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Short Communication

Department of Plant Pathology and Crop Physiology, LSU AgCenter, Baton Rouge, LA, USA

The Effect of the Sequence of Infection of the Causal Agents of Sweet Potato Virus Disease on Symptom Severity and Individual Virus Titres in Sweet Potato cv. Beauregard

Cecilia Mcgregor[1], Douglas Miano[1,2], Don La Bonte[1], Mary Hoy[3] and Christopher Clark[3]

Authors' addresses: [1]School of Plant, Soil and Environmental Sciences, LSU AgCenter, Baton Rouge, LA, USA; [2]Biotechnology Centre, Kenya Agricultural Research Institute, PO Box 14733 00800, Nairobi, Kenya; [3]Department of Plant Pathology and Crop Physiology, LSU AgCenter, Baton Rouge, LA, USA (correspondence to C. Clark. E-mail: cclark@agcenter.lsu.edu)

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Abstract

Sweet potato virus disease (SPVD) is caused by dual infection of plants with *Sweet Potato Feathery Mottle Virus* (SPFMV) and *Sweet Potato Chlorotic Stunt Virus* (SPCSV). Because SPFMV and SPCSV are transmitted by aphids and whiteflies, respectively, infection in nature occurs independently rather than simultaneously. To investigate the effect of consecutive infection on symptom development and individual virus titres, plants infected with a single virus were later inoculated with the second virus. Symptoms were significantly more severe in plants infected with SPCSV followed by SPFMV compared to plants infected with SPFMV followed by SPCSV. Virus titres were not significantly different for SPCSV, but SPFMV titres, in plants infected with SPCSV followed by SPFMV, were significantly higher than all other treatments. The results indicate that the sequence of infection of sweetpotato plants with the causal agents of SPVD influence the severity of symptoms and SPFMV titres in SPVD affected plants.

Introduction

Sweet Potato Virus Disease (SPVD) is the most important viral disease affecting sweetpotato production worldwide (Carey et al., 1999). Yield losses due to SPVD can reach 90% (Hahn, 1976; Ngeve, 1990; Gutiérrez et al., 2003). The disease develops due to a synergistic interaction between two viruses: *Sweet Potato Feathery Mottle Virus* (SPFMV) and *Sweet Potato Chlorotic Stunt Virus* (SPCSV). Plants affected with SPVD exhibit severe symptoms such as leaf strapping, vein clearing, leaf distortion, chlorosis, puckering, and stunting.

SPFMV is a member of the *Potyviridae* family and the *Potyvirus* genus. Plants infected with SPFMV alone are usually symptomless or show only mild symptoms and yield is relatively unaffected (Gutiérrez et al., 2003; Clark and Hoy, 2006). Similarly, the SPFMV titres in singly infected plants are low. However, when plants are infected with both SPFMV and SPCSV, the SPFMV titres are dramatically higher than in single infections (Karyeija et al., 2000; Kokkinos and Clark, 2006a).

Infection of plants with only SPCSV, a phloem-limited crinivirus (family *Closteroviridae*), can lead to yield losses of up to 43% (Gutiérrez et al., 2003), with accompanying moderate symptoms. The SPCSV titres in singly (SPCSV alone) and dually infected (SPCSV and SPFMV) sweetpotato plants are comparable (Karyeija et al., 2000), or in some cases lower virus titres have been observed in dually infected plants (Kokkinos and Clark, 2006a).

When studying SPVD, plants are usually inoculated with SPFMV and SPCSV at the same time. However, since SPFMV is transmitted by aphids [including *Aphis gossypii* (Glover) and *Myzus persicae* (Sulzer)] and SPCSV is transmitted by whiteflies [*Bemisia tabaci* (Gennadius) and *Trialeurodes abutilonea* (Haldeman)] simultaneous infection is unlikely to occur in nature. Our aim was to investigate how the development of SPVD symptoms and the titre levels of each virus are affected when plants already infected with one virus are infected with the second virus at a later time.

Materials and Methods

Plant materials and symptom observation

Virus-tested (VT) plants of sweetpotato cv. Beauregard were meristem-tip culture derived plantlets that

were found to be apparently free of viruses by three successive grafts to the standard indicator host, *Ipomoea setosa*. The VT plants were maintained in tissue culture by nodal propagation and established in the greenhouse for use as propagating material. VT plants were inoculated with SPCSV by grafting with a scion from a plant infected with isolate BWFT-3 of the US strain or with SPFMV by grafting with a scion infected with isolate 95-2 of the russet crack strain. Following inoculation, vine cuttings were taken from the infected stock plants to increase the number of plants available for experiments.

Single terminal vine cuttings from SPCSV-infected, SPFMV-infected or VT source plants were transplanted to 15-cm-diameter plastic pots containing a mix of 1:1:1 (v:v:v) river silt:sand:Redi-earth (Sun Gro Horticulture, Canada). These were allowed to grow for about three weeks and then were graft-inoculated with two-node scions to give the following combinations: SPCSV-infected stock grafted with SPFMV-infected scion (SPCSV/SPFMV), SPFMV-infected stock grafted with SPCSV-infected scion (SPFMV/SPCSV), VT stock grafted with SPCSV-infected scion (VT/SPCSV), VT stock grafted with SPFMV-infected scion (VT/SPFMV), ungrafted SPCSV-infected stock and ungrafted VT stock plants. Growth proliferating from the stock was trimmed to allow growth of only a single branch of vine. Beginning with the first appearance of symptoms at 12-days postinoculation (DPI), symptom severity was recorded every 2–3 days by recording symptoms on the second, fourth, and sixth open leaf from the apical terminal using a scale of 0–5, where: 0, no symptoms; 1, a few chlorotic spots or interveinal chlorosis; 2, mottling and/or chlorotic vein banding but no leaf distortion or stunting; 3, mosaic covering leaf but no leaf distortion or stunting; 4, fan-leaf or other moderate leaf distortion, moderate stunting of leaf, mosaic and/or general chlorosis; and 5, severe mosaic, leaf distortion, and stunting (Fig. 1). Immediately after symptom severity was rated at 42 DPI, the second, fourth, and sixth leaf from each plant was collected for estimation of virus titres. The plants were then cut back to leave approximately 3–4 nodes above the soil line and allowed to regrow from the axillary buds. Symptoms were recorded on the regrowth until 66 DPI when the experiment was terminated. A preliminary study was run from late May to August 2007 and the more detailed experiments described here were conducted from October to December 2007 and again from February to April 2008.

Fig. 1 Pairs of leaves from cv. Beauregard plants graft inoculated with *Sweet Potato Feathery Mottle Virus* and *Sweet Potato Chlorotic Stunt Virus* showing symptoms representing the different class intervals used in the SPVD symptom severity rating system. Each pair consists of an older leaf on the left and a younger leaf on the right. Top left pair = 0 rating, top middle = 1, top right = 2, bottom left = 3, bottom middle = 4, and bottom right = 5

Quantitative real-time PCR (Q-RT-PCR)

Leaf material harvested on day 42 was frozen with liquid nitrogen and ground to a fine powder with a mortar and pestle. Total RNA was isolated from *c*. 50 mg of powdered material using the Qiagen RNeasy Plant Mini Kit® (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions, including the optional DNase step. The quality and quantity of RNA were determined using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and all samples were diluted to a concentration of 10–60 ng/µl.

The primer and probe combinations for SPFMV and SPCSV described by Kokkinos and Clark (2006b) were used to determine individual virus titres using an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). An aliquot of 2.5 µl of the diluted RNA was amplified in a final volume of 25 µl that included 900 nM of each primer, 200 nM of the Taqman® probe and components from the TaqMan® One-Step RT-PCR Master Mix Reagents Kit (Applied Biosystems, Foster City, CA, USA) as instructed by the manufacturer. The Eukaryotic 18S rRNA Endogenous Control (FAM™ Dye/MGB Probe, Non-Primer Limited) (Applied Biosystems) was used to normalize for differences in RNA concentrations between samples.

Means of duplicates for every sample were used for analysis. The relative virus titre for each sample was calculated using the $\Delta\Delta$ct method (User Bulletin #2, Applied Biosystems). Analysis of variance (ANOVA) was used to determine statistical significance of the differences among treatments for the two viruses using MINITAB release 14.1 (Minitab Inc., State College, PA, USA). Differences were considered statistically significant if $P < 0.05$.

Results and Discussion

The trends observed in both experiments were similar and confirmed those obtained in the preliminary study. Data presented are from the February to April 2008 experiment. The first symptoms were observed on day 12 (Fig. 2). By day 18 symptoms observed in the SPCSV/SPFMV plants were significantly more severe than all other treatments. By day 29 the symptoms observed for SPFMV/SPCSV were also significantly more severe than those for singly

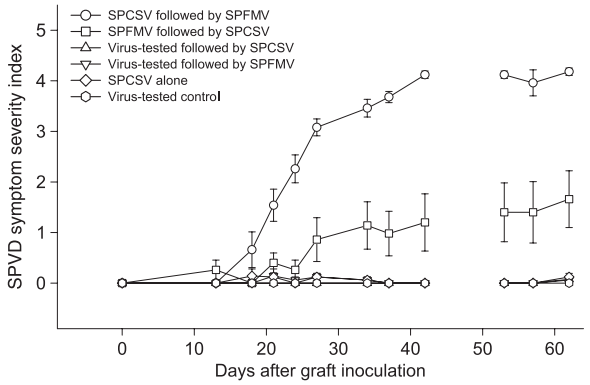

Fig. 2 Development of SPVD symptoms in plants initially infected with *Sweet Potato Chlorotic Stunt Virus* (SPCSV), followed by *Sweet Potato Feathery Mottle Virus* (SPFMV) and plants initially infected with SPFMV, followed by infection with SPCSV. Plants were cut back 42 days after graft inoculation. Bars indicate standard errors and data represented is from the 2008 experiment

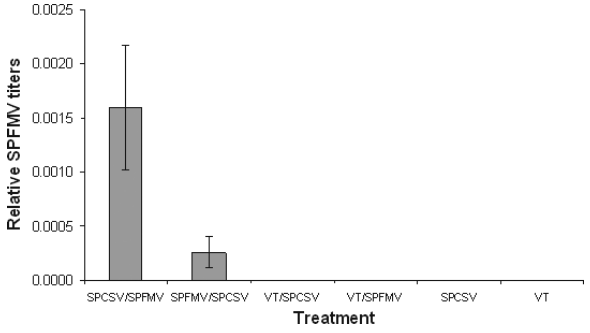

Fig. 4 Relative *Sweet Potato Feathery Mottle Virus* (SPFMV) titers 42 days after graft inoculation determined using Q-RT-PCR for plants initially infected with *Sweet Potato Chlorotic Stunt Virus* (SPCSV), followed by SPFMV (SPCSV/SPFMV) and plants initially infected with SPFMV, followed by infection with SPCSV (SPFMV/SPCSV) and singly infected controls at 42 DPI. Bars indicate standard errors and data represented is from the 2008 experiment

infected plants, but less severe than SPCSV/SPFMV plants. This trend continued for the rest of the study, even on regrowth after plants were cut back on day 42. A similar pattern of symptom development occurred on *Ipomoea setosa*, but virus titres were not determined in that preliminary test. There were no significant differences among relative SPCSV titres of any of the dually infected treatments and singly infected SPCSV treatments (Fig. 3). However, the SPFMV titres were significantly higher in the SPCSV/SPFMV plants (Fig. 4) compared to SPFMV/SPCSV plants, corresponding to differences in symptom severity.

The mechanism for the synergistic interaction between SPFMV and SPCSV that leads to SPVD has not been fully elucidated. It has been suggested that suppressors of RNA silencing present in SPCSV suppress the silencing mechanism that is responsible for SPFMV resistance (Kreuze et al., 2005). RNA silencing as a mechanism of plant virus resistance and the suppression of such silencing by viruses are well known (Waterhouse et al., 2002; Voinnet, 2005). However, it has not been proven that RNA silencing is indeed responsible for SPFMV resistance observed in many sweetpotato cultivars. In addition, it was recently reported that the SPCSV p22 protein previously thought to be involved in the synergistic interaction between SPCSV and SPFMV was absent in certain isolates of SPCSV despite the fact that these isolates were still capable of the synergistic interaction (Cuellar et al., 2008).

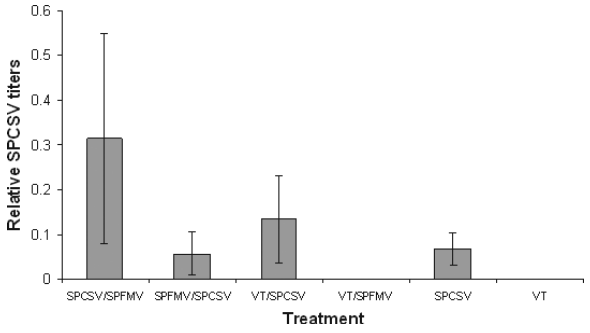

Fig. 3 Relative *Sweet Potato Chlorotic Stunt Virus* (SPCSV) titers 42 days after graft inoculation determined using Q-RT-PCR for plants initially infected with SPCSV, followed by *Sweet Potato Feathery Mottle Virus* (SPFMV) (SPCSV/SPFMV) and plants initially infected with SPFMV, followed by infection with SPCSV (SPFMV/SPCSV) and singly infected controls. Bars indicate standard errors and data represented is from the 2008 experiment

The fact that symptom severity remained low in SPFMV/SPCSV for several weeks, suggests that once the SPFMV resistance mechanism is established (plant initially infected with only SPFMV), it is not fully overcome when SPCSV infection takes place. Alternatively, if the plants are first infected with SPCSV, the SPFMV resistance mechanism appears to be already suppressed, so that when plants are later infected with SPFMV, very severe symptoms develop quickly, with comparably high SPFMV titre levels. Our results fit well into the model that SPFMV infection is usually suppressed by RNA silencing and that prior infection with SPCSV released this suppression by suppressing RNA silencing.

It remains to be seen whether this effect holds true for other sweetpotato cultivars. The drastic difference in disease development (symptoms and SPFMV titres) depending on the sequence of infection would mean that various infection scenarios would have to be tested to ensure that resistant cultivars developed under controlled conditions, will also be resistant in the field. Future research might try to exploit this phenomenon to ensure milder SPVD symptoms when susceptible plants are later (naturally) infected with SPCSV.

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