

## Chapter 5

### Detection and characterisation of sweet potato viruses

#### 5.1 Introduction

In many African countries, sweet potato (*Ipomoea batatas* Lam.) is an important crop for food security and also as a cash crop (Moyo *et al.*, 1999; Thompson *et al.*, 1999). Sweet potato viruses are grouped based on vector relationships: mainly aphid-borne and whitefly-borne viruses. The aphid-borne sweet potato feathery mottle potyvirus (SPFMV) is the most important and common virus infecting sweet potato wherever the crop is grown, including South Africa (Clark and Moyer, 1988; Moyer and Salazar, 1989; Jericho and Thompson, 2000). Sweet potato mild mottle ipomovirus (SPMMV) and possibly sweet potato latent potyvirus (SPLV), both of the family Potyviridae, have been reported to infect sweet potatoes in South Africa (Jericho and Thompson, 2000). The whitefly transmitted sweet potato chlorotic stunt crinivirus (SPCSV), family Closteroviridae, was reported to occur in the mainly tropical regions of the world (Gibson and Aritua, 2002). Symptoms caused by SPCSV alone are relatively mild in sweet potato and its relative, *Ipomoea setosa* Kerr. (Gibson *et al.*, 1998; Alicai *et al.*, 1999, Gibson and Aritua, 2002). When it occurs in mixed infection with SPFMV, SPCSV causes severe symptoms on infected plants (Karyeija *et al.*, 1998; Gibson and Aritua *et al.*, 2002). This devastating problem has not yet been reported to occur in South Africa (Jericho and Thompson, 2000). The potyviruses, sweet potato virus II (SPV II) and sweet potato virus G (SPV G), have also been recently reported to infect sweet potatoes (Salazar and Fuentes, 2000; Colinet *et al.*, 1994). Presently, more than 14 virus diseases of sweet potato have been reported (Moyer and Salazar, 1989; Brunt *et al.*, 1996; Di Feo *et al.*, 2000).

In South Africa, sweet potato viruses are difficult to identify in the field because of poor expression of symptoms possibly due to different climatic factors. In Chapter 3 of this thesis, it was indicated that the majority of rural farmers did not know or understand what sweet potato viruses were. Symptoms such as chlorosis and chlorotic spots, if present, were mistaken for ageing or sunburn by most rural farmers. In order for the

presence and identity of viruses infecting sweet potatoes in South Africa to be determined, indexing has to be carried out. This is also necessary to develop control strategies. The work reported here was undertaken to determine the identity and distribution of viruses infecting sweet potato grown by small-scale farmers in South Africa.

## **5.2 Materials and methods**

### **5.2.1 Collection of samples**

A total number of 673 samples were collected from small-scale farmers fields during the 2001/2003 survey in seven provinces (Gauteng, Limpopo, Mpumalanga, Kwazulu Natal, Western Cape, Eastern Cape and North West) of South Africa. Also, two commercial growers in Limpopo (Marble Hall) and several small-scale farmers in Mpumalanga (Hazyview, Numbi and Gutjwa) were visited in April 2003 as part of a tour with visiting virologists. A further 78 samples were collected. Symptom-bearing wild *Ipomoea* spp. were also collected during the survey for virus analysis. The cuttings of all collections consisted of an average of three nodes taken from the apical part of the vine and were representative of all varieties in the field. The samples were collected randomly to represent both symptomatic and non-symptomatic plants. The cuttings were taken to the ARC-Roodeplaat where they were planted in 15cm diameter pots using pasteurised media (Just Nature, South Africa). Planted cuttings were then maintained in the glasshouse at a temperature between 20-30°C and left to develop 10 nodes and more.

### **5.2.2 Biological indexing**

#### **5.2.2.1 Indexing**

All surviving plants (553) plus 51 plants from previous surveys (1996/1999) were used to conduct the study of sweet potato viruses in South Africa. Samples collected during the project team's visits were also used later. All living samples were side grafted on *I. setosa* at the 3-leaf stage. The graft was sealed with a latex bandage (Stericrepe) or Parafilm (American National Can<sup>TM</sup>) and transferred to a glasshouse maintained at a temperature of between 20-30°C. The plants were given a supplementary feeding on a weekly basis (for dosages, refer to Chapter 4). Insect pests were also monitored and sprayed as required. Symptom expression on *I.*

*setosa* was monitored and recorded over a period of about six weeks, depending on the season of the year and the glasshouse temperature. Non-symptomatic plants were also monitored and the graft was checked whether it had taken or not, and if not, grafting of that particular sample was repeated.

#### 5.2.2.2 Host range study

Eleven sweet potato isolates from the 1996/1999 survey and nine from the current (2001/2003) survey, infected with single infections of SPFMV and mixed infections of SPFMV, SPMMV and possibly SPLV were first grafted on *I. setosa* plants and monitored for symptoms over a period of six weeks. Before being used as sources of inoculum, these isolates were first tested again with NCM-ELISA to confirm the presence of viruses. Six test plants were used as indicator plants to conduct this study namely, *Ipomoea wrightii* Gray, *I. setosa* Taiwan, *I. setosa*, *Ipomoea nil* (L.) Roth., *Phaseolus vulgaris* L. (beans) and *Beta vulgaris* (Linn.) (beetroot). These test plants were sap-inoculated at the 3 to 4 leaf stage by grinding symptomatic leaves of *I. setosa* with a mortar and pestle in 0.06M phosphate buffer, pH 7.4, containing 1% sodium diethyldithiocarbamate (DIECA). Celite diatomaceous earth was added and the leaf extracts of chosen isolates were then rubbed by hand onto leaves of the six test plants. Inoculated leaves were rinsed with tap water and kept in the glasshouse. Symptom development was monitored and recorded for up to six weeks and longer after inoculations.

### 5.2.3 Serological characterisation

#### 5.2.3.1 Antisera

Serological analysis was aimed at detecting nine viruses, namely SPFMV, SPMMV, SPLV, SPCSV, sweet potato chlorotic fleck potyvirus (SPCFV), sweet potato caulimovirus (SPCaLV), sweet potato mild speckling virus (SPMSV), C-6 virus and cucumber mosaic cucumovirus (CMV). Two other potyviruses, SPV G and SPV II, were later included when antisera was made available by the Institut für Biochemie und Pflanzenvirologie, Braunschweig, Germany. Three types of enzyme linked immunosorbent assays (ELISA) were conducted: Nitrocellulose membrane based ELISA (NCM-ELISA), triple antibody sandwich ELISA (TAS-ELISA) and double antibody sandwich ELISA (DAS-ELISA), using polyclonal (PAb's) and monoclonal

(MAB's) antibodies, kindly supplied by the International Potato Center (CIP) Lima, Peru and the Institut für Biochemie und Pflanzenvirologie, Braunschweig, Germany. The CMV antiserum was produced by the ARC-Roodeplaat.

### 5.2.3.2 NCM- ELISA

NCM-ELISA was used to test for SPFMV, SPMMV, SPLV, SPCFV, SPMSV, SPCaLV, C-6 virus, CMV and later for SPV II. NCM-ELISA kits were kindly supplied by CIP, Peru. The test was carried out according to the instructions supplied. Symptomatic leaves were picked from the bottom, middle and top parts of the grafted *I. setosa* plants. Sweet potato leaves of isolates grafted to *I. setosa* were also analysed in order to determine the accuracy and the reliability of sweet potato leaves compared with *I. setosa* with regard to NCM-ELISA. Small leaf discs were homogenised in reagent tubes in 0.02M Tris buffer plus 0.50M sodium chloride (NaCl) (TBS, pH 7.5), containing 1.0g sodium sulphite as an extraction buffer. Homogenised samples were centrifuged for 2 min at 10 000rpm (Micro Spin 24S, Sorvall® Instruments, U.S.A). A piece of Hybond-C Extra membrane (Amersham Life Science), 3.5 x 10mm, was placed on a dry filter paper on a bench and 1 x 1mm squares were marked on the membrane with a pencil. One drop of 15 to 20µl of each supernatant was placed onto the marked square of the membrane using a micropipette and left to dry for 15 to 30 min, before being stored between clean filter paper until used. All blotted membranes, together with positive control strips were first blocked in the blocking solution consisting of TBS, pH 7.5, 2% Triton X-100 and 2% low fat milk powder and incubated on a shaker (Gerhardt, Bonn) at room temperature with the gentle agitation at 50rpm for 60 min. The blocking solution was discarded and membranes washed in TBS, pH 7.5 with 0.05% Tween 20 (T-TBS, pH 7.5) three times for 3 min.

Antibody solutions were prepared by diluting 1:300 dilution in TBS, pH 7.5. For SPCSV, healthy lyophilised or fresh sweet potato leaves were first homogenised in TBS, pH 7.5, containing 0.2% sodium sulphite, 2% milk powder and 0.02% sodium azide (1:25, w/v), and the sap expressed through cheesecloth. Then the polyclonal SPCSV Ky-CP antiserum was added at 1:300 dilution in 30ml of the sap and incubated at 37°C for one hour in order to initiate the absorption of the antiserum.

After blocking, the antisera were added to the blocked membranes and incubated overnight at 4°C.

The second antibody, goat anti-rabbit (GAR)/alkaline phosphatase conjugate (Sigma) was diluted in TBS, pH 7.5, at 1:300 for SPFMV, SPMMV, SPLV, SPCFV, SPMSV, SPCaLV, C-6 virus, at 1:1000 for CMV and 1:500 for SPV II. After washing, in T-TBS pH 7.5, four times for 3 min, with gentle shaking (100rpm), the conjugate solution was added and membranes were incubated for one hour with gentle shaking of 50rpm at room temperature.

The substrate buffer consisted of 0.1M Tris, 0.1M NaCl and 5mM  $MgCl_{2.6}H_{2}O$  (pH 9.5). For the colour development, 0.8ml of the coded SOLVENT solution was first added to the coded NBT and BCIP substances independently, to allow them to dissolve. Thereafter 0.1ml of NBT first, then 0.1ml BCIP was added independently into 25ml of substrate buffer. After incubation of the conjugates, membranes were washed as before in T-TBS pH 7.5 and the substrate solution was added and membranes were incubated in the solution for 30 min with gentle shaking (50rpm). Colour development was stopped by washing membranes in distilled water for 10 min and positive samples were identified by different grades of the purple colour reaction.

A different substrate was used initially consisting of 6mg/ml Fast Red TR salt (5-chloro-2-toluenediazonium chloride hemi) (Zinc chloride) (Sigma), dissolved in 0.2M Tris-HCl, pH 8.2, filtered through Whatman No.1 filter paper and mixed 1:1 (v/v) with 0.1% naphthol AS-MX phosphatase (free acid, Sigma) in 0.2M Tris-HCl, pH 8.2. The substrate was incubated with the membranes for 30 min at room temperature with gentle shaking (50rpm) and the reaction was stopped by washing in distilled water. This substrate solution will produce a red colour reaction for the positive samples.

### 5.2.3.3 DAS-ELISA and TAS-ELISA

DAS-ELISA was used to test for SPV II and SPV G. TAS-ELISA was used to test for SPCSV. In both ELISA methods, polystyrene microtitre plates (F96 cert

Maxisorp, Nalge Nunc International, Nunc™, Denmark) were coated with 100 or 200µl of polyclonal antibodies, against each virus tested, diluted at 1:1000 in coating buffer (0.05M sodium carbonate bicarbonate buffer, pH 9.6). SPV G antisera dilution was 1:500. The plates were incubated for 4 hr at 37°C or overnight at 4°C. After incubating, plates were washed in PBS-Tween three times for 3 min.

Sweet potato leaves, directly from the field and from the plants in the glasshouse, leaves from grafted *I. setosa*, positive controls (from leaves of *I. setosa* and sweet potato) for each virus tested and leaves of healthy *I. setosa* or sweet potato as negative controls were used to conduct these virus assays. Leaves were homogenised using a mortar and pestle, after adding six to 10ml of sample/conjugate buffer consisting of 0.02M phosphate buffered saline, pH 7.4, containing 0.05% Tween-20 (PBS-Tween), 2% polyvinylpyrrolidone (PVP) and 2% egg albumin. One hundred or 200µl sample extracts were added in duplicate, and incubated for 4 hr at 37°C or overnight at 4°C.

After incubating, plates were washed in PBS-Tween three times for 3 min. The third step for TAS-ELISA, consisted of adding the monoclonal antibodies, MAb mix 1 (East African strain) and MAb mix 2 (West African strain), at a dilution of 1:100 in sample/conjugate buffer. The third step for DAS-ELISA was carried out by adding SPV II and SPV G antibody conjugated to alkaline phosphatase (Sigma) at a dilution of 1:1000 and 1:500 respectively in sample conjugate buffer. One hundred or 200µl was dispensed in duplicate wells of the ELISA plates. All plates were incubated for three to 4 hr at 37°C or overnight at 4°C.

TAS-ELISA consisted of an extra step of adding the goat anti-mouse/alkaline phosphatase at a dilution of 1:10000 in sample/conjugate buffer. Plates were incubated for three to 4 hr at 37°C or overnight at 4°C.

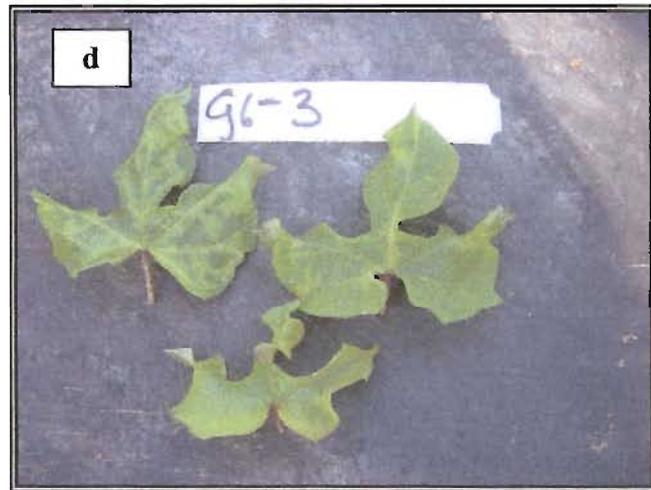
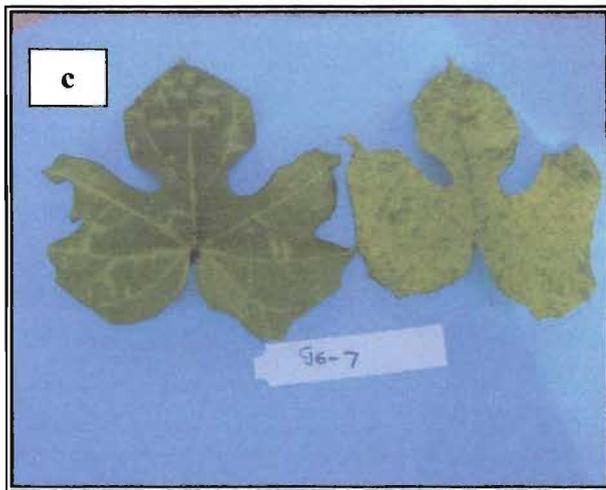
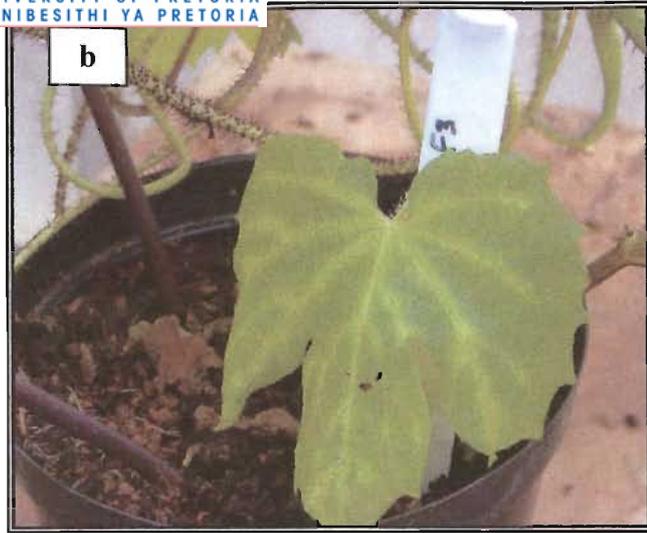
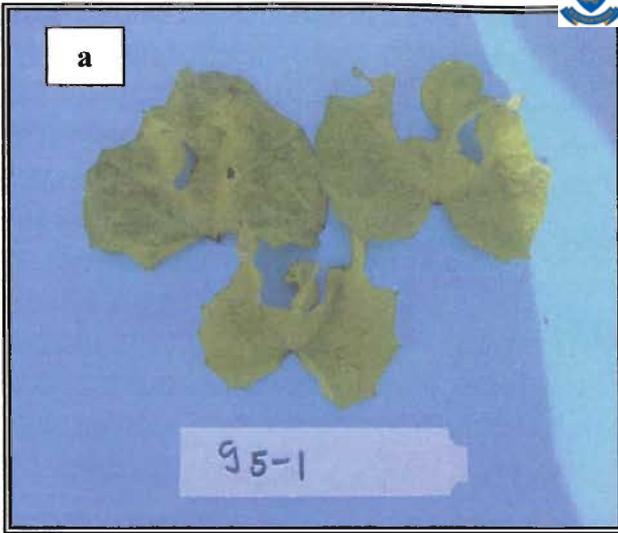
The positive or negative reaction of the sample was determined by adding 100 or 200µl of 4-nitrophenyl phosphate disodium salt hexahydrate (Fluka Biochemiko), diluted at 1mg/ml in 10% diethanolamine buffer, pH 9.8 to each well. After one hour of incubating in substrate solution at room temperature, readings were taken at

405nm using Flow Titertek® Multiskan Plus ELISA plate reader (Labsystems, Finland).

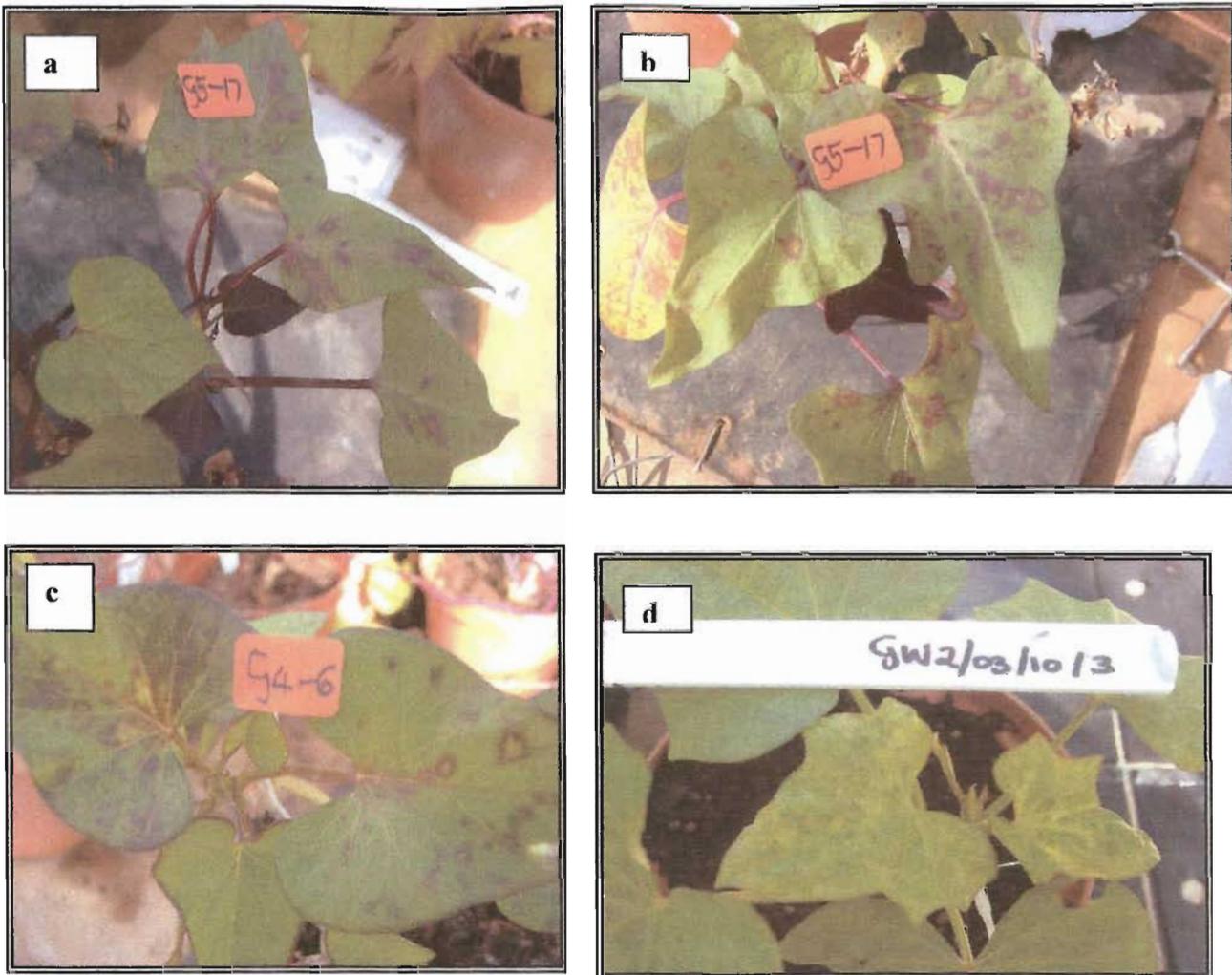
## 5.3 Results

### 5.3.1 Indexing

During the field surveys, although not prevalent, virus symptoms such as vein clearing, purple ring spots, vein banding and chlorotic spots were observed in Mpumalanga, Western Cape and Eastern Cape. The disease survey results given in Chapter 4, this thesis, confirm the fact that symptoms of virus infection are not readily seen on field-grown sweet potatoes in South Africa. After sweet potato cuttings had been grafted onto *I. setosa*, symptoms induced by the viruses included those typical of potyviruses (Moyer and Salazar, 1989). Symptoms included vein clearing, chlorotic spots, chlorosis, diffuse mottle and leaf distortion (Figure 5.1). Others symptoms observed on *I. setosa* included stunting, leaf deformation and mosaic, but were not as prevalent and severe as potyvirus associated symptoms. Symptoms on *I. setosa* were observed to differ from sample to sample. Some *I. setosa* plants expressed obvious severe vein clearing, diffuse mottle and chlorotic spots symptoms, while others expressed some mild diffused mottle, coupled with some chlorosis. Symptoms were also observed on sweet potato plants that were stored in the glasshouse after a certain period of time (Figure 5.2). After more than six weeks of monitoring, 19% of the 616 sweet potato samples indexed expressed no symptoms on *I. setosa* and they were taken to be virus free.



**Figure 5.1 Typical potyvirus symptoms shown by grated *I. setosa*.** (a) Vein clearing, diffuse mottle and leaf deformation, (b) severe vein clearing and mosaic (c) vein clearing and mosaic and (d) vein clearing and leaf curling



**Figure 5.2** Sweet potato plants exhibiting symptoms after they have been kept in the glasshouse. (a) and (b) show conspicuous purple ring spots, (c) purple ring spots and some chlorotic spots, (d) chlorotic spots and vein clearing

### 5.3.2 Host range study

Symptoms induced by 20 chosen isolates on six test plants are given in Table 5.1. Symptoms varied with test plants. Symptoms were mostly induced on *I. wrightii* and *I. nil* and the most common ones were vein clearing and leaf distortion. Obvious symptoms were also induced by a few isolates on the common *I. setosa* and the Taiwanese strain (Table 5.1). No symptoms were induced on beans and beetroot even after more than six weeks of monitoring. All symptomatic plants were further tested with NCM-ELISA to confirm the presence of viruses in the test plants. Due to problems that were experienced with the reagents, the results gave some false positive

reactions. However, once the problem had been solved, there was no leaf material left to repeat the test.

**Table 5.1 Symptoms induced by virus-infected sweet potatoes on host plants selected for host range study**

Isolates	Viruses	<i>I. wrightii</i>	<i>I. setosa</i> Taiwan	<i>I. setosa</i>	<i>I. nil</i>	<i>Phaseolus vulgaris</i>	<i>Beta vulgaris</i>
KS3-2/4	SPFMV	Leaf distortion	Chlorosis	-	Leaf distortion	-	-
KS2-1	SPFMV	Leaf distortion	-	-	Leaf distortion	-	-
M6/2/2 B	SPFMV	Leaf distortion	-	Puckering	Necrotic spots	-	-
M6/2/2 A	SPFMV	Vein clearing	-	-	Vein clearing	-	-
TO 3.4 Swazi	SPFMV/ SPMMV	Vein clearing	Vein clearing	Vein clearing	Vein clearing, chlorotic spots	-	-
Frank 5.2	SPFMV	-	Vein clearing	Vein clearing /severe	Vein clearing, puckering and some mosaic	-	-
TO 3.5	SPFMV	Vein clearing	-	Mosaic	Vein clearing, leaf distortion and mosaic	-	-
ARN 1/1/11	SPFMV	Leaf distortion	-	Mosaic	Vein clearing and leaf distortion	-	-
Cull 1/2/4	SPLV SPFMV	Leaf distortion	-	Necrotic spot	-	-	-
RS1-3	SPFMV	Leaf distortion	-	-	-	-	-
RS2-3	SPFMV	-	-	-	Chlorotic spots and leaf distortion	-	-
Cull 1/1/4	SPFMV	Leaf distortion	Puckering	-	Chlorosis	-	-
WC 10	SPFMV/ SPMMV	Leaf distortion	-	-	Leaf distortion	-	-
Cull 1/4/5	SPFMV	-	-	-	Leaf distortion	-	-
DDE 5/1/5A	SPFMV	Leaf distortion	-	-	Leaf distortion	-	-
PD 1/2/1	SPFMV	Leaf distortion	-	-	Necrotic spots	-	-
PD 1/2/1	SPFMV	Vein clearing	-	Puckering	Chlorosis and vein banding	-	-
TO 3.3 ENG	SPFMV/ SPMMV	-	-	-	Vein clearing, chlorosis and mosaic	-	-
TO 2.5 swazi	SPFMV	-	Leaf distortion and chlorosis	-	-	-	-
JIII	SPFMV	-	-	-	Stunting	-	-

SPFMV=sweet potato feathery mottle virus, SPMMV=sweet potato mild mottle virus, SPLV=sweet potato latent virus.

-- No symptoms

### 5.3.3 NCM, DAS- and TAS-ELISA

A summary of the results of serological tests and common viruses found in each province is given in Tables 5.2 and 5.3. Comprehensive tables of all tested isolates are given in Appendices 5.1-5.3. In all samples that were serologically analysed, 63% were found to be infected with SPFMV, occurring in all provinces. The potyviruses SPV II and SPV G were detected in 28% and 26% of samples, respectively. Unfortunately samples from the Limpopo province and from the 1998/1999

collections were not re-indexed to test for SPV II and SPV G due to time constraints. These two viruses were commonly detected in samples already infected with SPFMV and other viruses (Table 5.3).

During the surveys, suspected symptoms of sweet potato virus diseases (SPVD) such as severe vein clearing, leaf distortion, chlorosis, mosaic and stunting were observed on sweet potatoes in the field of small-scale farmers in Mpumalanga. TAS-ELISA confirmed the occurrences of low levels of SPCSV-EA (East African strain) after sweet potato samples were tested directly from the field. Approximately 2% of samples from the Limpopo, Mpumalanga, Kwazulu Natal and Western Cape provinces of South Africa were infected with East African strain of SPCSV.

Seventy-eight sweet potato samples collected during the sweet potato virus project team's visit in South Africa were also analysed for viruses directly from the field. DAS-ELISA and TAS-ELISA were used to test for the following viruses: SPFMV, SPMMV, SPV II, SPV G, SPMSV, SPCFV and SPCSV (antisera brought by the Biologische Bundesanstalt für Land-u, Braunschweig, Germany delegation). SPFMV, SPV II and SPV G were the most common viruses found. Sixty-seven samples were found to be infected with SPFMV, followed by 58 and 46 samples, which were infected with SPV G and SPV II, respectively. SPCFV was also detected in ten samples. TAS-ELISA confirmed the presence of SPCSV-EA and SPCSV-WA strains in eleven and seven samples, respectively. SPCSV-WA strain was detected only in samples from a commercial farm in Marblehall, in the Limpopo province (Appendix V). Out of 78 samples that were analysed for viruses, eight of them were found to be virus-free. It was found that SPCSV was not always transferred to *I. setosa*. Attempts to retest SPCSV infected plants using grafted *I. setosa* leaves gave negative reactions. However, SPCSV-EA infected sweet potato leaf materials from the glasshouse that were used for positive controls continued to give positive reactions.

Wild *Ipomoea* spp. collected during the field surveys were also infected with SPCSV-EA, SPFMV, SP VG and SPV II. The National Botanical Institute of South Africa later identified this species as *Ipomoea senensis* (Ders.) Choisy.

SPLV, SPCFV and SPMSV were also detected in a few samples from the Limpopo, Mpumalanga, Western Cape and Eastern Cape provinces. These viruses were detected most commonly in samples that were already infected with either SPFMV or other potyviruses and seldom as a single infection. CMV was detected in only one sample from the Western Cape province. SPCaLV and C-6 were not detected in all samples tested.

The use of sweet potato leaves from plants maintained in a glasshouse for virus analysis with NCM-ELISA was found to be unreliable. NCM-ELISA with sweet potato leaf materials normally resulted in faint or no reactions and sometimes some false positive reactions.

Generally, mixed infections were more prevalent and only 10% of samples were found to be singly infected. Only 19% of samples were found to be virus free after they had been grafted onto *I. setosa* and monitored for symptoms. Approximately 2% of the samples induced symptoms on *I. setosa* but did not react with any of the antisera used against sweet potato viruses (Appendix 5.3).

**Table 5.2 Summary of serological analysis of sweet potatoes collected in South Africa**

Prov.	Total	Nil	Neg Ser.	FMV	MMV	LV	CFV	C-6	CaLV	MSV	CMV	V-II	V-G	CSV-EA	CSV-WA	Single infection
GT	27	0	0	27	0	0	2	0	-	1	0	-	-	-	-	-
LP	144	15	5	113	3	8	1	0	0	3	0	24	1	3	0	36
MP	201	46	2	147	5	16	6	0	0	5	0	109	110	6	0	12
KZN	40	31	1	5	0	0	0	0	0	0	0	3	1	1	0	5
WC	89	19	2	63	0	2	2	0	0	2	1	39	42	1	0	16
EC	48	23	1	22	2	2	1	0	0	0	0	14	19	0	0	2
NW	16	0	0	14	0	0	0	0	0	0	0	6	13	0	0	2
Previous	51	0	0	51	10	9	4	0	0	1	0	-	-	-	-	-
<b>Total</b>	<b>616</b>	<b>134</b>	<b>11</b>	<b>442</b>	<b>20</b>	<b>37</b>	<b>16</b>	<b>0</b>	<b>0</b>	<b>12</b>	<b>1</b>	<b>195</b>	<b>186</b>	<b>11</b>	<b>0</b>	<b>73</b>
Percentage		19.0	1.6	62.8	2.8	5.3	2.3	0	0	1.7	0.1	27.7	26.4	1.6	0	10.4

GT=Gauteng, LP=Limpopo, MP=Mpumalanga, KZN=Kwazulu Natal, WC=Western Cape, EC=Eastern Cape and NW=North West provinces of South Africa. Nil=no symptoms on *I. setosa*, Neg, ser=negative serologically. Previous=samples collected during previous surveys. -=Not tested. FMV=sweet potato feathery mottle virus, MMV=sweet potato mild mottle virus, LV=latent virus, CFV=sweet potato chlorotic fleck, CaLV=sweet potato caulimovirus, MSV=sweet potato mild speckling, CMV=cucumber mosaic virus, V-II= sweet potato virus II, V-G=sweet potato virus G, CSV (EA and WA)=sweet potato chlorotic spot virus (East african and West african strain)

**Table 5.3 Summary of viruses found infecting sweet potatoes in each surveyed province of South Africa**

Province	District	Viruses (common)
Limpopo	Mianzwi	SPFMV + SPVII+ SPCSV-EA (SPVG not tested)
	Tshidane	SPFMV+SPVII (SPVG not tested)
	Tshiombo	SPFMV+SPVII (SPVG not tested)
	Klipspruit and Riverside	SPFMV (SPVII & SPVG not tested)
	Arthurstone and Dingleydale	SPFMV (SPVII & SPVG not tested)
Mpumalanga	Marblehall	SPFMV+ SPVII+ SPVG+SPCSV-EA/WA
	Beverbreed	SPFMV+SPVII +SPVG (SPLV + SPMMV)
	Gutjwa	SPFMV+SPVII+SPVG+ (SPLV+ SPCSV-EA)
	Hazyview	SPFMV+SPVII+SPVG (SPLV+SPCFV)
Western Cape	Tonga	SPFMV+SPVII +SPVG
	Ebeneser	SPFMV+SPVII +SPVG (SPMSV)
	Friemersheim	SPFMV+SPVG (SPCSV-EA)
	Goedverwacht	SPFMV (SPVG+SPVII)
	Pacalsdorp	SPFMV (SPVII+SPVG)
Eastern Cape	Saron	SPFMV+SPVG (SPVII)
	Alice and Burthust	SPFMV (SPVG+SPVII)
	Lower Mpako and Mpako	SPFMV+SPMMV (SPVG/SPVII)
Gauteng	Port Alfred and Thombo	SPFMV (SPVII & SPVG)
	Cullinen	SPFMV (SPVII & SPVG not tested)
Kwazulu Natal	Mvundleni	SPFMV
North West	Hamanskraal	SPFM (SPVG+SPVII)

SPFMV=sweet potato feathery mottle virus, SPMMV=sweet potato mild mottle virus, SPLV=latent virus, SPCFV=sweet potato chlorotic fleck, SPCaLV=sweet potato caulimovirus, SPMSV=sweet potato mild speckling, SPVII= sweet potato virus II, SPVG=sweet potato virus G, SPCSV (EA and WA)=sweet potato chlorotic spot virus (East african and West african strain)

## 5.4 Discussion and conclusion

Viruses of sweet potato have been identified and associated with poor sweet potato quality and low yield in South Africa (Joubert *et al.*, 1974; Laurie *et al.*, 2000). SPFMV has been reported to occur worldwide (Clark and Moyer, 1988; Moyer and Salazar, 1989). In South Africa, SPFMV, SPMMV and possibly SPLV have been identified and associated with symptoms on *I. setosa* as the important viruses of sweet potato (Jericho, 1999; Jericho and Thompson, 2000).

In the present study, almost all samples from seven provinces of South Africa proved to be infected with viruses after they had been grafted onto *I. setosa* plants and analysed for viruses with NCM-ELISA, DAS-ELISA or TAS-ELISA. Sixty three percent of samples infected by SPFMV in this study is a confirmation of the findings by Jericho (1999) that it is the most prevalent virus in most sweet potato growing areas of South Africa. Although it was detected in only a few samples (3%), these results support the previous analysis by Jericho (1999) that this virus does occur in South Africa. The low

percentage infection of SPMMV compared to that SPFMV could be due to its transmission by whiteflies, which are more limited in their distribution because of climatic conditions.

Analysis through DAS-ELISA confirmed the occurrence of potyviruses SPV II and SPV G. This is the first time that these two viruses were tested and detected in South Africa. These two viruses are most commonly found in synergism with SPFMV and other viruses and rarely as a single infection. Although their effect on yield has not been reported yet, SPV II and SPV G are also widely spread in South Africa. The two viruses seem to be a potential threat to sweet potato production following SPFMV, due to their occurrence in almost all samples tested.

This is also the first report of the occurrence of SPCSV-EA and SPCSV-WA strains in South Africa. Through virus analysis with TAS-ELISA, sweet potato samples suspected of having SPVD collected in Mpumalanga province proved to be infected with SPCSV-EA. SPCS-EA was also detected in few samples from the Limpopo, Kwazulu Natal and Western Cape provinces. The SPCSV-WA strain was also detected in samples from a single farm in the Limpopo province (Marble Hall). SPCSV is known to cause SPVD when it is in synergy with potyvirus SPFMV (Gibson *et al.*, 1998). Although suspected symptoms of SPVD were observed in few farmers' fields in Mpumalanga, the SPVD problem was not commonly observed in other provinces visited. In South Africa, climate seems to cause variation in virus concentration and the expression of symptoms on sweet potatoes in the field. Also, SPCSV positive samples were found to be infected with potyviruses SPFMV, SPV II and SPV G. However, the synergism of SPFMV and SPCSV did not always result in the devastating SPVD symptoms. The reason for this is yet unknown. It is unknown whether local sweet potato cultivars possess genes that enhance tolerance to mix virus infections. A thorough characterisation of SPCSV of the mechanism behind its synergism with SPFMV may provide answers of why the synergy does not always result in SPVD symptoms in South Africa.

The occurrence of SPCSV-EA strain in four provinces undoubtedly is an indication that it is widely distributed throughout the country although it occurs only in isolated fields. This indicates that the occurrence of SPCSV will have the potential of resulting in the

development of SPVD pandemic with time. Extensive surveys on SPCSV distributions still need to be carried out to determine the full extent of this danger.

SPLV, SPMSV and SPCFV were also detected in samples tested, and they were most commonly found in a mixed infection with potyviruses SPFMV, SPV II and SPV G and seldom as a single infection. The occurrence of SPLV is just a confirmation of what Jericho (1999) has also reported about SPLV occurring in South Africa. One sample from Western Cape was found to be infected with CMV. This could be possible because the Western Cape province is a large producer of cucumbers. However, this sample did not react positive with any of the whitefly transmitted virus antisera. It is reported that CMV, only occurs in sweet potato plants which carry whitefly-transmitted viruses (Cohen and Loebestein, 1991; Cohen *et al.*, 1992). This calls for further investigation on this particular sample to confirm the presence of CMV. Further tests must also be conducted to confirm the incidence of SPLV, SPMSV and SPCFV in South Africa using other techniques such as reverse transcription polymerase chain reaction (RT-PCR), ISEM and testing different host plants. SPCaLV and C-6 have not yet been found to infect sweet potato plants in South Africa.

Typical sweet potato virus symptoms were expressed on *I. setosa* plant. However, few symptomatic samples did not react with any of the antisera used against the sweet potato viruses tested. This might indicate that there are other uncharacterised viruses that are involved, which also calls for thorough research on these aspects.

Diagnosis of sweet potato viruses by serological analysis was sometimes found to be inconsistent and unreliable. Repeated tests had to be conducted in order to obtain accurate results. Testing for SPCSV from sweet potato leaves with TAS-ELISA was more reliable and accurate than with NCM-ELISA. Unfortunately, attempting to back test SPCSV from grafted *I. setosa* leaf materials was found to be unreliable. This aspect raises questions of whether the involvement of a potyvirus lowers SPCSV titres during translocation, making its detection by serological methods using *I. setosa* leaves difficult. Some serological inconsistency was also experienced when isolates that were tested initially with TAS-ELISA, using sweet potato leaves directly from the field, gave different reactions when they were later tested with NCM-ELISA using *I. setosa* leaves. Sweet potato leaves were also found to be unreliable for detecting sweet potato viruses

with NCM-ELISA. The important question is whether NCM-ELISA is a reliable technique for testing sweet potato viruses. The reasons behind the variations in virus reactions with sweet potato and *I. setosa* leaves and different serological techniques need to be resolved so that reliable methods can be developed for future research.

The induction of symptoms by viruses on *Ipomoea* spp. (*I. wrightii*, *I. nil* and *I. setosa*) used for host range study is a confirmation that *Ipomoea* spp. are hosts for sweet potato viruses. The occurrence of SPCSV-EA, SPFMV, SPV G and SPV II in wild *Ipomoea* spp. is also an indication that wild *Ipomoea* spp. have the potential of becoming reservoirs of viruses. For this reason, it should be crucial for farmers to control weeds and volunteer sweet potato plants in their fields in order to prevent viruses from spreading to new plants.

The fact that 19% of the samples collected were virus free plants might serve as a hope that selection of virus free planting materials by farmers can be possible. Unfortunately virus symptoms are not easily observed on sweet potato plants in the fields of South African farmers. The majority of farmers interviewed (Chapter 3) did not know what virus diseases were and how to control them. The poor expression of symptoms by plants in the field will make it difficult for farmers to know and familiarise themselves with symptoms and this will make the selection of planting material difficult. The high percentage of virus free plants found in the one area of Kwazulu Natal surveyed may be due to the establishment of a nursery in that area for the distribution of healthy propagation material.

Viruses have been recognised as the biggest threat to sweet potato production in South Africa (McClellan and Klesser, 1947). The problem of sweet potato virus diseases is perpetuated by its vegetative propagation means. When farmers were interviewed (Chapter 3), it was indicated that planting material was exchanged among friends and families and that traditional farming ways of using the old same planting material year after year was still practiced. These practices result in disseminating virus-infected materials from one place to another. In order to increase production of sweet potatoes, farmers need to be equipped with vital information that will enable them to control viruses in order to minimise yield and quality loss. In conclusion, it can be stated that the use of virus-free planting material is important and that it should be emphasised and

demonstrated to farmers as a starting point of their sweet potato production. Rouging should also be encouraged as an important and effective means of controlling sweet potato viruses. The use of transgenic sweet potato plants has been found to be tolerant to SPFMV-RC (Cipriani *et al.*, 2000). Any measures aimed at controlling viruses will help reduce their spread and if sustainable resistant varieties to other strains of SPFMV can be developed, the problem of fighting SPFMV will be minimised. Quarantine measures, in order to prevent the development of new SPFMV strains, should also be promoted.

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