 15,000 lx light intensity.

Virus detection. Serological detection of viruses was carried out by enzyme-linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA) (21). Polyclonal antibodies (produced at CIP) and goat anti-rabbit antibody conjugated with alkaline phosphatase (AP) (Bio-Rad Laboratories, Hercules, CA, USA) were used diluted 1:1,000 (vol/vol). Samples were collected from symptomatic leaves or a leaf from the basal, middle, and upper part of symptomless plants. Plants were serologically evaluated twice (21 and 42 dai) during primary and secondary infection. For this, stem cuttings from 4-month-old infected plants were grown to compare symptom evolution along two consecutive generations. Confirmation of infection in plants shown to be negative by NCM-ELISA was done by top grafting the sweet potato plants with healthy *I. setosa* or *I. nil.*

Serological assessment of virus accumulation. Accumulation of SPMMV and CMV in single, double (with SPCSV), and triple (with SPCSV+SPFMV) infected plants cv. Costanero was determined by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) (11) in three fully expanded leaves (third and fifth unfolded leaves from the top part of the plant) at 28 dai. Immunoglobulins for SPMMV and CMV (provided by CIP) and CMV-AP (Agdia Inc., IN, USA) were used diluted 1:1,000, 1:3,000, and 1:200, respectively. SPFMV accumulation on SPVD and triple (C-6+SPVD) infection was also assessed. Samples were diluted 1:25 (wt/vol) for SPMMV and CMV or 1:500 (wt/vol) for SPFMV. Viral titers were estimated by using a standard curve plotting DAS-ELISA absorbance values against known concentrations of each virus. For this, SPMMV, CMV, and SPFMV were purified following standard protocols (25,37,48). Purified viruses were diluted with sweet potato sap, processed in the ELISA plates and standard concentration curves determined with the R Statistical program (43). Data (two times, two replications each) of absorbance (A_{405}) values 30 min after adding the substrate for SPMMV and CMV, and 10 min for SPFMV were plotted and compared with viral titers using the nonparametric Krushkall-Wallis test at $P < 0.05$ (43). Additionally, variation of C-6 virus accumulation along the entire plant (leaves at positions 3, 6, 9, and 12 from top) was assessed in single, double (C-6 with SPFMV or SPCSV), and triple (with SPCSV+SPFMV) infected plants (35 dai) through NCM-ELISA.

Effect of viral synergisms on sweet potato yield. Synergistic diseases representing different virus groups were selected to evaluate their effect on yield under field conditions in cv. Costanero. Stem cuttings from healthy and infected (45 dai) plants were propagated in a greenhouse before planting them in a field in Quilmana District, Cañete Province, Peru, between October 2004 and March 2005. Eight types of viral infection (healthy and infected with SPMMV, CMV, C-6, SPCSV, SPCSV+SPMMV, SPCSV+CMV, and SPCSV+C-6) were evaluated in a randomized complete block design with three replications. The trial consisted of 24 plots 12.6 m^2 each (4 rows of 10 cuttings each, totaling 40 plants). The trial and plots (separated 3 m from each other) were isolated by planting natural barriers of maize and barley, respectively. Insecticides were applied to control whitefly and aphid populations and minimize virus transmission. Irrigation and fertilization of the crop were managed according to the farmer's common practices. During the trial, symptoms were recorded weekly for the first 2 months and compared with those expressed and recorded previously in greenhouse. Six different agronomic characteristics including groundcover, fresh foliage weight, and number and weight of total and marketable storage roots were also compared. Groundcover data were recorded as previously reported (21) from 18 plants selected at random per plot 50 days after planting (dap). Fresh foliage weight and number and weight of total and marketable storage roots (between 100 and 600 g) were recorded for all 40 plants from each plot at harvest (160 dap). Results were analyzed by one-factor ANOVA tests, followed by honestly significant differences (HSD) at $P < 0.05$ (43).

Table 1. Symptoms[y] in sweet potato cultivars caused by the co-infection of *Sweet potato chlorotic stunt virus* (genus *Crinivirus*) with different sweet potato viruses belonging to the genus *Potyvirus, Ipomovirus, Cucumovirus,* or *Carlavirus* (putative members)

		Interaction of SPCSV with					
		Potyvirus		*Ipomovirus*	*Cucumovirus*	*Carlavirus*	
Cultivar		SPLV	SPMSV	SPMMV	CMV	C-6	SPCFV
Paramonguino	21[z]	VC	VM	VM, CS	CS	IC	IC
	>35	M, LD, LR	M, LD, LR	M, LD, LR, S	CS	IY, N, D	NR, NS, N, D
Costanero	21	CR, VB	CS, VM	VM, CS, LD	M, B, CR	IC	VB, CS
	>35	M	Mo, LD	Mo, LD, LR, S	M, LD, LR, R	IY, N, D, S	NR, NS, N, D
Morada-INTA	21	VB	CS	VM, CS	CS	-	ICS
	>35	VB	CS	M, LD, LR, S	CS	-	M, IC
Jewel	21	VB	C	CR, VM	CS, CR, LD	IC	IC
	>35	M	C	Mo, LD, S	LD, S	VY, S	IC
Jonathan	21	VB	CS	VM, CS, LD	CS, CR	IY	ICS
	>35	M, LD	CS, LD	LR, LD, S	-	IY	IY
TIB-8	21	VB	VM	VM	CS, CR	ICS	ICS
	>35	M	VC, Mo	Mo, LD, S	M, LD, S	IY	IY

[y] B, blistering; C, chlorosis; CR, chlorotic rings; CS, chlorotic spots; D, defoliation; IC, interveinal chlorosis; ICS, interveinal chlorotic spots; IY, intervenial yellowing; LD, leaf deformation; LR, leaf reduction; M, mosaic; Mo, mottling; N, necrosis; NR, necrotic rings; NS, necrotic spots; R, rugosity; S, stunting; VB, vein banding; VC, vein chlorosis; VM, vein mosaic; VY, vein yellowing; -, no symptom observed.

[z] 21 and >35 indicate days after inoculation when symptoms were observed.

RESULTS

Symptoms and virus detection in single and mixed infections. Symptomatology observed in single- and mixed-infected

plants is summarized in Table 1. Single infections caused by SPLV, SPMSV, CMV, SPCFV, and SPFMV were symptomless in all tested cultivars grown in the greenhouse, and the viruses were not detected by NCM-ELISA. However, SPMMV caused mild vein chlorosis in leaves from the middle part of plants of cvs. Costanero, Paramonguino, and Jonathan at 15 dai, and C-6 caused small chlorotic spots in leaves from the basal part of plants in these cultivars at 30 dai (Fig. 1A and B). SPCSV caused mild stunting in all infected plants. Both SPMMV and C-6 were detected in symptomatic, but not in asymptomatic leaves, while SPCSV was detected in leaves from the middle part of all infected plants by NCM-ELISA. Infections of symptomless plants were confirmed by development of symptoms in healthy indicator plants that were top grafted onto the symptomless plants. SPFMV and SPMSV caused vein yellowing and leaf distortion on *I. setosa,* whereas SPLV and CMV caused vein banding and severe mosaic on *I. nil,* respectively.

SPFMV-associated co-infections did not generate any synergistic disease on sweet potato. When SPLV, SPMSV, or SPCFV were involved in co-infections, infected plants remained symptomless and viruses were detected by indicator plants but not by NCM-ELISA. In plants co-infected with SPMMV or C-6, no enhancement of symptom severity (same symptoms as in single infections) was observed, and SPMMV and C-6, but not SPFMV, were detected by NCM-ELISA on symptomatic leaves. Conversely, SPFMV+CMV was the only co-infection inducing temporary symptoms not observed in single infections with either virus. Thus, vein mosaic or mild mosaic was observed in cvs. Costanero, Paramonguino, and Jonathan at 15 dai, but no longer than 25 dai (Fig. 1C). SPFMV but not CMV was detected in these transient symptomatic leaves by DAS-ELISA.

SPCSV-associated co-infections showed different symptomatology (Figs. 2 and 3). Some of them caused leaf narrowing or distortion normally associated with SPVD. SPCSV in mixed infections with members of the *Potyviridae* (SPLV, SPMSV, and SPMMV) caused symptoms similar to vein chlorosis or mild mosaic in all combinations at 30 dai, but the severity was greater at 45 dai (Fig. 2). Leaf symptoms on SPCSV+SPMMV-infected plants became more severe (leaf deformation and narrowing, chlorosis, crinkle, and mild dark green areas in some cultivars) than in those with SPCSV+SPLV or SPCSV+SPMSV. Mixed infection of SPCSV+CMV invariably caused stunting, mosaic, and blistering as dark green islands in all cultivars, although severity varied from mild mosaic (in cv. Morada-INTA) to severe mosaic, rugosity, chlorotic rings, and leaf deformation (in cv. Costanero) (Fig. 3A to D). Plants showed varying degrees of recovery in some cultivars (Costanero, Paramonguino, and Jonathan) after 35 dai. Mixed infections of SPCSV with the putative carlaviruses SPCFV and C-6 caused symptoms of interveinal chlorosis, necrotic rings, yellowing, foliar necrosis, and defoliation (Fig. 3E to L). Symptoms began invariably in lower leaves 30 dai and spread acropetally toward higher leaves during the plant vegetative cycle. Eventually, the whole plant was chlorotic, and leaves from the lower and middle part of the plant showed necrosis. All viruses were detected with NCM-ELISA, and no changes in the reaction intensity in serological detection of SPCSV were observed.

SPVD-associated co-infections resulted in even more severe diseases in all cultivars, especially stunting and foliar reduction (Fig. 4A). Besides the induction of SPVD symptoms, usually at 15 dai, these infections generated certain characteristic symptoms that varied from each other. When mixed infections occurred jointly

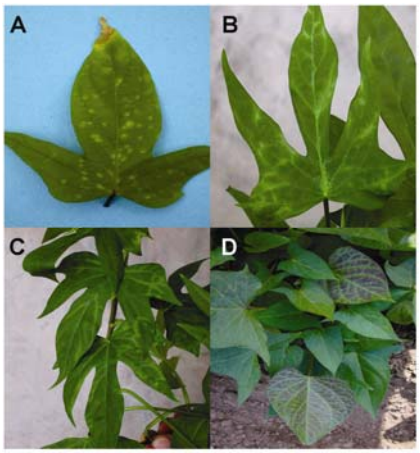

Fig. 1. Symptoms on sweet potato plants affected by viruses. **A,** Chlorotic spots caused by C-6 virus on cv. Paramonguino. **B,** Vein chlorosis caused by *Sweet potato mild mottle virus* on cv. Jonathan. **C,** Vein clearing caused by double infection of *Sweet potato feathery mottle virus* with *Cucumber mosaic virus* on cv. Jonathan. **D,** Purpling of lower leaves of cv. Costanero infected with *Sweet potato chlorotic stunt virus*.

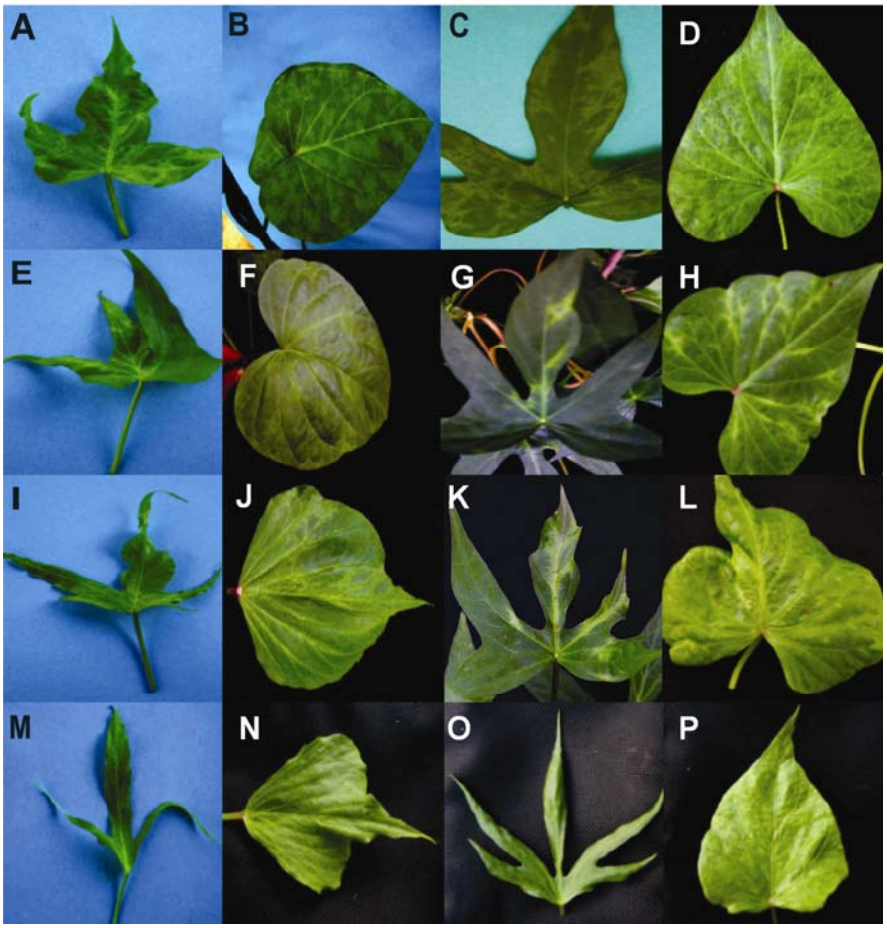

Fig. 2. Foliar symptoms on sweet potato plants cvs. Paramonguino, Costanero, Jonathan, and TIB-8 (from left to right, respectively) caused by synergistic co-infection of *Sweet potato chlorotic stunt virus* (SPCSV, genus *Crinivirus*) with viruses in the family *Potyviridae.* **A to D,** Mild leaf distortion, mild mosaic, vein banding, and mild mottle caused by SPCSV+*Sweet potato latent virus*. **E to H,** Leaf deformation and distortion, diffuse chlorosis, and vein chlorosis caused by SPCSV+*Sweet potato mild speckling virus*. **I to L,** Leaf deformation, distortion, and narrowing, vein chlorosis, and mild dark green islands caused by SPCSV+*Sweet potato mild mottle virus*. **M to P,** Leaf deformation, distortion, and narrowing, chlorosis, mottling, and crinkle, caused by SPCSV+*Sweet potato feathery mottle virus* (known as sweet potato virus disease, SPVD).

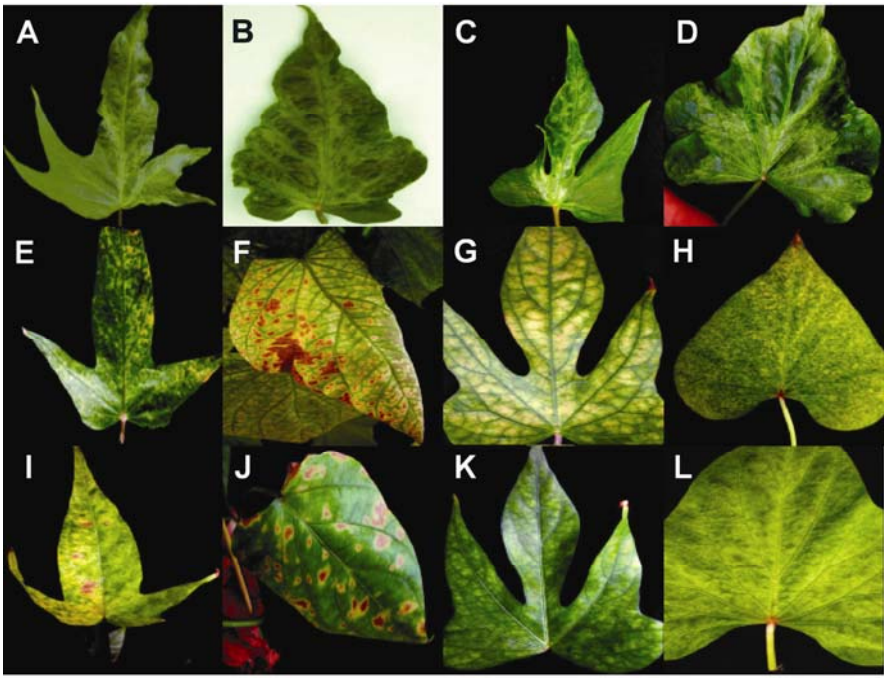

Fig. 3. Foliar symptoms in sweet potato plants cvs. Paramonguino, Costanero, Jonathan, and TIB-8 (from left to right, respectively) caused by synergistic co-infection of *Sweet potato chlorotic stunt virus* (SPCSV, genus *Crinivirus*) with viruses in genus *Cucumovirus* and *Carlavirus* (putative members). **A to D,** Rugosity, severe mosaic, blistering as dark green islands, and leaf distortion caused by SPCSV+*Cucumber mosaic virus*. **E to H,** Yellowing, necrotic spots, interveinal chlorosis, caused by SPCSV+C-6 virus. **I to L,** Yellowing, necrotic rings, interveinal chlorosis, and chlorosis caused by SPCSV+Sweet potato chlorotic fleck virus.

Fig. 4. Triple viral infections in sweet potato plants. **A,** (from left to right) Sweet potato virus disease (SPVD, *Sweet potato chlorotic stunt virus+Sweet potato feathery mottle virus*), SPVD+*Sweet potato mild mottle virus* (SPMMV), SPVD+*Cucumber mosaic virus* (CMV), and SPVD+C-6 virus in cv. Costanero. **B,** Dark green islands (arrow) in leaves from a secondary branch of a plant cv. Morada-INTA infected with SPVD+SPMMV. **C,** Severe mosaic and blistering as dark green islands (arrow) in leaves from secondary branch of cv. Costanero infected with SPVD+CMV. **D,** Leaf necrosis and defoliation in cv. Jonathan infected with SPVD+C-6.

with other potyviruses (SPLV or SPMSV), symptoms were similar to those caused by SPVD itself, although the severity of symptoms increased in secondary infections. SPVD+SPMMV-infected plants showed more severe symptoms than those caused by SPVD alone, with additional symptoms of dark green islands on leaves and more marked stunting of the plant (Fig. 4B) in primary infection. Leaves from secondary branches occasionally showed symptoms resembling SPCSV+SPMMV infection. This last phenomenon was also noted in SPVD+CMV-infected plants where severe mosaic resembling that caused by SPCSV+CMV infection was occasionally observed on leaves from some secondary branches (Fig. 4C), while leaves from main branches only showed SPVD-like symptoms. Mixed infections of SPVD+SPCFV and SPVD+C-6 caused similar lethal symptoms. In both cases, infected plants showed yellowing of the basal leaves 21 dai. Two weeks later, these leaves became necrotic and yellowing spread into the entire plant, progressing from lower to upper leaves. Eventually, defoliation was observed in affected plants, which finally died. When plants did not die after defoliation (Fig. 4D), cuttings from these plants did not survive more than 2 weeks after planting. Only plants of cv. Morada-INTA survived into the second vegetative generation.

Serological assessment of virus accumulation. SPMMV and CMV accumulated in much greater concentrations in triple and double infections than in singly infected leaves ($P < 0.05$; Table 2). An increase of 48- and 19-fold in the titers of SPMMV and CMV, respectively, occurred when co-infecting with SPCSV, but only an increase of 24- and 9.7-fold in the titers of the same viruses when co-infecting with SPCSV+SPFMV. While SPMMV and CMV did not increase as much, SPFMV accumulated in greater concentrations in triple infections (SPVD+SPMMV, SPVD+CMV, and SPVD+C-6) when compared to SPVD infection, reaching 1.3, 1.47, and 1.45 times higher, respectively. The increased accumulation of viruses corresponded to the presence of symptoms and/or increase in their severity in affected plants (Fig. 4A) and with the ease of their serological detection. C-6 virus was detected only in the lower leaves of plants singly infected or infected with SPFMV+C-6. In mixed-infected plants containing SPCSV, the C-6 virus was detected in all leaves by NCM-ELISA (Fig. 5). The intensity of the reaction in NCM-ELISA suggested that the concentration of the C-6 virus in leaves tested from plants affected by SPFMV+C-6 and SPCSV+C-6 was lower than in those from single-infected plants. However, a large increase in the concentration of C-6 virus was observed in all tested leaves of SPVD+C-6 infected plants when compared with the

SPCSV+C-6 infection, and there was a positive correlation with symptom severity and increase in virus accumulation.

Effect of viral synergisms on sweet potato yield. Under field conditions, single infections by SPMMV and CMV did not cause symptoms, whereas C-6 caused small chlorotic spots as observed under greenhouse conditions. SPCSV- and SPCSV-associated co-infections caused symptoms similar to those observed previously in the greenhouse, but additionally, purpling of lower leaves was observed under field conditions (Fig. 1D). Plants infected with SPCSV+CMV recovered 60 dap, a phenomenon also observed under greenhouse conditions.

There were no significant differences ($P < 0.05$) in the agronomic characteristics among healthy plants and the single infections; however, significant differences were observed between each of the SPCSV-associated co-infections and healthy plants or single infections (Table 3). Greater symptom expression correlated with lower values of agronomic characteristics in either single (C-6 and SPCSV) or mixed infections compared to healthy and symptomless infections. Groundcover of all mixed-infected plants was significantly different for SPCSV- and mixed-infected plants compared with the healthy and other single-infected plants. Weight and number of total storage roots were directly related to fresh weight of foliage. Single infection had no significant effect on yield of total storage roots, but the effect of mixed infections (synergistic viral interactions) was significantly different (Table 3, Fig. 6). Yield reduction of total storage root and fresh foliage ranged from 40.2 to 50.6% and 17.7 to 23.8%, respectively (Fig. 6). Variability coefficients ranged between 7.35 and 22.90% for groundcover and marketable storage roots, respectively (data not shown).

Isolation of the trial with natural barriers of maize and barley worked efficiently in minimizing movement of insect vectors (whiteflies and aphids) and transmission of undesirable viruses among treatments in the experimental parcel, as evidenced by the small number of plants contaminated with viruses (approximately 1%) from neighboring plots determined by visual and serological inspection. The contaminated plants were replaced with new ones grown as extra plants (each treatment) in the experimental trial.

DISCUSSION

Our study provides evidence of the existence of novel synergistic interactions involving SPCSV (a crinivirus) with viruses belonging to the virus genera *Potyvirus* (SPLV and SPMSV), *Cucumovirus* (CMV), *Ipomovirus* (SPMMV), and putative *Carlavirus* (SPCFV and C-6 virus). In nature, co-infection of two or more viruses in sweet potato is common, and as reported here, synergism of SPCSV with each of the above-mentioned viruses may occur in countries where these viruses are present (32). This is the case, for instance, of SPVD (21,26), SPCD (15), and SPSMD (39), where SPCSV—sometimes accompanied by SPFMV—is always present and interacting with different viruses. This work demonstrates and confirms that synergistic interactions occur between SPCSV and different viruses but not between SPFMV and the same viruses.

The results indicate that single infections by different viral species usually produce symptomless infection in plants of some sweet potato cultivars in which virus concentration is below the detection threshold of ELISA. Similar behavior for SPFMV and SPMMV has been observed by other researchers (4,21,44). We observed that some tested cultivars react with mild symptoms on leaves to single infection by SPMMV or C-6, which are serologically detectable from the symptomatic but not from the symptomless leaves in the same plants. When interacting with SPCSV, these viruses accumulate to levels high enough to facilitate their serological detection and cause visible symptoms in the infected plants. Thus, SPCSV (but not SPFMV) synergizes with the viruses studied, enhancing their symptom expression, replication, and/or movement in the plant. We observed that CMV synergizes with SPFMV temporarily during the early phase of the plant's growth, when symptoms were just visible and SPFMV was detected by DAS-ELISA. It is not known why SPFMV synergizes with *Sweet potato leaf curl virus* (a begomovirus), enhancing SPLCV titers in co-infected plants of cv. Beauregard (31).

Although plants affected with co-infections of SPCSV with *Potyviridae* (SPFMV, SPLV, SPMSV, or SPMMV) showed similar foliage symptoms, they differed in severity. Similar results were obtained in co-infection with other potyviruses (SPVG, IVMV, and SPVY) (4,50). In this study, SPVD (SPCSV+SPFMV) caused the most severe foliage symptoms, and stunting was more evident. Fan-shaped (in nonlobate) and filiform (in lobate) leaves were characteristic of plants infected with SPVD. These symptoms could be useful in differentiating SPVD from other double interactions of SPCSV with potyviruses. Co-infections of SPCSV with cucumovirus CMV or putative carlaviruses SPCFV or C-6 caused distinct symptoms from those associated with SPVD. The interaction of SPCSV with the carlaviruses is particularly noteworthy because they resulted in foliar necrosis and defoliation. We also observed that movement of the virus(es) within the plant is facilitated for the putative carlaviruses when interacting with SPCSV. Since co-infections of SPCSV with related potyviruses resulted in similar symptom expression (although with different severity), the similarity of symptom expression in co-infection of SPCSV with C-6 or SPCFV suggests that

Table 2. Estimate of concentrations of *Sweet potato mild mottle virus* (SPMMV), *Cucumber mosaic virus* (CMV), and *Sweet potato feathery mottle virus* (SPFMV) in plants of sweet potato cv. Costanero with single, double, and triple infections, determined using double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)

	Target virus (µg)[x]		
Virus infection[y]	SPMMV	CMV	SPFMV
SPMMV	1.57 ± 0.51 a[z]	…	…
CMV	…	2.94 ± 0.74 a	…
SPMMV + SPCSV	75.88 ± 11.92 b	…	…
CMV + SPCSV	…	56.07 ± 9.30 b	…
SPVD	…	…	69.29 ± 7.62 a
SPMMV + SPVD	37.82 ± 9.33 c	…	92.62 ± 4.79 b
CMV + SPVD	…	28.56 ± 4.71 c	102.02 ± 6.59 b
C-6 + SPVD	…	…	100.98 ± 6.78 b

[x] Means of the amount (µg) of virus per gram of leaf measured from the first three fully expanded leaves. ±, Standard error.

[y] SPCSV, *Sweet potato chlorotic stunt virus*; C-6, a new flexuous virus; SPVD, sweet potato virus disease (SPFMV + SPCSV).

[z] Within columns, values followed by a common letter do not differ significantly at $P < 0.05$ in the nonparametric Krushkall-Wallis test.

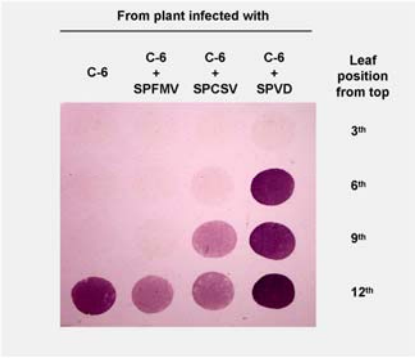

Fig. 5. Movement and accumulation of C-6 virus in leaves of sweet potato cv. Costanero affected by single, double, and triple infections at 35 days after inoculation, as detected by nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA). Purple color intensity is proportional to virus concentration in plant tissue. SPFMV, *Sweet potato feathery mottle virus*; SPCSV, *Sweet potato chlorotic stunt virus*; SPVD, sweet potato virus disease (SPFMV + SPCSV).

these two viruses are taxonomically related. There is evidence that SPCFV belongs to the carlavirus group (3). Although C-6 remains uncharacterized, our finding is interesting since C-6 virus is suspected to be a carlavirus, and a serological relationship between C-6 and *Potato virus S* (a carlavirus infecting potato) has been reported (32).

We also demonstrated that triple infections involving viruses from three different genera are even more severe, leading to a further increase of the potyvirus and putative carlavirus titers. The presence of SPMMV or CMV in SPVD increases the severity of symptoms shown by SPVD. The presence of C-6 or SPCFV caused foliar necrosis and defoliation in addition to the disease. Furthermore, the severity of these symptoms was enhanced, because leaf necrosis and defoliation occurred sooner than in double-infections, causing the premature death of the plant. Additive effects on symptoms due to synergism have been observed in some other crops (19,24). The results indicate that the diverse severity on plants showing SPVD-like symptoms in farmer's fields may be due to the presence of the viruses involved in the double and triple co-infections in the diseases, where SPCSV is a common element of the disease complex. Naming diseases to specify synergistic interactions is needed to differentiate them from each other.

Quantification of viral concentration in infected sweet potato plants cv. Costanero showed that both CMV and SPMMV concentrations increase when they interact with either SPCSV or SPCSV+SPFMV, although the increase is higher in double infections than in triple infections. Moreover, viral concentration of SPFMV is higher in triple infection than in double infection. These findings suggest that the expression of symptoms or the dramatic increase in symptom severity in co-infected plants may result, in part, from increased levels of viral accumulation in plant tissues. All viruses tested, when interacting with SPCSV, caused symptoms in affected plants, and they were serologically detected, indicating that an increase in their titers occurred in plant tissues. In contrast, SPCSV accumulation did not differ in singly or co-infected plants. Our results are consistent with those observed by others for SPFMV and SPMMV, which only attained high titers (more than 600- and 32-fold increase, respectively) and became readily detectable in plants co-infected with SPCSV, whose titer was not affected (26,39). On the other hand, a decline in virus accumulation and coincidental decline in symptom severity was also observed in plants infected with CMV+SPFMV. Previous work has shown that SPFMV is distributed in the whole plant as detected by nucleic acid spot hybridization

Table 3. Effect of *Sweet potato mild mottle virus* (SPMMV), *Cucumber mosaic virus* (CMV), C-6 virus (C-6), and *Sweet potato chlorotic stunt virus* (SPCSV) in single and double infections (with SPCSV) on some agronomic characteristics of sweet potato cv. Costanero, Quilmana, Cañete, Peru (October 2004 to March 2005)

		Infected with						
Agronomic characteristics[y]	**Healthy[z]**	**SPMMV**	**CMV**	**C-6**	**SPCSV**	**SPCSV + CMV**	**SPCSV + SPMMV**	**SPCSV + C-6**
Groundcover (cm^2/plant) at 50 dap	386.8 a	406.4 a	395.8 a	353.3 a	270.0 b	186.5 cd	152.4 d	243.7 bc
Weight fresh of foliage (kg/plot) at 160 dap	157.8 ab	167.9 a	162.2 ab	144.8 abc	135.3 abc	131.3 bc	120.4 c	121.4 c
Weight of TSR (kg/plot) at 160 dap	54.0 ab	67.4 a	60.5 ab	51.3 abc	38.7 bcd	32.3 cd	32.3 cd	26.7 d
Weight of MSR (kg/plot) at 160 dap	43.8 ab	47.7 a	45.1 ab	39.6 abc	24.8 bcd	21.4 cd	18.4 d	17.5 d
Number of TSR (kg/plot) at 160 dap	239.3 ab	266 a	258.0 ab	209.3 abc	166.3 bcd	159.3 cd	98.7 d	108.7 d
Number of MSR (kg/plot) at 160 dap	171.0 ab	182.7 a	177.7 ab	151.0 abc	103.3 bcd	84.0 cd	58.7 d	67.7 d

[y] dap = days after planting, TSR = total storage roots, and MSR = marketable storage roots (between 100 and 600 g).
[z] Healthy = virus free. Values are means of measurements recorded from 40 plants. Across rows, means with the same letter do not differ significantly ($P < 0.05$) in the honestly significant differences test.

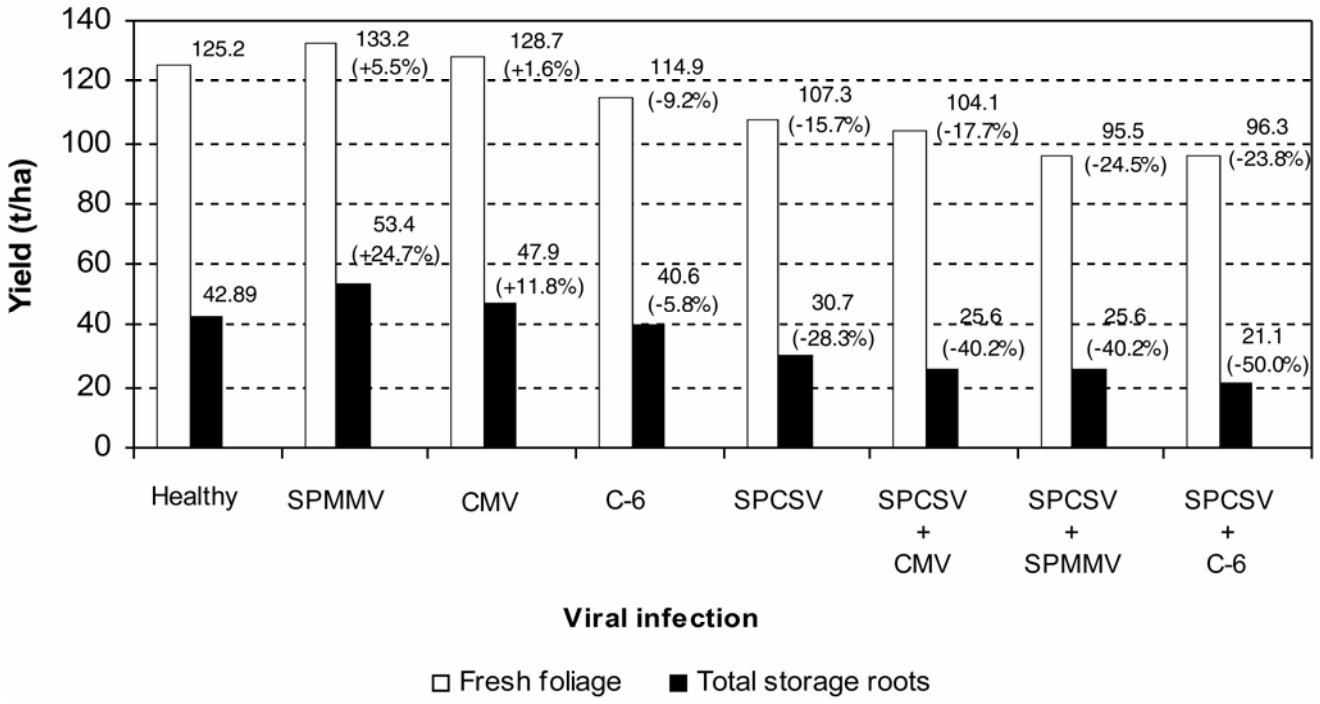

Fig. 6. Total storage roots and fresh foliage yield reductions of sweet potato cv. Costanero affected by single infections (*Sweet potato mild mottle virus*, SPMMV; *Cucumber mosaic virus*, CMV; C-6 virus; and *Sweet potato chlorotic stunt virus*, SPCSV) and mixed infections (SPCSV+CMV, SPCSV+SPMMV, and SPCSV+C-6) compared with healthy plants (controls). Figures in parentheses show yield reduction compared with the control.

(NASH) tests, but the virus is serologically detected mainly in symptomatic leaves (1,9,26). Therefore, recovery from infection was associated with a partial cleavage of the viral coat protein subunit by an enzyme present in extracts of infected symptomless leaves (46). It is possible that the virus moves as nucleic acid or as virions in very low concentration in symptomless leaves in the plant, but accumulation of detectable amounts of viral coat protein is directly associated with symptom expression. Previous work has shown that amino acid changes at specific sites in the CMV coat protein (51) or β-hexamer structure in assembled virions of *Cowpea chlorotic mottle virus* (a bromovirus) (54) modulate symptom expression. Additionally, symptoms are a function of the host genotype–virus strain or isolate–environment interaction. Cultivars used in this study were symptomless to SPFMV-RC. It is known that other genotypes are very susceptible to infection by other SPFMV strains and genotypes react with mild symptoms and the virus reaches high concentration (1,17).

Viral synergism was also expressed as an effect on yield of storage roots. Double infections of SPCSV with the evaluated viruses (SPMMV, CMV, or C-6) caused significant yield losses between 40.2 and 50.6%. This yield reduction was lower than that caused by SPVD (SPCSV+ SPFMV) in the same cultivar (70%), as previously reported (21). This was expected, as SPVD symptoms are also more severe than in the other virus interactions. Expression of symptoms caused by co-infections was more evident under field than in greenhouse conditions. The foliar chlorosis followed by necrosis and defoliation caused by SPCSV+C-6 resulted in the greatest yield reduction obtained in this study. Because the field experiment was protected from insect vectors and other external factors, our data closely estimate the negative effect that the virus diseases could cause naturally on sweet potato yield. Natural barriers (maize around the field and barley among plots) worked well in isolating the experiment and avoiding contamination with undesirable viruses from outside or within plots. Natural barriers have been demonstrated to reduce virus incidence (16,41), and they are suggested to be included as a component in integrated disease management of SPVD.

It is of epidemiological interest that CMV isolated from *A. xanthorrhiza* (*Umbelliferae*) was able to infect sweet potato plants without the need of a helper virus, as reported previously (13). Despite having used an isolate of CMV from another host, results show the possible interaction of this virus with SPCSV. Cohen and Loebenstein (13) obtained similar results when working with an isolate of CMV from cucumber and two other isolates from sweet potato. CMV strains appear to be nonspecific for infection in sweet potato. Growing unrelated crops known to be a host of CMV (8) close to each other could favor virus dissemination among them. This situation could be occurring with CMV in countries where the virus has become important (e.g., Canary Islands-Spain, Syria, Egypt) (13,32), probably due to its interaction with SPCSV. In the future, when information on CMV strains from sweet potato becomes available, the interaction of these isolates and other host's isolates with sweet potato viruses should be investigated. It is also of epidemiological importance that synergism affects virus accumulation in infected plants, and it could also affect virus transmission by insect vectors (55). It was demonstrated that SPFMV could be easily transmitted by *Myzus persicae* Sulz. from sweet potato plants co-infected with SPCSV and showing SPVD symptoms, but not from symptomless single-infected plants (44,47). Kennedy and Moyer (29) showed that SPFMV-RC was transmitted more frequently by *M. persicae* and *Aphis gossypii* Glover from symptomatic leaves than from symptomless leaves. We have demonstrated that symptom expression is correlated with high virus concentration in the plant tissues. Although virus accumulation of SPCSV is not affected in the synergisms (this study; 26), it is also probable that infected plants (showing chlorosis and mosaics among other symptoms) are more attractive to whiteflies, facilitating the transmission of SPCSV in sweet potato fields. It was reported that plants infected with *Tomato spotted wilt virus* were more attractive to its vector *Frankliniella occidentalis* (Pergande) than uninfected plants (34). Additionally, Rossel and Thottappilly (44) could transmit SPCSV by its vector from SPVD-infected sweet potato plants, but not from plants affected by SPCSV alone.

The irregular distribution of viruses in vines (1,20) and the low concentration of virus in single infections in the sweet potato plants (9,18,21) and resulting symptomless infection make the serological detection of viruses by ELISA more difficult. The natural plant resistance mechanism, suggested for SPFMV infection (26,39), seems also to operate for other single infections by members of the potyvirus, carlavirus, cucumovirus, and probably begomovirus genera (15,33,39). Such a resistance mechanism in sweet potato seems to be unaffected by co-infections involving SPFMV, but it is completely broken down by co-infections involving SPCSV (this study; 26,39). Certain proteins capable of suppressing posttranscriptional gene silencing (PTGS) have been considered as responsible for the occurrence of synergisms (2,28). This seems to be the case of some proteins encoded by SPCSV (30) involved in suppressing PTGS, while SPFMV appears not to produce proteins effective in suppressing PTGS as reported for other potyviruses (2,52,53). Whatever mechanism is involved, we observed that CMV was capable of temporarily overcoming such a mechanism when co-infecting plants with SPFMV. Although some researchers reported an increase of the potyvirus titer in co-infections with CMV (40,53), we only observed a transient increase of SPFMV, but not CMV, (data not shown) in transiently symptomatic leaves from co-infected plants of cv. Paramonguino, Costanero, and Jonathan.

In conclusion, this work demonstrates the occurrence of synergisms resulting from double or triple SPCSV-associated infections with members in different virus groups. Depending on the virus, the synergism was more evident in some cultivars than others and expressed as an increase in the severity of symptoms, virus accumulation and viral movement in plants, and reduction of sweet potato yield. It also emphasizes not only that synergistic interactions of viruses are an important factor of sweet potato decline but also the need to focus efforts on looking for natural or transgenic extreme resistance to SPCSV. A Peruvian landrace genotype with extreme resistance to SPCSV (consequently to SPVD) was identified in 2005 at CIP (S. Fuentes, *unpublished data*), and it could be used by breeding programs in different countries to improve their local cultivars.

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