

Survey and characterisation of sweet potato viruses in South Africa

by

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DECLARATION

I, Mapula Julia Domola, hereby declare that the dissertation/thesis, which I hereby submit for the degree M. Inst Agrar (Plant Protection) at the University of Pretoria, is my own work and has not previously been submitted by me for the degree at this or any other tertiary institution.

A handwritten signature in purple ink, appearing to be 'M.J. Domola', written over a horizontal line.

M.J. Domola

Dated this 03 day of December 2003.

SUMMARY

Sweet potato (*Ipomoea batatas* Lam.) is a crop which has been used for human consumption in South African rural communities for many years. The crop is widely grown on small-scale, primarily to help ensure the food security and as a cash crop for rural households. The ability of the crop to grow on marginal and degraded soil also adds to its advantages. A baseline survey on production and utilisation of sweet potato by small scale-farmers was conducted during 2001 to 2003 in six provinces of South Africa (Limpopo, Mpumalanga, Eastern Cape, Western Cape, KwaZulu Natal and Northwest). This revealed that farmers mostly harvested enough sweet potato to feed their families but under the small-scale production the yield was very low. During the baseline survey of the current study, sweet potato was ranked the third most important food crop of rural families in South Africa.

Virus diseases have been identified as one of the major constraints to sweet potato production. A survey to determine the incidence of pests and diseases of sweet potatoes grown by small-scale farmers was conducted in seven provinces of South Africa, namely Gauteng, Mpumalanga, Limpopo, KwaZulu Natal, North West, Western and Eastern Cape. Damage caused by insects was found to be more prevalent than any disease in all provinces. Plants showing symptoms of virus diseases were not frequently observed in fields. Representative samples of each province were selected randomly during surveys and cuttings were taken for virus analysis. Samples were indexed to *Ipomoea setosa* Kerr. and serological analysis were carried out aimed at detecting nine viruses, namely sweet potato feathery mottle potyvirus (SPFMV), sweet potato mild mottle ipomovirus (SPMMV), sweet potato latent potyvirus (SPLV), sweet potato chlorotic stunt crinivirus (SPCSV), sweet potato chlorotic fleck potyvirus (SPCFV), sweet potato caulimovirus (SPCaLV), sweet potato mild speckling potyvirus (SPMSV), C-6 virus and cucumber mosaic cucumovirus (CMV). Two other potyviruses, SPV G and SPV II, were later included when the 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 enzyme linked immunosorbent assay (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. CMV antiserum was obtained from the ARC-Roodeplaat.

Serological analysis showed that viruses are the biggest threat to sweet potato production in South Africa. Nine viruses were identified serologically and approximately 80% of sweet potato cuttings taken from the fields were found to be virus infected. SPFMV was found in 63%, SPMMV in 3% and SPLV in 5% of the samples. Five viruses were found for the first time in South Africa: SPCFV, SPMSV, SPCSV (East and West African strains), SPV II and SPV G. SPV II and SPV G were found in nearly 30% of the samples. Cucumber mosaic virus was found in a single sample. Mixed infections were detected in most samples and only 10% of the samples were infected by a single virus. The effects of virus infection on the yield of nine sweet potato cultivars and advanced breeding lines were studied over two seasons. The plants were infected with two combinations of viruses A) SPFMV, SPV II and SPV G and B) SPFMV, SPMMV, SPV II and SPV G. Healthy plants were infected by grafting to infected cuttings, multiplied and planted randomly in three replicated blocks. Plants from the first trial were kept and planted in the second year to determine the long-term effect of viruses on yield. Average total yield was significantly reduced by between 12 and 22% while the marketable yield was reduced by 21 to 38%. This was mainly caused by the increase in cracking (41-82%). Some cultivars were highly sensitive to virus infection while others showed a degree of tolerance. This study confirmed that cultivars that had been infected for more than one season showed a greater decrease in yield and increase in cracking than newly infected cultivars. This is the first comprehensive study to determine the effect of viruses on sweet potato yield in South Africa.

These findings will contribute to developing programmes aimed at controlling virus diseases that are feasible and more sustainable. It will also be of paramount importance that this valuable information be disseminated among small-scale farmers, so that some of the problems encountered can be minimised.

DEDICATION

I dedicate this work to my grandmother who God has spared to live to see the fruits of her hard work, unending love, sincere moral supports and words of wisdom. To my little girl, Thendo, hoping that this work will be a motivation and inspiration in her future life. Lastly, to my parents and my two siblings Ndivhuho and Happy.

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Chapter 1

Production and utilisation of sweet potato

1.1 General introduction to the sweet potato crop

Sweet potato (*Ipomoea batatas* Lam.), belonging to the family Convolvulaceae, originated in Central and South America (Steinbauer and Kushman, 1971; Yen, 1982; Thurston, 1984; Otterdijk, 1999). Sweet potato ranks as the world's seventh most important crop and the third most important tuber crop in sub-Saharan Africa after cassava (*Manihot esculenta* Crantz) and yam (*Dioscorea* spp. L.) (Karyeija *et al.*, 1998; Minde *et al.*, 1999; Anonymous, 2002a).

The spread of this crop has been very extensive and is now grown in tropical, subtropical and warmer temperate areas throughout the world (Steinbauer and Kushman, 1971). In Tanzania, Malawi, Mozambique, Zambia and Angola, sweet potato is an important food crop (Moyo *et al.*, 1999). Uganda is the largest African producer and the third largest producer in the whole world, growing approximately 2.2 million metric tones (Karyeija *et al.*, 1998). Nearly 90% of the total African output comes from eastern and southern Africa (Ewell and Mutuura, 1991; Kanju, 2000; Karyeija *et al.*, 2000). The crop was introduced into South Africa from Brazil shortly after the Dutch colonised the Cape in 1652 (Mynhardt and Joubert, 1982; du Plooy, 1986) and today it is grown virtually in all provinces (Laurie *et al.*, 1999; 2000). From these introductions, a few traditional cultivars such as Borrie and Six Months White originated (du Plooy, 1986). The crop is now grown by both small scale and commercial farmers with the main production areas being Limpopo, Mpumalanga, Western Cape, Eastern Cape, Kwazulu Natal and Free State Provinces of South Africa (Laurie, 1996; van der Mescht *et al.*, 1997). According to the official figures, the commercial sweet potato production is smaller than other vegetable crops (Thompson *et al.*, 1999). The average annual output of sweet potato storage roots for the 10 years from 1985/1986 to 1994/1995, was 58 000 tons (van der Mescht *et al.*, 1997; Thompson *et al.*, 1999). The commercial production has

stabilised around 60 000 tons per annum for the past decade and the crop is very popular amongst resource poor farmers. The contribution of this sector is estimated much higher than the figures indicate and is even produced partly for export (Thompson *et al.*, 1999; Minde *et al.*, 1999; Laurie *et al.*, 2000). According to the Directorate Agricultural Statistics of National Department of Agriculture (Anonymous, 2002b), 51 000 (1999/2000) and 53 000 tons (2000/2001) of sweet potato was produced commercially for export.

The crop is widely grown on small scale, primarily to help ensure the food security and as a cash crop of the rural household mostly in parts of central, eastern and southern Africa and it has been used for human consumption for many years (Steinbauer and Kushman, 1971; Scott *et al.*, 2000). Sweet potato is reliable by providing food on marginal and degraded soil with little labour and few or no inputs from outside the farm (Steinbauer and Kushman, 1971; Karyeija *et al.*, 1998; Anonymous, 2002a). It has received less research attention compared to other tropical food and cash crops (Kanju *et al.*, 2000; Scott *et al.*, 2000). According to the FAO's statistics for 1990-1998 over 6.2 million metric tons of sweet potato were harvested from 1.3 million ha in the sub-Saharan Africa (FAO, 1998), with the estimations that by the year 2020, sweet potato production will be 9.4 million metric tons (Scott *et al.*, 2000).

Over 70% of global root and tuber production is produced in developing countries and this includes 87% of sweet potato, 99% of yam and 100% of cassava (Kanju, 2000; Scott *et al.*, 2000). Internationally, 90% of sweet potato is grown in Asia with the major producing countries being China (harvesting 80% of global sweet potato production), Indonesia, India, Japan, Vietnam, the Philippines and the Republic of Korea (Kanju, 2000; Scott *et al.*, 2000). In rural Zambia, 71% of farmers also grow sweet potato (Kanju, 2000).

Root and tuber crops such as cassava and sweet potato produce large quantities of starch (edible energy) in relatively less time than other crops (Scott *et al.*, 2000).

The crop is efficient in the production of carbohydrates, protein, vitamins and cash income per unit of land and time (Steinbauer and Kushman, 1971; Ewell and Mutuura, 1991). Sweet potatoes traditionally grown in sub-Saharan Africa are white-fleshed varieties, which contain little or no beta-carotene (a precursor of vitamin A) (Anonymous, 2002c). Sweet potato varieties with high beta-carotene content (orange-fleshed ones) represent the least expensive source of dietary vitamins. Vitamin A deficiency in sub-Saharan Africa is a serious public health problem in central, eastern and southern Africa affecting young children (6 months to 6 years of age) and pregnant women (Ewell and Mutuura, 1991; Carey *et al.*, 1999; Simwambana *et al.*, 1999; Owour, 2000; Anonymous, 2002c). Eyes are adversely affected due to vitamin A deficiency causing a disease called xerophthalmia (Ewell and Mutuura, 1991; Carey *et al.*, 1999; Simwambana *et al.*, 1999; Owour, 2000). Sweet potatoes also provide vitamin C (ascorbic acid, 35mg/100gfw) whereas other cereal-based foods have none (Ewell and Mutuura, 1991).

The leaves are also a source of protein, containing 2.7-3.4g/100g of raw fresh leaves and are an important vegetable for most rural households in Malawi and other African countries (Ewell and Mutuura, 1991; Moyo *et al.*, 1999; Kanju, 2000) and are also used for animal feed (Steinbauer and Kushman, 1971). They also provide dietary fibre and contain significant amounts of several additional vitamins (thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, folic acid and tocopherol) and the minerals calcium, potassium, phosphorus, magnesium, iron and sodium (Kanju, 2000; Steinbauer and Kushman, 1971; Simwambana *et al.*, 1999). Together with potatoes, sweet potatoes contain an important amino acid, lysine, and are a source of energy (Kanju, 2000; Scott *et al.*, 2000).

Together with cassava, sweet potato is an important food crop with the advantage that it is tolerant to drought, has low demand for nutrients and is capable of providing reasonable yields in seasons where other crops would fail (Minde *et al.*, 1999). Another advantage is that it can be harvested piecemeal to provide daily

fresh food for the family (Karyeija *et al.*, 1998). The major problem of growing sweet potato is shortage of planting material, pests and diseases, in particular virus diseases (Moyo *et al.*, 1999).

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Chapter 2

A review of viruses infecting sweet potatoes

2.1 Introduction

Diseases are of great importance in sweet potato production, viruses being the biggest problem worldwide because sweet potatoes are very susceptible to viral diseases (Bolton and du Plooy, 1984; Owour, 2000). Since the early 1946-1950's, viral diseases have been recognised as causing deterioration in the quality and yield of sweet potatoes in South Africa in Brits, Nelspruit, Mpumalanga, Pretoria and Rustenburg (McClellan and Klessner, 1947). During a baseline survey on cassava and sweet potato which was conducted by ARC-Roodeplaat Vegetable and Ornamental Plant Institute in 1997, viral diseases were ranked high based on symptom expression in the field and results of indexing the collected vines to the indicator plant, *Ipomoea setosa* Kerr. (van der Mescht *et al.*, 1997). Production of sweet potato is greatly constrained by diseases that cause yield reduction by up to 98% (Mukasa, 2001), and viruses severely limit sweet potato production in the tropics and in Africa, accounting for over 50% of yield reduction in Nigeria and Uganda (Mukiibi, 1977). In South Africa the crop is sensitive to viral diseases in hot tropical regions such as the Lowveld of Mpumalanga and the Limpopo province (Coertze *et al.*, 1996). Data on yield loss caused by viruses is generally lacking in South Africa and in some other areas where the crop is grown due to the practice of piece-meal harvesting which makes it difficult to measure yield (Jericho, 1999).

A suspected virus disease of sweet potato was first reported in 1944, in South Carolina (Steinbauer and Kushman, 1971). 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). Studies indicate that there are five major potyviruses that affect sweet potato production: sweet potato feathery mottle potyvirus (SPFMV), sweet potato mild mottle ipomovirus (SPMMV), sweet potato latent potyvirus (SPLV), sweet potato vein mosaic potyvirus (SPVMV) and sweet potato yellow dwarf

ipomovirus (SPYDV) (Moyer and Salazar, 1989; Winter *et al.*, 1992; Chavi *et al.*, 1997; Di Feo *et al.*, 2000). In addition to these, viruses belonging to other taxonomic groups have been found to infect sweet potatoes such as sweet potato caulimovirus (SPCaLV), sweet potato chlorotic stunt crinivirus (SPCSV), sweet potato ring spot nepovirus (SPRSV), sweet potato leaf speckling luteovirus (SPLSV), and sweet potato chlorotic fleck potyvirus (SPCFV) (Winter *et al.*, 1992; Brunt *et al.*, 1996). SPFMV and SPMMV, and possibly SPLV have been reported in South Africa as the most important viruses of sweet potatoes (Thompson and Mynhardt, 1986; Jericho and Thompson, 2000). SPFMV in combination with SPCSV causes a devastating sweet potato virus disease (SPVD), which has been shown to be the principal virus disease of sweet potato in East Africa (Aritua *et al.*, 1998b; Gibson *et al.*, 1998, Karyeija *et al.*, 2000). A recently reported virus disease of sweet potato is sweet potato chlorotic dwarf disease (SPCDD), a synergistic combination of three viruses (SPFMV, SPMMV and SPCSV) found in Argentina (Di Feo *et al.*, 2000).

Although there are many sweet potato virus diseases described in the literature, the aetiology of many of these diseases has not been determined and reliable detection and characterisation procedures have not been documented (Moyer and Salazar, 1989). A summary of sweet potato viruses, their distribution and vectors is given in Table 2.1.

2.2 Aetiology of sweet potato viruses

2.2.1 Sweet potato feathery mottle potyvirus (SPFMV)

SPFMV is the most important and common virus infecting sweet potato and is found wherever sweet potato is grown, including South Africa (Clark and Moyer, 1988; Moyer and Salazar, 1989; Karyeija *et al.*, 1998a; Cipriani *et al.*, 2000; Jericho and Thompson, 2000; Kreuze *et al.*, 2000). Many strains have been identified and it has been referred to as russet crack virus, sweet potato virus A, sweet potato ring spot virus, sweet potato leaf spot virus and internal cork virus (Sheffield, 1957; 1958; Clark and Moyer, 1988; Moyer and Salazar, 1989;

Karyeija *et al.*, 1998a;). It has been detected in association with other viruses (Moyer and Salazar, 1989).

SPFMV is a member of the family Potyviridae, genus Potyvirus, the largest family of plant viruses (Clark and Moyer, 1988; Moyer and Salazar, 1989). Like other potyviruses, the virions are elongate, flexuous rods with a monopartite, single-stranded, positive sense RNA molecule (Karyeija *et al.*, 1998a; 1998b; Fauquet and Mayo, 1999), with a particle length of 830-850nm (Cohen *et al.*, 1988; 1997).

SPFMV is readily transmitted by aphids in a non-persistent manner through brief feeds of only 20-30 seconds (Schaefers and Terry, 1976; Clark and Moyer, 1988; Ames *et al.*, 1997; Owour, 2000). Aphids such as the green peach aphid (*Myzus persicae* Sulz.), cotton aphid (*Aphis gossipii* Glover) and groundnut aphid (*A. craccivora* Koch.) are reported to be the most efficient vectors of SPFMV (Schaefers and Terry, 1976; Karyeija *et al.*, 1998a). The virus is not seed-borne, but like many viruses infecting vegetatively propagated plants, it is also disseminated in tubers and vine cuttings (Cadena-Hinojosa and Campbell, 1981).

Symptoms on sweet potato leaves appear as faint to distinct, irregular chlorotic spots occasionally bordered by purplish pigmentation (Clark and Moyer, 1988; Cadena-Hinojosa and Campbell, 1981; Moyer and Salazar, 1989; Ames *et al.*, 1997). Diffuse mottle along the main veins and vein clearing can also be seen on infected leaves (Clark and Moyer, 1988; Moyer and Salazar, 1989; Karyeija *et al.*, 1998a; 1998b). Leaf symptoms vary with cultivar susceptibility, climatic condition, plant age and strain virulence (Thompson and Mynhardt, 1986; Clark and Moyer, 1988; Ames *et al.*, 1997). Some genotypes also exhibit external and internal root symptoms which include external cracking and internal necrosis depending on the cultivar and virus isolate (Cali and Moyer, 1981; Karyeija *et al.*, 1998a).

SPFMV is mostly restricted to members of the *Ipomoea*, which include *I. nil* L. (Roth), *I. setosa* and sweet potatoes (Thompson and Mynhardt, 1986; Clark and Moyer, 1988; Karyeija *et al.*, 1998a; 1998b). Single infections of SPFMV have been reported to cause severe symptoms, which are variable in *I. nil*, and *I. setosa* (Schaefer and Terry, 1976). Some isolates infect *Chenopodium amaranticolor* Coste & Reyn, *C. quinoa* Willd, or *Nicotiana benthamiana* Gray, but others seem to be restricted to *Ipomoea* species (Thompson and Mynhardt, 1986; Clark and Moyer, 1988; Karyeija *et al.*, 1998a; 1998b).

2.2.2 Sweet potato chlorotic stunt crinivirus (SPCSV)

It was formerly known as sweet potato sunken vein virus (Hoyer *et al.*, 1996; Milgram *et al.*, 1996; Ames *et al.*, 1997; Alicai *et al.*, 1999) and SPVD-associated closterovirus (Winter *et al.*, 1992). It is a member of the family Closteroviridae, genus Crinivirus (Aritua *et al.*, 1998a; Fauquet and Mayo, 1999; Karyeija *et al.*, 2001) with a positive-stranded RNA genome (Karyeija *et al.*, 2000; Gibson and Aritua, 2002). Early classification was based on particle lengths, long types with particles from 1.200 to 2.000nm and short types with particles from 700 to 800nm (Liu *et al.*, 2000). Karyeija *et al.* (2000) showed that SPCSV remains confined to the phloem and at similar or slightly lower titer in the SPVD-affected plants. SPCSV is transmitted semi-persistently by whitefly, *Bemisia tabaci* Genn. and *Trialeurodes abutilonea* Haldeman, and not by mechanical means (Winter *et al.*, 1992; Hoyer *et al.*, 1996; Gibson *et al.*, 1998; Alicai *et al.*, 1999; Gibson and Aritua, 2002). Although SPCSV can infect plants by itself, it has been identified as a component of synergistic complexes with other viruses such as SPFMV and SPMSV (Schaefer and Terry, 1976; Gibson *et al.*, 1998; Di Feo *et al.*, 2000; Gibson and Aritua, 2002).

Symptoms vary with plant genotypes (Gibson *et al.*, 1998). Symptoms caused by SPCSV alone are relatively mild in sweet potato and *I. setosa* and plants may become mildly stunted, chlorotic and purpling of leaves can occur (Aritua *et al.*, 1998a; 1998b; Gibson *et al.*, 1998; Alicai *et al.*, 1999, Gibson and Aritua, 2002).

Affected plants commonly produce less than half the tuberous root yield of symptomless ones (Aritua *et al.*, 1998b). SPCSV infects sweet potato (Kaitisha and Gibson, 1999; Karyeija *et al.*, 2000) and *I. setosa* (Gibson *et al.*, 1997; 1998). It was reported by Cohen *et al.* (2001) that SPCSV was also found to infect *Lisianthus* (*Eustoma grandiflorum* Raf. Shinn.). SPCSV is known to be distributed in Nigeria, Zambia and Tanzania (Gibson *et al.*, 1998; Kaitisha and Gibson, 1999), and it is also found in Kenya and the Caribbean (Ames *et al.*, 1997).

2.2.3 Sweet potato virus disease (SPVD)

SPVD is a name used to describe plants affected by a range of severe symptoms associated with a dual infection of SPCSV and SPFMV (Schaefer and Terry, 1976; Carey *et al.*, 1997; Gibson *et al.*, 1997; Aritua *et al.*, 1998a; 1998b; Karyeija *et al.*, 1998a; 1998b; Kaitisha and Gibson, 1999; Gibson and Aritua, 2002). The first report of SPVD may have been in the eastern Belgian Congo (DR Congo) in 1939 (Carey *et al.*, 1997; 1999). It is also the most serious disease of sweet potato in Africa, especially in Uganda (Karyeija *et al.*, 1998b; Alicai *et al.*, 1999). Co-infection between SPFMV and SPCSV results in the development of SPVD characterised by severe leaf distortion including narrowing (strap-like), vein clearing and crinkled leaves, chlorosis, discolouration and stunting of plants (Ngeve and Boukamp, 1991; Carey *et al.*, 1997; 1999; Alicai *et al.*, 1999; Kreuze *et al.*, 2000; Ndunguru and Aloyce, 2000; Karyeija *et al.*, 2001; Gibson and Aritua, 2002).

SPVD infection caused yield depression of up to 90% in sweet potato cultivars tested in 1986 in Ekona, Cameroon (Ngeve and Boukamp, 1991). Gibson *et al.* (1998) showed that sweet potato infected with this virus-complex produce *c.* 2% of the yield of unaffected sweet potato cuttings. The incidence of SPVD was revealed to be higher in fields planted as monocrop than in other cropping patterns (Ndunguru and Aloyce, 2000). Although it has been reported in East and West

Africa, it has not been reported in southern Africa (Chavi *et al.*, 1997; Jericho and Thompson, 2000).

2.2.4 Sweet potato mild mottle ipomovirus (SPMMV)

SPMMV is a member of the family Potyviridae, genus Ipomovirus (Hollings *et al.*, 1976; Fauquet and Mayo, 1999). It was described as an RNA-containing virus with filamentous particles c. 950nm long, found in East Africa and it was referred to as SPV-T in preliminary reports (Hollings *et al.*, 1976; Clark and Moyer, 1988; Brunt *et al.*, 1996). Like other Potyviruses, SPMMV induces cytoplasmic inclusions (Hollings *et al.*, 1976). The virus is transmitted by whitefly, *Bemisia tabaci*, in a persistent manner and by grafting but not by contact between plants or by seeds (Hollings *et al.*, 1976; Moyer and Salazar, 1989; Brunt *et al.*, 1996). The virus is readily transmitted to a fairly wide range of herbaceous plant species (Hollings *et al.*, 1976; Moyer and Salazar, 1989; Brunt *et al.*, 1996). The host range has been demonstrated to include 45 species in 14 plant families (Hollings *et al.*, 1976; Brunt *et al.*, 1996). *Chenopodium quinoa*, *I. setosa*, *Nicotiana tabacum* L., *N. benthamiana*, *N. glutinosa* L. and *N. clevelandii* L. are good local lesion hosts and are highly sensitive to infection by SPMMV (Hollings *et al.*, 1976; Clark and Moyer, 1988). Symptoms include leaf mottling and stunting (Brunt *et al.*, 1996; Ames *et al.*, 1997). Symptoms of SPMMV-infected *I. setosa* are similar to those induced by SPFMV, which include vein clearing and distortion (Clark and Moyer, 1988; Ames *et al.*, 1997). Loss of yield also occurs in SPMMV-infected plants (Hollings *et al.*, 1976). Different sweet potato genotypes differ greatly in susceptibility and reaction to the virus, some being symptomless and others apparently immune (Brunt *et al.*, 1996). SPMMV has been identified in Kenya, Uganda, Tanzania and Burundi and South Africa but yield effects are unknown (Brunt *et al.*, 1996; Ames *et al.*, 1997; Jericho and Thompson, 2000). Taking into consideration the wide host range of the virus and the polyphagous nature of its vector (*Bemisia tabaci*), it is probable that the geographical spread of SPMMV is much wider than presently known (Jericho, 1999).

2.2.5 Cucumber mosaic cucumovirus (CMV)

CMV was first observed in 1986 in Israel where it severely infected sweet potato fields (Cohen *et al.*, 1988). CMV was also found to infect sweet potatoes in Israel and the United States (Ames *et al.*, 1997). All CMV infected plants were also infected with SPFMV (Cohen *et al.*, 1988; Moyer *et al.*, 1989). For CMV to replicate in sweet potato a helper virus is required. CMV is easily transmitted to sweet potato plants mechanically and by aphid inoculation if the acceptor plant carries the whitefly-transmitted virus, which provides the necessary requirement for CMV replication (Cohen and Loebestein, 1991; Cohen *et al.*, 1992). Symptoms include stunting, chlorosis and yellowing (Cohen and Loebestein, 1991; Cohen *et al.*, 1992).

2.3 Other viral diseases of sweet potato

In addition to SPFMV, SPMMV, SPCSV and SPVD, there are other viruses reported to infect sweet potato: sweet potato leaf speckling luteovirus (SPLSV), sweet potato latent potyvirus (SPLV), sweet potato ring spot nepovirus (SPRSV), sweet potato caulimovirus (SPCaLV), sweet potato yellow dwarf ipomovirus (SPYDV), sweet potato vein mosaic potyvirus (SPVMV), sweet potato leaf curl badnavirus (SPLCV), sweet potato leaf curl geminivirus-US (SPLCV-US), *Ipomoea* crinkle leaf curl geminivirus (SPCLCV), and sweet potato phytoreovirus. Of all these viruses, only SPCFV, SPRSV, SPLV and SPCaLV have been reported to infect sweet potato in Africa (Atkey and Brunt, 1987; Brunt *et al.*, 1996; Carey *et al.*, 1997; Gibson *et al.*, 1997). Sweet potato chlorotic fleck virus (SPCFV) is a potyvirus, flexuous, with the length of 750-800nm and the vector for transmitting this virus is not yet known (Salazar and Fuentes, 2000).

Sweet potato chlorotic dwarf disease (SPCDD), a synergistic combination of SPFMV, SPMSV and SPCSV, is another virus complex, which was reported in Argentina (Di Feo *et al.*, 2000; Gibson and Aritua, 2002). SPCDD-affected plants are characterised by reduced leaf area, stunting and leaves show severe mosaic,

blisters, and distortion. The combination of three viruses and the different combinations of two of the three viruses account for the variability of SPCDD symptomatology observed in the field. This is very similar to the synergism of SPFMV and SPCSV causing the devastating SPVD-complex in Africa, except that in the case of SPCDD, three viruses are involved (Moyer and Salazar, 1989; Winter *et al.*, 1992). Sweet potato virus G (SPV G) has also been reported as another important virus of sweet potato (Colinet *et al.*, 1994a; Alvarez *et al.*, 1997). The length of the coat protein of SPVG is longer than that of SPFMV, but the virus is closely related to SPFMV and it is considered a potyvirus (Colinet *et al.*, 1994a; Brunt *et al.*, 1996). Sweet potato virus II (SPV II) has also been reported in Taiwan as another aphid-transmitted potyvirus that infects sweet potato (Salazar and Fuentes, 2000).

2.3.1 Sweet potato leaf speckling luteovirus (SPLSV)

SPLSV is a Luteovirus, which is transmitted only by the aphid, *Macrosiphum euphorbiae* Thomas (Fuentes *et al.*, 1996; Fauquet and Mayo, 1999). It infects sweet potato but it can infect 45 species of 14 plant families (Moyer and Salazar, 1989), including *I. nil* and *I. setosa* and symptoms include clear whitish flecks or specks on the leaves (Fuentes *et al.*, 1996).

2.3.2 Sweet potato latent potyvirus (SPLV)

SPLV is a member of the family Potyviridae, genus Potyvirus and was initially designated sweet potato virus N (Clark and Moyer, 1988; Moyer and Salazar, 1989; Brunt *et al.*, 1996; Fauquet and Mayo, 1999). It is a flexuous rod, 700-750nm long and it does not depend on another virus for replication (Clark and Moyer, 1988; Brunt *et al.*, 1990). It is transmitted by mechanical inoculation and no vector has been found to transmit the virus (Clark and Moyer, 1988; Moyer and Salazar, 1989; Brunt *et al.*, 1990; 1996). It is not transmitted by contact between plants nor by seed (Clark and Moyer, 1988; Brunt *et al.*, 1990; 1996). Infection of many sweet potato cultivars by SPLV does not result in obvious foliar symptoms (Clark and Moyer, 1988; Moyer and Salazar, 1989; Brunt *et al.*, 1996).

The host range includes many *Convolvulus*, *Chenopodium* and some *Nicotiana* spp. (Brunt *et al.*, 1996). Although SPLV induces mild symptoms in *I. setosa*, it is easily detected by serology (Clark and Moyer, 1988; Moyer and Salazar, 1989).

2.3.3 Sweet potato ring spot nepovirus (SPRSV)

SPRSV is a member of the family Comoviridae, genus Nepovirus (Brunt *et al.*, 1996). Virions are isometric, not enveloped and 28nm in diameter with conspicuous capsomere arrangements and the genome consists of single-stranded RNA (Brunt *et al.*, 1996). It was first reported in sweet potato cultivars imported from Papua New Guinea (Brunt *et al.*, 1996) and Kenya (Ames *et al.*, 1997). Symptoms on sweet potato include occasional chlorotic ring spots when temperature reach 26-28°C, but plants can be symptomless (Brunt *et al.*, 1996). The virus is transmitted by aphids (*Myzus persicae*) and mechanical inoculation but not by contact between plants (Loebestein and Harpaz, 1959; Brunt *et al.*, 1990; 1996). Susceptible hosts include plants from 3-9 families including *I. setosa* (showing faint systemic chlorotic leaf mottling), *Chenopodium quinoa*, some *Nicotiana* spp. and *Glycine max* L. (Brunt *et al.*, 1990; 1996).

2.3.4 Sweet potato caulimovirus (SPCaLV)

SPCaLV is a caulimovirus with a genome consisting of double stranded DNA with isometric particles of 50nm in diameter (Atkey and Brunt, 1987; Moyer and Salazar, 1989). The virus is not transmitted by either *Aphis gossypii* or *Myzus persicae* (Moyer and Salazar, 1989), nor by mechanical inoculation, contact between plants or seed (Brunt *et al.*, 1990; 1996). It is transmitted by grafting only (Brunt *et al.*, 1990; 1996). SPCaLV is found in the U.S.A, Puerto Rico, New Zealand, Kenya, Australia, Papua New Guinea, Solomon Islands, Tonga and Madeira (Atkey and Brunt, 1987; Clark and Moyer, 1988; Moyer and Salazar, 1989; Brunt *et al.*, 1990; 1996). Host plants include *I. setosa*, which shows chlorotic veinal flecks or circular interveinal chlorotic spots (Atkey and Brunt, 1987; Clark and Moyer, 1988; Brunt *et al.*, 1990; 1996). Symptoms on host plants

also include chlorosis resulting in wilting and premature death of the leaves (Clark and Moyer, 1988; Moyer and Salazar, 1989).

2.3.5 Sweet potato yellow dwarf ipomovirus (SPYDV)

SPYDV is an Ipomovirus of the family Potyviridae (Clark and Moyer, 1988; Brunt *et al.*, 1996; Fauquet and Mayo, 1999). Virions are long, flexuous rods with a modal length of 750nm (Clark and Moyer, 1988; Brunt *et al.*, 1996). Virion morphology and the vector of SPYDV are similar to that of SPMMV (Clark and Moyer, 1988; Moyer and Salazar, 1989). The virus is transmitted by whitefly (*Bemisia tabaci*) in a persistent manner and by mechanical inoculation (Clark and Moyer, 1988; Brunt *et al.*, 1990; 1996). Symptoms on sweet potato include mottling, stunting (dwarfing), chlorosis, and poor roots and tubers (Steinbauer and Kushman, 1971; Clark and Moyer, 1988; Moyer and Salazar, 1989; Brunt *et al.*, 1996). Poor soil fertility and low temperatures favour expression of symptoms (Clark and Moyer, 1988; Moyer and Salazar, 1989). The root systems of infected plants are poorly developed and the fleshy roots are not marketable (Clark and Moyer, 1988; Moyer and Salazar, 1989). SPYDV frequently occurs in combination with SPFMV (Clark and Moyer, 1988; Moyer and Salazar, 1989).

2.3.6 Sweet potato vein mosaic potyvirus (SPVMV)

SPVMV was first found in sweet potato in Argentina and it has been classified as a Potyvirus within the family Potyviridae (Clark and Moyer, 1988; Brunt *et al.*, 1990; 1996; Fauquet and Mayo, 1999). A data comparison of the particle morphologies of SPVMV and SPFMV indicate that SPVMV has a modal length of 761nm, which is significantly shorter than that of SPFMV (Moyer and Salazar, 1989; Brunt *et al.*, 1996). It is transmitted non-persistently by aphids (*Myzus persicae*) and by mechanical inoculation (Clark and Moyer, 1988; Brunt *et al.*, 1996). Host plants include *I. batatas* and other *Ipomoea* spp. (Clark and Moyer, 1988). Sweet potatoes infected with SPVMV are severely stunted and produce fewer roots (Clark and Moyer, 1988; Moyer and Salazar, 1989; Brunt *et al.*, 1996). The virus also causes severe foliar symptoms similar to those of SPFMV in

I. setosa (vein clearing, mosaic, chlorosis and twisting of leaves) (Clark and Moyer, 1988; Moyer and Salazar, 1989; Brunt *et al.*, 1996).

2.3.7 Sweet potato leaf curl badnavirus (SPLCV)

SPLCV is considered a member of the Badnavirus group with short, rod-shaped particles found in the cytoplasm of phloem-cells (Clark and Moyer, 1988). Its geographical distribution is limited to Japan and Taiwan (Clark and Moyer, 1988; Brunt *et al.*, 1996). The virus is transmitted by whitefly (*Bemisia tabaci*) in a persistent manner and by grafting (Clark and Moyer, 1988; Brunt *et al.*, 1990; 1996). It is not transmitted by contact between plants, mechanical inoculation nor by seed (Clark and Moyer, 1988; Brunt *et al.*, 1990; 1996). Its symptoms are typical leaf curl (Thottapilly and Rossel, 1988). Host plants include *I. batatas*, and *I. nil* (Clark and Moyer, 1988; Brunt *et al.*, 1990; 1996).

2.3.8 *Ipomoea* crinkle leaf curl geminivirus (ICLCV)

ICLCV is a geminivirus, which is transmitted by a whitefly *Bemisia argentifolii* Bellows and Perring in a persistent manner and by grafting onto *Ipomoea* spp. (Cohen *et al.*, 1997). ICLCV is not transmitted mechanically (Cohen *et al.*, 1997). It is associated with geminated particles, inducing distinct symptoms on several *Ipomoea* spp. (Cohen *et al.*, 1997). Symptoms are leaf curling, crinkling and sunken veins on *I. setosa*, *I. hederacea* Jacq., *I. trifida* Kunth G., *I. littoralis* Blume and *I. batatas* (Cohen *et al.*, 1997). Vein clearing can also be observed on *I. hederacea*, *I. trifida* (Cohen *et al.*, 1997).

2.3.9 Sweet potato leaf curl virus-US

SPLCV-US is another geminivirus, which was reported in the United States to infect sweet potatoes (Lotrakul *et al.*, 1998). The disease is transmitted by whitefly (*Bemisia tabaci*) and symptoms on *Ipomoea aquatica* Forssk., *I. cordatotriloba* Dennst. and *I. fistulosa* Mart. ex Choisy consist of yellow vein mottling and leaf curling. Infected *I. setosa* shows symptoms such as mild leaf curl, interveinal chlorosis and stunting.

2.3.10 Sweet potato (?) phytoeovirus

It is a Reoviridae virus, which was first reported in *I. aquatica* from material tested in the U.K. (Brunt *et al.*, 1996). The virus induces mild leaf chlorosis symptoms (Brunt *et al.*, 1996). The virus is restricted to members of the Convolvulaceae, more especially *I. setosa* (Brunt *et al.*, 1996).

Table 2.1 A summary of sweet potato viruses, their distribution and vectors

Virus	Known distribution	Vectors	References
SPFMV	Nigeria, Uganda, Kenya, Zimbabwe, Louisiana, U.S.A and South Africa	<i>Myzus persicae</i>	Brunt <i>et al.</i> , 1996; Chavi <i>et al.</i> , 1997; Gibson <i>et al.</i> , 1998; Kaitisha and Gibson, 1999; Jericho and Thompson, 2000.
SPMMV	Burundi, Kenya, Tanzania, Uganda and South Africa	<i>Bemisia tabaci</i>	Hollings <i>et al.</i> , 1976; Brunt <i>et al.</i> , 1996; Jericho and Thompson, 2000.
SPCSV	Nigeria, Uganda, Kenya, Zambia, and Israel	<i>Bemisia tabaci</i>	Winter <i>et al.</i> , 1992; Cohen <i>et al.</i> , 1992; Hoyer <i>et al.</i> , 1996; Brunt <i>et al.</i> , 1996; Ames <i>et al.</i> , 1997; Gibson <i>et al.</i> , 1998; Kaitisha and Gibson, 1999.
SPCFV	South East Africa, Indonesia, China, Japan, Peru, Philippines, Brazil, Cuba, Colombia, Bolivia and Central and South America	?	Ames <i>et al.</i> , 1997; Carey <i>et al.</i> , 1997; Gibson <i>et al.</i> , 1997; Salazar and Fuentes, 2000.
SPRSV	Papua New Guinea and Kenya	?	Brunt <i>et al.</i> , 1996.
SPLV	Taiwan, Japan, China and Israel	?	Clark and Moyer, 1988; Brunt <i>et al.</i> , 1990; 1996; Ames <i>et al.</i> , 1997.
SPCaLV	U.S.A., Puerto Rico, New Zealand, Kenya, Papua New Guinea, Australia, Solomon Island and Madeira	?	Atkey and Brunt, 1987; Moyer and Salazar, 1989.
CMV	Israel, Kenya and the United States	<i>Aphis gossypii</i>	Cohen and Loebestein, 1991; Ames <i>et al.</i> , 1997.
SPLSV	Peru	<i>Macrosiphum euphorbiae</i>	Clark and Moyer, 1988; Moyer and Salazar, 1989; Fuentes <i>et al.</i> , 1996.
SPVMV	Argentina	Aphids	Clark and Moyer, 1988; Moyer and Salazar, 1989; Brunt <i>et al.</i> , 1996.
SPYDV	Taiwan	<i>Bemisia tabaci</i>	Brunt <i>et al.</i> , 1996.
SPCDD	Argentina	<i>Bemisia tabaci</i> & <i>Myzus persicae</i>	Di Feo <i>et al.</i> , 2000.
ICLCV	North America	<i>B. argentifolii</i>	Cohen <i>et al.</i> , 1997.
SPLCV	Taiwan and Japan	<i>B. tabaci</i>	Clark and Moyer, 1988; Brunt <i>et al.</i> , 1996.
SPV G	China	?	Colinet <i>et al.</i> , 1994a.
SPV II	Taiwan	Aphids	Salazar and Fuentes, 2000.

? = The vector has not yet been identified

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2.4 Sweet potato virus detection and diagnosis

Detection and characterisation of sweet potato viruses is crucial in the understanding of the epidemiology of the disease(s) caused by these viruses, development of infectivity-based forecasting systems and control strategies (Joubert *et al.*, 1979; Jericho, 1999). Study of several of these diseases has been hampered by lack of simple detection techniques (Carey *et al.*, 1999). Sweet potato viruses have been detected by observing symptom expression in the field and host range studies (Chavi *et al.*, 1997) and some by their vector relationship (Schaefer and Terry, 1976). The primary tests to detect sweet potato viruses are bioassays on indicator plants by observing symptoms, vector transmission procedures and serology using enzyme linked immunosorbent assay (ELISA) (Moyer and Salazar, 1989; Chavi *et al.*, 1997).

2.4.1 Biological methods

2.4.1.1 Mechanical inoculation

Mechanical inoculation is the application of virus-bearing fluids (sap) to the surface of the leaves of an indicator plant in such a way that the virus can enter the cells and elicit a response or symptom in that plant (Sheffield, 1957a; Noordam, 1973; Chavi *et al.*, 1997). Mechanical inoculation is done by grinding sweet potato leaf tissue using a mortar and pestle in a phosphate buffer of the pH 7.2 containing 0.01M sodium diethyl dithiocarbamate (Cohen *et al.*, 1988; Chavi *et al.*, 1997; Jericho and Thompson, 2000). An abrasive such as carborundum or diatomaceous earth is normally added to the leaf extracts during mechanical inoculation to enhance the process (Cohen *et al.*, 1988; Chavi *et al.*, 1997; Jericho and Thompson, 2000). This method was first used by Sheffield when he transmitted sweet potato virus A and B mechanically, as a mixed infection, from naturally infected plants to healthy sweet potato plants and *Ipomoea* test plants (Sheffield, 1957).

Mechanical inoculations have been used to establish the host range of the different viruses including viruses infecting sweet potatoes (Sheffield, 1957;

1958; Walkey, 1991; Chavi *et al.*, 1997; Jericho and Thompson, 2000). Species in the family Convolvulaceae such as *I. nil* and *I. purpurea* L. (Roth) are most frequently used as host plants for mechanical inoculations. Other plant species such as *Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativus* L., *Datura stramonium* L., and *N. benthamiana* have also been used to transmit sweet potato viruses mechanically (Cali and Moyer, 1981; Cohen *et al.*, 1988; Chavi *et al.*, 1997). *I. nil* is more sensitive than *I. setosa* (Cohen *et al.*, 1997). Symptoms such as leaf puckering, vein clearing, chlorotic vein slashing and leaf distortion have been seen following graft and mechanical inoculation of *I. nil* and *I. setosa* with sweet potato cuttings from small-scale farmers in South Africa (Thompson and Mynhardt, 1986; Jericho and Thompson, 2000).

2.4.1.2 Grafting (Indexing)

This method is useful for viruses that cannot be mechanically transmitted (Walkey, 1991). This is done by grafting a two-leaf shoot of sweet potato onto *I. setosa* and monitoring for the development of symptoms (Anonymous, 1978). Attempts to transmit sweet potato virus A, transmitted by aphids (*Myzus persicae*), and sweet potato virus B, transmitted by whiteflies (*Bemisia tabaci*), were successful by cleft grafting sweet potato cuttings with infected scions (Sheffield, 1957; 1958). Virus A and B seemed to be readily transmitted to sweet potato and even to other species of *Ipomoea* (Sheffield, 1957; 1958). Plants for virus testing are grown in the greenhouse to produce stems, which are later assayed by grafting to *I. setosa* and to sweet potato clones because nearly all known viruses of sweet potato plants also infect *I. setosa* (Esbenshade and Moyer, 1982; Moyer and Salazar, 1989). Side grafting of sweet potato plants (cuttings) onto *I. setosa* induces symptoms such as vein clearing, puckering, leaf deformation and chlorotic spotting which start showing in 3-5 weeks, depending on temperature, age of the plant and the virus concentration (Clark and Moyer, 1988; Moyer and Salazar, 1989; Winter *et al.*, 1992; Gibson *et al.*, 1998; Jericho and Thompson, 2000).

Although *I. setosa* is susceptible to many viruses infecting sweet potato and is a good assay host, it is of no diagnostic value for some, such as cucumber mosaic virus (CMV) and tobacco streak virus (TSV) (Moyer and Salazar, 1989; Winter *et al.*, 1992). Although graft transmission is a reliable method for detection, it requires greenhouse space, labour, good insect control, and several weeks for reliable diagnosis (Karyeija *et al.*, 1998a).

2.4.2 Insect transmission

The most common vectors of sweet potato viruses are *Myzus persicae*, *Aphis gossypii*, *Macrosiphum euphorbiae* and *Bemisia tabaci* (Brunt *et al.*, 1996). Only whiteflies transmitted sweet potato virus B when species such as leafhoppers (unidentified), froghoppers (unidentified), mealybugs (*Planococcus kenyae* Lepelley) and mites (*Aceria* spp.) were experimentally tested for transmitting the virus (Sheffield, 1957; 1958). Various aphid species were tested for sweet potato virus A, but *Myzus persicae* transmitted the virus non-persistently and symptoms appeared 3-4 weeks after inoculation (Sheffield, 1957a; 1958). Insect vectors are allowed an acquisition access feeding and are then kept on the acceptor plant for inoculation of the virus (Winter *et al.*, 1992). Results are based on symptom expression (Winter *et al.*, 1992). Aphids (*Aphis gossypii*) can transmit CMV to cucumbers, *I. nil*, and to sweet potato plants if the acceptor plant carries the whitefly transmitted agent (Cohen *et al.*, 1988).

2.4.3 Serological detection

Enzyme-linked immunosorbent assay (ELISA) has been used many times to detect plant viruses since its introduction in 1976 (Voller *et al.*, 1976; Clark and Adams, 1977; Bar-Joseph *et al.*, 1979). It is based on the covalent linkage of an enzyme to an antibody, registering the occurrence of an antigen-antibody complex by rapid enzymatic development of a distinctly coloured product (Burrows *et al.*, 1984; Converse and Martin, 1990). Together with bioassay on indicator plants, ELISA is the primary test to detect plant viruses with polyclonal or monoclonal

antibodies (Voller *et al.*, 1976; Bar-Joseph *et al.*, 1979; Ben-Ze'ev *et al.*, 1988; Converse and Martin, 1990; Singh and Barker, 1991; Walkey, 1991).

Heterologous precipitin tests were used previously for detecting severe russet crack (SRC) and mild russet crack (MRS) strains of SPFMV (Cali and Moyer, 1981). Due to lack of sensitivity required, ELISA, which detected SPFMV in partially purified and symptomatic leaves of *I. batatas* and *I. incarnata* Choisy, was developed and was found to be a faster, convenient and more sensitive method to confirm SPFMV in sweet potato foliages and other *Ipomoea* spp. (Cadena-Hinojosa and Campbell, 1981; Thottapilly and Rossel, 1988). The most common ELISA methods that are used are double antibody sandwich-ELISA (DAS-ELISA) and indirect ELISA using the antisera specific for each virus (Hammond *et al.*, 1992; Kaitisha and Gibson, 1999). They have been used to examine the relationship between SPFMV, sweet potato latent virus (SPLV), and sweet potato mild mottle virus (SPMMV) and confirming that they are three distinct potyviruses (Hammond *et al.*, 1992). Triple antibody sandwich-ELISA is preferably used to test for SPCSV (Gibson *et al.*, 1998). Sap extract is prepared in a standard sample buffer of phosphate-buffered saline containing Tween and polyvinyl pyrolidone (PBS-Tween, + 2% PVP) (Abad and Moyer, 1991; Jericho and Thompson, 2000).

Nitrocellulose membrane-ELISA (NCM-ELISA) is also used for detecting viruses such as SPFMV and sweet potato chlorotic fleck virus (SPCFV) in sweet potato and *I. setosa* (Abad and Moyer, 1991; Karyeija *et al.*, 1998a; 2000; Jericho and Thompson, 2000). It produces results consistent to those obtained using triple antibody sandwich-ELISA (TAS-ELISA) (Gibson *et al.*, 1997). Monoclonal (Mabs) and polyclonal (Pabs) antibodies produced against purified sweet potato viruses have been used to detect sweet potato viruses (Cadena-Hinojosa and Campbell, 1981; Chavi *et al.*, 1997; Kaitisha and Gibson, 1999; Jericho and Thompson, 2000; Karyeija *et al.*, 2001). Results from naturally infected sweet potato and grafts indicated that SPFMV occurred in leaves of infected plants at

concentrations approaching the limits of ELISA (Esbenshade and Moyer, 1982). Thus, proper tissue selection and timing of the assay is critical (Esbenshade and Moyer, 1982). It is now widely accepted that SPFMV is most reliably detected by ELISA (Abad and Moyer, 1991). Advantages of ELISA are that it can detect viruses in small amounts or in low concentrations and speedy reaction, which is why ELISA is important in virus detection (Voller *et al.*, 1976; Clark and Bar-Joseph, 1984; Siitari and Kurppa, 1987; Walkey, 1991). But many serological methods such as most types of ELISA are not sensitive enough to detect antigens at low concentrations and those occurring in complexes as in the case of sweet potato (Voller *et al.*, 1976; Clark and Bar-Joseph, 1984; Siitari and Kurppa, 1987; Walkey, 1991). The high cost of good quality enzymes and their substrates can also prevent the widespread use of ELISA in developing countries (Singh and Barker, 1991).

2.4.4 Electron microscopy (EM) and immunosorbent electron microscope (ISEM)

EM is done to assign viruses according to a particular group and to confirm the presence of virus particles (Cohen *et al.*, 1992; Winter *et al.*, 1992). Viruses can be viewed either in leaf dip preparations or in ultra-thin sections of embedded material (Cali and Moyer, 1981; Cohen *et al.*, 1992; Winter *et al.*, 1992; Jericho and Thompson, 2000). Negative stains such as uranyl acetate have been used to observe long filamentous rod-shaped virus-like particles of SPCSV and other virus particles such as those of SPFMV in *I. setosa* and sweet potato plants (Cohen *et al.*, 1992; Winter *et al.*, 1992; Jericho and Thompson, 2000).

In order to be able to detect and determine the relationships among viruses, serological specific-electron microscope or immunosorbent electron microscope (ISEM) is used (Cadena-Hinojosa and Campbell, 1981; Hoyer *et al.*, 1996). It refers to the trapping of virus particles onto grids, which have been coated with specific virus antisera, to decorate the trapped virus particles, and observation using an electron microscope (Di Feo *et al.*, 2000; van der Merwe, 2001). Polyclonal antisera to viruses such as SPFMV-RC, SPFMV, SPMMV, SPLV,

SPCV and SPCSV have been used to detect these viruses (Hoyer *et al.*, 1996; Chavi *et al.*, 1997).

2.4.5 Polymerase chain reaction (PCR) and hybridisation

PCR is the use of synthetic nucleic acid probes or the *in vitro* amplification of the specific nucleic acid sequences (Chavi *et al.*, 1997). It involves making multiple copies of a particular sequence in a genome (virus genome) that are then used to identify the presence of a particular disease (Rosa, 2001). Lack of progress in virus identification and classification and due to the frequent occurrence of mixed infections and synergistic complexes in sweet potatoes (Clark and Moyer, 1988; Moyer and Salazar, 1989), PCR technology has been used for identifying and characterising members of the potyviruses infecting sweet potato (Chavi *et al.*, 1997; Colinet *et al.*, 1998). This method provides a convenient way of detecting mixed infections and unknown viruses without preliminary separation and purification of the components of the viral complexes (Colinet *et al.*, 1994b; 1998). Genus-specific PCR and subsequent molecular analysis of amplified regions thus comprises a powerful method for the rapid identification and differentiation of potyviruses infecting sweet potato and is the most suitable method for viruses which are difficult to purify or which occur in mixed infections (Colinet *et al.*, 1994b). Specific primers for detecting and differentiating SPFMV (-CHH and -CH2), SPLV, SPMMV and other viruses have been designed from nucleotide sequences of these viruses (Colinet *et al.*, 1994b; 1998; Chavi *et al.*, 1997). Antigen detection can be considerably enhanced by coupling serological trapping of viruses with PCR, the so-called immunocapture-PCR (Mumford *et al.*, 1994; Rosa, 2001). This provides sensitivity, rapidness and the ability to assay many samples.

Hybridisation is performed by extracting DNA or RNA from the samples, base-denaturing in NaOH, and directly blotting onto nylon membranes (Colinet *et al.*, 1994b; Cohen *et al.*, 1997; Lotrakul *et al.*, 1998). Membranes are then fixed with heat or ultraviolet (UV) radiation and then hybridised with a labelled probe

consisting of cDNA sequence of a particular virus (Colinet *et al.*, 1994b; Cohen *et al.*, 1997). If the virus is present, the probe will hybridise with its DNA extracts from the sample and if not, the probe is lost during the washing process of the membranes (Lotrakul *et al.*, 1998; van der Merwe, 2001). Western and Southern blottings have been successfully used to detect sweet potato sunken vein virus (SPSVV), which is now called SPCSV and SPLCV-US (Hoyer *et al.*, 1996; Lotrakul *et al.*, 1998). Southern blotting uses the DNA extracts to hybridise with the complimentary DNA probe whereas Western blotting refers to the transfer of proteins to the membranes and detecting with the antibody probes (Memelink *et al.*, 1994). The use of riboprobes complementary to the RNA or DNA of the virus was found to be more sensitive than serological assays, labelled cDNA and immunobinding assays due to specificity and the removal of non-hybridised probe to minimise non-specific background (Abad *et al.*, 1992). Non-radioactive methods such as horseradish peroxidase (HRP), dioxigenin-anti-digoxigenin (DIG) and the biotin-streptavidin systems to label nucleic acids that are used as probes are also used to detect plant pathogens (Karcher, 1994). Their use is due to the advantage that the probes generated are stable and require shorter exposure time to detect hybridised material (Karcher, 1994).

2.5 Management of sweet potato viruses

The fact that sweet potato in Africa is perceived as a crop for the poor, mainly grown by women, has many implications for cultivation of the crop (Karyeija *et al.*, 1998a; Owour, 2000). Traditional cultivation practices such as piecemeal harvesting and exchanging planting material freely between neighbouring farmers provides for the spread and perpetuation of virus infected material (Karyeija *et al.*, 1998a). Farmers obtain planting materials from mature crops which are normally not virus-free and on which pesticides are rarely used (Karyeija *et al.*, 1998a; Owour, 2000). These practices favour the survival and spread of SPFMV and other viruses (Karyeija *et al.*, 1998a; Owour, 2000).

Managing viruses infecting sweet potato will require knowledge on aetiology and ecology of the viruses. Information on the control of viruses and how viruses infect plants is lacking among resource-poor farmers in South Africa (Jericho, 1999). Three main control practices are used by African farmers to limit the effects of SPFMV and these are (a) selection of SPVD-resistant cultivars, (b) use of disease-free planting material and (c) removing all infected plants (Clark and Moyer, 1988; Karyeija *et al.*, 1998a). Efforts to control the spread of sweet potato viruses by controlling the vectors have not been successful (Clark and Moyer, 1988). Both SPFMV and SPCSV and other sweet potato viruses can be controlled using virus-free material and controlling weeds, which may serve as alternative hosts of insects and viruses, especially wild *Ipomoea* spp. in and around fields (Joubert *et al.*, 1974; Bolton and du Plooy, 1984; Thompson and Mynhardt, 1986; Karyeija *et al.*, 1998a). Isolating new crops a distance from the old mature crops will reduce virus incidence and result in high yielding crops (Gibson and Aritua, 2002). The use of intercropping to reduce the numbers of infectious vectors attacking the sweet potato crop can help reduce SPVD incidence by delaying SPVD vectors onset and build-up (Ndunguru and Alyoce, 2000). A sweet potato/maize cropping pattern was found to have a lower SPVD incidence and it can be an option to reduce SPVD damage in the traditional sweet potato farming system (Ndunguru and Alyoce, 2000). If volunteer sweet potato plants, which may have survived from previous crops, are removed and resistant varieties planted, viral diseases can be minimised (Joubert *et al.*, 1974; Bolton and du Plooy, 1984; Thompson and Mynhardt, 1986; Karyeija *et al.*, 1998a).

The development of transgenic sweet potato plants can be another method of controlling sweet potato viruses (Owour, 2000). The use of cysteine proteinase inhibitor gene (oryzacystatin I) proved to make some sweet potato cultivars tolerant to SPFMV-RC (Cipriani *et al.*, 2000). Although the transgenic lines can still be affected by SPFMV-RC through grafting with SPFMV-RC infected *I. setosa*, it was proven that the multiplication rate of the virus is reduced and the virus cannot be detected directly by either visual observation or by NCM-ELISA (Cipriani *et al.*,

2000). Genetic engineering of sweet potato may be possible but its extended application will be limited by resources, multiplication of viruses and their strains, and virus complexes that may alter virus-plant interactions and result in disease development (Mukasa, 2001).

Meristem-tip culture is a method for eliminating viruses from sweet potato cultivars and is based on the discovery that virus concentrations are lower in plant apices (Chiu *et al.*, 1982; Moyer *et al.*, 1989). Together with thermotherapy whereby sweet potatoes are grown at 38-40°C for four to 12 weeks, can possibly give rise to virus-free plants (Terry, 1982). Due to its importance, sweet potato germplasm free from known viruses is needed for commercial production (Moyer and Salazar, 1989), and cultivation practices neglected by many small-scale farmers need to be taken into consideration to prevent further spread.

In South Africa, sweet potato varieties are cleaned from viruses through virus-elimination and a sweet potato plant improvement scheme, which was initiated in the early seventies (Joubert *et al.*, 1974; Laurie and Stork, 1997). The scheme involves maintenance of disease-free mother stock of the varieties in an insect-free greenhouse and obtaining disease-free plantlets, which are supplied to registered sweet potato vine growers and sweet potato producers (Laurie and Stork, 1997). The scheme takes place at ARC-Roodeplaat and it plays an important role in securing the profitability of sweet potato in South Africa (Laurie and Stork, 1997).

2.6 Conclusion

Due to the increase in population growth in sub-Saharan Africa where economic growth is slow and poverty being the biggest problem, root crops such as cassava and sweet potato have drawn much attention. Sweet potato, especially in parts of central, eastern and southern Africa is important as a seasonal source of food, food security and cash (Scott *et al.*, 2000). Some sweet potato varieties contain high carbohydrate and vitamin contents (Ewell and Mutuura, 1991; Owour, 2000), and will thus continue to provide an affordable diet to rural households in many parts of

the continent (Karyeija *et al.*, 1998a; Madibela *et al.*, 1999; Owour, 2000). Improving sweet potato production and utilisation is often considered as a means to improve incomes and food security among the poorer of the rural populations (Anonymous, 2002). Unfortunately the crop is very susceptible to viral diseases, especially SPVD, resulting in deterioration of the quality and yield of many sweet potato cultivars (Joubert *et al.*, 1974; Karyeija *et al.*, 2000; Owour, 2000). Even though many of the sweet potato viruses are insect transmitted, wild plants such as *Ipomoea* spp. and their related genera, which act as host plants, contribute to making virus control difficult (Cadena-Hinojosa and Campbell, 1981; Karyeija *et al.*, 1998a).

Biological transmission of viruses to healthy plants, using vectors and grafting onto indicator plants has proven to be the most successful and efficient method of detecting viruses (Cohen *et al.*, 1992; 1997; Karyeija *et al.*, 1998a). It can be used more efficiently in places where other viral detection techniques are limited or inaccessible. Biological assays of sweet potato viruses have specific limitations because of co-infection by SPFMV as well as restricted host range (Moyer and Salazar, 1989), low concentration, uneven distribution in test plants and possible inhibitors of virus inoculation by plant tissue extracts (Chavi *et al.*, 1997). Although grafting is convenient, it requires time, greenhouse space, labour and large quantities of *I. setosa* (Cadena-Hinojosa and Campbell, 1981; Esbenshade and Moyer, 1982; Karyeija *et al.*, 1998a).

Although ELISA techniques has also proven to provide satisfactory results in detection of sweet potato viruses, the sensitivity and specificity depend on the antibodies used (Clark and Bar-Joseph, 1984).

Because of its ability to rapidly identify and differentiate potyviruses infecting sweet potato, PCR has become a powerful tool in detecting sweet potato viruses (Colinet *et al.*, 1994b; 1998). But in Africa, neither riboprobes nor PCR-based methods were reported to be used to detect SPFMV due to the fact that facilities and

materials required are rare (Karyeija *et al.*, 1998a). Although there could be some financial constraints in other cases, all methods described above are useful in order to obtain satisfactory results.

Management of sweet potato viruses should be directed towards the analysis of the constraints that farmers encounter. Lack of knowledge results in farmers using the same planting material year after year (without any crop protection measures) and ignoring other important agronomical aspects such as weeding and plant protection. Supplying farmers with the necessary training can help minimise virus infections.

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Chapter 3

Survey of utilisation, constraints and perception of sweet potato among small-scale farmers in South Africa

3.1 Introduction

Sweet potato (*Ipomoea batatas* Lam, Convolvulaceae) is an important food source to many rural families, primarily ensuring food security for the poor and as a cash crop in most parts of the world, including South Africa (Steinbauer and Kushman, 1971; Scott *et al.*, 2000). Apart from being high in carbohydrates and vitamin A (orange-fleshed cultivars), the crop is easy to grow even in poorly productive soil (Steinbauer and Kushman, 1971; Karyeija *et al.*, 1998; Ewell and Mutuura, 1991; Anonymous, 2002). Unfortunately, the crop is very susceptible to viral diseases, which have been recognised in South Africa since 1940's as the cause of yield and quality loss (McClellan and Klessner, 1947). It was later discovered by Thompson and Mynhardt (1986) that local cultivars were infected with a flexuous rod-shaped virus, which was serologically related to sweet potato feathery mottle virus (SPFMV). Although sweet potato viruses have been recognised for many years (Joubert *et al.*, 1974; Thompson and Meynhardt, 1986), most small-scale farmers still do not know or understand what sweet potato viruses are.

The first baseline survey on root crops such as sweet potato and cassava was conducted in 1996/1997 in four areas of Kwazulu Natal province, namely Hlabisa, Port Durnford, Makhathini and Mandlazini (van der Mescht *et al.*, 1997). During that survey, sweet potato was found to be the most important root crop in some areas and the second most important crop after maize (van der Mescht *et al.*, 1997; Thompson *et al.*, 1999). Farmers were found to be using their own vine cuttings and none of them were using virus-free planting materials. This common cultural practice of sharing vine cuttings, as planting materials seemed to promote the spread of infected material from one farmer to another (Karyeija *et al.*, 1998).

The current baseline survey was done in order to include as many provinces as possible so that a true reflection of sweet potato production by small-scale farmers can be obtained based on extensive information. The objective of the survey was to obtain information from farmers on the importance and role of sweet potato in their households. It was also important to know farmer cultivation practices and how they influence virus and diseases spread and to establish the farmers' knowledge on sweet potato viruses, production and constraints. This will also help in understating the farmers' way of farming and the reasons behind their production practices so that a means of improving their sweet potato production can be developed.

3.2 Materials and methods

3.2.1 Survey of farmer's knowledge

3.2.1.1 Approach

A baseline questionnaire to gather information was designed by Sunette Laurie (ARC-Roodeplaas) during the 1996/1997 survey. It was designed to gather information on production and uses of sweet potato. Also the current status of sweet potato production, local farming systems, varietal and taste preferences and constraints to the production of sweet potatoes were addressed. Other aspects such as production importance of the crop versus others crops, production costs, income and marketing were also addressed. This questionnaire was modified for use in the current survey to also establish the farmer's knowledge of virus diseases. The questionnaire is given in Appendix 3.1.

Through collaboration with extension officers of the Provincial Department of Agriculture, areas and farmers growing sweet potatoes within the province were identified. Seven provinces of South Africa (Limpopo, Mpumalanga, Western Cape, Eastern Cape, and Kwazulu Natal, Gauteng and North West province) were identified as sweet potato growing areas. From February 2001 to April 2003, both small scale and medium scale farmers were visited and questionnaires were administered only in six provinces (Limpopo, Mpumalanga, Western Cape, Eastern Cape, Kwazulu Natal and North West). Interviews were not conducted in Gauteng province due to time constraints.

3.2.1.2 Analysis

All data was entered in the Microsoft EXCEL spreadsheet programme in order to group or arrange and to calculate means and percentages.

3.3 Results

3.3.1 Area surveyed

The baseline survey was done in six provinces, namely Limpopo, Mpumalanga, Eastern Cape, Western Cape (Figure 3.1), Kwazulu Natal and Northwest provinces of South Africa. See Table 3.1 for the number of questionnaires conducted. Extension services in the Free State and Northern Cape claimed that very little or no sweet potatoes are grown in their provinces. Therefore these provinces were not surveyed.

Table 3. 1 Areas included during the baseline survey of farmers growing sweet potatoes in South Africa

Province	Limpopo	Mpumalanga	Gauteng	Eastern Cape	Western Cape	Kwazulu-Natal	North West
Areas	Venda Bushbuckridge Nebo	Hazyview Tonga Beyersbreed Gutjwa	Cullinan	Alice Port Alfred Bathurst Port St Johns Umtata	Ebenezer Saron Goedverwacht Freemeshin Pacalsdorp	Pietermaritzburg	Hamman-skraal
No. of questionnaires	46	22	0	10	7	3	1

In all the provinces surveyed, most rural farmers did not own a large piece of land for their agricultural activities. The largest farms were 55-100ha but 39% of farmers had $\leq 500\text{m}^2$ and another 38% had between 5500m^2 and 15000m^2 (1.5ha). Most farmers did not know exactly how big their land size was. Sweet potatoes were found to be cultivated on any piece of land available in the home yard. In most cases, 54.9% grew sweet potato on a land size between 100m^2 and 5000m^2 . Other farmers (29.6%) planted between 1-1.5ha and the largest fields were between 20-30ha (5.6%). The average areas where sweet potatoes were found to be cultivated per province were 6631m^2 in Limpopo (n=42), 30506m^2 in Mpumalanga (n=14), 8993m^2 in Western Cape (n=7) and 5000m^2 in North West (n=1) with the overall average of 6679m^2 / farmer. The sweet potato field size was an average 59% of the total field size.

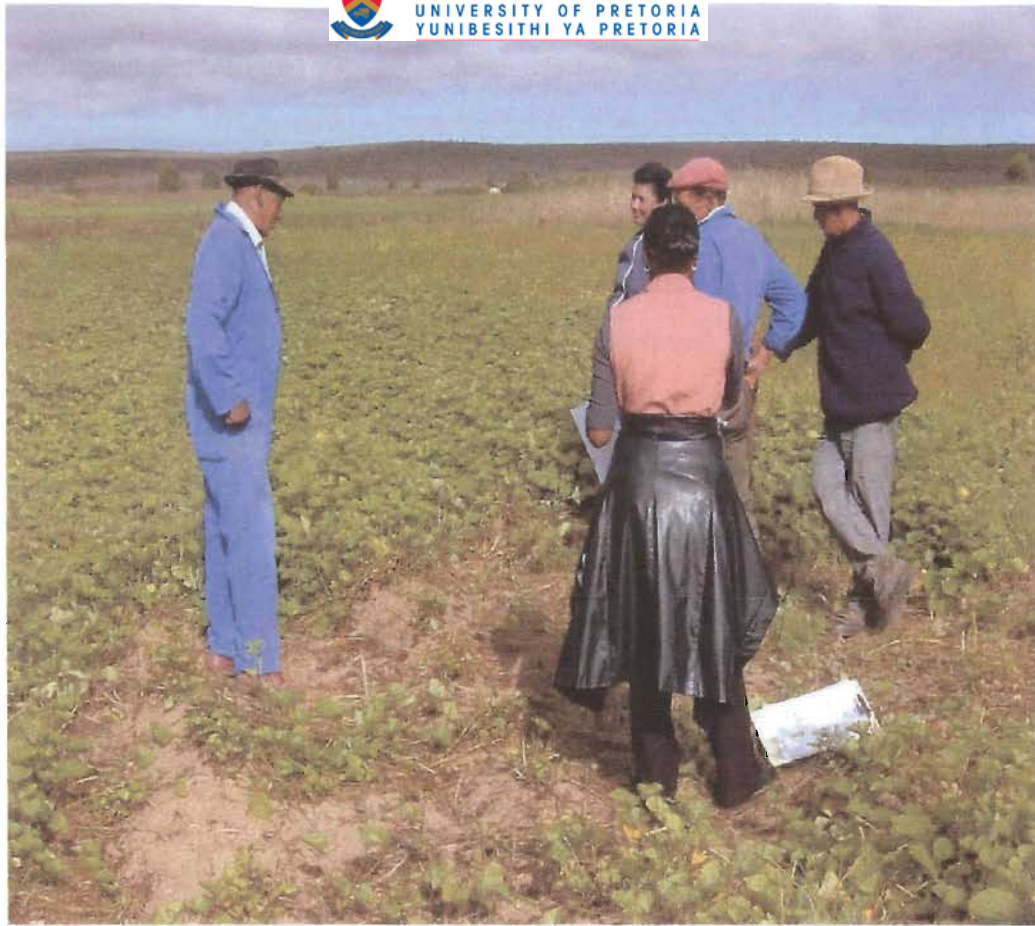


Figure 3.1 Julia conducting a baseline survey of production and utilisation of sweet potato in Western Cape province

3.3.2 Socio economic importance

3.3.2.1 Biographic profile

In all provinces where questionnaires were completed, the majority of respondents were women, comprising 62%, while only 38% were males. Many of the respondents were middle aged, between 41-50 years (38.5%), 31-40 years (27.7%) and between 51-60 years (17%). The oldest were between 61-70 (4.6%) and greater than 70 years (6.1%). The youngest group (6.1%) was between 20-30 years old.

The majority (95%) of farmers grew their crops subsistently and they depended on agricultural activities for their household food supply. Many (73%) of the respondents had been growing sweet potatoes for many years or for their whole lives. Others (19.3%) had been growing it for less than five years.

3.3.2.2 Production objectives

Sweet potato was regarded as a traditional crop by most farmers (40%), whereas 23% grew it for food, while others (25%) saw it as an income-generating crop (Table 3.2). It was also mentioned that the crop was grown for its drought tolerance and adaptation to rough climatic condition characteristics, ease to get planting material and that there is a market for it. Most importantly, the crop played an important role for many farmers by providing food and income generation. For most farmers (81%) the purpose of growing sweet potato was for both consumption and selling to generate income. Only 2% grew the crop for income generation (Table 3.3).

Table 3.2. Reasons for farmers to grow sweet potato

Reasons	LP	MP	KZN	W.C	E.C	N.W	Total	%
Tradition	29	15	3	3	8	1	59	40
Income	19	10	3	3	1	1	37	25
Food	25	7	0	0	1	0	33	23
Climate/drought tolerance	3	1	0	0	4	0	8	5.5
Easy to get planting material	2	0	0	0	2	0	4	3
Good market	1	0	0	2	0	0	3	2
Good at it	0	0	0	1	0	0	1	0.7
Increase soil fertility	1	0	0	0	0	0	1	0.7
Total no. of responses	79	33	6	9	16	2	145	

LP=Limpopo, MP=Mpumalanga, KZN=Kwazulu Natal, W.C=Western Cape, E.C=Eastern Cape and N.W=North West province.

Table 3.3 Purpose of sweet potato in the household (Selling, own consumption or income generating)

Purpose	LP	MP	KZN	W.C	E.C	N.W	Total	%
Sell to generate income	0	0	2	0	0	0	2	2.2
Own consumption	1	9	0	5	0	0	15	16.5
Both	45	13	5	5	3	3	72	81
Total no. of responses	46	22	7	10	3	3	89	

LP=Limpopo, MP=Mpumalanga, KZN=Kwazulu Natal, W.C=Western Cape, E.C=Eastern Cape and N.W=North West province.

3.3.2.3 Importance of sweet potato, compared to other crops

As expected, grain crops such as maize were still rated the most important crops for food supply by the majority of farmers (89%) in all provinces surveyed, followed by vegetables with 56% farmers indicating that it was important in their daily diet (Table 3.4). Sweet potato was the third most important food crop, with 48% of farmers showing their need to always have a small patch of the crop in the yard, which was why they did not harvest everything in their fields. Although potatoes and fruits were also mentioned as part of their daily food sources, these two were rated as fourth and fifth most important respectively.

For cash crops, vegetables were rated to be the most important by 79.3% of farmers. Grain crops also brought high returns if climatic conditions such as good rainy seasons were favourable, with 77.7%, farmers indicating that maize was an important cash crop to them. Sweet potato was also an important cash crop to 47% of farmers. Some farmers mentioned that they also exchanged sweet potato for maize. Potatoes and fruits were also sold for cash, but not to the extent that vegetables, grain crops and sweet potatoes were sold.

Table 3.4 Comparison of sweet potato with other crops as food/cash crop in percentages

Crops	As food	As cash
Grain crops	89%	77.7%
Vegetables	56%	79.3%
Sweet potato	48%	47.3%
Potato	23%	10.7
Fruits	10%	12.4%

3.3.2.4 Income

Although the majority of farmers in rural households who were visited indicated that they produced sweet potato normally for their own consumption, it was also discovered that sweet potato could have a potential market in the future. If production constraints such as costs and lack of land were minimised, sweet potatoes could be a good means of increasing household income. The income mentioned by farmers if they were selling their produce is given in Table 3.5. The highest, not usually expected in rural farming, was from Mpumalanga with one farmer claiming that he could get as high as R15000 in a growing season, if production constraints were minimised. For all provinces, the potential average income from sweet potato was found to be R685, a reasonable figure for covering some of the day-to-day expenses that rural people are unable meet.

Table 3.5 Potential income that can be obtained from sweet potato production

Income R	LP	MP	KZN	W.C	E.C	N.W	Total	%
<R100	4	0	0	0	1	0	5	904
R100-R499	14	4	3	0	0	0	21	39.6
R500-R999	11	0	0	0	0	0	11	20.7
R1000-R1999	12	0	0	0	1	0	13	24.5
R2000-R2999	0	0	0	1	1	0	2	3.8
R15000	0	1	0	0	0	0	1	1.9
Total no. of responses	41	9	3	1	3	0	53	

LP=Limpopo, MP=Mpumalanga, KZN=Kwazulu Natal, W.C=Western Cape, E.C=Eastern Cape and N.W=North West province.

3.3.2.5 Yield

Farmers normally harvested enough to feed their family, leaving the plants in the field in order to prolong the availability of sweet potato for the family until the next season. When they sold their products, buckets of between 5kg and 10kg or 20kg crates were used. A very low yield was measured (Table 3.6) with 64% of farmers producing less than 1.0 tons/ha. Some farmers in Mpumalanga achieved a yield as high as 11-20t/ha in a season.

Table 3.6 Yield in t/ha obtained from sweet potato production

Tons/ha	LP	MP	KZN	W.C	E.C	N.W	Total	%
0.1-0.5	11	1	0	1	0	0	13	27
0.6-1.0	17	0	0	0	1	0	18	37
1.1-1.5	1	0	0	0	1	0	2	4
1.6-2.0	3	0	0	0	0	0	3	6
2.1-5.0	4	1	0	0	0	0	5	10
6-10	3	2	0	0	1	0	6	12
11-20	0	2	0	0	0	0	2	4
Total no. of responses	39	6	0	1	3	0	49	
Average	1.6	8.5	0	0.47	3.0	0		

LP=Limpopo, MP=Mpumalanga, KZN=Kwazulu Natal, W.C=Western Cape, E.C=Eastern Cape and N.W=North West province.

3.3.2.6 Marketing

Sales in rural areas were very irregular. It sometimes depended on whether the farmer had enough for both consumption and selling. Some farmers only harvested as required by the family (piecemeal) at a time and did not consider selling. Those who sold their fresh sweet potato, indicated that prices varied depending on the quantity, size of the storage roots, the place and the people they were selling to and if there were any, inputs price can also be a deciding factor on the price. The price per kg of sweet potato is given in Table 3.7. Most of them (54%) sold at a price between R0.50-R1.00/kg, others (23%) sold at between R1.1-R1.50/kg, while 19% are sold at between R1.60-R2.00/kg. It was only in North West province where 3% of farmers were found sell at a price of R3.00/kg. The overall average price sweet potato was sold for by rural people in all provinces was found to be R1.11/kg.

The majority of farmers (49%) sold to their neighbours, while others (30%) sold to the local markets. Some (15.6 %) sold door-to-door, a way of taking the product to the people. Although it was also mentioned that tourists were also their target markets, it

was not common or practical to the majority of farmers due to lack of transportation of their products to these markets, where they claimed they would sell at better prices. Hawkers, local communities and pensioners also supported rural farmers by coming and buying from them.

Table 3.7 Selling price for sweet potato

Price/kg	LP	MP	KZN	W.C	E.C	N.W	Total	%
R0.50-R1.00	4	7	0	1	2	0	14	54
R1.1-R1.50	1	1	0	4	0	0	6	23
R1.60-R2.00	1	1	3	0	0	0	5	19.2
R2.10-R2.50	0	0	0	0	0	0	0	0
R3.00	0	0	0	0	0	1	1	3.8
Total no. of responses	6	9	3	5	2	1	26	

LP=Limpopo, MP=Mpumalanga, KZN=Kwazulu Natal, W.C=Western Cape, E.C=Eastern Cape and N.W=North West province.

3.3.2.7 Consumption and utilisation

Sweet potato was normally consumed as a fresh product in rural household with farmers getting their sweet potato to eat from their local markets (32%) and hawkers (44%) when they did not have any available in their fields or home yards. Other sources of sweet potatoes for consumption mentioned were friends and families, shops, neighbours, and other farmers having their own supply in their home yards. Sweet potato was boiled and eaten when cold with tea by 58% of respondents in rural communities (households). It was also consumed when first boiled, mashed and formed part of a main meal. Leaves were also cooked and eaten as vegetables in the main meal by some people. Fresh sweet potato tubers were also mashed and eaten with rice, while other farmers boiled them, mashed them, and mixed with them with groundnuts or maize meal. Boiled tubers are also eaten fresh as a main meal. Chips were also mentioned to be some ways of preparing sweet potato.

3.3.3 Cultivation practices

3.3.3.1 Planting material

Sweet potato was a household crop for many rural families, and although some farmers bought their planting material, 47% got their planting materials from neighbours and friends and 30% get them from relatives. Some of the planting materials were kept by farmers (14%) for many years. Although 4% and 2% of the farmers mentioned that they bought their planting materials from shops and vine growers respectively, it is was clear that rural farmers mainly exchanged their

planting material among friends, relatives and neighbours and new varieties that were diseases-free were not used or known. Sources of planting materials are given in Table 3.8. If planting materials were bought, the average cost was up to R35 for a 50kg maize meal bag full of cuttings. Since farmers exchanged planting materials among each other, 69% of farmers did not have a problem in getting planting materials while 31% indicated that it was a constraint because of the high cost involved in getting planting materials. In some areas in Eastern Cape, Mpumalanga and Limpopo provinces, sweet potato is not a common crop and it is not easily accessible. Only 5% of farmers indicated that they preserved their planting materials by storing tubers, planting few tubers in beds and putting some tubers in cool temperatures of $\pm 15-20^{\circ}\text{C}$ and used them as planting materials for the next season. The most common harvesting practice was digging sweet potatoes out by hand or lifting them up with a fork.

The majority of farmers (92%) had never received formal training on how to produce or preserve their own planting materials. ARC-Roodeplaat has started at target sites to train people in these aspects.

Farmers were found to plant between two and six varieties. Selection of planting materials was not practiced. Although cultivars such as Bosbok (n=1), Mafutha (n=1), Blesbok (n=3) were mentioned, 89% of farmers used their local land races which they shared among each other. Given a choice, farmers (22%) would prefer varieties with good taste, disease and pest tolerance (15%), high yielding (14%), drought tolerance (14%) and having both high yielding and good taste characteristics. Characteristics such as taste and less fibrous, fast growing and early maturing, medium sized, less cracking, longer storage period, high consumer demand, shape and good skin colour were pointed out to be desirable by farmers. All farmers interviewed showed interest in getting varieties that were improved and virus-free with the hope that they would taste better, yield better, and that they would be able to make money.

Table. 3.8 Sources of sweet potato planting materials

Sources of plant material	LP	MP	KZN	W.C	E.C	N.W	Total	%
Own	6	1	0	5	7	0	18	14
Relatives	38	8	0	1	2	0	39	30
Neighbours/Friends	40	17	0	2	2	0	61	47
Shop	9	4	0	0	1	0	5	4
Vine growers	6	0	0	2	0	1	3	2
ARC-Roodeplaat	0	0	3	0	0	0	3	2
Total no. of responses	99	30	3	10	12	1	129	

LP=Limpopo, MP=Mpumalanga, KZN=Kwazulu Natal, W.C=Western Cape, E.C=Eastern Cape and N.W=North West province.

3.3.3.2 Land preparation

In all provinces surveyed, 59% of farmers ploughed their fields with a tractor while 32% cultivated with a hand hoe. In Limpopo, for example, to hire a tractor was mentioned to be a constraint since it could cost an average price of R540/ 1000m², varying from R125 for 2000m² to R700 for 12000m². Animal traction and spades were also used for cultivating the land. Although few farmers indicated what spacing they used, spaces between the plants and between rows was not a common thing to do in rural farming, and when it was practiced, farmers estimated their spacing using their feet. The general spacing used for sweet potato was 0.3m (60.8%) to 0.2m (30.4%) between plants and 1m (39%) to 2m (57%) between rows.

3.3.3.3 Irrigation

Sweet potato was irrigated by 54% of farmers, but for the rest, it was rainfed in many rural farming systems. The furrows system was most commonly practiced by 49%, followed by 22% of farmers using flood irrigation. Watering cans, hosepipes, overhead sprinklers and micro-irrigations were also used by some farmers.

3.3.3.4 Fertilisation

Applying fertilisers to sweet potato was not commonly practiced by 53.3% of small scale farmers interviewed. Only farmers (46.6%) who had been exposed to some modern farming practices applied fertilisers sometimes. The average price of fertiliser in Limpopo was R122/50kg bag. Many farmers believed that applying fertilisers on sweet potato would affect its taste by making it too watery after cooking. Inorganic fertilisers such as LAN, 2:3:2, super phosphate, 2:3:4, potash nitrate and MAP and organic fertilisers such as kraal manure were mentioned to be used by farmers who fertilised their soils, as a single treatment or in a combination.

3.3.3.5 Beds types

Ridges, flats and mounds were the type of beds that farmers were planting on. Although some of the farmers used a single bed type or a combination of two or three methods, the most common was to plant on ridges, with 63% of farmers choosing it, followed by 33% of farmers planting on flats. A minority of farmers (3%) used the mounds bed type.

3.3.3.6 Weeding and plant protection

Most farmers (69%) in rural areas used hoes, others (30%) weeded by hand, because normally their fields were small. Farmers in Limpopo (n=1) and Eastern Cape (n=1) indicated that they did not weed their fields. In all provinces visited, herbicides were not mentioned to be used to control weeds.

The majority of farmers (90%) did not practice plant protection and those (10%) who did sprayed only for pests.

3.3.3.7 Intercropping and crop rotation

Intercropping was also not a common practice, with 80% farmers not applying it. The few who practiced intercropping used crops such as maize, pumpkins, groundnuts, bambara groundnuts and potatoes.

Crop rotation was found to be practiced by over half of the farmers (54%) interviewed in all provinces. Crops such as maize, pumpkins, groundnuts, cabbage, bambara groundnuts, potatoes, beans, garlic, lucern and onions were mentioned to be used in rotation with the sweet potato crop.

3.3.4 Virus diseases

The majority of rural farmers (96%) had no knowledge of sweet potato viruses, neither knowing what symptoms looked like or how they spread. Although few claimed to have seen symptoms, common insects that spread viruses such as whiteflies and aphids were not known, which makes it clear that rural farmers are highly lacking in knowledge on these aspects.

3.3.5 Constraints to production and utilisation

The major constraints in the production of sweet potato mentioned were pests such as moles and caterpillars. Constraints in production are given in Table 3.9. Over half of the farmers (60 %) indicated that insects that infest storage roots threatened their production by degrading sweet potatoes in such a way that they became unmarketable. Due to lack of proper fencing in some of the households, animals such as goats also tended to bother farmers by eating the leaves. Irrigation water was also a big problem if it was not a good rainy season. Equipment (tractors), planting material, production costs, storage places and markets for selling their products were given as other constraints. As already indicated, land for agricultural activities was found to be small in most of the areas visited, which was also a constraint to production.

Table 3.9 Major constraints in the production of sweet potato

Constraints	LP	MP	KZN	W.C	E.C	N.W	Total	%
Insects	39	13	3	0	3	0	58	60
Diseases	1	0	0	0	0	1	2	2
Irrigation water	9	1	0	0	0	0	10	10
Equipments	6	1	0	0	1	1	8	8.2
Production costs	0	3	0	1	0	0	4	4.1
Expensive planting materials	2	0	0	0	0	0	2	2
Animals (eating leaves)	0	0	0	1	1	1	2	2
Market (for selling the produce)	5	3	0	0	0	0	8	8.2
Land	0	0	0	0	0	1	1	1
Storage places	0	0	0	1	0	0	1	1
Yield	0	0	0	1	0	0	1	1
Total no. of responses	62	21	0	4	6	6	97	

LP=Limpopo, MP=Mpumalanga, KZN=Kwazulu Natal, W.C=Western Cape, E.C=Eastern Cape and N.W=North West province.

3.4 Discussion and conclusion

Although sweet potato has been regarded as a low value crop and has received very low attention in research as a cash crop, our results indicate that it is a very important crop to many rural households. Like in other African countries such as Malawi (Moyo *et al.*, 1999), sweet potato is still perceived as a woman's crop in South Africa. Women play an important role in making sure the crop is available for the family. In order to make other people aware of the importance of sweet potato, varieties that provide important nutrients such as Vitamin A (orange-fleshed) need to be introduced to rural people. It was stipulated that the crop is not only important in consumption, but it helps in generating income and also as a food security crop. Although maize and other grain crops are still the major crops for food supply, sweet potato was ranked the third most

important crop for food supply. The requirements of few inputs for production and easy to manage characteristics add to its advantage as a cash crop.

Although they still use their traditional farming methods, farmers in all provinces indicated their lack of knowledge in making planting material and preserving them as a major constraint. Dissemination of planting material among farmers (friends and families) was found to be common practice in all provinces surveyed. And since most of them harvest piecemeal, the practice results in using the same varieties that are not improved nor virus tested planting material. This aids in spreading diseases and producing low yielding and poor quality crops.

Sweet potato viruses are the most important diseases that threaten the production of sweet potato by lowering the yield and causing cracks in some sensitive cultivars, making them unmarketable. Sweet potato feathery mottle virus (SPFMV) infects sweet potatoes worldwide (Moyer and Salazar, 1989). SPFMV, together with sweet potato mild mottle virus (SPMMV) and possibly sweet potato latent virus, has been reported to occur in South Africa (Jericho and Thompson, 2000). Farmers in all provinces surveyed indicated that they did not know what virus diseases were. This confirms the need for farmers to be trained on plant diseases and how to prevent their spread. Farmers need to be shown through demonstration trials that a better quality sweet potato is produced from virus tested propagation materials. Although land for agricultural activities is still a problem, low yield is also discouraging farmers in continuing growing sweet potatoes.

In order to increase yield, farmers urgently need knowledge in selecting planting materials that are diseases free. Although ARC-Roodeplaat has already started with the initiative of supplying farmers in target rural communities with improved and diseases free sweet potatoes cuttings and teaching them how to grow it, as part of empowering them, there is still much to be done as the knowledge is not yet widely spread. Government extension officers can also play a vital role in disseminating this knowledge. Outreach programmes of teaching farmers some other methods of processing sweet potato will also help by making the consumption of the crop desirable, at the same time increasing its market. Farmers need to be taught to integrate their traditional farming systems with some of the modern methods of farming. Half of the farmers in all areas visited, in all provinces, grow sweet potato on a dry land farming

system. The use of fertilisers before planting (for example) and crop rotation will prevent the depletion of nutrients from the soil, making it possible for the crop to survive and still produce in times of poor rains. Research on cost effective production inputs such as compost and plant extracts that can help control pests still needs to done.

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Chapter 4

Incidence of diseases and pests in sweet potato fields

4.1 Introduction

Sweet potato, like other crops is prone to pest and diseases, which affect its production. The incidence of pest and diseases on sweet potatoes is not largely known in South Africa. The first survey was conducted in Kwazulu Natal and Mpumalanga provinces of South Africa in 1996/1997 (van der Mescht *et al.*, 1997; Thompson *et al.*, 1999). Based on field observation judgements, insect damage was the most common problem of sweet potatoes. Sweet potato weevils, stem borers and beetles are important insects of sweet potatoes (Ames *et al.*, 1997). Aphids and whiteflies as virus vectors (Brunt *et al.*, 1996) were also reported to lower the production of sweet potatoes by reducing the yield of the crop.

It was also observed that infected sweet potatoes rarely express virus symptoms in the field and when present, symptoms are seen on older leaves (van der Mescht *et al.*, 1997; Thompson *et al.*, 1999). The current survey is a continuation of the previous one because only few provinces were covered during that survey.

The main objectives of the study was to establish the incidence of pests and diseases in different provinces and to observe the prevalent sweet potato virus symptoms found in each province. However, not all nine provinces were covered in this current survey due to the fact that sweet potato is not a common crop in some provinces and that access to areas can only be gained through extension worker and community members and getting such cooperation was sometimes a problem. In these two surveys, the incidence and importance of pests and diseases was found to vary from region to region. It is important to establish the distribution and relative importance of these pests and diseases in order to orientate research priorities and develop control strategies.

4.2 Materials and methods

A survey of pests and diseases in sweet potato fields was carried out in seven provinces of South Africa (Limpopo, Mpumalanga, Gauteng, Western Cape, Eastern Cape, Kwazulu Natal and North West). All fields were surveyed on a W-pattern method of sampling. Thirteen sampling points were selected at random along this pattern in each field. The plants were rated for leaf spots or blights, stem spots or blights, virus symptoms, insect damage, root rot and wilts.

Diseases ratings were as follows:

- 0 - Disease/pest free
- 0.1 - 0.5 - Plants slightly affected or infected
- 1 - Approximately 1/5 of plants affected
- 2 - Approximately 2/5 of plants affected
- 3 - Approximately 3/5 of plants affected
- 4 - Approximately 4/5 of plants affected
- 5 - Whole plant affected/ all plants infected

Samples representing different cultivars found in each farm were collected at every sampling point. Collected vines were brought to ARC-Roodeplaat where they were grown in pots of 15 and 18cm diameter, using pasteurised media (Just Nature, South Africa). Planted cuttings were maintained in a glasshouse at temperatures of 20-30°C. Plants were given a supplementary feeding containing 19.0% nitrogen, 8.2% phosphorus, 15% potassium, 350ppm zinc, 1000ppm boron, 70ppm molybdenum, 750ppm iron, 300ppm manganese, 75ppm copper and 900ppm magnesium (Multifeed P, Plaasskem, Pty, LTD) on a weekly basis. Plants were also monitored for insect pests and sprayed with chemicals as required.

4.3 Analysis

The data was entered in an EXCEL spreadsheet program and the statistical averages of the Provinces and of the whole country was calculated.

4.4 Results

As in 1996/1997, the prevalence of diseases and pests varied between provinces and due to climatic factors. The results for severity ratings for pests and diseases incidences assessed during the survey are given in Appendix 4.1 and a summary is given in Table 4.1. Although some leafspots and blights were observed on sweet potato leaves in the fields in some provinces, the average incidence for leafspots for the country was very low (0.2). Stemspots and blights, root rot and wilt were not observed on sweet potatoes in all areas surveyed. Although not prevalent, virus symptoms were observed during field surveys (Figure 4.1) but not in all areas of the provinces visited. The average virus incidence was 0.3 (less than one) for the country. Although it had the small average of 0.5 (less than one), Mpumalanga was the only province that had the highest number of plants with conspicuous symptom expression. The most prevalent symptoms were vein clearing, crinkling, chlorotic spots, and purple ring spots on older leaves in areas such as Beverbreed and Hazyview. Suspected symptoms of sweet potato virus diseases (SPVD) such as severe vein clearing, leaf distortion, chlorosis, mosaic and stunting were also observed in Hazyview (Ntsikadzi) and Gutjwa. Typical sweet potato feathery mottle virus symptoms such as vein clearing, purple ring spots and chlorotic spots were also observed in the Western Cape (Goedverwacht and Ebenezer). In the Eastern Cape, the incidence of virus symptoms was also very low, with the average of 0.1. Virus symptoms were rarely observed in the Limpopo, Gauteng and Kwazulu Natal provinces. Symptoms such as vein clearing were also observed on wild *Ipomoea* species such as *Ipomoea sinensis* (Ders.) Choisy (identified by National Botanical Institute of South Africa), which were found to be growing with sweet potatoes in the Mpumalanga and Western Cape province. The overall average virus disease incidence of 0.3 for the whole country showed that affected sweet potatoes did not readily express virus symptoms.

Insect damage on leaves was the major problem in all areas of the provinces visited. The overall average incidence for insect damage for the provinces surveyed was 1.4. Gauteng and Kwazulu Natal provinces had the highest insect damage incidences average of 3.8 and 3.5 respectively compared to other provinces. Although leaf damage was of lesser important to farmers, it was indicated that the most problematic insect pests to their production were weevils and caterpillars that fed on leaves. Weevils were

also mentioned to lower the production of sweet potato by eating holes in exposed tubers. Sweet potato virus transmitters such as whiteflies (on sweet potatoes) (Figure 4.2) and aphids (on weeds) were also observed in some fields in the Mpumalanga and Western Cape provinces.

Table 4.1 A summary of the average incidence of pests and diseases of sweet potato for the provinces and the country

Ratings	Provinces							Country
	E. Cape	W. Cape	MP	Gauteng	KZN	N. West	L.P	
Leaf spots and blights	0.2	0.6	0	0.5	0.5	0	0.03	0.2
Stem spots and blights	0	0	0	0	0	0	0	0
Virus diseases	0.1	0.2	0.5	0.4	0	0.2	0.01	0.3
Insects damage	2.2	2.1	0.6	3.8	3.5	2	0.9	1.4
Root rot	0	0	0	0	0	0	0	0
Wilts	0	0	0	0	0	0	0	0

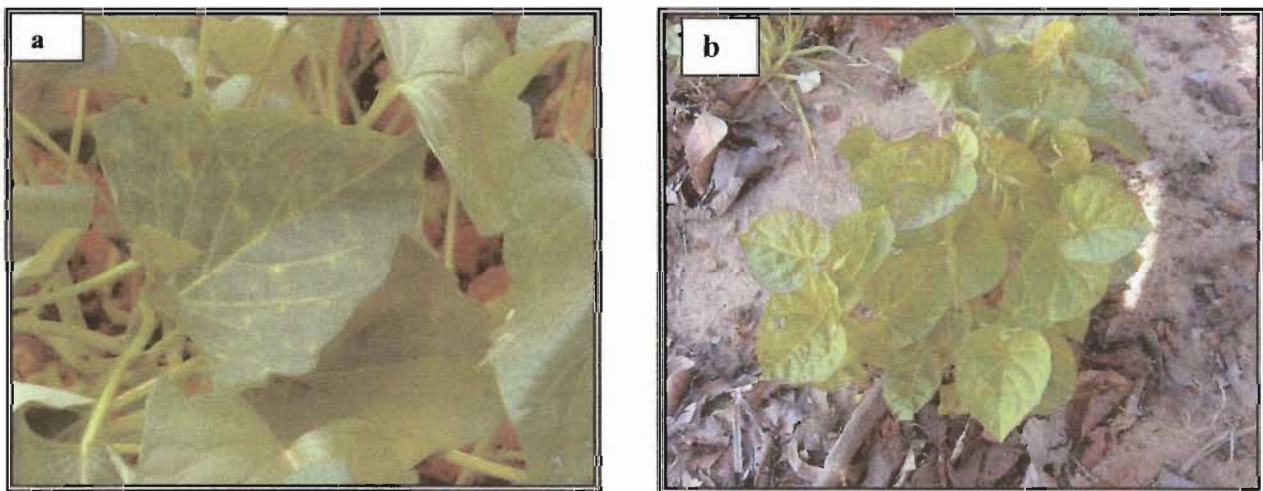


Figure 4.1 Sweet potato plants/leaves showing (a) chlorotic spots on older leaves and (b) vein clearing and stunting symptoms in the fields



Figure 4.2 Whiteflies on sweet potato leaf during field survey in the Western Cape province

4.5 Discussion and conclusion

During the 1996/1997 survey, leaf spots were found to be more prevalent than stem spots and the incidence was found to be less than 10% (van der Mescht *et al.*, 1997; Thompson *et al.*, 1999). Fungal organisms were also diagnosed from samples tested (van der Mescht *et al.*, 1997; Thompson *et al.*, 1999). It was also found during the current survey that leaf spots and blights, stems spots and blights, roots rots and wilt are not as important to sweet potato production as virus diseases and insect damage. Although their effect on sweet potato production is not as high as that of virus diseases, leaf spots and blights, stems spots and blights, roots rots and wilt also need to be controlled to prevent them from becoming threats to production in future.

Insects damage on leaves was also found to be prevalent during the 2001/2003 survey and the 1996/1997 (van der Mescht *et al.*, 1997) survey. Insects such as cutworms,

millipedes, sweet potato weevils, bollworms and in some areas locusts were mentioned to be problematic by some farmers who had been interviewed. However, most farmers did not consider leaf damage by insects a limiting factor to their production because only few farmers indicated that they were controlling (apply chemicals) insects pests on sweet potato crops. It should be brought to the government's attention that, affordable control measures need to be researched and made accessible to both small-scale and medium scale farmers in order to enhance pests control all over the country.

Sweet potato virus symptoms are rarely seen in the field in most areas in South Africa. This is due to the fact that the prevalence of diseases (or insect damage) is greatly influenced by weather conditions. It can also be speculated that the local varieties have some tolerant genes that enhance the suppression of symptoms under field conditions. Aphids and whiteflies have been reported to spread sweet potato virus diseases such as sweet potato feathery mottle virus (SPFMV), sweet potato mild mottle virus (SPMMV) and sweet potato chlorotic stunt virus (SPCSV) (Moyer and Salazar, 1989; Gibson *et al.*, 1998). The presence of aphids and whiteflies in some areas in the provinces is an indication that high levels of viruses could be found within the plants and that the spread of the most important viruses in South Africa such as SPFMV and SPMMV (Jericho and Thompson, 2000) could increase with time.

In Chapter 3 of this thesis, it was indicated that farmers did not know what aphids and whiteflies were nor their effect on sweet potato production. The vegetative propagation of sweet potato and sharing planting materials among farmers will result in the uncontrollably spread of sweet potato viruses. The non-persistent modes of transmission of SPFMV by aphids (Moyer and Salazar, 1989) also make control impossible. During the baseline survey (Chapter 3, this thesis), farmers also indicated that disease and pests control measures are rarely practised. Farmers need to be taught about the use of virus free planting materials and how to select them, not only to prevent the spread of viruses, but also to produce high yielding crops with good quality. However the low incidence of virus symptoms in the field makes the selection of virus free material difficult. It should be considered that the incidence and severity of diseases and pests during all surveys (1996/1997 and 2001/2003) only covered seven provinces and that surveys were conducted at a certain period of time. The effect of pests and viruses within the country could still be underestimated and the results might not be a true reflection of the

real incidence of pests and diseases in the country. The true reflection can only be obtained if extensive research on incidences of diseases or pests can be conducted in all seasons, since the prevalence is influenced by weather conditions.

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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 CanTM) 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 $MgCl_{2.6}H_2O$ (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). Other 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.

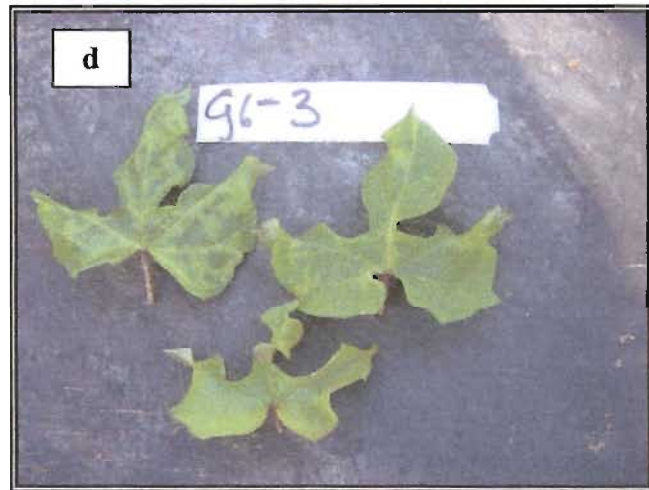
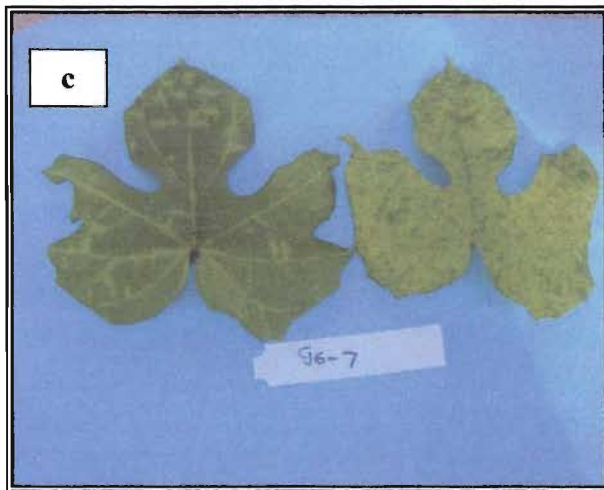
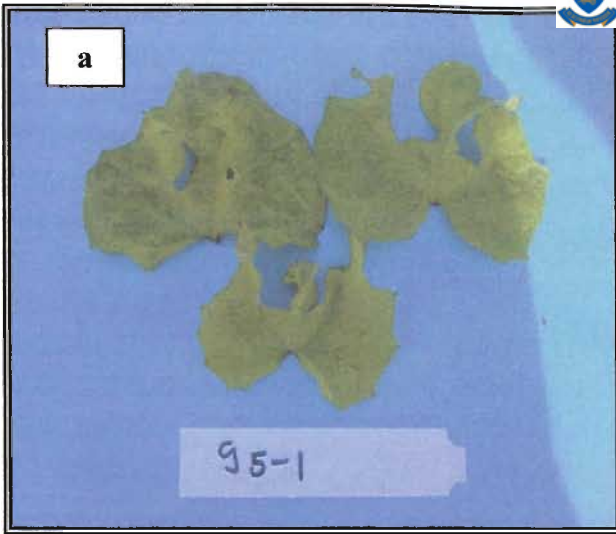


Figure 5.1 Typical potyvirus symptoms shown by grated *I. setosa*. (a) Vein clearing, diffuse mottle and leaf deformation, (b) severe vein clearing and mosaic (c) vein clearing and mosaic and (d) vein clearing and leaf curling

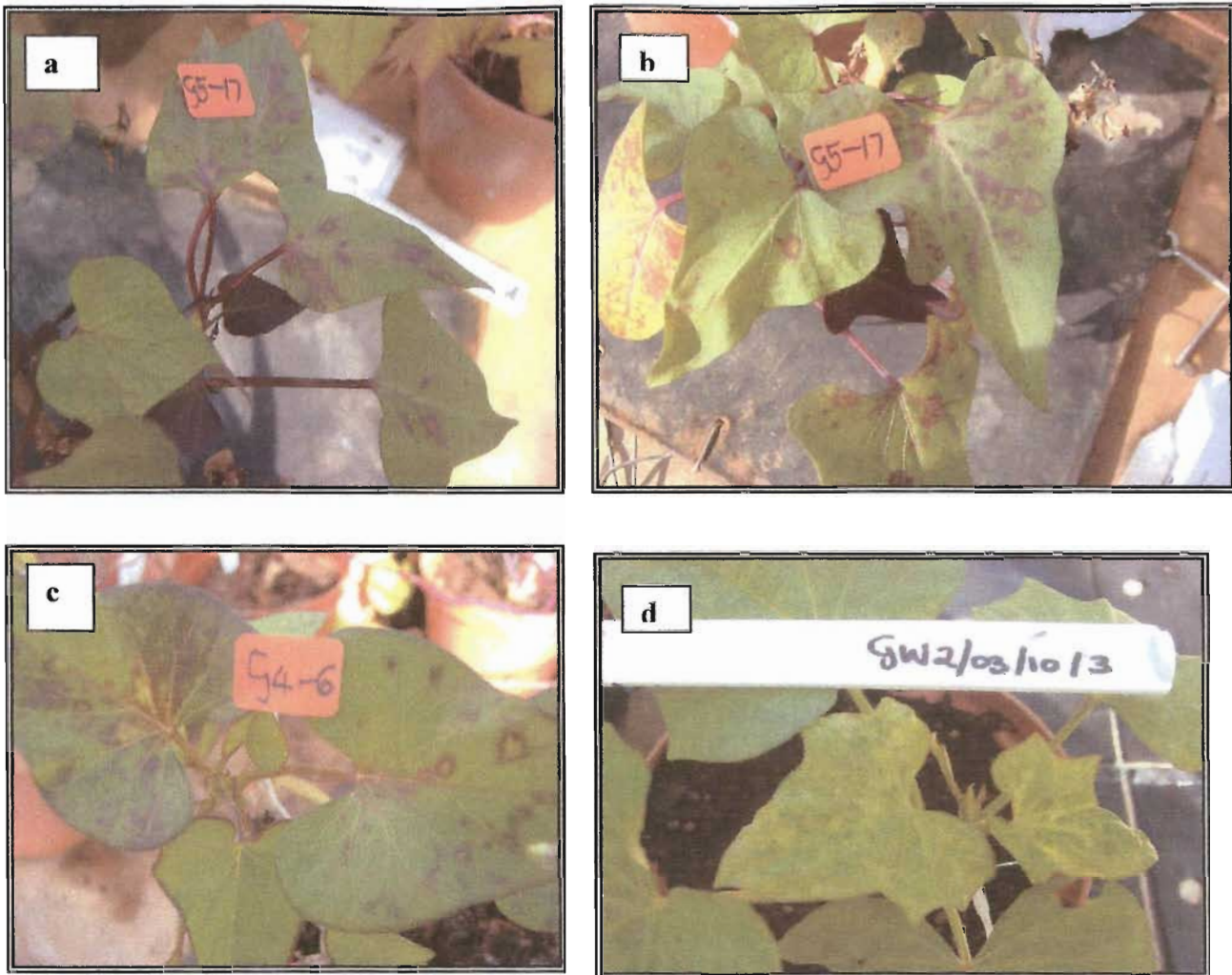


Figure 5.2 Sweet potato plants exhibiting symptoms after they have been kept in the glasshouse. (a) and (b) show conspicuous purple ring spots, (c) purple ring spots and some chlorotic spots, (d) chlorotic spots and vein clearing

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	-	-	-	-	-
Total	616	134	11	442	20	37	16	0	0	12	1	195	186	11	0	73
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)
Western Cape	Hazyview	SPFMV+SPVII+SPVG (SPLV+SPCFV)
	Tonga	SPFMV+SPVII +SPVG
	Ebeneser	SPFMV+SPVII +SPVG (SPMSV)
	Friemersheim	SPFMV+SPVG (SPCSV-EA)
	Goedverwacht	SPFMV (SPVG+SPVII)
Eastern Cape	Pacalsdorp	SPFMV (SPVII+SPVG)
	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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Chapter 6

Assessment of the effects of virus infection on the yield of sweet potato cultivars

6.1 Introduction

Sweet potato (*Ipomoea batatas* Lam.) is the most important crop of rural households as it is easy to grow even in harsh environments. Unfortunately it is prone to virus infections that result in cultivar decline and deterioration in root yield and quality. The influence of viruses infecting sweet potatoes is largely known. Since the 1960's, yield and quality degeneration was experienced by commercial producers in South Africa in such levels that it was uneconomical to grow sweet potato (Joubert *et al.*, 1974; Laurie *et al.*, 2000). Although virus symptoms were seldom evident, the decline was associated with infection by viruses (Laurie *et al.*, 2000). Thompson and Mynhardt (1986) showed that the old South African cultivars were infected with a flexuous rod-shaped virus, which was serologically related to sweet potato feathery mottle potyvirus (SPFMV). Non-symptomatic sweet potato samples, which were collected during the 1997/1998 surveys, confirmed that SPFMV was the most prevalent virus infecting sweet potatoes in South Africa (Jericho, 1999; Jericho and Thompson, 2000). Sweet potato mild mottle virus (SPMMV) was also detected to be another important virus of sweet potato in South Africa (Jericho, 1999; Jericho and Thompson, 2000). Potyviruses such as sweet potato virus II (SPV II) and sweet potato virus G (SPV G) have been reported to infect sweet potatoes in Taiwan (Salazar and Fuentes, 2000), Uganda, Egypt, India and China (Colinet *et al.*, 1994; Salazar and Fuentes, 2000). These viruses have never been characterised in South Africa. The results given in Chapter 5 indicate that these two viruses are prevalent in all provinces surveyed. The two viruses were found to infect sweet potatoes in a combination with other viruses and rarely as a single infection. Like SPFMV, these two viruses show the potential of becoming a threat to sweet potato production in South Africa. Results obtained in South Africa several years ago showed that elimination of viruses led to increases in yields of up to 80% (Joubert *et al.*, 1974). Marketable yield obtained from virus-free materials was found to have increased by 53% for the four cultivars tested (Joubert *et al.*, 1974; Laurie *et al.*, 2000). However,

these experiments were done comparing the virus-free plants with plants that had been infected with viruses over an extended period. Also the viruses were not identified at that stage.

The objective of the current study was to establish the effects of the most common viruses, either as single infections or in combination on the yield of local cultivars. It was also attempted to determine if the effects of virus infection increased with time.

6.2 Materials and methods

6.2.1 Source of material

Ten sweet potato cultivars and ARC breeding lines, which looked promising for use by resource-poor farmers after one season's off-station trials, were selected for use in these trials. These were: Bosbok, Mafutha and Natal Red (South African cultivars), 1984-10-340, 1994-5-1, 1989-17-1 and 1995-13-2 (ARC breeding lines), Excel, CN 1656-97, and Xushu 18 (germplasm imported from the International Potato Center, Lima, Peru). Some of these breeding lines have been renamed as follows, 1995-13-2=Ndou, 1989-17-1= Monate and 1984-10-340=Mamphenyane. The first trial was conducted in 2001/2002 and the second in 2002/2003. The cultivar Mamphenyane was excluded from the first trial as there was not enough planting material. Also, planting material for Excel cultivar was insufficient when the 2002/2003 trial was conducted. The results are explained according to the year the cultivars and lines were inoculated. The first trial was designated 2001 trial. The trial conducted using virus-infected sweet potato planting materials of the previous trial/season (2001 x2) is explained as secondary infection. The newly inoculated cultivars and lines of the 2002 trial are explained as primary infection.

6.2.2 Inoculation

Infected sweet potatoes collected from small-scale farmers during the 1997/1998 surveys were used as sources of inoculum. Cuttings from sweet potato plants designated PD 1/2/1, MD 1, T1/1, Frank 5.2 max, were used as a source of inoculum for SPFMV as a single infection. Cuttings from sweet potato plants designated WC 10, TO 3.4 and TO 2.5 Swazi were used as a source of inoculum for SPFMV and SPMV as mixed infection. Cuttings of these virus-infected plants were side grafted

onto healthy cuttings of the above-mentioned cultivars and lines. After two to three months, cuttings of grafted cultivars were indexed by grafting onto *Ipomoea setosa* Kerr. The indicator plants were monitored for symptoms and tested using nitrocellulose enzyme linked immunosorbent assay (NCM-ELISA). The positive plants were multiplied and transplanted to the field towards the end of October 2001 in a multiplication block.

When conducting the second trial, healthy cuttings of the cultivars and lines used in the 2001/2002 trial, were selected in April 2002 except that Mampheyane was substituted for Excel. Cuttings of these healthy cultivars and lines were inoculated by grafting them onto SPFMV and SPFMV/SPMMV (combination) infected sweet potato cuttings and kept in the glasshouse for two to three months. The same sweet potato plants as used previously for the sources of inoculum were used. After harvesting the 2001/2002 trial, cuttings of all cultivars were taken and kept in the glasshouse at a temperature of 20-30°C and their storage roots were stored to be used as planting material for the second trial. This was done in order to determine the accumulative effect of virus infection over time. In July 2002, both the newly infected and the previously infected sweet potato cultivars and lines were first grafted onto *I. setosa* and monitored for symptoms for six weeks and more. Symptomatic plants were first tested by NCM-ELISA for the presence of viruses and positive plants were selected for further multiplication in August 2002 in seedling trays and kept in the glasshouse at a temperature of 20-30°C. The plants were transplanted to the field in October 2002 in a multiplication block.

Antisera for SPV II and SPV G were introduced later from Biologische Bundesanstalt für Land-u. Braunschweig, Germany. When back testing sweet potato materials from the field trial during the second trial, it was discovered that all plants were also infected with SPV II and SPV G in combination with SPFM and SPFMV/SPMMV. This compelled the renaming of the virus combinations to be as follows, A=SPFMV/SPV II/SPV G, B=SPFMV/SPMMV/ SPV II/SPV G.

6.2.3 Field trial

Cuttings from multiplication blocks were used to establish the trials. The first trial was established in December -January 2001/2002. The second trial was established in January 2003. There were three replicates of each of the three treatments in both trials: 1) A=SPFMV/SPV-II/SPV-G, 2) B=SPFMV/SPMMV/SPV-II/SPV-G, and 3) C=control/healthy plants. Each plot consisted of 30 plants. Cultivars were planted in a randomised block design in both trials. Maize was planted in border rows between each replicate in both trials to prevent the spread of aphids or whiteflies moving to the other replicates.

The first trial was planted in clay loam soil. The herbicides Eptan (3l/ha) was applied before planting and 1.8l/ha of Afulon was applied after planting. Before planting, the soil was fertilised with the following fertilisers at the following rates: 1000kg/ha of 2:3:4 (30%), 150kg/ha of LAN and 125kg/ha of KNO₃. The total amount of nutrients applied was 160kg N, 90kg P and 178kg K. The trial was irrigated with overhead sprinkler irrigation according to crop factors. The total amount of irrigation water applied during the 2001/2002 season was 611mm. The plants had a growth period of five months and were harvested at the end of May 2002.

The second trial was also planted in clay-loam soil. Before planting, the soil was fertilised with the following fertilisers at the following rates: 800kg/ha of 2:3:4 (30%), 300kg/ha of LNA and 125kg/ha of KNO₃. For top dressing, the following fertilisers were used: 125kg/ha of LAN, 125kg/ha of LAN and 125kg/ha of KNO₃-(24). The total amounts of nutrients applied were 136kg N, 80kg P and 144kg K. The trial was also irrigated with overhead sprinkler irrigation according to crop factors. Total irrigation water applied during the 2002/2003 seasons was 690mm. The plants had a growth period of five months and were harvested in mid June 2003. Both trials were planted at a spacing of 1m between the rows and 0.3m between the plants.

6.2.4 Statistical analysis

Data was analysed using the statistical package Genstat. An analysis of variance (ANOVA) was done to obtain the mean values, least significant differences (LSD) and

coefficient of variation (CV%) for the yield variables, namely, percentage cracks, marketable yield, and total yield, average root mass and percentage marketable yield.

6.3 Results

Only percentage cracks, marketable yield and total yield showed significant differences between the treatments and were used to explain the results. The results showed that virus treatments decreased yields significantly compared to the control treatment after only one season of infection and even more so after two seasons of virus build-up. Statistical analysis could not be done to compare the 2001 x2 with 2002 since that would have added another error term. Mean values for yield variables of the first trial (2001) and second trials (2001 x2 and the 2002) are given in Table 6.1.

In the first trial (2001), the marketable yield of cultivars infected with virus treatment A and B was decreased by 15.2t/ha (33%), and 14.4 t/ha (31%) respectively (Table 6.1). The total yield for virus treatment A and B was decreased by 13.8t/ha (21%) and 14.6t/ha (22%) respectively (Table 6.1). On cultivar bases, the greatest yield decrease was 40.8t/ha for marketable yield and 41.6t/ha for total yield. The yield data also showed that most sweet potato cultivars/lines were sensitive to virus treatment A. Only the ARC cultivar Monate and the USA cultivar Excel were tolerant. Likewise most cultivars were also sensitive to virus treatment B. Only the ARC line 1994-5-1, the Taiwanese cultivar CN1656-97 and the local traditional cultivar Natal Red, were tolerant. Prevalence of viruses also increased cracking significantly. The cracking increased by 11.9 % (or an increased of 82% above the control) with virus treatment A and by 8.8 % (61 % above control) with virus treatment B (Table 6.1).

The second trial was conducted to compare the previously infected plants with the newly infected ones and healthy plants. It was proven from this trial that the two virus treatments (A and B) decreased marketable and total yield significantly in both seasons. Virus treatment A and B decreased the marketable yield of the secondary (two seasons of virus build ups-2001x2) infected cultivars by 7.4t/ha (26%) and 10.7t/ha (37.5%) respectively (Table 6.1). Also, the marketable yield of primary infection (newly infected plants-2002) was decreased by 9.1t/ha (30%) and 6.4t/ha (21.2%) with virus treatments A and B respectively (Table 6.1). The total yield of the secondary infections

was decreased by 5.7t/ha (11.5%) and 9.1t/ha (18.3%) with virus treatments A and B respectively (Table 6.1). Primary infections also decreased the total yield by 7.4t/ha (14.8%) and 6.5t/ha (13%) with virus treatment A and B respectively (Table 6.1). Percentage cracks increased significantly with both virus treatments compared to healthy treatments. Percentage cracks increased by 13.5% with virus treatments A and by 11.0% with virus treatment B after two seasons of virus build-up (secondary infection) (Table 6.1). Newly infected plants were also affected by virus infections. Primary infections increased percentage cracks by 10.4% and 8.8% with virus treatment A and B respectively (Table 6.1). The percentage increase in cracks was found to be higher mostly with virus treatment A, after two seasons of virus build-up than the one season. The results therefore show higher occurrence of cracks after a longer period of virus accumulation compared to single season infection. The control treatment also had an average of 23.4% cracks from the secondary infection compared to 21.9% of the primary infection.

Almost all cultivars except Bosbok and Mamphenyane, showed sensitivity to both virus treatments. Cultivar Monate was sensitive to both virus treatments but the marketable yield and total yield of this cultivar was decreased significantly by treatment A after two seasons of virus build-up. Cultivars Mafutha, CN1656-97 and Xushu 18 also showed some sensitivity to virus treatments, especially in terms of cracking. Both virus treatments greatly increased cracks in these cultivars after two seasons of virus build-up. Cracks in Mafutha increased by 30.07% and 23.4% with virus treatment A and B and for Xushu 18, cracks increased by 29.6% and 30.08% with virus treatment A and B respectively. Cultivar CN1656-97 also had an increase in cracks of 44.4% and 20.02% with virus treatment A and B respectively. Virus treatment A did not affect cultivar Excel in either cracks, marketable and total yield, even after two seasons of virus build-up. This cultivar showed some sensitivity towards virus treatment B after two seasons of virus build-up but only marketable yield was significantly affected. Unfortunately it was not possible to reconfirm its tolerance to virus treatment A with primary infection because there was not enough material.

Table 6.1 The effect of virus infection on the yield of sweet potato cultivars

Treatment	Cultivar	Cracks*						Marketable yield						Total yield						Reaction to Treatments
		2001		2001 x 2		2002		2001		2001 x 2		2002		2001		2001 x 2		2002		
		%	Change	%	change	%	change	t/ha	Change	t/ha	change	t/ha	change	t/ha	change	t/ha	change	t/ha	change	
C	Monate	2.9		5.10		12.8		57.5		51.7		46.2		84.1		71.5		68.7		
A	Monate	7.8	4.9	8.03	2.9	6.94	-5.9	57.4	0.0	42.4	-9.2**	44.8	-1.4	76.1	-8.0	59.4	-12.1**	66.7	-2.0	
B	Monate	2.8	-0.1	8.66	3.6	19.04	6.2	41.6	-15.8**	31.1	-20.6**	36.4	-9.8**	50.7	33.4**	48.1	-23.4**	57.4	-11.3**	
C	1994-5-1	13.2		11.3		13.6		65.4		44.2		35.3		81.9		57.0		47.8		
A	1994-5-1	26.4	13.2**	24.6	13.3	8.0	-5.7	26.6	-40.8	29.7	-14.5**	45.3	10.0**	40.3	-41.6	45.2	-11.8**	62.2	14.4**	
B	1994-5-1	9.0	-4.2	15.2	3.9	22.2	8.6	64.2	-1.2	33.1	-11.11*	36.8	1.5	75.6	-6.3	52.4	-4.6	58.7	10.9	
C	Ndou	0.0		2.27		0.0		69.7		50.3		44.6		82.7		63.3		68.0		
A	Ndou	7.6	7.6	6.06	3.8	23.89	23.9**	53.1	16.5**	44.6	-5.6	28.4	-16.2**	67.4	-15.3**	62.7	-0.6	51.3	16.7**	
B	Ndou	0.0	0.0	6.14	3.9	8.6	8.6	48.0	-21.6**	32.4	-17.9**	43.6	-0.9	6.05	-22.2**	51.8	-11.6**	60.0	-8.0	
C	Bosbok	3.8		2.8		5.0		53.6		22.3		39.6		62.8		30.1		49.8		
A	Bosbok	5.7	2.0	0.0	-2.8	0.0	-5.0	34.3	-19.4**	30.1	7.7	12.0	-27.6**	44.9	-17.9**	37.1	7.0	18.2	-13.6**	
B	Bosbok	0.0	-3.8	0.0	-2.8	0.0	-5.0	38.1	-15.6**	21.3	-1.0	22.7	-16.9**	46.3	-16.5**	35.0	4.9	32.7	17.1**	
C	CN1656-97	19.1		26.26		12.80		26.8		18.5		19.4		50.0		36.0		33.4		
A	CN1656-97	53.3	34.2**	70.69	44.4**	6.96	-5.8	8.4	-18.4**	1.1	-17.4**	1.0	-18.5**	41.2	-8.8	29.9	-6.1	31.5	-1.9	
B	CN1656-97	28.9	9.8	46.49	20.02**	19.04	6.2	22.6	-4.1	7.5	-11.1**	0.7	-18.8**	44.2	-5.8	31.0	-5.1	20.8	-12.5**	
C	Excel	10.6		23.10		-	-	31.8		31.1		-	-	42.9		48.6		-	-	
A	Excel	14.5	4.0	16.35	-6.8	-	-	36.9	5.2	27.6	-3.5	-	-	50.5	7.6	50.6	2.0	-	-	
B	Excel	38.6	28.0**	36.21	13.1	-	-	16.4	-15.3**	17.1	-14.0**	-	-	38.0	-5.0	38.3	-10.3	-	-	
C	Mafutha	6.3		35.29		26.52		38.5		18.0		24.5		52.9		35.6		35.5		
A	Mafutha	18.5	12.2**	66.03	30.07**	41.43	14.9**	32.5	-6.1	2.8	-15.2**	12.5	-12.0**	47.2	-5.7	21.2	-14.4**	28.9	-6.6	
B	Mafutha	26.0	19.7**	58.72	23.4**	27.12	0.6	12.6	-26.0**	2.3	-15.8**	17.2	-7.3	23.1	-29.8**	15.9	-19.7**	29.9	-5.7	
C	Natal Red	48.8		65.40		76.90		23.9		6.7		2.3		62.3		55.3		45.6		
A	Natal Red	58.2	9.4	78.51	13.1	78.27	1.4	9.6	-14.2**	0.9	-5.8	1.0	-1.3	45.4	-16.9	36.9	-18.4**	34.5	-11.1	

6.4 Discussion and conclusion

Sweet potato viruses have been associated with the deterioration in the quality and yield of sweet potatoes in South Africa for many years (McClean and Klesser, 1947). SPFMV and SPMMV have been identified and reported as the most important viruses of sweet potatoes in South Africa (Jericho and Thompson, 2000). Sweet potato viruses seem to be the most important problem in sweet potato production worldwide. In our findings, almost all cultivars except Mamphenyane and Bosbok showed sensitivity to virus treatments based on reduction in marketable and total yield. Differences were also found in the reaction of some cultivars with different virus combinations. Cracks were also induced in some sensitive cultivars by virus treatments. The high percentage increase in cracks of the 2001 infection, compared to the 2002 infection could be due to the fact that the trial was planted earlier, and also in good soil. A higher percentage increase in cracking was prevalent with virus treatment A (SPFMV, SPV II and SPV G) than with B (SPFMV, SPMMV, SPV II and SPV G). This could mean that the presence of SPMMV had a suppressing effect on virus treatment A. Viruses did not influence the average root mass and percentage marketable yield significantly.

However, among the different variables the trend was that cultivars in which the virus concentration had built up for two seasons showed a greater decrease in yield and increase in cracks than the newly infected ones. Virus treatments A and B significantly decreased both marketable yield, percentage marketable yield and resulted in an increase of cracks in some sensitive sweet potato varieties.

Although yield loss and quality of tubers was determined with only two virus treatments, it was evident that viruses, when present, lowered the quality of sweet potatoes by increasing cracks and reducing marketable and total yield. This is the first extensive report to show the effect of viruses on sweet potato yield in South Africa.

Sweet potato seems to have a potential in future markets because of its importance as food security as well as a cash crop. Its nutritional value, especially of orange-fleshed cultivars because of high beta-carotene content (Pro-vitamin A) is important to humans because vitamin A deficiency is a serious public health problem in South Africa (SAVACG, 1996). Based on these facts, increasing sweet potato production is of paramount importance. Different agronomic aspects need to be integrated in order to sustain high yielding cultivars. From this study, it is evident that viruses are the biggest threat to sweet potato production. It is crucial to prevent the spread of viruses, and farmers, most importantly small-scale farmers, need to be equipped with the knowledge of producing sweet potato and how to combat viruses. This lack of valuable information has resulted in farmers using the infected planting materials for a longer time and sharing among each other (Chapter 3, this thesis). Farmers need to be shown the importance of using virus free planting materials and to change planting materials after using them for a period of one or two years. Farmers, both commercial and small-scale, need to take into consideration the importance of nursery blocks (with clean materials) situated far away from the production areas to prevent the spread of viruses. The other effective way of overcoming yield decline is crop rotation. This cultural practice was recommended because it breaks the cycle of pest and disease build-up (Lian, 2000). The development of resistant cultivars and sanitation could also contribute in the control of viruses resulting in an increase in sweet potato production.

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Chapter 7

General discussion

During the survey on root crops such as sweet potato and cassava conducted in South Africa in 1996/1997 (van der Mescht *et al.*, 1997), sweet potato was found to be the most important root crop in some areas and the second most important crop after maize (van der Mescht *et al.*, 1997; Thompson *et al.*, 1999). The aim of the present study was to determine the importance of sweet potato to rural households and the constraints that limit their production. The most important objective was to determine the incidence of pest and diseases, most importantly, viruses in terms of their geographical distribution. Most farmers regarded sweet potato as a traditional crop and it was grown as a food source for the family. Although yield obtained was to some extent low, it was indicated that sweet potato was also used for income-generation. The majority of rural farmers (96%) did not know what sweet potato viruses were and how they spread. The tradition of using the same planting material year after year was highly practised by most rural farmers and plant protection measures were seldom practised. The other main constraints to sweet potato production were pests such as moles, weevils and caterpillars.

The survey of pest and disease incidence during the 1997/1998 and 2001/2003 indicated that the prevalence of diseases and pests varied between provinces and was influenced by climatic factors. The average virus incidence was found to be very low for the country. This confirms that virus symptoms are rarely seen in fields of sweet potatoes in South Africa. Although virus symptoms were rarely observed in the field during surveys, variations in symptom expression were found when samples were indexed to *I. setosa*. Analysis by NCM-ELISA, DAS-ELISA and TAS-ELISA demonstrated that approximately 80% of samples were infected with viruses. In this study, nine viruses were found to infect sweet potatoes in South Africa, namely: SPFMV, SPMMV, SPLV, SPCFV, SPMSV, SPV G, SPV II, CMV and SPCSV (EA and WA strains). Viruses were

commonly found in mixed infections and rarely as a single infection. This is the first report of the following viruses: SPV II, SPV G, SPCSV (EA and WA strains) and SPMSV, SPCFV and possibly CMV infecting sweet potatoes in South Africa. SPFMV was found in 63% of samples tested, confirming that it is the most prevalent virus of sweet potato in South Africa. SPV G and SPV II were detected in 28 and 26% of samples respectively. Following SPFMV, these two viruses have the potential of becoming a threat to production of sweet potato in South Africa. It can also be speculated that South Africa could be the only country having a high incidence of these two viruses. SPMV is still an important virus of sweet potato and its occurrence in few samples could be influenced by weather conditions limiting the distribution of its vector, whiteflies.

Occurrence of potyviruses, SPFMV, SPV II and SPV G, in such high percentages calls for immediate and effective means of controlling them. It was also found that viruses significantly decreased both marketable and total yield and also increased the amount of cracking in storage roots. Wild *Ipomoea* spp. are also reservoirs of viruses and together with volunteer plants, they should be eradicated as soon as they are seen in the field. Rouging is an effective tool to eliminate viruses. The poor expression of symptoms on infected sweet potato under field condition makes this form of virus control difficult. It is not possible for farmers to familiarise themselves with virus symptoms and rouging of infected plants cannot be efficiently practised. Fortunately, SPCSV only occurs sporadically and its control can be based on preventing it from spreading to provinces where it is not yet prevalent.

The use of virus free planting material was found to give higher yields compared to infected ones. The occurrence of a high number of negative samples in Kwazulu Natal Province is evidence that the use of virus free material is important. Kwazulu Natal is one of the provinces that the ARC-Roodeplaat targeted when they started with the initiative of providing rural farmers with healthy and improved cuttings as part of empowering them so that they can start their own nursery blocks of clean cuttings. Breeding of resistant cultivars against potyviruses will have a tremendous contribution in reducing

sweet potato viruses. The development of broad-spectrum resistance using transgenic sweet potato will also help increase yield and quality of virus sensitive cutlivars.

7.1 References

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Appendices

Chapter 3

Appendix 3.1 A questionnaire form that was used during the baseline survey interviews of farmers in the communities

INTRODUCTION

The survey aims at collecting information on the current status of sweet potato production in target communities in order to be able to measure the effects of research projects after a period of two years. The main thrust being to increase the production and utilisation of sweet potato in South Africa.

Name of interviewer

Production region (Province)

Date of interview

Respondent name (group)

Name of production locality

[Where applicable, mark by “x”. If more than one option is applicable, mark more than one]

Biographic profile of respondent

1. Gender: Male Female

2. Age of respondent

3. What is your major livelihood/source of income?

Agricultural activities Other Employment (Please specify)

Other

.....
.....

Agricultural profile

4. If various agricultural activities are practiced, please rank these activities according to their economic importance.

1	4
2	5
3	6

5. What is the size of land available for agricultural activities?.....unit.....

C. Importance and role of sweet potato

6. Which crops do you rank as the 5 most important for household food security and for cash sale as income generation? Ranking (1 - 5)

Crop	Food	Cash
Grain crops
Irish potato
Sweet potato
Fruits
Vegetables
Other

7. Why do you choose to produce sweet potato? (i.e. tradition, good at it, right climate, soil, good income, crop rotation? etc.)

.....

.....

.....

8. For what purpose do you use your sweet potato crop?
 Sell to generate income Own consumption Both

9. If you are selling your sweet potato crop to what extent does sweet potato contribute to your household income?
 Small Average Large
 Percentage contribution%

10. What is the annual income of your household from sweet potato?
 < R100 R100 - R400 R500 - R900 More (specify)

11. How often do you eat root crops?
 Less than once a week Once a week
 Twice a week More (specify)

.....

12. Where do you get sweet potato to eat?
 Local market Hawkers
 Shop Other

13. How do you eat it?
 As vegetable in main meal Cold with tea
 Half of main meal Chips
 Leaves as vegetable Baking products

Other

D. Sweet potato cultivation practices



14. How many years have you been actively producing sweet potato? (years of experience in SP production)

15. Have you ever received formal training in the cultivation of sweet potato?

Yes No

If yes, from whom and when did you receive training?

.....

17. What is the average size of land you with sweet potato?
 ha/morgans/number of plots of m x m.

18. What do you use to plough/cultivate your land?

Hand hoe Animal traction
 Tractor Other.....

19. What is the plant spacing applied for sweet potato cultivation?

spacing.....cm between plants in rows
 spacing.....cm between rows

20. Are you irrigating your sweet potato crop? Yes No

If yes, specify the type of irrigation used and how often

.....

21. Are you applying fertiliser? Yes No

If yes, please specify the type and quantity of fertiliser applied

.....

22. What type of beds do you plant on? Flat Ridges Mounds

23. What type of weeding practices are implemented?

Hand Hoe Other.....

24. Are you practising plant protection? Yes No

If yes, what method or type of protection is used?.....



25. What are the production costs for sweet potato associated with each of the activities in sweet potato production?

Labour	R...../unit.....
Planting material	R...../unit.....
Fertilisers	R...../unit.....
Pest and disease management	R...../unit.....
Weeding	R...../unit.....
Irrigation	R...../unit.....
Other:.....	R...../unit.....
.....	R...../unit.....
.....	R...../unit.....

Total cost? R.....
Cost / unit R...../Unit.....

26. Are you practising intercropping with regard to sweet potatoes?

Yes No If yes, please list other crops
.....

27. Do you rotate sweet potato crop with others ?

Yes No
If yes , what crop ?.....

28. What yield do you get from sweet potato?

..... ton or kg per area
..... crates per area (Size of crates:)
..... buckets per area (Size of buckets:)

29. If you are selling your excess produce, please indicate to whom you are selling your produce.

Neighbours Door to door Local market
Fresh produce market Other, please specify
.....

30. What is the average price obtained for the produce sold?

.....R perunit.....

E. Information with regard to cutting material

31. Where do you obtain your planting material from?

Own	<input type="checkbox"/>	Relatives	<input type="checkbox"/>
Neighbours/Friends	<input type="checkbox"/>	Shop	<input type="checkbox"/>
Vine growers	<input type="checkbox"/>	Other.....	

IF NOT MAKING OWN MATERIAL

32. Is it easy to get hold of planting material? Yes No

If not, give reason

33. How many farmers produce and sell cuttings in this area?

IF MAKING OWN PLANTING MATERIAL

34. If you grow your own material, how do you preserve your material (during winter time)?

.....
.....

35. Did you ever receive any formal training in making sweet potato planting material?

Yes No

36. If yes, from whom and when did you receive the training?

.....
.....

F. Virus questions

37. Do you know (understand) what virus disease are ?

Yes No

38. (If yes), How did you acquire the knowledge ?

Friends

Extension workers

ARC

Other.....

(If no) Show pictures of sweet potato plant with virus symptoms if there are no virus-diseased plants in the field/ plants showing symptoms.

39. Have you ever seen these symptoms in your field ?

Yes No

40. If no, give a brief explanation

41. (If yes) What do you call the symptoms? (Local name).....

42. What causes or spreads the disease?

Planting material

Insects (aphids, whiteflies)

Don't know

Other.....

43. Which season of the year is the disease/symptoms more severe?

Show pictures of insects (aphids, whiteflies)

44. Do you regularly see these insects in your field/plot ?
Yes No

F. Varietal choice of root crops

The objective of the question is to establish what characteristics producers are valuing as important in choosing a variety to plant and the reasons why they prefer certain characteristics. Use real roots or use pictures to visualise the different characteristics. Try to identify farmers' criteria for evaluating varieties. Mark those in ranking matrix. Then let them rank the criteria according to importance. Do not force interview into this set of criteria and try to use their own wordings.

Possible phrase for question: What characteristics do you look for in a sweet potato cultivar when deciding on a cultivar to plant and give a reason for the specific choice? (Indicate with ✕ the criteria chosen in the matrix and state reason for choice)

Value these criteria indicated on a scale of 1-5 on the level of importance when choosing a variety (1 = very important; 5 = just of little importance)

45. Criteria applied in cultivar choice matrix

Criteria:	✕	Level of importance	Reasons for preference of characteristic
<i>Foliage</i>			
Colour of vines (sweet potato)			
Leaf shape (sweet potato)			
Length of vines (sweet potato)			
<i>Roots</i>			
Root shape			
Root skin colour			
Root flesh colour			
Root yield			
Root size			
Occurrence of defects: Veins Grooves Cracks Constrictions			
<i>Other characteristics</i>			
Taste			
Storage quality			
Drought tolerance			
Disease resistance			
Cooking quality			
Consumer demand			
Other:			

46. How many varieties do you plant?
Sweet potato: varieties.....

Name	Description

47. What do you consider good varieties? (E.g. specific colour or shape; good yield - use characteristics in ranking matrix as guideline)

.....
.....

48. Which characteristics are needed to improve current varieties used?

.....
.....

49. Are you interested in obtaining new sweet potato varieties for cultivation?

Yes No

Reason for answer.....

G. Major constraints

50.. What are the main problems you encounter in producing sweet potato?

.....
.....

Thank you for your valuable time and inputs

Chapter 4

Appendix 4.1 Pests and diseases of sweet potato-survey results

Mpumalanga

Ratings	Areas surveyed																					
	GW1	GW2	GW3	GW4	GW5	GW6	B1	B2	B3	B4	B5	B6	T1	T2	T3	HV1	HV2	HV3	HV4	HV5	HV6	AVG
LS/B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.04	0	0	0.00
SS/B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
Viruses	0.19	0.65	0.46	0.62	0.55	0.23	0.75	0.6	0.31	0	0	0.11	0.35	0.4	0.35	3	0.58	0.54	0.35	0.66	0.35	0.53
ID	0.42	0.35	0.69	0.15	0	0	0.25	0.6	0.85	0.96	0.25	0.5	0.55	0.32	0.88	3.7	0.27	0.38	0.38	0.27	0.12	0.57
Root rot	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wilt	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

LS/B-Leaf spot and blight; SS/B-Stem spot and blight & ID-Insects damage

GW-Gutjwa, B-Beverbreed, T-Tonga, HV-Hazyview

Limpopo

Ratings	Areas surveyed										
	Tshid 1	MIW1	MIW2	Tshiombo	MP	RS	GS	DDE1	DDE2	ARN1	AVG
LS/B	0.03	0.14	0	0	0.1	0	0	0	0	0.05	0.03
SS/B	0	0	0	0	0	0	0	0	0	0	0
Viruses	0	0	0	0	0.1	0	0	0	0	0	0.01
ID	0.85	0.4	0.7	0.58	1.4	1.17	1.4	0.8	0.9	0.33	0.85
Root rot	0	0	0	0	0	0	0	0	0	0	0
Wilt	0	0	0	0	0	0	0	0	0	0	0

LS/B-Leaf spot and blight; SS/B-Stem spot and blight & ID-Insects damage

Tshid-Tshidane, MIW-Mianzwi, MP-Maseromola Park, RS-Riverside, GS-Greenside, DDE-Dingleydale and ARN-Arthurstone

Eastern Cape

Ratings	Areas surveyed													AVG
	A1	TM1	TM2	TM3	LM1	MK1	PA2	NC1	NC2	BT1	BT2	BT3	MQ1	
LS/B	2.5	0	0	0	0	0	0.14	0	0.05	0.01	0	0	0	0.21
SS/B	0	0	0	0	0	0	0	0	0	0.01	0	0	0	0.00
Viruses	0	0.03	0	0	0	0	0	0	0	0.01	0.1	0	0.7	0.06
ID	1.5	4	1.9	0.1	3.8	0.1	3.5	3	4	0.09	2.4	2.2	1.5	2.2
Root rot	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Wilt	0	0	0	0	0	0	0	0	0	0	0	0	0	0

LS/B-Leaf spot and blight; SS/B-Stem spot and blight & ID-Insects damage

A-Alice, TM-Thombo, LM-Lower Mpako, MK-Mpako, PA-Port Alfred, NC-Ncambendlana, BT-Buthurst and MQ-Mqanduli

Western Cape

Ratings	Areas surveyed										AVG
	S1	S2	S3	FH1	E1	E2	E3	GT1	GT2	PD1	
LS/B	0.1	0.57	0.58	0	0.12	0	1.4	0.6	2.13	0.15	0.57
SS/B	0.1	0	0	0	0	0	0	0	0	0	0.01
Viruses	0	0	0	0	0	0	0.2	0.43	2.27	0.15	0.31
ID	1	1.33	4.17	1.7	1.46	1.2	5.0	1.87	1.63	1.92	2.13
Root rot	0	0	0	0	0	0	0	0	0	0	0
Wilt	0	0	0	0	0	0	0	0	0	0	0

LS/B-Leaf spot and blight; SS/B-Stem spot and blight & ID-Insects damage

S-Saron, FH-Fremersheim, E-Ebeneser, GT-Goedverwacht and PD-Pacalsdorp

Average for the whole country

Ratings	Average			Average
	GT	KZN	NW	
				0.170
LS/B	0.46	0.54	0	0.002
SS/B	0	0	0	0.274
Viruses	0.42	0	0.15	1.388
ID	3.8	3.54	2	0
Root rot	0	0	0	0
Wilt	0	0	0	

LS/B-Leaf spot and blight; SS/B-Stem spot and blight & ID-Insects damage

GT-Gauteng, KZN-Kwazulu Natal and NW-North West Provinces

Chapter 5

Appendix 5.1 ELISA results of sweet potato samples from seven provinces of South Africa (LP-Limpopo, MP-Mpumalanga, GT, Gauteng, WC-Western Cape, EC-Eastern Cape, KZN-Kwazulu Natal and NW-North West)

a. SPV G not tested

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
2MIW 7	L.P	Mianzwi	VC	LP	+	+	-	-	-	-	-	-	-		-	-
2 MIW 6	L.P	Mianzwi	LD	N	-	-	-	-	-	-	-	-	+		-	-
2 MIW 8	L.P	Mianzwi	VC	LD	+	-	-	-	-	-	-	-	?		-	-
2 MIW 9	L.P	Mianzwi	VC	N	+	-	-	-	-	-	-	-	-		-	-
C MIW 2	L.P	Mianzwi	VC/CS	N	+	-	-	-	-	-	-	-	-		-	-
MIW 2	L.P	Mianzwi	VC/F	N	+	-	-	-	-	-	-	-	+		-	-
MIW 3	L.P	Mianzwi	VC	N	+	-	-	-	-	-	-	-	+		-	-
MIW 4	L.P	Mianzwi	VC-severe	N	-	-	?	-	-	-	-	-	-		+	-
MIW 5	L.P	Mianzwi	CS/M	N	+	-	-	-	-	-	-	-	+		-	-
MIW 6	L.P	Mianzwi	VC	N	+	-	-	-	-	-	-	-	-		-	-
MIW 8	L.P	Mianzwi	VC	N	+	-	-	-	-	-	-	-	-		-	-
MIW 9	L.P	Mianzwi	VC	N	+	-	-	-	-	-	-	-	+		-	-
MIW 10	L.P	Mianzwi	VC/CS	N	+	-	-	-	-	-	-	-	+		-	-
MIW 11	L.P	Mianzwi	VCLD	N	+	-	-	-	-	-	-	-	+		-	-
MIW 12	L.P	Mianzwi	VC	N	+	-	-	-	-	-	-	-	+		-	-
MIW 13	L.P	Mianzwi	VC	N	-	-	-	-	-	-	-	-	+		-	-
MIW 14	L.P	Mianzwi	VC	N	+	-	-	-	-	-	-	-	+		-	-
MIW 15	L.P	Mianzwi	VC	N	+	-	-	-	-	-	-	-	-		+	-
MIW 16	L.P	Mianzwi	VC	N	?	-	-	-	-	-	-	-	+		-	-
MIW 17	L.P	Mianzwi	VC	N	+	+	-	-	-	-	-	-	-		+	-
M MIW 1	L.P	Mianzwi	VC	N	+	-	-	-	-	-	-	-	-		-	-
M MIW 4	L.P	Mianzwi	VC/severe	N	+	+	-	-	-	-	-	-	+		-	-
Tshidane 1/11	L.P	Tshidane	CV/LP	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/5	L.P	Tshidane	VC/S	VC	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/46	L.P	Tshidane	VC/LD	VC	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/7	L.P	Tshidane	VC/LD	N	+	-	-	-	-	-	-	-	+		-	-

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
Tshidane 1/48	L.P	Tshidane	VC	VC	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/41	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	+		-	-
Tshidane 1/1	L.P	Tshidane	VC/LD	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/18	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/24	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/12	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/16	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/19	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/23	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/40	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/44	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/34	L.P	Tshidane	VC/F	N	+	-	-	-	-	-	-	-	+		-	-
Tshidane 1/38	L.P	Tshidane	VC	CS	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/16	L.P	Tshidane	VC/CS	VC/LP	+	-	-	-	-	-	-	-	+		-	-
Tshidane 1/39	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/3	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	+		-	-
Tshidane 1/14	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	+		-	-
Tshidane 1/4	L.P	Tshidane	VC	N	-	-	-	-	-	-	-	-	+	+	-	-
Tshidane 1/22	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/27	L.P	Tshidane	VC/CS	N	+	-	-	-	-	-	-	-	+		-	-
Tshidane 1/30	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/28	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-		-	-
Tshidane 1/33	L.P	Tshidane	VC	CS	-	-	-	-	-	-	-	-	+		-	-
Tshidane 1/21	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	+		-	-
Tshidane 1/29	L.P	Tshiombo	VC	N	+	-	-	-	-	-	-	-	-		-	-
1 Tshiombo 1/1	L.P	Tshiombo	VC	N	-	+	-	-	-	-	-	-	-		-	-
1 Tshiombo 1/9	L.P	Tshiombo	DM	N	-	-	-	-	-	-	-	-	+		-	-
1 Tshiombo 1/15	L.P	Tshiombo	VC/LP/VB	N	+	-	-	-	-	-	-	-	-		-	-
1 Tshiombo 1/13	L.P	Tshiombo	LD	N	+	-	-	-	-	-	-	-	+		-	-
1 Tshiombo 1/10	L.P	Tshiombo	VC	N	+	-	-	-	-	-	-	-	-		-	-
1 Tshiombo 1/3	L.P	Tshiombo	LD/VC	N	+	-	-	-	-	-	-	-	-		-	-
1 Tshiombo 1/8	L.P	Tshiombo	VC	N	+	-	-	-	-	-	-	-	-		-	-

Isolates name	Province	Region/Subregion	Symp on I set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
Tshiombo 1/45	L.P	Tshiombo	VC/F	N	+	-	-	-	-	-	-	-	+		-	-

Symptoms→VC=vein clearing, CS=chlorotic spots, LP=leaf puckering, F=feathering, VB=vein banding, DM=diffuse mottle, LD=leaf distortion, M=Mosaic, S=stunting & N=no symptoms
+=Virus detected, -=Virus not detected, ?=Faint reaction/unsure, *=positive when tested with sweet potato material from the field.

b. All 12 viruses were tested

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
B1-5	MP	Beverbreed	VC/DM	N	+	-	+?	+	-	-	-	-	++	-	-	-
B1-6	MP	Beverbreed	DM/VC	N	+	+	-	-	-	-	-	-	+	++	-	-
B1-7	MP	Beverbreed	DM	N	+	-	-	-	-	-	-	-	++	-	-	-
B1-8	MP	Beverbreed	VC/CS	N	+	-	-	-	-	-	-	-	+	-	-	-
B1-3	MP	Beverbreed	VC	N	+	-	-	-	-	-	-	-	++	-	-	-
B1-1	MP	Beverbreed	VC	N	+	-	-	-	-	-	-	-	++	-	-	-
B1-4	MP	Beverbreed	DM/VC	N	+	-	-	-	-	-	-	-	++	-	-	-
B2-4	MP	Beverbreed	DM/M	N	+	-	+?	-	-	-	-	-	+	+	-	-
B2-8	MP	Beverbreed	VC/DM	N	+	-	-	-	-	-	-	-	+	-	-	-
B2-7	MP	Beverbreed	LD/DM	N	++	-	-	-	-	-	-	-	-	-	-	-
B2-6	MP	Beverbreed	VC/DM	N	+	-	+	-	-	-	-	-	-	++	-	-
B3-2	MP	Schuzendal	VC	N	+	-	-	-	-	-	-	-	+	-	-	-
B3-6	MP	Schuzendal	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
B3-5	MP	Schuzendal	VC	N	+	-	+	-	-	-	-	-	+	-	-	-
B4-1	MP	Beverbreed	CS-older leaves	N	-	-	+	+	-	-	-	-	+	+	-	-
B4-6	MP	Beverbreed	DM/CS/F	N	+	++	-	-	-	-	-	-	+	-	-	-
B4-4	MP	Beverbreed	DM	N	++	-	-	-	-	-	?	-	-	++	-	-
B4-3	MP	Beverbreed	VC/DM	N	+	-	+	+?	-	-	-	-	-	+++	-	-
B4-8	MP	Beverbreed	LD/DM	N	+	-	+	-	-	-	-	-	+	++	-	-
B4-7	MP	Beverbreed	VC/DM	N	+	-	-	-	-	-	-	-	++	+	-	-
B5-4	MP	Beverbreed	VC/DM	N	+	-	-	-	-	-	-	-	-	+	-	-
B5-3	MP	Beverbreed	VC	N	+	-	+	-	-	-	-	-	-	-	-	-
B5-2	MP	Beverbreed	DM	N	+	-	-	-	-	-	-	-	+	-	-	-
B5-1	MP	Beverbreed	VC/DM	N	+	-	-	-	-	-	+	-	+	++	-	-
B5-5	MP	Beverbreed	VC/DM	N	+	-	-	-	-	-	-	-	++	-	-	-
B5-6	MP	Beverbreed	VC/F	N	-	+	-	-	-	-	-	-	?	?	-	-
B5-7	MP	Beverbreed	VC	N	+	-	-	-	-	-	-	-	-	-	-	-
B6-1	MP	Beverbreed	DM	N	+	+	+?	-	-	-	-	-	+	+	-	-
B6-5	MP	Beverbreed	VC	N	+	-	-	-	-	-	-	-	-	-	-	-
B6-4	MP	Beverbreed	VC/CS	N	+	-	+	-	-	-	-	-	+	-	-	-
B6-2	MP	Beverbreed	VC/DM/CS	N	+	+	-	-	-	-	-	-	+	+	-	-

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
GW1-6	MP	Gutjwa	CS	N	-	-	-	-	-	-	-	-	+	-	-	-
GW1-5	MP	Gutjwa	VC/DM	N	+	-	+?	-	-	-	-	-	++	+	-	-
GW1-4	MP	Gutjwa	VC	N	+?	-	-	-	-	-	-	-	+	?	-	-
Gw1-1	MP	Gutjwa	DM	N	+	-	-	-	-	-	-	-	++	-	-	-
GW5-5	MP	Gutjwa	CS	N	-	-	-	-	-	-	-	-	-	+	-	-
GW5-6	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
GW5-7	MP	Gutjwa	VC/DM/LP	N	++	-	-	-	-	-	-	-	+	++	-	-
G3-10	MP	Gutjwa	VC/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
G3-9	MP	Gutjwa	VC/DM/M	N	+	-	-	-	-	-	-	-	+	++	-	-
G3-3	MP	Gutjwa	VC/CS	N	+	-	-	-	?	-	-	-	+	-	-	-
G4-1	MP	Gutjwa	VC/	N	+	-	-	-	-	-	-	-	+?	-	-	-
G4-2	MP	Gutjwa	VC/CS	N	+	-	-	-	-	-	-	-	+	-	-	-
G4-3	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	+?	-	+	+	-	-
G4-4	MP	Gutjwa	VC/CS	N	+	-	-	-	?	-	-	-	+	+	-	-
G4-5	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	++	+	-	-
G4-6	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	-	+	-	-
G6-2	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
G6-3	MP	Gutjwa	CS/VC	N	+	-	-	-	-	-	-	-	+	-	-	-
G6-7	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	+	-	-	-
G6-5	MP	Gutjwa	CS	N	-	-	-	-	-	-	-	-	+	+	-	-
G6-4	MP	Gutjwa	VC/CS	N	+	-	-	-	-	-	-	-	++	-	-	-
G5-8	MP	Gutjwa	VC/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
G5-9	MP	Gutjwa	VC/DM/M	N	++	-	+	+	-	-	-	-	-	-	-	-
G5-10	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
G5-12	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	++	++	-	-
G5-13	MP	Gutjwa	VC/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
G5-15	MP	Gutjwa	DM	N	+	-	-	-	-	-	-	-	-	+	-	-
G5-14	MP	Gutjwa	DM	N	+	-	-	-	-	-	-	-	+	-	-	-
G5-16	MP	Gutjwa	VC/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
G5-5	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	+	-	-	-
G5-4	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
G5-3	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
G5-2	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
G5-1	MP	Gutjwa	DM	N	+	-	-	-	-	-	-	-	+	+	-	-
G5-6	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
G5-7	MP	Gutjwa	VC/DM/F	N	+	-	-	-	-	-	-	-	+	-	-	-
HV1-1	MP	Gutjwa	VC	VC	+	-	-	-	-	-	-	-	+	-	-	-
HV1-2	MP	Hazyview	DM/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
HV1-4	MP	Hazyview	VC/CS	N	+	-	-	+	-	-	-	-	+	-	-	-
HV1-6	MP	Hazyview	VC	N	+	-	+	+	-	-	-	-	-	-	-	-
HV1-7	MP	Hazyview	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
HV1-8	MP	Hazyview	VC/CS	VC	+	-	-	-	-	-	-	-	+	+	-	-
HV1-9	MP	Hazyview	VC	N	+	-	-	-	-	-	-	-	?	-	-	-
HV1-10	MP	Hazyview	VC/DM	CS	+	-	+	-	-	-	-	-	-	-	-	-
HV2-5	MP	Numbi	VC/DM	N	+	-	+	+	-	-	-	-	++	+	-	-
HV2-3	MP	Numbi	VC/CS	N	+	-	-	-	-	-	-	-	-	+	-	-
HV2-6	MP	Numbi	VC	N	+	-	-	-	-	-	-	-	+	++	-	-
HV2-7	MP	Numbi	VC/CS	N	++	-	+	-	-	-	-	-	+	++	-	-
HV2-4	MP	Numbi	VC	N	-	-	-	-	-	-	-	-	-	++	-	-
HV2-8	MP	Numbi	VC/DM	N	+	-	-	-	-	-	-	-	-	++	-	-
HV3-3	MP	Hazyview	VC/DM	N	+	-	-	-	-	-	-	-	-	+	-	-
HV3-1	MP	Hazyview	VC/DM/LP	N	+	-	-	-	-	-	+	-	-	++	-	-
HV4-1	MP	Burgerhall	VC/CS	N	+	-	-	-	-	-	-	-	++	-	-	-
HV4-3	MP	Burgerhall	VC/DM	N	+	-	?	-	-	-	-	-	+	+	-	-
HV4-5	MP	Burgerhall	DM	N	+	-	-	-	-	-	-	-	-	+	-	-
HV6-1	MP	Ntsikazi	VC/DM	VC	+	-	-	-	-	-	-	-	+	+	-	-
HV6-5	MP	Ntsikazi	VC/DM	VC	+	-	?	-	-	-	-	-	+	-	-	-
HV6-4	MP	Ntsikazi	VC/CS	N	+	-	-	-	-	-	+	-	+	+	-	-
HV6-13	MP	Ntsikazi	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
HV6-11	MP	Ntsikazi	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
HV6-10	MP	Ntsikazi	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
HV6-14	MP	Ntsikazi	VC/DM	N	+	-	-	-	-	-	-	-	++	++	-	-
HV7-2	MP	Hazyview	VC/DM-severe	N	++	-	+	-	-	-	-	-	+	++	-	-
HV7-3	MP	Hazyview	VC/DM/CS	N	++	-	-	-	-	-	-	-	+	++	-	-

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
HV7-4	MP	Hazyview	VC/DM	N	+	-	-	-	-	-	-	-	++	-	-	-
T1-4	MP	Tonga	DM/CS-mild	N	+	+	-	-	-	-	-	-	-	++	-	-
T1-8	MP	Tonga	VC/DM	N	+	-	-	-	-	-	-	-	+	++	-	-
T1-7	MP	Tonga	VC/DM	N	+	-	-	-	-	-	-	-	+	-	-	-
T1-9	MP	Tonga	VC/CS	N	+	-	-	-	-	-	+	-	-	-	-	-
T1-5	MP	Tonga	DM	N	+	-	-	-	-	-	-	-	+	-	-	-
T1-3	MP	Tonga	DM/CS	N	+	-	-	-	-	-	-	-	+	++	-	-
T1-2	MP	Tonga	VC/CS	N	+	+	-	-	-	-	-	-	-	++	-	-
T1-6	MP	Tonga	VC/DM	N	+	-	-	-	-	-	-	-	+	++	-	-
T1-1	MP	Tonga	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
T2-3	MP	Tonga	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
T2-2	MP	Tonga	DM/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
T2-7	MP	Tonga	VC/DM-severe	N	++	-	-	-	-	-	-	-	++	-	-	-
T2-4	MP	Tonga	VC	N	+	-	-	-	-	-	-	-	++	++	-	-
T3-5	MP	Tonga	DM/VC/M	N	+	-	-	-	-	-	-	-	-	++	-	-
T3-4	MP	Tonga	VC/DM	N	+	-	-	-	-	-	+	-	-	++	-	-
T3-3	MP	Tonga	VC/DM	N	+	-	-	-	-	-	-	-	-	+	-	-
T3-9	MP	Tonga	VC/DM/CS	N	++	-	+	-	-	-	+	-	-	+	-	-
T3-8	MP	Tonga	VC/DM	N	+	-	-	-	-	-	-	-	-	-	-	-
GW2/03/1/1	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	+	++	-	-
GW2/03/1/2	MP	Gutjwa	VC/DM/CS	CS	+	-	-	-	-	-	-	-	++	++	-	-
GW2/03/1/3	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	-	++	-	-
GW2/03/2/1	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	++	++	-	-
GW2/03/2/2	MP	Gutjwa	VC/DM	N	+	-	+	-	-	-	-	-	+	++	-	-
GW2/03/2/3	MP	Gutjwa	CS/DM	N	+	-	-	-	-	-	-	-	-	+	-	-
GW2/03/2/4	MP	Gutjwa	VC/CS-severe	N	++	-	-	-	-	-	-	-	+	+	-	-
GW2/03/2/5	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	+	++	-	-
GW2/03/3/1	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	+	++	-	-
GW2/03/3/2	MP	Gutjwa	VC/DM	N	+	-	+	-	-	-	-	-	-	++	-	-
GW2/03/3/3	MP	Gutjwa	VC/DM	N	+	-	+	-	-	-	-	-	-	++	-	-
GW3/03/4/1	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	++	++	-	-
GW2/03/4/2	MP	Gutjwa	VC/CS	CS	+	-	-	-	-	-	-	-	-	++	-	-

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
GW2/03/4/3	MP	Gutjwa	VC/CS	VC/CS	+	-	-	-	-	-	+	-	+	++	-	-
GW2/03/5/1	MP	Gutjwa	VC/LD/CS	Chlrs/VB	+	-	-	-	-	-	-	-	-	-	++	-
GW2/03/5/2	MP	Gutjwa	VC	Chlrs /VB	+	-	-	-	-	-	-	-	+	+	-	-
GW2/03/5/3	MP	Gutjwa	VC/DM/CS	Chlrs /VB	+	-	-	-	-	-	-	-	-	+	++	-
GW2/03/6/1	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	++	+	-	-
GW2/03/6/2	MP	Gutjwa	VC/DM/CS	N	+	-	-	-	-	-	-	-	-	?	-	-
GW2/03/6/3	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
GW2/03/6/4	MP	Gutjwa	VC	N	++	-	-	-	-	-	-	-	-	++	-	-
GW2/03/7/1	MP	Gutjwa	DM/CS	N	+	-	-	-	-	-	-	-	++	+	-	-
GW2/03/7/2	MP	Gutjwa	DM/CS	N	+	-	-	-	-	-	-	-	+	++	-	-
GW2/03/7/3	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	-	++	-	-
GW2/03/7/4	MP	Gutjwa	VC/CS/DM	N	++	-	-	-	-	-	-	-	+	++	-	-
GW2/03/8/1	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	-	+	-	-
GW2/03/8/2	MP	Gutjwa	VC/LD/S	CS/LD	+	-	-	-	-	-	-	-	+	++	-	-
GW2/03/8/3	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	+	++	-	-
GW2/03/8/4	MP	Gutjwa	VC/CS	N	+	-	-	-	-	-	-	-	++	++	-	-
GW2/03/8/5	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	++	+	-	-
GW2/03/9/1	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
GW2/03/9/2	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	++	++	-	-
GW2/03/9/3	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	-	++	-	-
GW2/03/9/4	MP	Gutjwa	VC/CS	N	+	-	-	-	-	-	-	-	+	++	-	-
GW2/03/10/1	MP	Gutjwa	VC/DM/LD	CS/M/VB	+	-	-	-	-	-	-	-	+	++	++	-
GW2/03/10/2	MP	Gutjwa	VC/LD/S	CS/M/VB	+	-	-	-	-	-	-	-	++	++	++	-
GW2/03/10/3	MP	Gutjwa	VC/LD	CS/M/VB	+	-	-	-	-	-	-	-	++	++	++	-
GW2/03/10/4	MP	Gutjwa	VC/CS/M	CS/M/VB	+	-	-	-	-	-	-	-	++	++	++	-
E1-1	W. Cape	Ebenezer	VC	N	+	-	-	-	-	-	-	-	+	-	-	-
E1-3	W. Cape	Ebenezer	VC/CS	N	+	-	-	-	-	-	-	-	-	+	-	-
E1-6	W. Cape	Ebenezer	VC	N	+	-	-	-	-	-	-	-	+	-	-	-
E1-8	W. Cape	Ebenezer	VC/CS	N	-	-	-	-	-	-	-	-	+	+	-	-
E1-7	W. Cape	Ebenezer	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
E1-9	W. Cape	Ebenezer	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
E1-5	W. Cape	Ebenezer	VC	N	+	?	-	-	-	-	-	-	+	+	-	-

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
E1-10	W. Cape	Ebenezer	VC	N	+	-	-	-	-	-	-	-	+	-	-	-
E1-11	W. Cape	Ebenezer	VC	N	+	-	-	-	-	-	-	-	-	?	-	-
E1-13	W. Cape	Ebenezer	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
E2-1	W. Cape	Ebenezer	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
E2-3	W. Cape	Ebenezer	VC	N	+	-	-	-	-	-	+	-	-	+	-	-
E2-4	W. Cape	Ebenezer	VC/CS	VC	+	-	-	-	-	-	+	-	+	+	-	-
E3-2	W. Cape	Friemmersheim	DM/CS	N	+	-	+	+	-	-	-	-	-	+	-	-
FH1-8	W. Cape	Friemmersheim	VC/DM	N	+	-	-	-	-	-	-	-	-	++	-	-
FH2-10	W. Cape	Friemmersheim	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
FH1-11	W. Cape	Friemmersheim	VC	N	+	-	-	-	-	-	-	-	+	++	-	-
FH1-12	W. Cape	Friemmersheim	DM/CS/M	N	++	-	-	-	-	-	-	-	-	++	+	-
FH1-5	W. Cape	Friemmersheim	VC/CS	N	+	-	-	-	-	-	-	-	-	++	-	-
FH1-6	W. Cape	Friemmersheim	DM	N	+	-	-	-	-	-	-	-	-	++	-	-
FH1-1	W. Cape	Goedverwacht	DM	N	+	-	-	-	-	-	-	-	-	++	-	-
GT-1	W. Cape	Goedverwacht	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
GT-2	W. Cape	Goedverwacht	VC	VC	+	-	-	-	-	-	-	-	+	-	-	-
GT-3	W. Cape	Goedverwacht	DM	N	+	-	?	?	-	-	-	-	+	+	-	-
GT-7	W. Cape	Goedverwacht	VC	N	+	-	-	-	-	-	-	-	-	-	-	-
GT-5	W. Cape	Goedverwacht	VC/DM/CS	N	++	-	+	-	-	-	-	+	-	++	-	-
GT-10	W. Cape	Goedverwacht	VC	N	+	-	-	-	-	-	-	-	-	++	-	-
GT-6	W. Cape	Goedverwacht	CS	N	+	-	-	-	-	-	-	-	-	-	-	-
GT-8	W. Cape	Goedverwacht	DM/CS	N	+	-	-	-	-	-	-	-	+	++	-	-
GT-4	W. Cape	Goedverwacht	VC/CS	N	+	-	-	-	-	-	-	-	+	++	-	-
GT-1	W. Cape	Goedverwacht	CS/DM	N	+	-	-	-	-	-	-	-	-	-	-	-
GT1-7	W. Cape	Goedverwacht	CS	N	-	-	-	-	-	-	-	-	+	+	-	-
GT1-6	W. Cape	Goedverwacht	VC	N	+	-	-	-	-	-	-	-	-	?	-	-
GT1-12	W. Cape	Goedverwacht	VC/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
GT1-8	W. Cape	Goedverwacht	VC	N	-	-	-	-	-	-	-	-	+	+	-	-
GT1-14	W. Cape	Goedverwacht	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
GT1-3	W. Cape	Goedverwacht	VC/Cs	N	+	-	-	-	-	-	-	-	+	+	-	-
GT1-1	W. Cape	Goedverwacht	CS	N	+	-	-	-	-	-	-	-	-	-	-	-
GT1-13	W. Cape	Goedverwacht	VC	N	-	-	?	+	-	-	-	-	-	-	-	-

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
GT1-5	W. Cape	Goedverwacht	VC/LD	N	+	-	-	-	-	-	-	-	-	++	-	-
GT-9	W. Cape	Pacalsdorp	VC	N	+	-	?	-	-	-	-	-	++	-	-	-
PD1-10	W. Cape	Pacalsdorp	VC	N	+	-	?	-	-	-	-	-	+	-	-	-
PD1-5	W. Cape	Pacalsdorp	VC/chlrs	CS	+	-	-	-	-	-	-	-	+	-	-	-
PD1-7	W. Cape	Pacalsdorp	VC	N	+	-	-	-	-	-	-	-	-	-	-	-
PD1-11	W. Cape	Pacalsdorp	DM/CS	N	+	-	-	-	-	-	-	-	-	-	-	-
PD1-6	W. Cape	Pacalsdorp	VC/CS	N	+	-	-	-	-	-	-	-	++	-	-	-
PD1-1	W. Cape	Pacalsdorp	VC	N	+	-	-	-	-	-	-	-	?	-	-	-
PD1-15	W. Cape	Pacalsdorp	VC	N	+	-	-	-	-	-	-	-	+	-	-	-
PD1-13	W. Cape	Pacalsdorp	VC/CS	N	+	-	-	-	-	-	-	-	?	-	-	-
PD1-4	W. Cape	Pacalsdorp	VC/DM	CS	+	-	-	-	-	-	-	-	+	+	-	-
PD1-3	W. Cape	Saron	VC	N	+	-	-	-	-	-	-	-	+	++	-	-
S1-4	W. Cape	Saron	DM	N	-	-	-	-	-	-	-	-	+	-	-	-
S1-5	W. Cape	Saron	CS	N	-	-	-	-	-	-	-	-	+	-	-	-
S1-3	W. Cape	Saron	VC/CS	N	++	-	-	-	-	-	-	-	-	-	-	-
S1-6	W. Cape	Saron	VC/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
S1-7	W. Cape	Saron	VC	N	+	-	-	-	-	-	-	-	-	-	-	-
S2-4	W. Cape	Saron	VC/CS	N	+	-	-	-	-	-	-	-	+	-	-	-
S2-3	W. Cape	Saron	DM/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
S2-2	W. Cape	Saron	VC/DM	N	+	-	-	-	-	-	-	-	+	++	-	-
S2-5	W. Cape	Saron	Chlrs	N	?	-	-	-	-	-	-	-	-	++	-	-
S2-1	W. Cape	Saron	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
S2-9	W. Cape	Saron	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
S2-8	W. Cape	Saron	VC	N	+	-	-	-	-	-	-	-	-	-	-	-
S2-7	W. Cape	Saron	VC	N	+	-	-	-	-	-	-	-	-	++	-	-
S3-4	W. Cape	Saron	VC/LP	N	+	-	-	-	-	-	-	-	+	+	-	-
S3-3	W. Cape	Saron	DM	N	+	-	-	-	-	-	-	-	+	+	-	-
S3-5	W. Cape	Saron	F/VC	N	+	-	-	-	-	-	-	-	-	+	-	-
S3-1	W. Cape	Saron	DM	N	+	-	-	-	-	-	-	-	+	+	-	-
A1	E. Cape	Alice	CS	N	+?	-	-	-	-	-	-	-	-	+	-	-
A2	E. Cape	Alice	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
A3	E. Cape	Alice	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
A4	E. Cape	Burthust	VC/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
BT1-4	E. Cape	Burthust	VC/DM	N	+	-	+	-	-	-	-	-	+	+	-	-
BT1-3	E. Cape	Burthust	VC/DM	N	++	-	+	-	-	-	-	-	-	++	-	-
LM1-5	E. Cape	Lower Mpako	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
LM1-2	E. Cape	Lower Mpako	VC/DM	N	+	-	-	-	-	-	-	-	-	+	-	-
LM1-3	E. Cape	Lower Mpako	CS	N	+	-	-	-	-	-	-	-	-	+	-	-
LM1-4	E. Cape	Lower Mpako	CS	N	+	+	-	-	-	-	-	-	-	+	-	-
LM1-1	E. Cape	Mpako	DM/CS	N	+	-	-	-	-	-	-	-	-	-	-	-
MP1-1	E. Cape	Mpako	VC	CS	+	-	-	-	-	-	-	-	++	-	-	-
MP1-2	E. Cape	Mpako	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
MP1-4	E. Cape	Mpako	VC	N	+	-	-	-	-	-	-	-	+	++	-	-
MP1-3	E. Cape	Ncambedlana (Umtata)	VC	N	+	-	-	-	-	-	-	-	-	+	-	-
NC2-2	E. Cape	Port Alfred	VC/CS	N	+	-	-	+	-	-	-	?	+	+	-	-
PA1-2	E. Cape	Port Alfred	VC/DM	N	+	+	-	-	-	-	-	-	?	+	-	-
PA1-1	E. Cape	Port Alfred	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
PA2-1	E. Cape	Port Alfred	VC	N	+	-	-	-	-	-	-	-	-	-	-	-
PA2-4	E. Cape	Port Alfred	DM/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
PA2-5	E. Cape	Port Alfred	VC/DM	N	+	-	-	-	-	-	-	-	-	+	-	-
PA2-3	E. Cape	Tombo	Chlrs	N	-	-	-	-	-	-	-	-	+	+	-	-
TM1-1	E. Cape	Thombo	VC	N	+	-	-	-	-	-	-	-	+	-	-	-
TM2-1	E. Cape	Thombo	VC	N	+	-	-	-	-	-	-	-	+	-	-	-
VK 46	KZN	Mvundleni	VC	N	+	-	-	-	-	-	-	-	-	-	-	-
VK 8	KZN	Mvundleni	VC	N	+	-	-	-	-	-	-	-	+	-	+	-
VK 2	KZN	Mvundleni	VC	N	+	-	-	-	-	-	-	-	-	-	-	-
VK 3	KZN	Mvundleni	VC	N	-	-	-	-	-	-	-	-	+	-	-	-
VK 24	KZN	Mvundleni	VC	N	+	-	-	-	-	-	-	-	-	-	-	-
VK 25	KZN	Mvundleni	VC	N	-	-	-	-	-	-	-	-	+	-	-	-
VK 9	KZN	Mvundleni	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
VK 39	KZN	Mvundleni	VC/CS	N	+	-	-	-	-	-	-	-	-	-	-	-
MGL 6	N. West	Magolego	VC	N	+	-	-	-	-	-	-	-	+	++	-	-
MGL 21	N. West	Magolego	VC	N	+	-	-	-	-	-	-	-	+	++	-	-
MGL 1	N. West	Magolego	VC	N	+	-	-	-	-	-	-	-	-	+	-	-

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCSV WA
MLG 2	N.West	Magolego	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
MGL 3	N.West	Magolego	CS	N	+	-	-	-	-	-	-	-	-	+	-	-
MGL 7	N.West	Magolego	DM	N	?	-	-	-	-	-	-	-	+	-	-	-
MGL 9	N.West	Magolego	VC/DM	N	+	-	-	-	-	-	-	-	-	++	-	-
MGL 15	N.West	Magolego	VC/CS	N	+	-	-	-	-	-	-	-	-	+	-	-
MGL 12	N.West	Magolego	DM/CS	CS	+	-	?	-	-	-	-	-	+	+	-	-
MGL 8	N.West	Magolego	Chlrs	N	+	-	-	-	-	-	-	-	-	+	-	-
MGL 14	N.West	Magolego	VC	N	+	-	-	-	-	-	-	-	-	+	-	-
MGL 11	N.West	Magolego	VC	N	+	-	-	-	-	-	-	-	-	+	-	-
MGL 17	N.West	Magolego	DM/CS	N	+	-	-	-	-	-	-	-	-	-	-	-
MGL 18	N.West	Magolego	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
MGL 13	N.West	Magolego	VC	N	+	-	-	-	-	-	-	-	-	+	-	-

Symptoms→VC=vein clearing, CS=chlorotic spots, LP=leaf puckering, F=Feathering, VB=vein banding, DM=diffuse mottle, LD=leaf distortion, M=Mosaic, S-stunting, Chlrs=chlorosis & N=no symptoms
+=Virus detected, ++=highly positive, -=Virus not detected, ?=Faint reaction, *=sample positive when tested with sweet potato material from the field.

c. SPV G and SPCSV not tested

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII
Tshidane 1/37	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-
Tshidane 1/47	L.P	Tshidane	VC	N	+	-	+	-	-	-	-	-	-
Tshidane 1/13	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-
Tshidane 1/10	L.P	Tshidane	VC	N	+	-	+	-	-	-	-	-	-
Tshidane 1/15	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-
Tshidane 1/8	L.P	Tshidane	VC	N	+	-	+	-	-	-	-	-	-
2 MIW 7	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-
I MIW 3	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-
C MIW 3	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-
1 Tshiombo 1/2	L.P	Tshidane	VC	N	+	-	-	-	-	-	-	-	-

Symptoms→VC=vein clearing & N=no symptoms
+=Virus detected, -=Virus not detected.

d. SPVII, SPVG and SPCSV not tested

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV
KS2 3/3	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS2 3/2	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS2 3/5	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS2 3/1	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS2 2/2	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS 1-3/1	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS1 1/2	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS1-2/2	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS3 -2/1	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS1-2/5	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS2-2/1	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS1 2/4	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS3-1	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS1- 2/1	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS1-3/2	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS1-3/4	L.P	Klipspruit	VC	N	-	-	-	-	?	-	-
KS1-1/1	L.P	Klipspruit	VC	N	-	-	-	-	-	-	-
KS1-3/3	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS1-2/3	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS2-2/2	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
KS3-2/4	L.P	Klipspruit	VC	N	+	-	+	-	?	-	-
KS2-1	L.P	Klipspruit	VC	N	+	-	-	-	-	-	-
RS2-1/3	L.P	Riverside	VC	N	+	-	-	-	-	-	-
RS1 -1/1	L.P	Riverside	VC	N	+	-	-	-	-	-	-
RS2-2	L.P	Riverside	VC	N	+	-	-	-	-	-	-
RS2 1/2	L.P	Riverside	VC	N	+	-	-	-	-	-	-
RS2-4	L.P	Riverside	VC	N	+	-	-	-	-	-	-
RS1-2/1	L.P	Riverside	VC	N	+	-	+	-	-	-	-
RS1-1/2	L.P	Riverside	VC	N	+	-	-	+	?	-	-
RS2-5/3	L.P	Riverside	VC	N	+	-	-	-	-	-	-
RS2-2/1	L.P	Riverside	VC	N	+	-	-	-	-	-	-
RS1-2	L.P	Riverside	VC	N	+	-	-	-	-	-	-

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV
RS1-4	L.P	Riverside	VC	N	+	-	-	-	-	-	-
RS2-3	L.P	Riverside	VC	N	+	-	-	-	-	-	-
RS1-3	L.P	Riverside	VC	N	+	-	-	-	-	-	-
RS2-5/1	L.P	Riverside	VC	N	+	-	-	-	-	-	-
RS2-5/2	L.P	Riverside	VC	N	+	-	-	-	-	-	-
ARN 1/1/10	L.P	Athurstone	VC	N	+	-	+	-	-	-	-
ARN 1/1/2	L.P	Athurstone	VC	N	+	-	-	-	-	-	-
ARN 1/1/7	L.P	Athurstone	VC	N	+	-	-	-	-	-	-
ARN 2/1/3	L.P	Athurstone	VC	N	+	-	-	-	-	-	-
ARN 2/1/4	L.P	Athurstone	VC	N	+	-	-	-	-	-	-
ARN 1/1/1	L.P	Athurstone	VC	N	+	-	+	-	-	-	-
ARN 2/1/1	L.P	Athurstone	VC	N	+	-	-	-	-	-	-
ARN 2/1/5	L.P	Athurstone	VC	N	+	-	-	-	-	-	-
ARN 1/1/11	L.P	Athurstone	VC	N	+	-	+	-	-	-	-
ARN 2/1/6	L.P	Athurstone	VC	N	+	-	-	-	-	-	-
ARN 2/1/7	L.P	Athurstone	VC	N	+	-	-	-	-	-	-
DDE 4/1/3	L.P	Dingledale	VC	N	+	-	-	-	-	-	-
DDE 2/1/1	L.P	Dingledale	VC	N	+	-	-	-	-	-	-
DDE 5/1/12	L.P	Dingledale	VC	N	+	-	-	-	-	-	-
DDE 2/1/3	L.P	Dingledale	VC	N	+	-	-	-	-	-	-
DDE 5/1/6	L.P	Dingledale	VC	N	+	-	-	-	-	-	-
DDE 3/1/2	L.P	Dingledale	VC	N	+	-	-	-	-	-	-
DDE 5/1/4	L.P	Dingledale	VC	N	+	-	-	-	-	-	-

Symptoms→VC=vein clearing & N=no symptoms
+=Virus detected, -=Virus not detected

e. SPVIL, SPVG and SPCSV not tested

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV
Cull 1/5/3	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/2/5	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/1/2	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/5/4	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/1/1	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/5/5	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/5/1	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/4/2	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/4/1	Gauteng	Cullinen	VC	N	+	-	-	+	-	-	-
Cull 1/2/1	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/5/2	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/4/3	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/2/2	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/3/1	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/4/4	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/2/3	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/3/3	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/3/5	Gauteng	Cullinen	VC	N	+	-	-	+	?	-	-
Cull 1/1/3	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/3/5	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/1/4	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/2/4	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/4/5	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/3/2	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/1/1	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/5/5	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-
Cull 1/5/1	Gauteng	Cullinen	VC	N	+	-	-	-	-	-	-

Symptoms→VC=vein clearing & N=no symptoms

+ =Virus detected, - =Virus not detected & ? = Faint reaction

f. Old isolates from the previous survey (1997/1998)- SPVII, SPVG and SPCSV not tested

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV
TO 5.3	M.P	Tonga	VC	N	+	+	-	-	-	-	-
TO 3.3 ENGLAND	M.P	Tonga	VC	N	+	+	-	-	-	-	-
TO 3.4 SWAZI	M.P	Tonga	VC	N	+	+	-	-	-	-	-
TO 2.5 SWAZI	M.P	Tonga	VC	N	+	-	-	-	-	-	-
TO 3.7	M.P	Tonga	VC	N	+	-	+	-	-	-	-
TO 1/1	M.P	Thulamahashe	VC	N	+	-	-	+	?	-	-
TO 3.8 MOZA	M.P	Tonga	VC	N	+	-	-	-	-	-	-
TO 7(2)	M.P	Tonga	VC	N	+	-	-	-	-	-	-
TO 3.5 SWAZI	M.P	Tonga	VC	N	+	-	-	-	-	-	-
TO 2.1	M.P	Tonga	VC	N	+	-	-	-	-	-	-
T ½ SPMAFUTHA	M.P	Thulamahashe	VC/LD	N	+	-	-	-	-	-	-
M2/2/2	KZN	Mamfene	VC	N	+	-	-	+	-	-	-
M3/2/1	KZN	Mamfene	VC	N	+	-	-	-	-	-	-
M4/2/2	KZN	Mamfene	VC	N	+	-	-	-	-	-	-
M6/2/1	KZN	Mamfene	VC	N	+	-	+	+	-	-	-
M6/2/2	KZN	Mamfene	VC	N	+	-	-	-	-	-	-
M5/2/1	KZN	Mamfene	VC	N	+	-	-	-	-	-	-
M5/2/3	KZN	Mamfene	VC	N	+	+	-	-	-	-	-
M2/2/1	KZN	Mamfene	VC	N	+	-	-	-	-	-	-
THOMAS 1.6.A	N/A	N/A	VC	N	+	+	-	-	-	-	-
THOMAS 1.6.AMX	N/A	N/A	VC	N	+	-	-	-	-	-	-
THOMAS 1.6.CMX	N/A	N/A	VC	N	+	+	-	-	-	-	-
THOMAS 1.6.BMX	N/A	N/A	VC	N	+	-	+	-	-	-	-
THOMAS 1.6.B MX 600	N/A	N/A	VC	N	+	-	+	-	-	-	-
FRANK 5.2(MX)	N/A	N/A	VC	N	+	-	-	-	-	-	-
FRANK 5.2	N/A	N/A	VC	N	+	-	-	-	-	-	-
MB4 b	N/A	N/A	VC/LD	VB	+	+	-	-	-	-	-
MD1/1	N/A	N/A	VC	N	+	-	-	-	-	-	-
MD3	N/A	N/A	VC	N	+	-	+	-	-	-	-
MD1	N/A	N/A	VC	N	+	-	-	-	-	-	-
MD2	N/A	N/A	VC	N	+	-	-	-	-	-	-
PD1/2	N/A	N/A	VC	N	+	-	-	-	-	?	-

Isolates name	Province	Region/Subregion	Symp on I.set	Symp on SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV
PD1/2/1	N/A	N/A	VC	N	+	-	-	-	-	-	-
H2	L.P	Hoxane	VC	N	+	-	-	-	-	-	-
H16	L.P	Hoxane	VC	N	+	-	-	-	-	-	-
H3	L.P	Hoxane	VC	N	+	-	-	-	-	-	-
JIII	N/A	N/A	VC	N	+	-	-	-	-	-	-
J5/1	N/A	N/A	VC	N	+	-	+	-	-	-	-
VTF4C tshimbupfe	L.P	Tshimbupfe	VC	N	+	-	+	-	-	-	-
VTSB TSHILOMBO V	L.P	Tshiombo	VC	N	+	-	+	-	-	-	-
TSHILOMBO IV	L.P	Tshiombo	VC	N	+	+	-	-	-	-	-
TSHILOMBO IV I-1	L.P	Tshiombo	VC	N	+	-	-	-	-	-	-
TSHILOMBOIII VT-3	L.P	Tshiombo	VC	N	+	-	-	-	-	-	-
TSHILOMBO III VT-3A	L.P	Tshiombo	VC	N	+	-	-	-	-	-	-
TSHILOMBO V	L.P	Tshiombo	VC	N	+	+	-	-	-	-	-
TSHILOMBO IV VT-4B	L.P	Tshiombo	VC	N	+	-	-	-	-	-	-
MALAWUVE IVVM-4	L.P	N/A	VC	N	+	-	-	-	-	-	-
MALAWUVE III VM 3B	L.P	N/A	VC	N	+	+	-	-	-	-	-
MAVUNDLA1.9.2	L.P	N/A	VC	N	+	-	+	+	-	-	-
MAVUNDLA 1.9.2 MAX	L.P	N/A	VC	N	+	-	-	-	-	-	-
WC10	N/A	N/A	VC	N	+	+	-	-	-	-	-

Symptoms→VC=vein clearing, VB=vein banding, LD=leaf distortion & N=no symptoms
 +=Virus detected, -=Virus not detected, ?=Faint reaction
 N/A=Province or region not known

g. Samples collected with the EU-Sweet potato virus project team (2003)

* Sweet potato plants from the field

Isolates	Province	Region/sub region	Symptoms on sweet potato	SPCSVEA	SPCSVWA	SPMM V6F11	SPFMV1C 4	SPFMV 46B	SPF MV C CIP	SPV II	SPLV CIP	SPV G	SPMSV	SPCFV
GWA1	MP	Gutjwa	VC, CS	-	-	-	-	+	+	++	-	+	-	-
GWA10	MP	Gutjwa	Chlrs, CS	-	-	-	-	++	-	++	-	-	-	-
GWA11	MP	Gutjwa	M, LD	-	-	-	-	++	-	++	-	+	-	+
GWA12	MP	Gutjwa	M, CS	-	-	-	-	++	+	++	-	++	-	+
GWA2	MP	Gutjwa	VC, CS	-	-	-	-	+	-	++	-	++	-	-
GWA3	MP	Gutjwa		-	-	-	-	-	+	++	-	-	-	-
GWA4	MP	Gutjwa	VC	-	-	-	-	++	+	++	-	+	-	-
GWA5	MP	Gutjwa	CS	-	-	-	-	++	+	++	-	++	-	-
GWA6	MP	Gutjwa	Chlrs, LD	-	-	-	-	++	+	++	-	+	-	++
GWA7	MP	Gutjwa		-	-	-	-	+	-	++	-	-	-	-
GWA8	MP	Gutjwa		-	-	-	-	++	-	++	-	+	-	+
GWA9	MP	Gutjwa		-	-	-	-	++	-	++	-	++	-	++
GWB1	MP	Gutjwa	VC	-	-	-	-	++	+	++	-	+	-	-
GWB10	MP	Gutjwa	Chlrs, M	-	-	-	-	+	+	+	-	++	-	-
GWB11	MP	Gutjwa	VC, M, LD	-	-	-	-	+	+	+	-	++	-	-
GWB12	MP	Gutjwa	VC, LD, Chlrs	++	-	-	-	++	+	++	-	+	-	-
GWB13	MP	Gutjwa	VC	++	-	-	-	++	+	++	-	+	-	-
GWB14	MP	Gutjwa	VC, M, P	-	-	-	-	+	+	++	-	++	-	-
GWB15	MP	Gutjwa	LD, M,	-	-	-	-	++	++	++	-	++	-	-
GWB16	MP	Gutjwa	VC	-	-	-	-	++	+	+	-	++	-	-
GWB18	MP	Gutjwa	Wild <i>Ipomoea</i> , Chlrs, CS	-	-	-	-	+	+	-	-	++	-	-
GWB19	MP	Gutjwa	Wild <i>Ipomoea</i> -LD, S	-	-	-	-	+	+	+	-	++	-	-
GWB2	MP	Gutjwa	weed-LC	-	-	-	-	-	-	-	-	-	-	-
GWB20	MP	Gutjwa	Cucumber, CMV?	-	-	-	-	-	+	-	-	-	-	-
GWB3	MP	Gutjwa	M, Chlrs, M	-	-	-	-	++	+	-	-	+	-	-
GWB4	MP	Gutjwa	S, VC, LD	-	-	-	-	++	++	-	-	+	-	-
GWB5	MP	Gutjwa	VC, Chlrs, M	-	-	-	-	++	++	-	-	+	-	-
GWB6	MP	Gutjwa	CS	++	-	-	-	++	+	++	-	++	-	-
GWB7	MP	Gutjwa		++	-	-	-	++	++	-	-	+	-	-
GWB7	MP	Gutjwa	VC, LD and M	-	-	-	-	-	-	-	-	-	-	-

Isolates	Province	Region/sub region	Symptoms on sweet potato	SPCSVEA	SPCSVWA	SPMM V6F11	SPFMV1C 4	SPFMV 46B	SPF MV C CIP	SPV II	SPLV CIP	SPV G	SPMSV	SPCFV
GWB8	MP	Gutjwa	LD, VC, S	++	-	-	-	++	+	++	-	++	-	-
GWB8	MP	Gutjwa		+	-	-	-	++	+	++	-	++	-	-
GWB9	MP	Gutjwa	Wild <i>Ipomoea</i>	++	-	-	-	-	-	-	-	-	-	-
GWC1	MP	Gutjwa	VC and M	+	-	-	-	++	++	++	-	++	-	-
GWC2	MP	Gutjwa	VC	-	-	-	-	+	-	-	-	+	-	-
GWC3	MP	Gutjwa	VC	++	-	-	-	-	-	-	-	+	-	-
GWC4	MP	Gutjwa	VC, LD, S	-	-	-	-	++	+	++	-	+	-	++
GWC5	MP	Gutjwa	no symptoms	-	-	-	-	-	-	-	-	-	-	-
GWC6	MP	Gutjwa	M	-	-	-	-	-	-	-	-	-	-	-
HVA1	MP	Hazyview	CS	-	-	-	-	++	-	++	-	-	-	-
HVA2	MP	Hazyview	Chlrs	-	-	-	-	+	-	++	-	+	-	-
HVA3	MP	Hazyview	CS	-	-	-	-	++	-	+	-	-	-	-
HVA4	MP	Hazyview	CS	-	-	-	-	+	+	++	-	+	-	-
HVA5	MP	Hazyview	CS, pallor	-	-	-	-	+	-	++	-	++	-	++
HVB1	MP	Hazyview		-	-	-	-	+	-	-	-	++	-	-
HVB2	MP	Hazyview		-	-	-	-	+	-	+	-	+	-	-
HVB3	MP	Hazyview		-	-	-	-	++	-	-	-	+	-	-
HVB4	MP	Hazyview	cucumber	-	-	-	-	+	+	++	-	++	-	-
HVB5	MP	Hazyview		-	-	-	-	+	+	++	-	++	-	-
HVC6	MP	Hazyview		-	-	-	-	+	-	++	-	-	-	-
MH1	LP	Marblehall		-	-	-	-	-	-	-	-	-	-	-
MH2	LP	Marblehall		-	-	-	-	++	+	-	-	-	-	-
MH3	LP	Marblehall	Wild <i>Ipomoea</i>	-	-	-	-	-	-	-	-	-	-	-
MH4	LP	Marblehall		++	-	-	-	+	-	-	-	-	-	-
MH7	LP	Marblehall		-	-	-	-	-	+	-	-	-	-	-
MH8	LP	Marblehall		-	-	-	-	-	+	-	-	-	-	-
MH9	LP	Marblehall		+	-	-	-	-	-	-	-	-	-	-
MHB 9	LP	Marblehall	Chlrs	-	-	-	-	++	+	-	-	++	-	-
MHB1	LP	Marblehall		++	-	-	-	++	++	-	-	-	-	-
MHB10	LP	Marblehall	S, Chlrs, VC, LD	++	-	-	-	++	++	++	-	++	-	-
MHB11	LP	Marblehall	CS	-	-	-	-	++	+	+	-	-	-	-
MHB12	LP	Marblehall	CS	-	-	-	-	++	++	+	-	+	-	-

Isolates	Province	Region/sub region	Symptoms on sweet potato	SPCSVEA	SPCSVWA	SPMM V6F11	SPFMV1C 4	SPFMV 46B	SPF MV C CIP	SPV II	SPLV CIP	SPV G	SPMSV	SPCFV
MHB13	LP	Marblehall	M, LD and VC	-	+	-	-	++	+	+	-	++	-	-
MHB14	LP	Marblehall	LC, CS, VC	-	-	-	-	+	+	-	-	-	-	-
MHB15	LP	Marblehall	LC	-	-	-	-	-	-	-	-	-	-	-
MHB16	LP	Marblehall	Volunteer weed	-	-	-	-	++	++	-	-	++	-	-
MHB2	LP	Marblehall		-	++	-	-	++	++	++	-	++	-	-
MHB3	LP	Marblehall	VC, LD, Chlrs	-	+	-	-	++	++	++	-	++	-	-
MHB4	LP	Marblehall	LC	-	+	-	-	++	++	-	-	-	-	-
MHB5	LP	Marblehall	Chl, CSD, M, Chlrs	-	-	-	-	++	++	+	-	++	-	-
MHB6	LP	Marblehall		-	+	-	-	++	++	+	-	++	-	-
MHB7	LP	Marblehall		-	++	-	-	++	++	-	-	-	-	-
MHB8	LP	Marblehall	VC	-	+	-	-	++	+	+	-	++	-	-
NA1	MP	Numbi		-	-	-	-	+	-	-	-	++	-	-
NA2	MP	Numbi		-	-	-	-	-	-	-	-	+	-	-
NA3	MP	Numbi		-	-	-	-	++	-	++	-	+	-	-
NA4	MP	Numbi		-	-	-	-	-	-	++	-	+	-	-
NB1	MP	Numbi		-	-	-	-	++	++	+	-	++	-	++
NB2	MP	Numbi	weed	-	-	-	-	-	-	-	-	-	-	-
NB3	MP	Numbi		-	-	-	-	-	-	-	-	+	-	-

Symptoms→VC=vein clearing, CS=chlorotic spots, LC=leaf curl, LP=leaf puckering, VB=vein banding, DM=diffuse mottle, LD=leaf distortion, M=Mosaic, S=stunting, Chlrs=chlorosis & N=no symptoms
+=Virus detected, ++=highly positive, -=Virus not detected, ?=Faint reaction

* After they have been grafted to *I. setosa*

Isolates name	Province	Region/Subregion	Symp on I.set	Sympon SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCAV WA
GWA 1	MP	Gutjwa	VC/CS	VC	+	-	-	-	-	-	-	-	++	++	-	-
GWA 6	MP	Gutjwa	VC/DM	N	+	-	+	-	-	-	-	-	-	?	-	-
GWA 4	MP	Gutjwa	VC/CS	N	?	-	-	-	-	-	-	-	-	++	-	-
GWA 5	MP	Gutjwa	VC/DM	CS	+	-	-	-	-	-	-	-	++	++	-	-
GWA 7	MP	Gutjwa	VC/CS	VC	+	-	-	-	-	-	-	-	++	++	-	-
GWA 10	MP	Gutjwa	VC/CS/DM	N	+	-	-	-	-	-	-	-	++	++	-	-
GWA 11	MP	Gutjwa	VC/DM	VC	+	-	-	-	-	-	-	-	++	++	-	-
GWA 8	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	+	+	-	-
GWA 3	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	-	+	-	-
GWA 2	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	+	+	-	-
GWA 12	MP	Gutjwa	VC/CS	N	+	-	-	+	-	-	-	-	+	+	-	-
GWB 6	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	++	++	+	-
GWB 16	MP	Gutjwa	VC/DM/CS	N	+	-	+	-	-	-	-	-	++	++	-	-
GWB 10	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	-	++	-	-
GWB 11	MP	Gutjwa	VC/chlrs	N	+	-	-	-	-	-	-	-	++	++	-	-
GWB 8	MP	Gutjwa	VC	N	+	-	-	-	-	-	-	-	++	++	-	-
GWB 1	MP	Gutjwa	VC/DM	N	+	-	-	-	-	-	-	-	-	+	-	-
GWC 1	MP	Gutjwa	LD/CS-severe	N	+	-	-	-	-	-	-	-	+	++	-	-
GWC 2	MP	Gutjwa	VC/DM	VC	+	-	-	-	-	-	-	-	++	-	-	-
GWC 3	MP	Gutjwa	VC/DM	N	-	-	-	-	-	-	-	-	++	-	-	-
GWC 4	MP	Gutjwa	DM/CS	N	+	-	-	-	-	-	-	-	-	++	-	-
GWC 6	MP	Gutjwa	DM	N	+	-	-	-	-	-	-	-	-	++	-	-
GWC 5	MP	Gutjwa	VC/CS	N	+	-	-	-	-	-	-	-	+	++	-	-
HVA 4	MP	Gutjwa	VC/CS	N	+	-	-	-	-	-	-	-	+	+	-	-
HVA 2	MP	Hazyview	VC	CS	+	-	-	-	-	-	-	-	+	++	-	-
HVA 1	MP	Hazyview	VC	N	+	-	-	-	-	-	-	-	+	++	-	-
HVB 1	MP	Hazyview	VC/CS	VC	-	-	-	-	-	-	-	-	-	-	-	-
HVB 2	MP	Hazyview	VC/DM	N	+	-	-	-	-	-	-	-	++	-	-	-
HVB 14	MP	Hazyview	VC	N	+	-	-	-	-	-	-	-	-	+	-	-
HVB 5	MP	Hazyview	VC	CS	+	-	+	-	-	-	-	-	+	+	-	-
HVB 4	MP	Hazyview	VC/DM	N	+	-	-	-	-	-	-	-	+	++	-	-
HVC 6	MP	Hazyview	VC/chlrs	N	+	-	-	-	-	-	-	-	+	++	-	-

Isolates name	Province	Region/Subregion	Symp on I.set	Sympon SP	SPFMV	SPMMV	SPLV	SPCFV	C-6	CaLV	SPMSV	CMV	SPVII	SPVG	SPCSV EA	SPCAV WA
NA1	MP	Numbi	VC	N	+	-	-	-	-	-	-	-	-	+	-	-
NA3	MP	Numbi	VC/CS	N	-	-	-	-	-	-	-	-	+	-	-	-
NA4	MP	Numbi	VC	N	+	-	-	-	-	-	-	-	-	++	-	-
NB 3	MP	Numbi	DM/CS	N	+	-	-	-	-	-	-	-	-	++	-	-
NB1	MP	Numbi	DM/VC	N	+	-	-	-	-	-	-	-	-	-	-	-
MH 7	L.P	Marblehall	VC	N	+	-	-	-	-	-	-	-	+	-	-	-
MHB 9	L.P	Marblehall	DM	N	+	-	-	-	-	-	-	-	-	+	-	-

Symptoms → VC=vein clearing, CS=chlorotic spots, DM=diffuse mottle, LD=leaf distortion & N=no symptoms
 +=Virus detected, ++=highly positive, -=Virus not detected, ?=Faint reaction.

Appendix 5.2 Sweet potato plants that were grafted on *I. setosa* and showed no symptoms

Isolates name	Province	Region/Subregion	Isolates name	Province	Region/Subregion	Isolates name	Province	Region/Subregion
2 MIW 1	L.P	Mianzwi	E1-2	W. Cape	Ebenezer	VK 6	KZN	Mvundleni
2 MIW 2	L.P	Mianzwi	E2-5	W. Cape	Ebenezer	VK 7	KZN	Mvundleni
2 MIW 5	L.P	Mianzwi	E3-5	W. Cape	Ebenezer	VK 10	KZN	Mvundleni
T MIW 3	L.P	Mianzwi	FH1-13	W. Cape	Friemmersheim	VK 1	KZN	Mvundleni
Tshidane 1/9	L.P	Tshidane	GT1-11	W. Cape	Goedverwacht	VK 4	KZN	Mvundleni
Tshidane 1/31	L.P	Tshidane	GT1-10	W. Cape	Goedverwacht	VK 5	KZN	Mvundleni
Tshidane 1/43	L.P	Tshidane	GT1-9	W. Cape	Goedverwacht	VK 26	KZN	Mvundleni
Tshidane 1/32	L.P	Tshidane	GT1-2	W. Cape	Goedverwacht	VK 27	KZN	Mvundleni
Tshidane 1/26	L.P	Tshidane	PD1-12	W. Cape	Pacalsdorp	VK 28	KZN	Mvundleni
1 Tshiombo 1/14	L.P	Tshiombo	PD1-2	W. Cape	Pacalsdorp	VK 29	KZN	Mvundleni
1 Tshiombo 1/6	L.P	Tshiombo	PD1-14	W. Cape	Pacalsdorp	VK 21	KZN	Mvundleni
1 Tshiombo 1/11	L.P	Tshiombo	PD1-9	W. Cape	Pacalsdorp	VK 22	KZN	Mvundleni
1 Tshiombo 1/7	L.P	Tshiombo	S1-1	W. Cape	Saron	VK 23	KZN	Mvundleni
Tshiombo 1/12	L.P	Tshiombo	S1-2	W. Cape	Saron			
B1-2	MP	Beverbreed	S1-8	W. Cape	Saron			
B2-2	MP	Beverbreed	S1-10	W. Cape	Saron			
B2-1	MP	Beverbreed	S1-9	W. Cape	Saron			
B2-5	MP	Beverbreed	S3-2	W. Cape	Saron			
B2-3	MP	Beverbreed	BT1-1	E. Cape	Burthust			
B3-9	MP	Schuzendal	BT1-2	E. Cape	Burthust			
B3-1	MP	Schuzendal	BT1-5	E. Cape	Burthust			
B3-4	MP	Schuzendal	LM2-4	E. Cape	Lower Mpako			
B3-3	MP	Schuzendal	LM2-3	E. Cape	Lower Mpako			
B4-2	MP	Beverbreed	LM2-2	E. Cape	Lower Mpako			
B4-5	MP	Beverbreed	LM2-1	E. Cape	Lower Mpako			
B6-3	MP	Beverbreed	MP1-5	E. Cape	Mpako			
B6-6	MP	Beverbreed	MP2-3	E. Cape	Mpako			
GW 1-8	MP	Beverbreed	MP2-1	E. Cape	Mpako			
GW1-7	MP	Gutjwa	NC1	E. Cape	Ncambelana (Umtata)			
GW5-4	MP	Gutjwa	NC1-1	E. Cape	Ncambelana (Umtata)			

Isolates name	Province	Region/Subregion	Isolates name	Province	Region/Subregion	Isolates name	Province	Region/Subregion
GW5-8	MP	Gutjwa	NC1-3	E. Cape	Ncamedlana (Umtata)			
G3-2	MP	Gutjwa	NC2-1	E. Cape	Ncamedlana (Umtata)			
G3-1	MP	Gutjwa	NC2-3	E. Cape	Ncamedlana (Umtata)			
G3-5	MP	Gutjwa	PA1-3	E. Cape	Port Alfred			
G6-6	MP	Gutjwa	PA2-2	E. Cape	Port Alfred			
G5-20	MP	Gutjwa	PA2-5	E. Cape	Port Alfred			
G5-19	MP	Gutjwa	TM1-2	E. Cape	Tombo			
G5-18	MP	Gutjwa	TM1-3	E. Cape	Thombo			
G5-11	MP	Gutjwa	TM2-2	E. Cape	Thombo			
HV1-5	MP	Hazyview	TM3-1	E. Cape	Thombo			
HV2-1	MP	Numbi	TM3-2	E. Cape	Thombo			
HV2-2	MP	Numbi	TM3-3	E. Cape	Thombo			
HV3-4	MP	Hazyview	VK 48	KZN	Mvundleni			
HV3-5	MP	Hazyview	VK 49	KZN	Mvundleni			
HV4-12	MP	Burgerhall	VK 50	KZN	Mvundleni			
HV4-11	MP	Burgerhall	VK 41	KZN	Mvundleni			
HV4-2	MP	Burgerhall	VK 42	KZN	Mvundleni			
HV4-8	MP	Burgerhall	VK 44	KZN	Mvundleni			
HV6-12	MP	Ntsikazi	VK 36	KZN	Mvundleni			
HV6-9	MP	Ntsikazi	VK 38	KZN	Mvundleni			
HV7-1	MP	Hazyview	VK 31	KZN	Mvundleni			
HV7-5	MP	Hazyview	VK 32	KZN	Mvundleni			
T2-1	MP	Tonga	VK 33	KZN	Mvundleni			
T2-5	MP	Tonga	VK 17	KZN	Mvundleni			
T2-6	MP	Tonga	VK 18	KZN	Mvundleni			
T3-2	MP	Tonga	VK 19	KZN	Mvundleni			
T3-1	MP	Tonga	VK 11	KZN	Mvundleni			
T3-6	MP	Tonga	VK 12	KZN	Mvundleni			
T3-10	MP	Tonga	VK 13	KZN	Mvundleni			
T3-7	MP	Tonga	VK 14	KZN	Mvundleni			

LP (Limpopo)=14, MP (Mpumalanga)=46, Western Cape=18, Eastern Cape=24 and KZN (Kwazulu Natal)=31

Appendix 5.3 Sweet potato samples that showed symptoms on *I. setosa*, but tested negative with serological analysis

Isolates	Province	Region	Symtoms on <i>I. setosa</i>
2miw 10	LP	Mianzwi	VC
2MIW 4	LP	Mianzwi	VC
Tshid 1/20	LP	Tshidani	VC/DM
Tshid 1/17	LP	Tshidani	VC
Tshid 1/25	LP	Tshidani	VC
B3-10	MP	Beverbreed	VC/DM
T2-6	MP	Tonga	VC/CS
FH1-9	W.C	Fremeshein	VC/DM
PD1-8	W.C	Pacalsdorp	VC
BT2-3	E.C	Burthust	VC
VK 37	KZN	Mvundleni	VC
MGL 16	N.W	Magolego	VC

LP=Limpopo, MP=Mpumalanga, W.C=Western Cape, E.C=Eastern Cape, KZN=Kwazulu Natal and N.W=North West provinces of South Africa.